The 14th Biennial Conference of the International Association for Research on Epstein-Barr Virus & Associated Diseases



4-7 September 2010
University of Birmingham, UK

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Contents

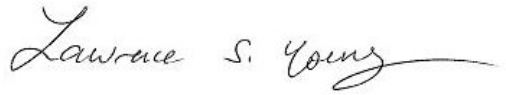
The 14th Biennial Conference of the International Association for Research on Epstein-Barr Virus and Associated Diseases

Welcome

Welcome to the 14th Biennial Conference of the International Association for Research on Epstein-Barr Virus and Associated Diseases. We are excited to be hosting this conference at the University of Birmingham where EBV research has been a major focus for over 25 years. The Association exists to promote and stimulate the exchange of ideas, knowledge and research materials among researchers throughout the world who study EBV and its related diseases. By organizing and conducting an International Symposium on EBV research every two years, the Association promotes and encourages co-operation between institutions, organizations and societies which have interests in common relating to basic and applied research on EBV and associated diseases. This conference provides an important opportunity to celebrate Alan Rickinson's retirement and acknowledge his outstanding contributions to our field. The Henle Lecture is awarded at each conference to a distinguished scientist who has made an enduring contribution to our field. This year we honour David Thorley-Lawson who has worked on various aspects of EBV biology and immunology for over 30 years. We are also honoured by the attendance of Harald zur Hausen whose seminal work on the association of EBV with nasopharyngeal carcinoma in 1970 was instrumental in establishing the oncogenic role of the virus beyond B lymphocytes.

We look forward to welcoming delegates from a variety of countries and with a range of backgrounds and expertise. We have made a determined effort to maximise the number of presentations at this conference and to provide adequate time for the viewing and discussion of posters. While offering a forum for established researchers, we also hope that this conference will provide an opportunity for junior fellows and post-graduate students to learn more about EBV.

When I originally suggested Birmingham as a venue for this conference, the Governing Board of the EBV Association were not convinced! I hope that having had an opportunity to visit the University and the city centre, you will appreciate the location and the ambition of the UK's second city.



Lawrence S. Young
President of the International Association for Research on Epstein-Barr Virus and Associated Diseases

Conference programme at a glance

	Saturday 4th	Sunday 5th	Monday 6th	Tuesday 7th
8.30am		Session 1 Latency I (abstracts 1-8)	Session 5 Immunology and Immunotherapy (abstracts 29-36)	Session 8 Epithelial cells Infection, Carcinoma (abstracts 50-57)
9.00am				
9.30am				
10.00am				
10.15am				
10.30am		Refreshments	Refreshments	Refreshments
10.45am				
11.00am		Session 2 Immunology (abstracts 9-14A)	Session 6 Virus Replication (abstracts 37-42)	Prof zur Hausen Lecture
11.30pm				
12.00pm				Session 9 Therapy (abstracts 58-61)
12.30pm			Lunch EBV Governors' Meeting	
12.45pm		Lunch		
1.00pm				Lunch EBV Association General Meeting
1.30pm				
2.00pm		Session 3 Genetics, Epigenetics, Non-coding RNA (abstracts 15-22)	Session 7 Latency II (abstracts 43-49)	
2.15pm				Session 10 Lymphoma (abstracts 62-68)
2.30pm				
3.00pm				
3.15pm				
3.30pm				
3.45pm			Refreshments	
4.00pm	Welcome Reception	Refreshments		Refreshments
4.15pm			Henle Lecture	
4.30pm		Session 4 Signalling (abstracts 23-28)		Session 11 Infection and Reactivation (abstracts 69-72)
4.45pm				
5.00pm			Poster Session & Reception	
5.15pm				
5.30pm				Alan Rickinson Lecture
6.00pm				
6.30pm				
7.00pm				
7.30pm		Social Evening Thinktank	Free Time	Conference Dinner Town Hall
8.00pm				
9.00pm				
10.00pm				
11.00pm				

EBV Association Governing Board

Martin Allday	UK
Yu-Sun Chang	Taiwan
Lori Frappier	Canada
Stephen Gottschalk	USA
Bettina Kempkes	Germany
Patrice Morand	France
Erle Robertson	USA
Rosmary Rochford	USA
George Sai-Wah Tsao	China
Alison Sinclair	UK

Local Organising Committee

Prof Lawrence Young	Head of College of Medical and Dental Sciences
Prof Paul Murray	Professor of Molecular Pathology, School of Cancer Sciences
Prof Martin Rowe	Professor of Tumour Virology, School of Cancer Sciences
Dr Andrew Bell	School of Cancer Sciences
Dr Chris Dawson	EBV:NPC Group, School of Cancer Sciences
Dr David Blackbourn	School of Cancer Sciences
Dr John Arrand	School of Cancer Sciences
Prof John Gordon	Head of MDS College Graduate School, Director (non-Clinical) Wellcome Trust Training Programme

Scientific Programme Committee

Martin Allday	UK
Paul Farrell	UK
Lori Frappier	Canada
Steve Gottschalk	USA
Margaret Gulley	USA
Ruth Jarrett	UK
Irene Joab	France
Bettina Kempkes	Germany
Shannon Kenney	USA
Rajiv Khanna	Australia
Paul Lieberman	USA
Maria Lung	Hong Kong
Maria Masucci	Sweden
Jaap Middeldorp	Netherlands
Georgios Mosialos	Greece
Erle Roberston	USA
Rosemary Rochford	USA
Alison Sinclair	UK
George Tsao	Hong Kong
Joanna Wilson	UK

Welcome to the 14th Biennial Conference of the International Association for Research on Epstein-Barr Virus and Associated Diseases

Meeting Venue

University of Birmingham
College of Medical and Dental Sciences
Vincent Drive
Edgbaston
Birmingham
B15 2TT

Email: med-cpdbookings@contacts.bham.ac.uk
Tel: +44 (0)121 414 8608

Campus Accommodation

Shackleton
The Vale
Edgbaston Park Road
Birmingham
B15 3SZ

Tel: +44 (0)121 415 8520

Internet access available in all rooms, please ask at Shackleton reception 24 hour reception

Lucas House
48 Edgbaston Road
Birmingham
B15 2RA

Tel: +44 (0)121 414 6232

24 hour reception

Breakfast will be served from 6.30am, (7am at Lucas House) coaches will then transfer delegates staying at campus accommodation to the conference, these will leave at 7.45am please wait at reception.

Name Badges
Please wear your name badge at all times during the conference, to the Social Evening and Conference Dinner.

Social Evening
Sunday 5th September, 7.45pm – 11pm, Thinktank, Millennium Point, Curzon Street, Birmingham, B4 7XG – smart casual

Conference Dinner
Tuesday 7th September, 7.30pm - 11pm, Town Hall, Victoria Square, Birmingham, B3 3DQ – smart casual

Transport
For those staying at Lucas House and Shackleton coaches have been arranged to transport delegates between accommodation and conference venues. Please wait for the coach in reception, at the times stated on the agenda.

A return coach from the Social Evening on Sunday 5th has been arranged for those staying in Birmingham City Centre. This will make one stop near the Hyatt Hotel on Broad Street.

Taxis - TOA – 0121 427 8888

University Attractions

Winterbourne House & Garden

Restored to its Edwardian Arts and Craft splendour, Winterbourne House is a unique heritage attraction set within seven acres of beautiful botanic gardens. Winterbourne is a hidden gem, home to beautiful antiques and over 6,000 plant species from around the world. Wander along the woodland walk, stroll through the hazelnut tunnel, cross the 1930's Japanese Bridge or simply soak up the tranquillity of this perfectly English Edwardian home.

Located on Edgbaston Park Road, a few minutes' walk from Lucas House and Shackleton.

Opening Times 10am – 6pm

EBV Badge Holders will be able to gain entry to Winterbourne House and Garden for a discounted rate of £3 per person.

Barber Institute of Fine Arts

Situated on the main campus of the University of Birmingham is the Barber Institute of Fine Arts.

The Barber Institute of Fine Arts contains one of the finest small collections of European art in the UK. Featuring works from 13th to the 20th century, it is a near-perfect gathering of some of the most influential artists of the previous millennium. The collection is made up of paintings, drawings, prints and sculpture. Among the artists represented are Bellini, Botticelli, Rubens, Van Dyck, Rembrandt, Monet, Degas, Matisse just to name a few.

Located near the East Gate on Main Campus

Opening Times: Monday - Saturday 10:00am – 5:00pm, Sunday 12:00pm – 5:00pm
Admission is Free

Travel Directions and Maps

By motorway

Approaching from the north west or south east along the M6:

- Leave at Junction 6 (signposted Birmingham Central) to join the A38(M)
- At the end of the motorway, keep to the right, go over a flyover, then through some underpasses to join the A38 Bristol Road
- The University is on your right, two and a half miles from the city centre

Approaching from the M42 north:

Leave at Junction 8 to join the M6 northbound and follow the instructions above

- Approaching from the south west:
- Leave the M5 at Junction 4 signposted Birmingham SW) to join the A38
- The University is approximately eight miles from the motorway

Approaching from the M40:

- It is easier to turn south on the M42 and leave at Junction 1, heading north on the A38 Bristol Road
- The University is approximately eight miles from the motorway

By rail

Most cross-country services to Birmingham arrive at New Street Station. Up to six trains an hour depart for the University on the cross-city line (ten minutes to University station, final destination Longbridge or Redditch). The centre of the main campus is a five-minute walk from University Station.

By coach

There are frequent express coach services to Birmingham from London, Heathrow and Gatwick Airports, and many UK cities. The long-distance coach station is in Digbeth in the city centre.

By bus

Numbers 61, 62 and 63 travel to the University's Edgbaston and Selly Oak campuses, while the 21 and 44 serve the Medical School and Queen Elizabeth Hospital. The services all run frequently from the city centre. There is a travel information office outside New Street Station, where you can obtain bus timetables and departure point information. Maps can be found throughout the city centre indicating bus stop locations.

By taxi

There are taxi ranks at New Street Station and throughout the city centre. The journey to the University takes about ten minutes.

By air

Birmingham International Airport has direct flights from locations in the UK, as well as from the USA, Canada, Europe and the Middle East.

The journey by taxi from the airport to the University takes approximately half an hour. Alternatively, Air-Rail Link provides a free, fast connection between the airport terminals and Birmingham International railway station. Air-Rail Link operates every two minutes (journey time 90 seconds). Birmingham International railway station has frequent services to New Street Station in the city centre (journey time around 15 minutes).

If you are arriving at London, there is a frequent train service from London Euston railway station to New Street Station (journey time around 1 hour 30 minutes).

- From Heathrow Airport. Take the Heathrow Express train to Paddington Station and then the Underground or a taxi to Euston Station. Alternatively, an Airbus runs from Heathrow Airport direct to Euston Station
- From Gatwick Airport. Take the Airport Express train to Victoria Station and then the Underground or a taxi to Euston Station

Oral Presentations

Oral presentations are strictly limited to 12 minutes, allowing an additional 3 minutes for questions.

Both PC and Macintosh computers are available for oral presentations in the Leonard Deacon Lecture Theatre, where the presentations will be made.

Powerpoint presentations should be brought on USB stick and loaded by the times stated below.

Identify the presentation with the abstract number, given previously, and with the last name of the presenting author.

To facilitate smooth running of the scientific sessions, *the use of personal laptops for presentations is actively discouraged.*

Timetable for uploading presentations to the podium computers in the Leonard Deacon lecture theatre:

Session	Abstract numbers	Upload by
1	1-8	During Registration
2	9-14A	11:00am, Sun 5th Sept
3	15-22	2:00pm, Sun 5th Sept
4	23-28	4:30pm, Sun 5th Sept
5	29-36	8:30 am, Mon 6th Sept
6	37-42	11:00am, Mon 6th Sept
7	43-49	2:00 pm, Mon 6th Sept
8	50-57	8:30am, Tues 7th Sept
9	58-61	12:00pm, Tues 7th Sept
10	62-68	2:15pm, Tues 7th Sept
11	69-72	4:30pm, Tues 7th Sept

Poster Viewing and Poster Sessions

The poster board dimensions are 1m x 1m. Please identify in the poster title the abstract number, given previously.

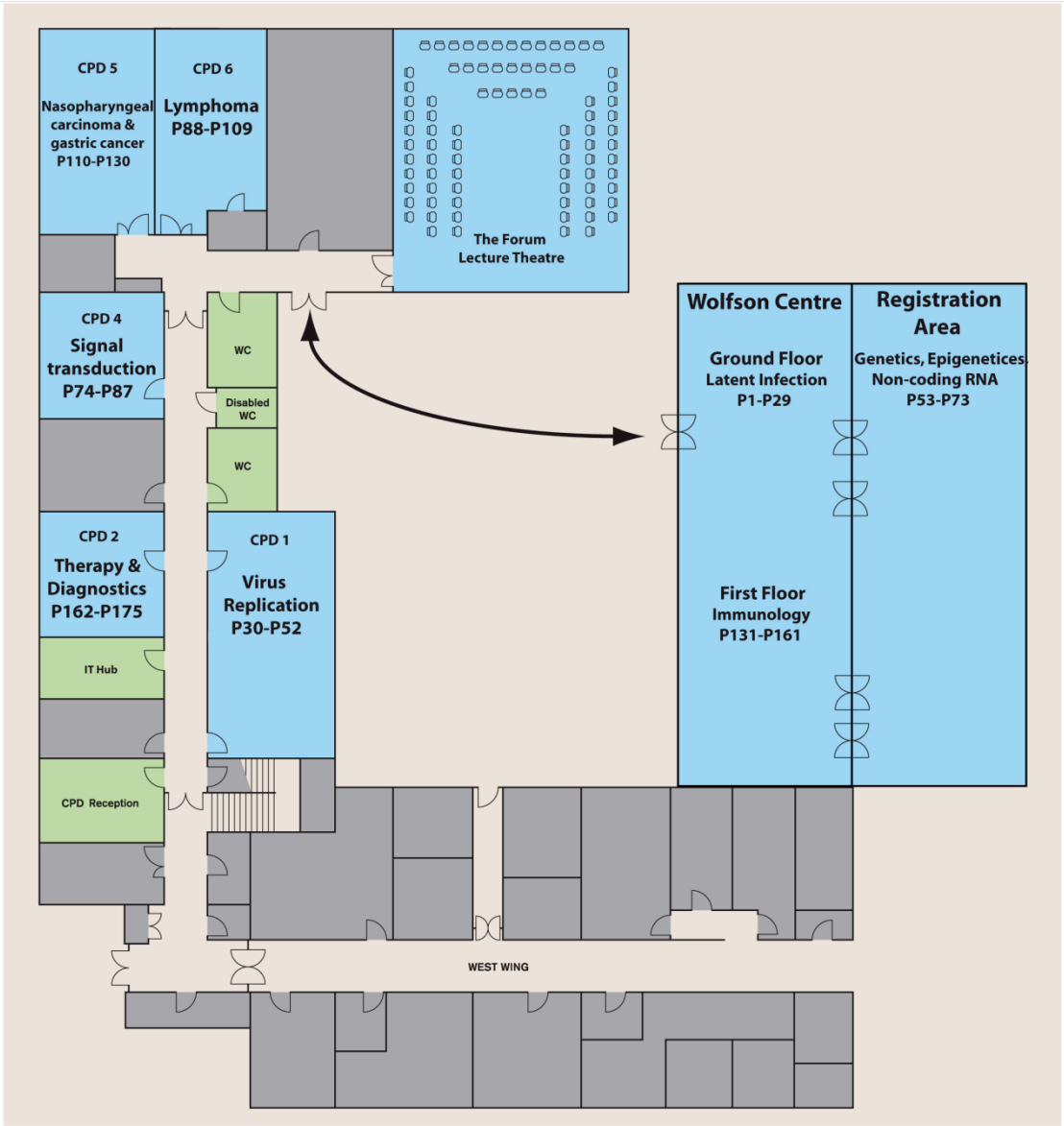
Posters can be placed on Sunday 5th September from 8:00am. The poster boards are located on the Ground and First Floor in the Wolfson Centre and in the Centre for Professional Development and will be available for viewing throughout the conference. Posters should be removed by 4:30pm Tuesday 7th September.

Poster Reception

Monday 6th September, 5:00pm – 7:30pm

Poster Areas

The poster boards are located on the Ground and First Floor in the Wolfson Centre and in the Centre for Professional Development and will be available for viewing throughout the conference.



Agenda

Date	Time	Event
Saturday 4th	4:00 – 7:00pm	Welcome Reception, The Great Hall, University Campus
	4:00 – 7:00pm	*Shuttle coach between Lucas House, Shackleton & The Great Hall*
Sunday 5th	*7:45am*	*Coach pick up at Lucas House & Shackleton*
	8:30 – 10:30am	Session 1 – Latency I (abstracts 1-8)
	10:30 – 11:00am	Break
	11:00am – 12:45pm	Session 2 – Immunology (abstracts 9-14A)
	12:45 – 2:00pm	Lunch
	2:00 – 4:00pm	Session 3 – Genetics, Epigenetics, Non-coding RNA (abstracts 15-22)
	4:00 – 4:30pm	Break
	4:30 – 6:00pm	Session 4 – Signalling (abstracts 23-28)
	6:15pm	*Coach pick up at College of Medical and Dental Sciences (MDS) to Lucas House & Shackleton*
	7:30pm	*Coach pick up at Lucas House & Shackleton*
	7:45 – 11:00pm	Social Evening, Thinktank
Monday 6th	*7:45am*	*Coach pick up at Lucas House & Shackleton*
	8:30 – 10:30am	Session 5 – Immunology and Immunotherapy (abstracts 29-36)
	10:30 – 11:00am	Break
	11:00am – 12:30pm	Session 6 – Virus Replication (abstracts 37-42)
	12:30 – 2:00pm	Lunch & EBV Governors' Meeting
	2:00 – 3:45pm	Session 7 – Latency II (abstracts 43-49)
	3:45 – 4:15pm	Break
	4:15 – 5.00pm	Henle Lecture
	5:00 – 7:30pm	Poster Session & Reception
	7:30pm	*Coach pick up at MDS to Lucas House & Shackleton*

Tuesday 7th	*7:45am*	*Coach pick up at Lucas House & Shackleton*
	8:30 – 10:30am	Session 8 – Epithelial cells, Infection, Carcinoma (abstracts 50-57)
	10:30 – 11:00am	Break
	11:00am – 12:00pm	Prof zur Hausen Lecture – Engagement of infectious Agents in Human Cancers
	12:00 – 1:00pm	Session 9 – Therapy (abstracts 58-61)
	1:00 – 2:15pm	Lunch & Optional EBV Association General Meeting (Leonard Deacon Lecture Theatre)
	2:15 – 4:00pm	Session 10 – Lymphoma (abstracts 62-68)
	4:00 – 4:30pm	Break
	4:30 – 5:30pm	Session 11 – Infection and Reactivation (abstracts 69-72)
	5:30 – 6:00pm	Alan Rickinson Lecture
	6:15pm	*Coach pick up at MDS to Lucas House & Shackleton*
	7:15pm	*Coach pick up at Lucas House & Shackleton*
	7:30 – 11:00pm	Conference Dinner, Town Hall

Detailed Scientific Meeting Agenda

Session 1. Latency I

Sunday 5th September 8:30am-10:30am

Session Chairs: Bettina Kempkes and Erle Robertson

Abstract #	Title	Author
1	**EBNA1 DISRUPTS PML NUCLEAR BODIES THROUGH A DIRECT INTERACTION WITH PROTEIN KINASE CK2**	Jennifer Yinuo Cao, Nirojini Sivachandran and Lori Frappier
2	**FUNCTIONAL DIFFERENCES BETWEEN TYPE 1 AND TYPE 2 EBV EBNA2**	Laila Cancian, Walter Lucchesi, Claudio Elgueta Karstegl and Paul J. Farrell
3	**EBNA2 DRIVES IMMORTALISATION THROUGH PTEFB RECRUITMENT TO CP AND LONG-RANGE SERINE 2 PHOSPHORYLATION OF THE RNA POLYMERASE II CTD**	Richard D. Palermo, Helen M. Webb and Michelle J. West
4	**THE UBIQUITIN C TERMINAL HYDROLASE L1 IS INDUCED BY EBNA2 IN TRANSFORMED B-LYMPHOCYTES AND IS ASSOCIATED WITH MITOTIC SPINDLE**	Anjali Bheda, Julia Shackelford, Joseph S Pagano
5	**KINETICS OF EBV INFECTION OF B CELLS BY KNOCKOUT VIRUSES WITH DEFECTS IN THE LATENT MEMBRANE PROTEIN 2 GENE**	Laura Wasil, Shushen Xu, Monica Tomaszewski, and David T. Rowe
6	**CELL GENE REGULATION ASSOCIATED WITH EBER EXPRESSION**	Goran Gregorovic, Rachel Bosshard, Claudio Elgueta, Oliver Dittrich-Breiholz, Michael Kracht, Rainer Russ and Paul J. Farrell
7	**EBV IMMORTALIZATION ALTERS TELOMERE CHROMATIN STRUCTURE AND TERRA TRANSCRIPTION**	Deng, Z., Wang, P., Tsai, K., and Lieberman, P. M.
8	**AN ATM/CHK2-MEDIATED DNA DAMAGE RESPONSIVE SIGNALING PATHWAY SUPPRESSES EPSTEIN-BARR VIRUS TRANSFORMATION OF PRIMARY HUMAN B CELLS**	Pavel A. Nikitin, Chris Yan, Eleonora Forte, Alessio Bocedi, Jay Tourigny, Amee Patel, Sandeep Dave, William Kim, Katherine Hu, Jing Guo, David Tainter, Olena Rusyn, and Micah Luftig

Session 2. Immunology

Sunday 5th September 11:00am-12:45pm

Session chairs: Maria Masucci and Rajiv Khanna

Abstract #	Title	Authors
9	**THE EBV-ENCODED BILF1 PROTEIN MODULATES IMMUNE RECOGNITION OF ENDOGENOUSLY PROCESSED ANTIGEN BY TARGETING MHC CLASS I MOLECULES TRAFFICKING ON BOTH THE EXOCYTIC AND ENDOCYTIC PATHWAYS**	Jianmin Zuo, Jennifer Tamblyn, Wendy Thomas and Martin Rowe
10	**EPSTEIN-BARR VIRUS BNLF2A-MEDIATED T-CELL EVASION DURING PRODUCTIVE INFECTION**	Daniëlle Horst, Vincenzo Favaloro, Fabio Vilardi, Stephen High, Bernhard Dobberstein, Andrew Hislop, Alan Rickinson, Maaike Ressing and Emmanuel Wiertz
11	**PURIFIED HEXAMERIC SBARF1 PROTEIN IS A DECOY RECEPTOR FOR MACROPHAGE COLONY STIMULATING FACTOR AND INTERFERES WITH MACROPHAGE DIFFERENTIATION AND ACTIVATION, BUT HAS NO DETECTABLE MITOGENIC ACTIVITY**	EK Hoebe, T Le Large, SJ Stevens, AE Greijer and JM Middeldorp.
12	**EPSTEIN-BARR VIRUS LYTIC-PHASE PROTEIN BGLF5 REDUCES TOLL-LIKE RECEPTOR 9 EXPRESSION DURING PRODUCTIVE INFECTION**	Michiel van Gent, Bryan Griffin, Femke Berkhoff, Femke Stalpers, Daphne van Leeuwen, Franca Hartgers, Emmanuel Wiertz, Maaike Ressing
13	**REGULATION OF THE IMMUNO –INHIBITOR B7-H1 EXPRESSION IN EPSTEIN BARR VIRUS INFECTED B-CELLS**	Stéphanie Durand-Panteix, Pauline Rouaud, Amandine David, Nathalie Faumont, Jean Feuillard, Chantal Jayat-Vignoles
14	**IMPAIRED EPSTEIN-BARR VIRUS-SPECIFIC CD8+ T CELL FUNCTION IN X-LINKED LYMPHOPROLIFERATIVE DISEASE IS RESTRICTED TO SLAM FAMILY POSITIVE B CELL TARGETS**	Andrew D. Hislop, Umaimainthan Palendira, Alison M. Leese, Peter D. Arkwright, Pierre S. Rohrlich, Stuart G. Tangye, H. Bobby Gaspar, Arjan C. Lankester, Alessandro Moretta, Alan B. Rickinson
14A	**LMP1 CAUSES CHRONIC INFLAMMATION PRIOR TO NEOPLASIA IN VIVO**	Asif Qureshi, Adele Hannigan & Joanna B.Wilson

Session 3. Genetics, Epigenetics, Non-coding RNA

Sunday 5th September 2:00pm-4:00pm

Session chairs: Paul Farrell and Paul Lieberman

Abstract #	Title	Authors
15	**EBV'S CHROMATIN IN A CLOSE-UP: A FURTHER STEP TOWARDS UNDERSTANDING EBV'S LIFE CYCLE**	Anne Schmeinck, Martin Bergbauer, Markus Kalla, and Wolfgang Hammerschmidt
16	**POLYCOMB-MEDIATED REPRESSION OF THE PRO-APOPTOTIC TUMOR SUPPRESSOR GENE *BIM* BY EBNA3A AND EBNA3C**	Kostas Paschos, Robert E White, Jade Yee and Martin J Allday
17	**CELLULAR MICRORNAS 200B AND 429 REGULATE THE EBV LATENT-LYTIC SWITCH VIA ZEB1 AND ZEB2**	A.L. Ellis, I. Xu, T. Iempridee, X. Yu, and J.E. Mertz
18	**UPREGULATION OF MIR155 IN NASOPHARYNGEAL CARCINOMA RESULTS DOWNREGULATION OF JMJD1A, A NEGATIVE PROGNOSTIC MARKER**	Zi-Ming Du, Li-Fu Hu, Hai-Yun Wang, Li-Xu Yan, Yi-Xin Zeng, Jian-Yong Shao, Ingemar Ernberg
19	**IDENTIFYING MRNA TARGETS FOR EBV-DERIVED AND CELLULAR MICRORNAS EXPRESSED IN LYMPHOBLASTOID CELL LINES**	Rebecca Skalsky, David Corcoran, Eva Gottwein, Markus Hafner, Jeff Nusbaum, Micah Luftig, Uwe Ohler, Thomas Tuschl, and Bryan Cullen
20	**EBV-ENCODED MICRO RNAS PROMOTE CELL CYCLE PROGRESSION AND PREVENT APOPTOSIS OF PRIMARY HUMAN B CELLS**	Eri Seto, Andreas Moosmann, Sebastian Grömminger, Nicole Walz, Adam Grundhoff, and Wolfgang Hammerschmidt.
21	**INHIBITION OF APOPTOSIS BY EBV BART MIRNAS IN GASTRIC CARCINOMA**	Aron R. Marquitz, Cyd Stacy Nam, Anuja Mathur and Nancy Raab-Traub
22	**FUNCTIONAL DELIVERY OF EBV-MIRNAS VIA EXOSOMES**	D. Michiel Pegtel, Kat Cosmopoulos, David A. Thorley-Lawson, Monique v. Eijndhoven, Erik Hopmans, Tanja D. de Gruijl, Jelle L. Lindenberg, Thomas Würdinger and Jaap M. Middeldorp

Session 4. Signalling

Sunday 5th September 4:30pm-6:00pm

Session chairs: George Tsao and Irene Joab

Abstract #	Title	Authors
23	**ARGININE DIMETHYLATION OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 2 IS REQUIRED FOR PROMOTER BINDING AND ASSOCIATION WITH CELL PROTEINS**	Henrik Gross, Stephanie Barth, Richard D. Palermo, Alfredo Mamiani, Christine Hennard, Ursula Zimber-Strobl, Michelle J. West, Marlies Sauter, Nikolaus Mueller-Lantzsch, Elisabeth Kremmer, and Friedrich A. Grässer
24	**ACTIVATION OF TOLL-LIKE RECEPTOR 3 SIGNALING BY EBER CONTRIBUTES TO GASTRIC CARCINOGENESIS**	Dai Iwakiri, Takeharu Minamitani, Kenzo Takada
25	**SUBTRACTIVE PROTEOMICS IDENTIFIES AN ESSENTIAL ROLE OF A STE20 GERMINAL CENTER KINASE IN JNK SIGNALING BY LMP1**	Anna Shkoda, Jennifer Town, Janine Griese, Fabian Giehler, Cinthia Briseño, Arnd Kieser
26	**IDENTIFICATION OF TMEM134 AS A NOVEL LMP1-BINDING PROTEIN USING BIMOLECULAR FLUORESCENCE COMPLEMENTATION AND AN ENHANCED RETROVIRAL MUTAGEN**	Amanda Emery, Pooja Talaty, and David N. Everly Jr.
27	**THE EPSTEIN-BARR VIRUS ENCODED PROTEIN COMPLEX BDLF2/BMRF2 INDUCES MORPHOLOGICAL CHANGES BY A PKC-RELATED INHIBITION OF ERM-PRITEINS**	J. Mühe, K. Ritter, and M. Kleines
28	**EPSTEIN-BARR VIRUS UTILIZES EXOSOMES FOR INTERCELLULAR COMMUNICATION**	David G. Meckes, Jr., Kathy H.Y. Shair, Aron R. Marquitz, Che-Pei Kung, Rachel H. Edwards, and Nancy Raab-Traub

Session 5. Immunology and Immunotherapy

Monday 6th September 8:30am-10:30am

Session chairs: Emmanuel Wiertz and Eva Klein

Abstract #	Title	Authors
29	**GENE EXPRESSION SIGNATURE OF PRIMARY EBV INFECTION DERIVED FROM A PROSPECTIVE STUDY**	Kristin A. Hogquist, Samantha K. Dunmire, Jean L. Porter, Oludare A. Odumade, and Henry H. Balfour Jr
30	**MALARIA CO-INFECTIONS SELECTIVELY EDIT IMMUNITY TO EBV LEADING TO DIFFERENTIAL EVOLUTION OF LYTIC AND LATENT EPITOPE-SPECIFIC MEMORY $CD8^+$ T CELLS**	Pratip K. Chattopadhyay, Kiprotich Chelimo, Paula B. Embury, Emma Gostick, Mario Roederer, David A. Price, Ann M. Moormann
31	**BROAD TARGETING OF THE $CD4^+$ T CELL RESPONSE TOWARDS THE EPSTEIN-BARR VIRUS (EBV) LYTIC CYCLE ANTIGENS**	Heather M. Long, Alison M. Leese, Odette L. Chagoury, Shawn R. Connerty, Jared Quarcoopome, Laura L. Quinn, Alan B. Rickinson
32	**CD8+ CYTOTOXIC T CELLS SPECIFIC FOR LATE LYTIC INFECTION PROTEINS ARE PRESENT IN RHESUS MACAQUES WITH PERSISTENT LYMPHOCRYPTOVIRUS INFECTION**	Nina Orlova, Angela Carville, Fred Wang, and Mark Fogg
33	**INDUCTION OF EBV-SPECIFIC IMMUNE RESPONSES BY VIRUS LIKE PARTICLES**	Romana Ruiss, Wolfgang Hammerschmidt, Reinhard Zeidler
34	**EBV-SPECIFIC T CELL RECEPTOR GENE TRANSFER TO TARGET NASOPHARYNGEAL CARCINOMA**	Yong Zheng, Lee Machado, Beatrice Johnson, Christine James, Gregory Parsonage and Steven P. Lee
35	**DEVELOPMENT OF CHIMERIC T CELLS AGAINST EBV-ASSOCIATED TUMOURS**	I. Johannessen, S.J. Talbot, D.H. Crawford
36	**ADVANCES IN T CELL THERAPY FOR EBV TYPE 2 LATENCY LYMPHOMAS**	Cliona Rooney, Catherine Bollard, Stephen Gottschalk, Ann Leen, Adrian Gee, Malcolm Brenner and Helen Heslop

Session 6. Virus Replication

Monday 6th September 11:00am-12:30pm

Session chairs: Alison Sinclair and Shannon Kenney

Abstract #	Title	Author
37	**DYNAMIC CHROMATIN STRUCTURE AT EBV ORIGIN OF LYTIC REPLICATION AND EARLY PROMOTERS DURING EBV REPLICATION**	Sharada Ramasubramanyan, Kay E Osborn and Alison Sinclair
38	**THE CELLULAR STRESS SENSOR, OCT-1, COOPERATES WITH THE IMMEDIATE-EARLY PROTEIN, BRLF1, TO INDUCE EBV LYTIC REACTIVATION**	Amanda R. Robinson, Swee Sen Kwek, Shannon C. Kenney
39	**EPSTEIN-BARR VIRUS LF2 PROTEIN REGULATES VIRAL REPLICATION BY ALTERING RTA SUBCELLULAR LOCALIZATION**	Andreas M. F. Heilmann, Michael A. Calderwood, and Eric Johannsen
40	**EBV RTA INHIBITS PROTEIN KINASE CK2**	Vineetha Raghavan, Jill Countryman, Duane Shedd, and George Miller
41	**EB2, THE EBV VIRAL MRNA EXPORT PROTEIN INTERACTS WITH SRP20, A FACTOR IMPLICATED IN CELLULAR MRNA SPLICING AND EXPORT**	Franceline Juillard, Quentin Bazot, Fabrice Mure, Lionel Tafforeau, Chantal Rabourdin-Combe, Vincent Lotteau, Evelyne Manet, Henri Gruffat
42	**EPSTEIN-BARR VIRUS SM PROTEIN MODULATES RNA SPLICING BY INTERACTING WITH SPLICING FACTOR SRP20**	Dinesh Verma, Swarna Bais, Melusine Gaillard and Sankar Swaminathan

Session 7. Latency II

Monday 6th September 2:00pm-3:45pm

Session chairs: Martin Allday and Joanna Wilson

Abstract #	Title	Author
43	**MOLECULAR MECHANISM OF EPIGENETIC GENE SILENCING BY EBNA-3A**	Marie Hertle and Bettina Kempkes
44	**EPSTEIN-BARR VIRUS NUCLEAR PROTEIN EBNA3C CONTRIBUTES TO THE GROWTH OF EBV-TRANSFORMED B CELLS THROUGH INHIBITING INK4A/ARF EXPRESSION**	Seiji Maruo, Elliott Kieff, and Kenzo Takada
45	**THE CELL-CYCLE CONTROL GENE RGC-32IS UPREGULATED BBY EBNA 3C AND SELECTIVELY TRANSLATED IN LATENCY III CELLS**	Sandra N. Schlick, Helen M. Webb, C. David Wood, Andrea Gunnell and Michelle J. West
46	**EBNA 3A REPRESSES CDKN2A AND CDKN2B EXPRESSION THROUGH INTERACTION WITH MIZ-1**	Quentin Bazot, Lionel Tafforeau, Chantal Rabourdin-Combe, Vincent Lotteau, Henri Gruffat, Evelyne Manet
47	**BNLF2A AND BNLF2B ARE EXPRESSED AS LATENT GENES IN EBV-INFECTED B LYMPHOCYTES AND EPITHELIAL CELLS**	Hironori Yoshiyama, Asuka Nambo, and Kenzo Takada
48	**LMP1 INDUCED NFKB PROMOTES GLUCOSE METABOLISM VIA GLUT1 MEMBRANE TARGETING**	Thomas Sommermann, Kathleen O'Neill, Ellen Cahir-McFarland
49	**THE EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN-1 (EBNA1) ACTIVATES ALTERNATIVE LENGTHENING OF TELOMERE VIA INDUCTION OF OXIDATIVE STRESS**	Siamak Akbari Kamranvar, and Maria G. Masucci

Session 8. Epithelial cells, Infection, Carcinoma

Tuesday 7th September 8:30am-10:30am

Session chairs: Maria Lung and Margaret Gulley

Abstract #	Title	Authors
50	**IMPORTANT BUT DIFFERENTIAL ROLES FOR ACTIN IN TRAFFICKING OF EBV IN B CELLS AND EPITHELIAL CELLS**	Sarah M. Valencia and Lindsey M. Hutt-Fletcher
51	**EPSTEIN-BARR VIRUS INFECTION OF POLARISED EPITHELIAL CELL LAYERS BY B CELL-MEDIATED TRANSFER INFECTION: COMPONENTS OF THE VIROLOGICAL SYNAPSES**	Claire Shannon-Lowe, Alan Rickinson and Martin Rowe
52	**EBV-POSITIVE NPC TUMOURS CONTAIN CELLS DISPLAYING A STEM CELL PHENOTYPE AND SHOW DEREGULATED EXPRESSION OF THE HEDGEHOG SIGNALLING**	Rebecca Port, Chunfang Hu, Khilan Shah, Lawrence S. Young, John R. Arrand and Christopher W. Dawson
53	**EPSTEIN-BARR VIRUS-ENCODED LMP2A INDUCES AN EPITHELIAL-MESENCHYMAL TRANSITION AND INCREASES THE NUMBER OF SIDE POPULATION STEM-LIKE CANCER CELLS IN NASOPHARYNGEAL CARCINOMA**	Qing-Li Kong, Li-Huan Hu, Jing-Yan Cao, Li-Hua Xu, Yi-Liang, Xing Zhang, Man-Zhi Li, Yun-Fei Xia, Li-Bing Song, Yi-Xin Zeng and Mu-Sheng Zeng
54	**ABERRANT NOTCH3 EXPRESSION IN EBV-ASSOCIATED NASOPHARYNGEAL CARCINOMA**	Cheuk-Him Man, Samantha Wei-Man Lun, Jan Wai-Ying Hui, Chit Chow, Kwong-Wai Choy, Ka-Fai To, Pierre Busson, and Kwok-Wai Lo
55	**FUNCTIONAL INVESTIGATION OF TUMOR AND ANGIOGENESIS SUPPRESSIVE CANDIDATE TUMOR SUPPRESSOR, *CYSTEINE-RICH INTESTINE PROTEIN 2* IN NASOPHARYNGEAL CARCINOMA**	Arthur Kwok, Leung Cheung, Josephine Mun Yee Ko, Hong Lok Lung, Eric J. Stanbridge, Eugene R. Zabarovsky, Dora Li-wan Kwong, Daniel Chua[1], Sai Wah Tsao, and Maria Li Lung
56	**FUNCTIONAL EPIGENOMICS IDENTIFIES UBE2L6 AS A TUMOR SUPPRESSOR GENE IN HUMAN NASOPHARYNGEAL CARCINOMA**	Xiaoying Zhou, Jiazhang Wei, Qian He, Xue Xiao, Shumin Wang, Longde Lin, Guangwu Huang and Zhe Zhang
57	**EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 IS NOT ASSOCIATED WITH VESSEL DENSITY NOR WITH HYPOXIA INDUCIBLE FACTOR 1 ALPHA EXPRESSION IN NASOPHARYNGEAL CARCINOMA TISSUE**	Alexander A. Benders, Weihua Tang, Jaap M. Middeldorp, Astrid E. Greijer, Leigh B. Thorne, William K. Funkhouser, W. Kimryn Rathmell, Margaret L. Gulley

Session 9 Therapy

Tuesday 7th September 12:00pm-1:00pm

Session chairs: Jaap Middeldorp and Steve Gottschalk

Abstract #	Title	Author
58	**A XENOTRANSPLANT MODEL OF CHRONIC ACTIVE EPSTEIN-BARR VIRUS (EBV) INFECTION BY USE OF NOG MICE**	Ken-Ichi Imadome, Misako Yajima, Ayako Arai, Atsuko Nakagawa, Fuyuko Kawano, Sayumi Ichikawa, Hiroyuki Nakamura, Osamu Miura, Mamoru Ito, Norio Shimizu, Naoki Yamamoto, and Shigeyoshi Fujiwara
59	**EBV-POSITIVE DLBCL OF THE ELDERLY IS A DISTINCT CLINICO-BIOLOGICAL ENTITY WITH POOR OUTCOME IN CHOP-R TREATED PATIENTS, WITH PROPERTIES LIKELY AMENABLE TO ANTI-EBV TARGETING**	Do Nguyen-Van, Colm Keane, Jamie P. Nourse, Erica Han, Nathan Ross, Kimberley Jones, Pauline Crooks, and Maher K. Gandhi
60	**THERAPEUTIC VACCINATION TO TREAT EPSTEIN-BARR VIRUS-POSITIVE MALIGNANCY: PHASE I CLINICAL TRIALS.**	Graham Taylor, Hui Jia, Edwin Hui , Anthony Chan, Kevin Harrington, Lip Wai Lee, Alan Rickinson, Neil Steven
61	**CLINICAL TRIAL DESIGN IN EBV-SPECIFIC IMMUNOTHERAPY FOR NASOPHARYNGEAL CARCINOMA**	Mark Fogg, Lori Wirth, Fred Wang, and Marshall Posner

Session 10. Lymphoma

Tuesday 7th September 2:15pm-4:00pm

Session chairs: Ruth Jarrett and Rosemary Rochford

Abstract #	Title	Author
62	**NONINVASIVE IDENTIFICATION OF EBV-INFECTED LYMPHOCYTE SUBTYPES IN EBV-ASSOCIATED T/NK LYMPHOPROLIFERATIVE DISEASES**	Hiroshi Kimura, Yoshinori Ito, Shinji Kawabe, Kensei Gotoh, Seiko Iwata, and Yukihiro Nishiyama
63	**DIFFERENTIAL REGULATION OF BLIMP1 ISOFORMS BY THE EPSTEIN-BARR VIRUS; IMPLICATIONS FOR VIRUS PERSISTENCE AND THE PATHOGENESIS OF EBV-ASSOCIATED LYMPHOMAS**	Katerina Vrzalikova, Martina Vockerodt, Sarah Leonard, Ciaran Woodman, Martin Rowe, Paul Murray.
64	**C-MYC IMPAIRS AN INTERFERON INDUCING ACTIVITY IN EBV POSITIVE BURKITT LYMPHOMA CELLS**	Sebastian Grömminger, Martin Schlee, Rainhard Mailhammer, Gerhard Laux and Georg Bornkamm
65	**A C-MYC INDUCED GENE EXPRESSION SIGNATURE IN HUMAN GERMINAL CENTRE B CELLS PREDICTS SUBTYPES OF AGGRESSIVE NON-HODGKIN LYMPHOMA**	Kube D, Schrader A, Bentink S, Spang R, Hummel M, Kuo M Arrand JR, Murray P, Trümper L, Vockerodt M
66	**INVESTIGATING THE ROLE OF VIRAL AND CELLULAR BCL2 PROTEINS IN BURKITT LYMPHOMA USING THE EμMYC TRANSGENIC MOUSE MODEL**	Gemma Kelly, Marco Herold, Stefan Glaser, Stephanie Grabow, Leah Fitzsimmons, David Huang, Philippe Bouillet, Martin Rowe, Andreas Strasser
67	**EBNA3B IS A TUMOUR SUPRESSOR**	Robert E White, Patrick C Rämer, Kikkeri Naresh, Sonja Meixlsperger, Ernest Turro, Laurie Pinaud, Cliona Rooney, Barbara Savoldo, Christian Münz and Martin J Allday
68	**ENHANCED TUMOURIGENESIS OF EBNA3B DEFICIENT EBV IN-VIVO**	Patrick C Rämer, Robert E White, Sonja Meixlsperger, Kikkeri Naresh, Laurie Pinaud, Cliona Rooney, Barbara Salvodo, Martin J Allday and Christian Münz

Session 11. Infection and Reactivation

Tuesday 7th September 4:30pm-5:30pm

Session chair: Lawrence Young

Abstract #	Title	Author
69	**PRIMARY EBV INFECTION IN EARLY INFANCY RESULTS IN POORLY CONTROLLED EBV VIRAL LOAD OVER TIME**	Erwan Piriou, Amolo Asito, Nancy Fiore, Peter S. Odada, Jaap M. Middeldorp, Ann M. Moormann, Robert Ploutz-Snyder, Rosemary Rochford
70	**HERPESVIRUS KINASES ACTIVATE TIP60: A CONSERVED PATHOGEN-HOST INTERACTION PROMOTING VIRUS REPLICATION**	Renfeng Li, Jian Zhu, Zhi Xie, Gangling Liao, Shaohui Hu, Crystal Woodard, Jiang Qian, Gary S Hayward, Heng Zhu, and S. Diane Hayward
71	**SCREENING OF CELLULAR FACTORS THAT ENHANCE REACTIVATION FROM THE EPSTEIN-BARR VIRUS LATENCY**	Takayuki Murata & Tatsuya Tsurumi
72	**THE EPSTEIN-BARR VIRUS ENHANCES INTERLEUKIN-8 DEPENDENT EXPRESSION OF MATRIX METALLOPROTEASES AND VASCULAR ENDOTHELIAL GROWTH FACTORS IN CARCINOMAS INDUCED IN SCID MICE**	Hratch Arbach, Aurore Rampanou, Samia Mourah, Olivier Deas, Ibrahim Casal, Robert Strieter, Sébastien Jauliac, Vanessa Ramirez, Carine Chavey6, Gwendal Lazennec, Chantal Cochet, Marie-Pierre Podgorniak, Fabien Calvo, and Irène Joab

Session 1 abstracts 1-8

Latency I

Latency I **Abstract 1**

EBNA1 DISRUPTS PML NUCLEAR BODIES THROUGH A DIRECT INTERACTION WITH PROTEIN KINASE CK2

Jennifer Yinuo Cao, Nirojini Sivachandran and Lori Frappier

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Abstract

Proteomics profiling of EBNA1-host interactions in human cells previously identified an interaction with protein kinase CK2 (formerly known as Casein Kinase 2), an enzyme comprised of catalytic (CK2α or α') and regulatory (CK2β) subunits that regulates many cellular processes. To better understand the significance of this interaction, we examined its mechanism and consequences. In both GST pull-down assays and glycerol gradient sedimentation analyses with purified proteins, EBNA1 bound CK2β but not CK2α , indicating that EBNA1 binds directly to CK2 through the regulatory subunit. By co-immunoprecipitation of EBNA1 mutants with CK2 from human cells, we mapped the CK2 binding region of EBNA1 and showed that deletion of EBNA1 residues 387-394 selectively disrupted CK2 binding. We have previously shown that EBNA1 disrupts PML nuclear bodies in nasopharyngeal carcinoma cells and others have shown that CK2 promotes the polyubiquitylation and degradation of PML proteins by phosphorylating them at S517. Therefore we investigated the requirement of the CK2-EBNA1 interaction for EBNA1-mediated PML disruption. We found that the ability of EBNA1 to disrupt PML bodies was abrogated by silencing of CK2_, by chemical inhibition of CK2 catalysis or by deletion of the CK2 binding site in EBNA1. In addition, we showed that EBNA1 increases both the association of CK2 with PML proteins and the phosphorylation of PML S517. Taken together, the results indicate that EBNA1 promotes the loss of PML bodies in part by increasing the occupancy of CK2 at these bodies, where CK2 phosphorylates and triggers the degradation of the PML proteins.

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Latency I **Abstract 2**

FUNCTIONAL DIFFERENCES BETWEEN TYPE 1 AND TYPE 2 EBV EBNA2

Laila Cancian, Walter Lucchesi, Claudio Elgueta Karstegl and Paul J. Farrell

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Abstract

EBV strains are classified as type 1 or type 2 according to the sequence of the EBNA2 gene. Type 1 EBV immortalises B lymphocytes much more efficiently than type 2 EBV, a difference previously mapped to the EBNA2 locus.

We developed a system that functionally distinguishes between type 1 and type 2 EBNA2 using the ability of the EBNA2 allele to support proliferation of EREB2.5 cells. By testing a panel of type 1/type 2 EBNA2 chimaeras in this assay, we have now mapped the EBNA2 sequences that may account for the greater ability of type 1 EBNA2 to immortalise B cells. Substitution of the C terminal third of the type 1 protein into the type 2 EBNA2 is sufficient to confer the type 1 growth phenotype in the EREB2.5 assay. Within this region, the RG, CR7 and TAD domains are the minimum type 1 sequences required. Sequencing the C terminus of EBNA2 from additional EBV isolates showed high sequence identity within type 1 isolates and within type 2 isolates, indicating that the functional differences mapped are typical of type sequences.

Differential regulation of the viral oncogene LMP1 in Daudi cells correlates with the greater transforming activity of type 1 EBNA2. This is also being analysed in primary B cells infected with recombinant viruses, containing either type 1 or type 2 EBNA2 and a transfection assay has been developed to study how type 1 and type 2 EBNA2 differentially regulate the LMP1 promoter from type 1 or type 2 EBV.

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Latency I **Abstract 3**

EBNA 2 DRIVES IMMORTALISATION THROUGH PTEFB RECRUITMENT TO CP AND LONG-RANGE SERINE 2 PHOSPHORYLATION OF THE RNA POLYMERASE II CTD

Richard D. Palermo, Helen M. Webb and Michelle J. West.

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Abstract

EBV-driven transformation and the continuous proliferation of infected cells requires EBNA 2 expression. EBNA 2 and EBNA-leader protein are initially expressed from an early cellular factor-driven promoter (Wp) and then mediate promoter switching through activation of the main latency promoter C (Cp). Cp then drives transcription of the long message (~120 Kb) encoding the remaining EBNAs required for cellular transformation. We have previously shown that EBNA 2-activated transcription is dependent on pTEFb (CDK9/cyclin T1) kinase activity. Here we show that pTEFb is recruited to Cp in the presence of EBNA 2, stimulating high-level pTEFb-dependent serine 2 phosphorylation at distal genome regions up to 75 Kb downstream from the transcription start site. We also demonstrate high-level association of the pTEFb-regulated elongation complexes, Negative elongation factor (NELF) and DRB sensitivity-inducing factor (DSIF) with Cp. In contrast, pTEFb, NELF and DSIF recruitment to two other key EBNA 2 viral target genes (latent membrane proteins 1 and 2A) was very low-level and significantly lower increases in CTD phosphorylation were observed. Taken together, our data suggest that specific high-level recruitment of pTEFb to Cp promotes long-range transcription of essential immortalising genes during infection by (i) phosphorylating the pol II CTD on serine 2 at distal regions and (ii) phosphorylating NELF/DSIF to overcome promoter-proximal pausing.

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Latency I **Abstract 4**

THE UBIQUITIN C-TERMINAL HYDROLASE L1 IS INDUCED BY EBNA2 IN TRANSFORMED B-LYMPHOCYTES AND IS ASSOCIATED WITH MITOTIC SPINDLE

Anjali Bheda, Julia Shackelford, Joseph S Pagano.

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Abstract

Deubiquitinating enzymes (DUBs) have been increasingly implicated in regulation of cellular processes by virus infections (J. Shackelford et al, 2004, 2007). UCH L1 is expressed in a number of malignancies suggesting that this protein might be involved in oncogenic processes, and increased expression and activity of UCH L1 have been detected in EBV-immortalized cell lines. We recently identified UCH L1 as a factor in intracellular signaling pathways that promote proliferation and invasiveness of transformed cells (A. Bheda et al, 2009).

We now provide evidence that EBNA2 activates the UCH L1 promoter through Pu.1 transcription factor. RNAi studies indicate that UCH L1 expression is required for proliferation and invasive properties of EBV-transformed cells. We also show that UCH L1 expression is increased during mitosis, and it is tightly associated with mitotic spindle in EBV as well as other transformed cells. Further, we show that UCH L1 also exists as a dimer in cells and in its dimeric form possesses an E3 ubiquitin ligase activity. Finally, UCH L1 ubiquitinates components of microtubules and regulates microtubule dynamics by depolymerization. These findings point to novel roles of UCH L1 in fundamental processes in normal and transformed cells in the cell cycle and specifically during EBV infection.

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Latency I **Abstract 5**

KINETICS OF EBV INFECTION OF B CELLS BY KNOCKOUT VIRUSES WITH DEFECTS IN THE LATENT MEMBRANE PROTEIN 2 GENE

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Abstract

The LMP2 gene produces two protein isoforms (LMP2A and LMP2B) that are made by *in vitro* immortalized B cells and play a role in a number of EBV-associated tumors. LoxP recombination and site specific mutations were used generate EBV-BAC plasmids which had LMP2A knocked out (LMP2A-KO), LMP2B knocked out [either by loxP recombination (LMP2B-KOD) or via an M to C mutation (LMP2B-KOc)], and double knockouts (LMP2A/B-KO). Recombinant viruses contained an EGFP marker and were produced in 293-SL cells and titered for Green-Inducing Units (GIUs) on Raji cells. LMP2A-KO and both LMP2B-KOs were able to efficiently infect cells with a frequency of infection similar to *wt*. Infection kinetics revealed three distinct phases – activation, proliferation, and establishment – in which LMP2 mutants differed from *wt*. All viruses activated B cells (cells became + for CD23, CD71, Ki67) although LMP2A-KO mutants had slower and lower activation levels. By Day 4 post infection wt, LMP2B-KOD, and LMP2B-KOc infected cells began to proliferate, while LMP2A-KOs did not. Kinetics of LMP2B-KOc infections were slightly accelerated compared to *wt* although both viruses established Lymphoblastoid Cell Lines (LCLs) with similar efficiencies. After showing essentially *wt* kinetics for activation and proliferation, LMP2B-KOD infections failed to establish LCLs regardless of the multiplicity of infection used. These results reveal the existence of a 'late acting' event in EBV immortalization of B cells and suggest that LMP2 gene products play a crucial role in the establishment of immortalized LCLs from primary B cells after EBV infection.

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Latency I **Abstract 6**

CELL GENE REGULATION ASSOCIATED WITH EBER EXPRESSION

Goran Gregorovic[1], Rachel Bosshard[1], Claudio Elgueta Karstegl[1], Oliver Dittrich-Breiholz[2], Michael Kracht[2], Rainer Russ[3] and Paul J. Farrell[1]

[1]Section of Virology, Faculty of Medicine, Imperial College London, Norfolk Place, London W2 1PG, UK [2]Institute of Pharmacology, Medical School Hannover, Carl-Neuberg Strasse 1, D-30625 Hannover, Germany [3]Genedata AG, Margarethenstrasse 38, CH-4053 Basel, Switzerland.

Abstract

Microarray gene expression profiling has been used to investigate cell genes whose regulation correlates with expression of the Epstein-Barr virus EBER RNAs. EBV mutants with deletions of EBER1 or EBER2 were constructed using BAC mutagenesis in *E. Coli* and stably transfected into 293 cells. Multiple cell lines containing wild type B95-8 EBV BAC, EBER1 or EBER2 deletion mutants and the respective revertants were cloned and characterised. Recombinant EBVs were then produced upon lytic replication induction and the resulting viruses, which carry a GFP marker, were titred in Raji cells. Equal titres of the virus stocks were used to infect primary human B cells isolated from peripheral blood resulting in the formation of lymphoblastoid cell lines (LCLs).

Cytoplasmic RNA isolated from LCLs representing each of the wt, mutant and revertant EBVs (up to 6 LCLs in each case) was used in microarray profiling. Bioinformatic analysis of the data showed that the patterns of cell gene expression of the different LCL groups could be distinguished by cluster analysis. A number of genes reproducibly differed in expression by greater than 5-fold in EBER-deletion background in comparison to the B cells infected with wt EBV. Differential expression pattern between different EBER mutants has been confirmed for numerous genes with RT-PCR and Western blotting. Several of the EBER-regulated genes whose expression was rescued in the EBV revertant background might be used as markers for elucidating the mechanism of EBER action and understanding the role that EBERs play in EBV-associated cancers.

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Latency I **Abstract 7**

EBV IMMORTALIZATION ALTERS TELOMERE CHROMATIN STRUCTURE AND TERRA TRANSCRIPTION

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Abstract

Telomere length and integrity must be maintained in continuously proliferating cells to avoid replicative senescence. Epstein-Barr Virus (EBV) has been shown to induce several changes in telomere maintenance during the immortalization of primary B-lymphocytes. We now show that telomere chromatin structure and transcriptional activity undergo dynamic changes in response to EBV-induced proliferation signals. EBV primary infection of peripheral blood B-cells results in an increase in telomere heterochromatin modifications and a decrease in the expression of telomere repeat containing RNA, referred to as TERRA. These events can lead to telomere dysfunction, but do not cause telomere-associated cellular senescence. EBV immortalized lymphoblastoid cell lines (LCLs) containing the estrogen-regulated EBNA2 gene (EREB2) lose telomeric heterochromatin and upregulate TERRA RNA upon the inactivation of EBNA2. The same effect on telomeres could be induced by serum starvation indicating that telomere heterochromatin and TERRA expression respond dynamically to changes in growth proliferation signals. We also present evidence that TERRA RNA can accumulate in large perinuclear bodies and exosomes during growth arrest, suggesting that TERRA may provide some form of stress response signal that is inactivated by viral-induced proliferation. Preliminary data indicates that TERRA may modulate autocrine and paracrine Toll-receptor signaling. The interplay between EBV infection, immortalization, and TERRA-associated stress response will be discussed.

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Latency I **Abstract 8**

AN ATM/CHK2-MEDIATED DNA DAMAGE RESPONSIVE SIGNALING PATHWAY SUPPRESSES EPSTEIN-BARR VIRUS TRANSFORMATION OF PRIMARY HUMAN B CELLS

Pavel A. Nikitin[1], Chris Yan[1], Eleonora Forte[1], Alessio Bocedi[1], Jay Tourigny[1], Amee Patel[2], Sandeep Dave[2], William Kim[2], Katherine Hu[1], Jing Guo[1], David Tainter[1], Olena Rusyn[1], and Micah Luftig[1]*

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Abstract

Epstein-Barr virus infection of primary B cells leads to the outgrowth of indefinitely proliferating lymphoblastoid cell lines (LCLs). However, the efficiency of immortalization is less than 10% of infected cells. We hypothesize that a robust innate tumor suppressor response prevents long-term outgrowth of the majority of infected cells. In this study we identify the DNA damage response (DDR) as a major component of this response. EBV infection of primary B cells activated hallmarks of the DDR including phosphorylated ATM, Chk2, g-H2AX, and 53BP1 foci. DDR activation was not due to lytic viral DNA replication nor did its marks co-localize with latent viral episomes. Rather, EBV induced a period of hyper-proliferation early after infection responsible for DDR activation. Microarray data supported the transient activation and subsequent attenuation of proliferation and DDR-associated mRNAs during LCL outgrowth. Importantly, activation of this pathway suppressed transformation as small molecule antagonism of the DNA damage responsive kinases ATM and Chk2 increased EBV transformation efficiency. Thus, we propose a model whereby EBV infection initially drives aberrant cellular DNA replication activating an anti-proliferative DNA damage response. Long-term outgrowth depends on attenuation of this hyper-proliferative signal through full latency III gene expression.

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Session 2 abstracts 9-14A

Immunology

Immunology **Abstract 9**

THE EBV-ENCODED BILF1 PROTEIN MODULATES IMMUNE RECOGNITION OF ENDOGENOUSLY PROCESSED ANTIGEN BY TARGETING MHC CLASS I MOLECULES TRAFFICKING ON BOTH THE EXOCYTIC AND ENDOCYTIC PATHWAYS

Jianmin Zuo, Jennifer Tamblyn, Wendy Thomas and Martin Rowe

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Abstract

The gamma-herpes virus, EBV, has successfully colonized more than 90% of the adult human population, despite the presence of strong immune responses. A number of EBV-encoded immunoevasin proteins have been identified that cooperate to interfere with MHC class I (MHC-I) and class II (MHC-II) antigen presentation pathways. Here we have studied the BILF1 protein, which was recently identified as an immuno-evasion that functioned by enhancing internalization of MHC-I from the cell surface, and targeting MHC-I for degradation *via* lysosomes. We now demonstrate that BILF1 also targets newly-synthesised MHC-I/peptide complexes *en route* from the ER to the cell surface. The effects of BILF1 on the excocytic and endocytic pathways can be genetically separated with mutant BILF1 genes. Disruption of the 'DRY' signalling motif with a K122A mutation was shown to impair the enhanced endocytosis of surface MHC-I, whilst the sorting of internalised MHC-I for lysosomal degradation was abrogated by deletion of the 21 aa C-termus tail of BILF1. The BILF1 domain responsible for diverting MHC-I away from the cell surface on the exocytic pathway did not require either the intact DRY signalling motif or the C-terminus. Importantly, although the diversion of MHC-I on the exocytic pathway caused a relatively modest reduction in total cell surface MHC I, the effect on presentation of endogenously processed target peptides to $CD8^+$ effector T cells was substantial. This study therefore extends our initial observations on BILF1 to show that this immunoevasin can impair the antigen presentation on both the exocytic and endocytic trafficking pathways.

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Immunology **Abstract 10**

EPSTEIN-BARR VIRUS BNLF2A-MEDIATED T-CELL EVASION DURING PRODUCTIVE INFECTION

Daniëlle Horst[1,2], Vincenzo Favaloro[3], Fabio Vilardi[3], Stephen High[4], Bernhard Dobberstein[3], Andrew Hislop[5], Alan Rickinson[5], Maaike Ressing[1,2] and Emmanuel Wiertz[1,2]

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Abstract

During productive EBV infection, the immune evasion protein BNLF2a inhibits peptide transport into the ER via the Transporter associated with Antigen Processing (TAP). As a consequence, antigen presentation via HLA class I molecules is impaired and T-cell recognition is avoided. In virus-producing B cells, the timing of BNLF2a expression allows for immediate interference with the presentation of viral antigens by HLA class I molecules; BNLF2a is highly expressed early upon EBV reactivation and declines during progression of viral replication.

BNLF2a displays characteristics of a tail-anchored (TA) protein: we find the viral protein to be inserted into membranes post-translationally, with its hydrophobic C-terminal domain functioning as a membrane anchor. This topology leaves BNLF2a's N-terminal domain exposed in the cytosol, where it inhibits TAP function. BNLF2a interacts with Asna1, a cellular protein involved in membrane insertion of TA proteins. Asna1 facilitates membrane insertion of BNLF2a and is required for efficient HLA class I downregulation by the EBV protein. These results illustrate how an EBV-encoded protein exploits a cellular pathway for TA protein biogenesis for evasion of the host immune response.

In conclusion, EBV has acquired an immune evasion molecule that blocks peptide transport via TAP. Interestingly, BNLF2a is unrelated to other viral TAP inhibitors discovered so far, both in mechanism and structure. By impairing TAP-mediated peptide transport and thereby T-cell detection of EBV-producing cells, BNLF2a could contribute to creating a window for undetected virus production, thus facilitating the spread of EBV to new hosts.

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Immunology **Abstract 11**

PURIFIED HEXAMERIC SBARF1 PROTEIN IS A DECOY RECEPTOR FOR MACROPHAGE COLONY STIMULATING FACTOR AND INTERFERES WITH MACROPHAGE DIFFERENTIATION AND ACTIVATION, BUT HAS NO DETECTABLE MITOGENIC ACTIVITY

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Abstract

Epstein Barr virus (EBV) has acquired numerous mechanisms for subverting immune surveillance and stimulating cell growth. This study aims to investigate the putative role(s) of secreted native BARF1 protein (sBARF1) on the microenvironment in EBV-positive carcinomas.
Glycosylated hexameric, NPC-derived sBARF1 was purified from the supernatant of stably transfected human 293 cells. We determined its dose-effect on M-CSF and GM-CSF stimulated growth and differentiation of myeloid cells and its proposed mitogenic effect on multiple cell types of lymphoid and epithelial background.
sBARF1 inhibited M-CSF but not GM-CSF dependent growth of the myeloid MUTZ3 cell line. Antibodies specific to hexameric sBARF1 were able to block this effect. M-CSF was shown to co-immunoprecipitate with sBARF1, which was lost when sBARF1 was mutated at defined sites. Analysis of activation of M-CSF receptor *c-fms* and its downstream kinase pathways showed that sBARF1 prevents M-CSF induced phosphorylation. Since M-CSF is important in macrophage differentiation, the effect of sBARF1 on maturation and function(s) of human monocyte-derived macrophages was evaluated, showing inhibitory effects of sBARF1 on macrophage survival and morphology. Macrophages treated with sBARF1 showed impaired responses to LPS and phagocytosis of apoptotic cells was reduced. We could not detect any mitogenic effect of purified sBARF1 on multiple non-myeloid cell types, which contrasts with previous findings.
In conclusion, EBV encoded sBARF1 produced by epithelial cells is a potent decoy receptor for M-CSF but has no detectable mitogenic activity. These results suggest a role for sBARF1 in carcinogenesis by modifying innate and adaptive immune responses in the tumour microenvironment.

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Immunology **Abstract 12**

EPSTEIN-BARR VIRUS LYTIC-PHASE PROTEIN BGLF5 REDUCES TOLL-LIKE RECEPTOR 9 EXPRESSION DURING PRODUCTIVE INFECTION

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Abstract

Viruses employ a wide range of strategies to evade elimination by the host immune system. The human γ-herpesvirus Epstein-Barr virus (EBV), causative agent of infectious mononucleosis and several malignant tumors, can subvert adaptive immune responses. Less is known about interference by EBV with innate immunity at the level of Toll-like receptor (TLR)-mediated pathogen recognition. The viral double-stranded DNA sensor TLR9 is expressed on B cells, a natural target of EBV infection. Here, we show that EBV particles trigger innate immune signaling pathways through TLR9. Using an *in vitro* system for productive EBV infection, it was possible to compare the expression of TLRs by EBV-negative and EBV-positive human B cells during latent and lytic phases of infection. Several TLRs were found to be differentially expressed either in latently EBV-infected cells or after induction of the lytic cycle. In particular TLR9 expression was profoundly decreased at both the RNA and protein levels during productive EBV infection. We identified the EBV lytic-phase protein BGLF5 as a protein contributing to these downregulated TLR9 levels. Reducing the levels of a pattern-recognition receptor capable of sensing the presence of EBV provides a mechanism by which the virus could obstruct host innate antiviral responses.

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Immunology **Abstract 13**

REGULATION OF THE IMMUNO –INHIBITOR B7-H1 EXPRESSION IN EPSTEIN BARR VIRUS INFECTED B-CELLS

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Abstract

Esptein-Barr virus (EBV) is known to have developed mechanisms to escape to immune system. Immunological tolerance is mainly due to inhibitor signals, through co-signalling molecules. B7-H1, also called PD-L1 (Program-Death Ligand 1 or CD274) is a co-signalling molecule of the B7 family, which can inhibit activated effector T cells by interaction with its receptor PD-1 (Program-Death-1). We observed an increase in B7-H1 expression at both the membrane and RNA levels of EBV-infected B lymphocytes. By contrast, intracellular expression of the protein remained unchanged when compared to uninfected cells. We found that inhibition of c-Myc led to decrease of B7-H1 expression at the RNA and membrane protein levels but not in the cytoplasm. Induction of c-Myc in EBV infected cells led also to a decrease of NF-kB binding activity. But inhibition of c-Myc in EBV-negative cells led also to increase of B7-H1. Therefore, we conclude that B7-H1 is regulated at RNA and membrane levels, but not at the cytoplasm level. We also concluded that NF-κB is likely to up-regulate B7-H1 expression while c-Myc would repress it either directly or by inhibition of NF-κB. To precisely understand mechanisms of this regulation, we are currently studying the cellular compartments of the protein (cytosol, cellular membranes, exosomal fraction,..). Moreover, cDNA of B7-H1 was also cloned into an inducible expression vector in order to examine its trafficking. This study will contribute to understand the regulation of B7-H1 in EBV-immortalised B-cells.

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Immunology **Abstract 14**

IMPAIRED EPSTEIN-BARR VIRUS-SPECIFIC CD8+ T CELL FUNCTION IN X-LINKED LYMPHOPROLIFERATIVE DISEASE IS RESTRICTED TO SLAM FAMILY POSITIVE B CELL TARGETS

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Abstract

X-linked lymphoproliferative disease (XLP) is a condition associated with mutations in the signaling lymphocytic activation molecule (SLAM) associated protein (SAP/SH2D1A). SAP functions as an adaptor, binding to and recruiting signaling molecules to SLAM family receptors expressed on T and NK cells. XLP is associated with extreme sensitivity to primary Epstein-Barr virus (EBV) infection, often leading to a lethal infectious mononucleosis. To investigate EBV-specific immunity in XLP patients, we studied five individuals who had survived EBV infection and found CD8+ T cell responses numerically comparable to healthy donors. However further investigation of in vitro derived CD8+ T cell clones established from two of these donors showed they efficiently recognized SLAM-ligand-negative target cells expressing EBV antigens, but showed impaired recognition of EBV-transformed, SLAM-ligand-positive, lymphoblastoid cell lines (LCLs). Importantly, LCL recognition was restored when interactions between the SLAM receptors CD244 and NTBA and their ligands on LCLs were blocked. We propose that XLP patients' particular sensitivity to EBV, and not to other viruses, reflects at least in part EBV's strict tropism for B lymphocytes and the inability of the CD8+ T cell response often to contain the primary infection of SLAM ligand-expressing target cells.

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Immunology **Abstract 14A**

LMP1 CAUSES CHRONIC INFLAMMATION PRIOR TO NEOPLASIA IN VIVO

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Abstract

It has long been postulated that chronic inflammation can be tumourigenic but the exact mechanisms involved are poorly understood. Nasopharyngeal carcinoma, like many cancers, is heavily infiltrated with inflammatory cells, which could be contributory to tumourigenesis. To explore the role of LMP1 induced inflammation and oxidative stress in EBV associated malignancies, we utilize a transgenic mouse model of epithelial carcinogenesis in which $LMP1^{CAO}$ is expressed in the epidermis. The skin of these mice undergoes progressive inflammatory pathology prior to neoplasia and is infiltrated with T-cells, mast cells and neutrophils. Affected tissues show upregulation of several cytokines and inflammation associated proteins including CD30, CD30L, CD40, L-Selectin, IL-3, IL-1β and s100A9. LMP1D6-null mice (D6: decoy receptor for inflammatory chemokines) show acceleration of inflammatory processes, suggesting a role of chemokines in LMP1 induced cancer predisposition.

The preneoplastic skin also shows dermal deposition of immunoglobulins and increased levels of complement component-3 (C-3). The critical role of mature B and/or T-cells in the advancing pathology is evidenced by their elimination, which limits the pathology to an early benign stage.

By proteomic analysis, we have demonstrated upregulation of enzymatically inactive chitinase-like proteins, chitinase-3-like-4 (chi3l4/YM2), chitinase-3-like-1 (chi3l1/YKL40) and chitotriosidase (chit1), in the affected skin, suggesting a role in chronic inflammation.

We have found a decrease in SOD1 levels and increase in H_2O_2 in preneoplastic skin indicating that the tissue is also under oxidative stress.

Dissecting the mechanisms by which LMP1 induces inflammation prior to neoplasia may provide new insights for EBV associated cancer therapies.

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Session 3 abstracts 15-22

Genetics, Epigenetics, Non-coding RNAs

Genetics, Epigenetics, Non-coding RNAs **Abstract 15**

EBV'S CHROMATIN IN A CLOSE-UP: A FURTHER STEP TOWARDS UNDERSTANDING EBV'S LIFE CYCLE

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Abstract

Epigenetic mechanisms are essential for mammalian gene regulation in all thinkable scenarios, from development to cancer progression. For example, DNA methylation at CpG-sites is among other things a defense mechanism against incoming DNA from pathogens to downregulate expression of potentially harmful genes. However, EBV has evolved to take advantage of this system to regulate its life cycle. Surprisingly, genes essential for EBV's lytic phase depend on CpG methylated promoters for their induction by BZLF1, the master gene for the switch from latent to lytic phase. However, these experiments were conducted *in vitro* on epigenetically unmodified DNA, the relevance in living cells is still unclear, due to missing information about EBV's chromatin state.

Therefore I analyzed DNA methylation of about 20 % of the Raji genome, covering every important latency region as well as early and late lytic promoters by deep-bisulfite-sequencing. Furthermore, I investigated the positions of nucleosomes EBV-genome wide by MMD-on-Chip (MNase protected mononucleosomal DNA). These experiments reveal the importance of epigenetics for EBV's regulation and open the question how regions like the EBER locus are kept free from repressing epigenetic marks, and how the early lytic genes can be activated from a closed chromatin state.

Furthermore, I analyzed the formation of nucleosomes and DNA methylation on EBV's DNA in a time course. This experiment reveals a clear hierarchy of epigenetic events occurring after infection, which suggests a model, how EBV uses these modifications not only for the switch to the lytic phase, but also for the establishment of latency.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 16**

POLYCOMB-MEDIATED REPRESSION OF THE PRO-APOPTOTIC TUMOR SUPPRESSOR GENE *BIM* BY EBNA3A AND EBNA3C

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Abstract

Regulation of *BIM* is uniquely important in the pathogenesis of Burkitt's lymphoma (BL), since in this childhood cancer the *MYC* gene is deregulated by chromosomal translocation and MYC can induce apoptosis via BIM. Latent EBV represses BIM expression via the functional interaction of nuclear oncoproteins EBNA3A and EBNA3C. Analyses of the histone modification H3K27-Me3 (trimethylation of histone H3 lysine 27) and CpG methylation at loci throughout the *BIM* promoter region suggested that in EBV-positive B cells repression of *BIM* is initially associated with this repressive epigenetic histone mark and can gradually be followed by DNA methylation at CpG dinucleotides. Using B cell lines generated with EBNA3A-, EBNA3B-, EBNA3C- and triple EBNA3-knockout viruses we show that both EBNA3A and EBNA3C are necessary for the association of SUZ12 and EZH2 (components of the polycomb PRC2 complex) with the *BIM* promoter and the establishment of the H3K27-Me3 mark. However, EBV does not appear to alter the steady state levels of PRC2 proteins or the histone demethylase JMJD3. Neither EBNA3A nor EBNA3C appeared to affect the distribution of H3K4-trimethylation or the recruitment of RNA Polymerase II (Pol II) – these remained at relatively constant levels on the *BIM* promoter, suggesting a 'bivalent' chromatin organization similar to that of many polycomb-regulated genes in stem cells. The data indicate that here regulation by EBV is at the level of transcriptional elongation or pausing rather than initiation. By inducing heritable repression of *BIM,* EBV increases the likelihood of B lymphomagenesis in general and BL in particular.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 17**

CELLULAR MICRORNAS 200B AND 429 REGULATE THE EBV LATENT-LYTIC SWITCH VIA ZEB1 AND ZEB2

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Abstract:
We previously reported that EBV containing a 2-bp substitution mutation in the ZEB1-binding ZV element of the BZLF1 promoter, Zp, spontaneously reactivates out of latency into lytic replication at a higher frequency than WT (Yu et al., PLoS Pathogens 3:3194, 2007). Using siRNA and shRNA technologies, we recently showed that ZEB1 is, indeed, a key player in maintaining EBV latency in many epithelial and B-cell lines. However, in others, ZEB2/SIP1, which can also bind the ZV element, is the key player. Thus, we concluded that either ZEB1 or ZEB2 can play a central role in the maintenance of EBV latency, doing so in a cell type-dependent.
Here, we report that the levels of the cellular miRNAs 200b and 429 in epithelial and B-lymphocytic EBV-infected cell lines strongly negatively correlate with ZEB1 and ZEB2 levels and strongly positively correlate with the degree of EBV lytic-gene expression. Addition of either miRNA 200b or 429 to EBV-positive cells leads to EBV lytic reactivation in a ZEB-dependent manner; inhibition of these miRNAs leads to decreased EBV lytic-gene expression. The degree of latent infection by an EBV mutant defective in the ZV element of Zp was not affected by addition of these miRNAs. Thus, we conclude that the cellular miRNAs 200b and 429 are key regulators of the EBV latent-lytic switch via their effects on expression of ZEB1 and ZEB2. The existence of miRNAs with such properties suggests new strategies for treating patients with EBV-associated diseases.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 18**

UPREGULATION OF MIR155 IN NASOPHARYNGEAL CARCINOMA RESULTS DOWNREGULATION OF JMJD1A, A NEGATIVE PROGNOSTIC MARKER

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Abstract

MicroRNA-155 (miR-155) was detected by in situ hybridization (ISH) in nasopharyngeal carcinoma (NPC) formalin fixed, paraffin embedded tissue (FFPE) samples. Compared to the adjacent normal epithelial cells, miR-155 was found to be upregulated in NPC tumor cells. In addition miR-155 was also upregulated in the NPC derived cell lines CNE1 and TW03, compared to NP69 cells from normal nasopharyngeal epithelium. JMJD1A and BACH1 were identified as putative targets of miR-155 in a bioinformatics screen. We showed that overexpression of miR-155 downregulated a luciferase transcript fused to the 3'UTR of JMJD1A and BACH1. MiR-155 mimic could downregulate the expression of JMJD1A and BACH1 in CNE1, TW03 and NP69 cells, while miR-155 inhibitor could upregulate JMJD1A expression in CNE1 and TW03 cells. The expression level of JMJD1A and BACH1 were downregulated in NPC CNE1 and TW03 cells and NPC clinical samples. Moreover, downregulation of JMJD1A was significantly correlated with N stage (p = 0.025), a lower five-year survival rate (p = 0.018), and a lower five-year disease-free survival rate (p = 0.041) of NPC patients. EBV encoded LMP1 and LMP2A enhanced further the expression of miR-155 in NPC CNE1 and TW03 cells. Taken together, we show that miR155 expression is very high in NPC, further upregulated by LMP1 and LMP2A, and it results in downregaultion of JMJD1A and BACH1, which is associated with N stage and poor prognosis of NPC patients.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 19**

IDENTIFYING MRNA TARGETS FOR EBV-DERIVED AND CELLULAR MICRORNAS EXPRESSED IN LYMPHOBLASTOID CELL LINES

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Abstract

miRNAs are ~ 22 nt small RNAs that post-transcriptionally silence gene expression by guiding the RNA-induced silencing complex (RISC) to complementary sequences on target mRNAs. EBV encodes 25 pre-miRNAs that are differentially expressed during latent infection. A number of cellular miRNAs, including miR-155, are also strongly upregulated in response to EBV infection. The mRNA targets for most viral and cellular miRNAs are not yet known. To determine EBV and cellular miRNA targets in EBV-infected lymphoblastoid cell lines on a transcriptome-wide level in a high-throughput manner, we implemented a cross-linking immunopurification method to directly analyze RISC-bound mRNAs. Briefly, cells were cultured in the presence of a photoactivatable nucleoside analog, crosslinked, and cDNA libraries prepared from immunopurified RISC-bound RNAs. The incorporation of the photoactivatable nucleoside analog marks the site of RISC:RNA crosslinking, and aids in determining the location of miRNA target sites within mRNAs. Deep sequencing combined with bioinformatics revealed over 400 cellular transcripts as EBV miRNA targets, including MICB, BACH1, and IPO7. Intriguingly, we also identified cellular miRNA target sites in EBV latent transcripts, suggesting a complex host-virus interaction at the small RNA level. Work is ongoing to identify miRNA targets in EBV+ AIDS-related DLBCLs and to determine the combinatorial role that cellular and viral miRNAs have in EBV pathogenesis.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 20**

EBV-ENCODED MICRO RNAS PROMOTE CELL CYCLE PROGRESSION AND PREVENT APOPTOSIS OF PRIMARY HUMAN B CELLS

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Abstract

EBV encodes 44 microRNAs (miRNAs), which are clustered in the non-coding regions of the *BHRF1* and *BART* genes. Only a few cellular or viral targets of EBV´s miRNAs have been identified so far and their function(s) in the life cycle of EBV has remained elusive.
To assess the role of EBV´s miRNAs in EBV-mediated B-cell activation, we systematically introduced mutations in EBV's precursor miRNA transcripts of the prototypic B95.8 strain (wt EBV) to prevent their subsequent processing into mature miRNAs. Viral mutants deficient in all miRNAs of the BHRF1 locus (DmirBHRF1 EBV) or devoid of all known viral miRNAs (DmirALL EBV) were used to infect primary human B cells, followed by phenotypic analyses at early time points after infection. As compared to wt EBV, the numbers of outgrowing B cells were dramatically reduced when infected with DmirBHRF1 and DmirALL EBVs within a period of 12 days post infection. Cells infected with both mutant EBVs progressed less efficiently through the cell cycle and died by apoptosis more often than cells infected with wt EBV. Only the altered cell cycle distribution was maintained in long-term cultured cells suggesting that BHRF1 miRNAs are critical in primary B cells early after infection but dispensable in established LCLs. We did not observe an apparent role of EBV´s miRNAs in maintaining viral latency. Our findings indicate that EBV´s miRNAs contribute to EBV-associated cellular transformation rather than regulate viral genes of EBV's lytic phase.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 21**

INHIBITION OF APOPTOSIS BY EBV BART MIRNAS IN GASTRIC CARCINOMA

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Abstract

MicroRNAs (miRNAs) are small, noncoding RNAs of approximately 22 nucleotides that regulate gene expression by blocking translation of target mRNAs through binding to complementary sequences most often in the 3' untranslated region of the target message. EBV encodes three clusters of miRNAs, two of which are located within the introns of the Bam HI region rightward transcripts (BARTs) and are referred to as the BART miRNAs. Expression of these miRNAs mirrors the BARTs themselves, which show highest expression in latency pattern II seen in nasopharyngeal carcinoma, gastric carcinoma, and Hodgkin's disease. In this study, stable expression of either cluster of BART miRNAs in the EBV negative gastric carcinoma cell line, AGS, resulted in cells that grow more effectively in soft agar and were resistant to apoptosis induced by the DNA damaging agent etoposide. A combination of expression microarray and bioinformatic analysis suggested multiple members of the pro-apoptotic BH3-only, BCL2 family members as potential targets of the BART miRNAs in AGS cells. A previous report identified one such family member, PUMA, as a direct target of miR-BART5, however this regulation could not be detected in AGS cells expressing miR-BART5 at physiological levels. Another BH3-only protein, Bim contains target sites for multiple BART miRNAs and was downregulated in cells expressing both the miRNA clusters and several individual miRNAs. Taken together, these results suggest that the BART miRNAs promote transformation by multiple mechanisms, including the inhibition of apoptosis through the downregulation of BH3-only family member proteins.

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Genetics, Epigenetics, Non-coding RNAs **Abstract 22**

FUNCTIONAL DELIVERY OF EBV-MIRNAS VIA EXOSOMES

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Abstract

Non-coding regulatory microRNAs (miRNAs) of cellular- and viral origin control gene expression by repressing the translation of messenger RNAs (mRNAs) into protein. Surprisingly, we found that small RNAs are highly enriched in secreted nano-sized vesicles from EBV-infected B cell blasts (LCLs) commonly referred to as exosomes. To investigate whether transferred EBV-miRNAs may act in uninfected recipient cells, we mimicked miRNA transference using a co-culture model in vitro, luciferase miRNA-based reporter assays and a quantitative multiplex RT-PCR approach for EBV-miRNA detection.

We demonstrate that EBV-encoded miRNAs are secreted in large copy numbers by LCLs through exosomes. The released exosomes are efficiently internalized by neighboring recipient cells, including primary monocyte-derived dendritic cells (MoDC). To confirm that miRNA-mediated gene silencing has a role in inter-cellular communication, we designed reporter-assays in which we express full-length 3'-UTR luciferase target constructs in recipient cells. Accumulation of EBV-miRNA copy numbers in recipient cells was measured by quantitative RT-PCR and lead to a dose-dependent, miRNA-mediated repression of the EBV-miRNA target gene CXCL11/ITAC, an immuno-regulatory gene commonly down-regulated in primary EBV-associated lymphomas. Finally, we demonstrated that Epstein Barr virus (EBV) BART-miRNAs are expressed by infected circulating B cells in humans but are also present in circulating non-infected non-B cells.

Collectively, these studies show that primary MoDCs, upon exosome internalization transport exogenous miRNAs to cellular compartments for miRNA-dependent gene silencing. Thus, viral miRNAs may exploit an existing miRNA-mediated intercellular communication pathway between circulating immune cells via transference of exosomes.

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Session 4 abstracts 23-28

Signalling

Signalling **Abstract 23**

ARGININE DIMETHYLATION OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 2 IS REQUIRED FOR PROMOTER BINDING AND ASSOCIATION WITH CELL PROTEINS

Henrik Gross[1], Stephanie Barth[1], Richard D. Palermo[2], Alfredo Mamiani[1], Christine Hennard[3], Ursula Zimber-Strobl[4], Michelle J. West[2], Marlies Sauter[1], Nikolaus Mueller-Lantzsch[1], Elisabeth Kremmer[3], and Friedrich A. Grässer[1]

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Abstract

The EBV-encoded nuclear antigen 2 (EBNA2) is essential for transformation and activates gene expression by association with DNA-bound transcription factors. EBNA2 contains an Arginine-Glycin (RG)-repeat the deletion of which results in a reduced ability of the virus to immortalise B-cells. EBNA2 contains asymmetrically (aDMA) and symmetrically dimethylated Arginine (sDMA) residues but neither non-methylated (NMA) Arginines nor Citrulline residues. Only aDMA-containing EBNA2 is found in a complex with DNA-bound RBPJκ *in vitro* and aDMA-modified EBNA2 is preferentially found at the LMP1, LMP2A and C promoters *in vivo*. Inhibition of methylation in EBV-infected cells results in reduced expression of the EBNA2-regulated LMP1, providing additional evidence that methylation is a prerequisite for DNA-binding. The sDMA-modified EBNA2 is associated with the survival motor neuron protein (SMN), a factor essential for spliceosome formation but also involved in transcriptional activation. We set out to identify cellular target genes binding to the aDMA-form of EBNA2. We found that the NP9 protein encoded by the endogenous retrovirus HERV K101 is induced by EBV-immortalization and that it binds to EBNA2. Binding was confirmed by co-immunoprecipitation and confocal laser scanning microscopy. A GST-pull-down analysis indicated that NP9 binds preferentially to the RG-repeat. Binding of NP9 to EBNA2 reduced DNA-binding in vitro and inhibited the transactivation of the viral CP and LMP2A but not the LMP1 promoter in vivo. We hypothesise that induction of NP9 by EBV and the concomitant down-regulation of EBNA2 represents a feed-back mechanism to safeguard the cell from the toxic effects of EBNA2 over-expression.

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Signalling **Abstract 24**

ACTIVATION OF TOLL-LIKE RECEPTOR 3 SIGNALING BY EBER CONTRIBUTES TO GASTRIC CARCINOGENESIS

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Abstract

EBV is associated with various malignancies including gastric cancer. We have recently reported that EBER, which has a double stranded (ds)RNA-like structure is released from EBV-infected cells and activates toll-like receptor (TLR)3-mediated signaling. We also reported that EBER exists in the sera of patients suffering from Infectious mononucleosis (IM), EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) and chronic active EBV infection (CAEBV) and induces innate immune responses via TLR3, suggesting that EBER-mediated activation of TLR3 signaling contribute to the pathogenesis of active EBV infectious diseases. Here we demonstrated that activation of TLR3 signaling by EBER occurs in EBV-positive gastric epithelial cells. EBER is released from EBV-positive gastric epithelial cells into the culture supernatant. In vitro-synthesized EBER induced type I interferon (IFN) and inflammatory cytokine production in gastric epithelial cells in a TLR3 dependent manner. The downstream TLR3 intracellular signaling molecules including IRF3 and NF-κB were activated by synthesized EBER, suggesting that in EBV-positive gastric epithelial cells, TLR3 signaling is constitutively activated by released EBER. Moreover, EBER induces insulin-like growth factor (IGF)1 that acts as an autocrine growth factor of EBV-positive gastric epithelial cells, whereas knockdown of TLR3 resulted in downregulation of IGF1 in EBER-positive but not in EBER-negative gastric epithelial cells. These findings strongly suggest that activation of TLR3 signaling by EBER is involved in the development of EBV-associated gastric cancer.

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Signalling **Abstract 25**

SUBTRACTIVE PROTEOMICS IDENTIFIES AN ESSENTIAL ROLE OF A STE20 GERMINAL CENTER KINASE IN JNK SIGNALING BY LMP1

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Abstract

The latent membrane protein 1 (LMP1) is essential for cell transformation by EBV, activating NF-kappaB, MAPK, PI3-kinase and JNK signaling. LMP1 assembles a unique signaling complex involving TRAFs and TRADD. Here, we have developed a subtractive proteomics approach that allowed us to identify novel components of the native LMP1 complex in LCLs. Primary B-cells were transformed with a recombinant EBV in which the endogenous LMP1 gene had been replaced by a HA-tagged LMP1 version carrying a TEV protease cleavage site between its transmembrane and signaling domains. After immunoprecipitation of the LMP1 complex, proteins interacting with the LMP1 signaling domain were released by TEV cleavage and analyzed by mass spectrometry versus a control sample. Thereby, we identified a member of the STE20 germinal center kinase family with so far largely unknown functions as a novel component of the LMP1 complex. This STE20 kinase is recruited to LMP1 by the C-terminal activator region 2 (CTAR2). CTAR2 also mediates translocation of the kinase into lipid rafts. We identified TRAF6, an essential signaling mediator of CTAR2, as a novel and direct interaction partner of the STE20 kinase. Studies in TRAF6-/- MEFs revealed that TRAF6 mediates recruitment of the kinase to LMP1. Also TAK1 and TAB2 were identified as novel interaction partners of this STE20 kinase in 293 cells and LCLs. RNAi-mediated knockdown of the STE20 kinase by siRNA and shRNA blocked JNK signaling by LMP1. In summary, we have for the first time identified a STE20 kinase as an essential mediator of LMP1 signaling.

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Signalling **Abstract 26**

IDENTIFICATION OF TMEM134 AS A NOVEL LMP1-BINDING PROTEIN USING BIMOLECULAR FLUORESCENCE COMPLEMENTATION AND AN ENHANCED RETROVIRAL MUTAGEN

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Abstract

Protein complementation is a novel technique to examine protein-protein interaction through the assembly of functional fluorescent proteins or enzymes from inactive fragments. In the present study, bimolecular fluorescence complementation (BiFC) was used to study the assembly of the latent membrane protein 1 (LMP1) signaling complex within the plasma membrane of mammalian cells. LMP1 of Epstein-Barr virus induces constitutive signaling in infected cells. LMP1 signaling requires oligomerization of LMP1 via its transmembrane domain, localization to lipid rafts in the membrane, and association of the LMP1 cytoplasmic domain to adaptor proteins, such as the tumor necrosis factor receptor associated factors (TRAFs). Previous studies in our lab demonstrated that oligomerization of LMP1 with itself induced complementation and BiFC was observed between LMP1 and TRAF2 or TRAF3. Fluorescence was observed in perinuclear and membrane locations, sites consistent with previously described LMP1 signaling locations. Importantly LMP1-BiFC constructs induced fibroblast transformation indicating that they are not significantly impaired in signaling. The LMP1-BiFC construct was used as bait in a genome-wide screen with an enhanced retroviral mutagen to identify new LMP1-binding proteins. In our pilot screen Tmem134 was identified as a new LMP1-binding protein which was subsequently confirmed in pull-down experiments. Tmem134 is a highly conserved transmembrane protein of unknown function. Tmem134 is a candidate oncogene that is amplified in breast cancer cells lines. Data concerning the role of Tmem134 in LMP1 signaling will be presented. Together these data suggest that BiFC is a unique and novel platform to identify proteins recruited to the LMP1-signaling complex.

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Signalling **Abstract 27**

THE EPSTEIN-BARR VIRUS ENCODED PROTEIN COMPLEX BDLF2/BMRF2 INDUCES MORPHOLOGICAL CHANGES BY A PKC-RELATED INHIBITION OF ERM-PROTEINS

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Abstract

The recently characterised glycoprotein-complex BDLF2/ BMRF2 of the Epstein-Barr virus localises on the cytoplasma membrane of mammalian cells and induces significant morphological changes in adherent cells in an actin-dependent manner. These changes include disassembly of actin stress fibers and formation of cellular protrusions. We have previously shown that the BDLF2/BMRF2-triggered phenotype is induced via the RhoA-signaling pathway.

Now we demonstrate that RhoA itself is not involved in this process. Phosphorylation levels of the ezrin/radixin/moesin-(ERM-) proteins, which are RhoA downstream effectors serving as linker-proteins between the cytoplasma membrane and the cytoskeleton, decreased in BDLF2/BMRF2-co-expressing cells. Expression of the C-terminal and N-terminal domains of ezrin, which were shown to inhibit physiological activity of the ERM-proteins, induced morphological changes similar to the BDLF2/BMRF2-associated phentotype. Thus, inhibition of ERM-proteins is sufficient to explain the BDLF2/BMRF2-triggered morphological changes.

Co-expression-analyses of BDLF2/BMRF2 and ezrin, radixin, or moesin displayed a co-localisation of ERM-proteins and the viral protein-complex at the cell membrane. However, the BDLF2/BMRF2-complex does not bind to ERM-proteins directly. Analyses of total activity-levels of well known mediators between RhoA and the ERM-proteins like ROCK, PKC and PIP_2, revealed no effect of BDLF2/BMRF2-expression, indicating a different mode of ERM-inhibition. Interestingly, we could show that the BDLF2/BMRF2-complex binds directly to PKCα, which leads to reduced interaction between PKCα and the ERM-proteins and, as a result, to reduced phosphorylation of ezrin, radixin, and moesin.

Taken together, our data suggest that the EBV encoded BDLF2/BMRF2-protein complex severely modifies the cellular morphology by sequestering PKC-activity and thereby inhibiting ERM-proteins.

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Signalling **Abstract 28**

EPSTEIN-BARR VIRUS UTILIZES EXOSOMES FOR INTERCELLULAR COMMUNICATION

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Abstract

Regulation of the tumor microenvironment is an important facet of tumor progression that is needed to promote cell growth, angiogenesis, and impair recognition by the immune system. Vesicular exosomes secreted from most cell types contain proteins, mRNAs, and miRNAs that can be taken up by neighboring or distant cells and have been found in many biological fluids. Recent compelling evidence suggests that exosomes represent a novel mechanism of intercellular communication through the transfer of bioactive components between cells. In this study, exosomes secreted from nasopharyngeal carcinoma (NPC) cells harboring latent Epstein-Barr virus (EBV) were found to contain the major viral oncogene latent membrane protein 1 (LMP1), signal transduction molecules, and virus-encoded miRNAs that activated MAPK/ERK and PI3K/AKT growth signaling pathways in non-infected cells following exosome transfer experiments. Interestingly, viral miRNAs were enriched and selectively packaged into NPC exosomes that are distinct in comparison for those identified in LCL-derived exosomes. These differences may contribute to cell-type specific functions in recipient cells. Additionally, the stable expression of LMP1 in an EBV negative epithelial cell line resulted in its secretion into exosomes and also increased the exosomal release and uptake of epidermal growth factor receptor (EGFR). These findings suggest that EBV can manipulate the tumor microenvironment to influence the growth of neighboring cells by intercellular transfer of exosomes containing a viral transforming protein, cellular signaling molecules, and viral miRNAs.

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Session 5 abstracts 29-36

Immunology and Immunotherapy

Immunology and Immunotherapy **Abstract 29**

GENE EXPRESSION SIGNATURE OF PRIMARY EBV INFECTION DERIVED FROM A PROSPECTIVE STUDY

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Abstract

We are performing a prospective study of primary infection in EBV-naïve freshmen at the University of Minnesota. Frequent clinical sampling (every 8 weeks) and a relatively high rate of infection (22% per year) allowed us to study the immune response to primary EBV infection, independent of the severity of clinical symptoms. We used this resource to define common gene expression changes that occur in the blood of individuals experiencing natural primary infection. We performed microarray analysis of total peripheral blood mononuclear cells (PBMC) from 8 individuals prior to and during acute infection with EBV. This analysis identified 465 genes significantly up or down regulated in response to infection. Many of these were IFN regulated genes, or genes associated with cell proliferation/metabolism, as expected. Interestingly, a large fraction of the gene changes were not observed during primary infection with influenza, RSV, rhinovirus, or yellow fever vaccination, suggesting an "EBV unique" aspect to the signature. Furthermore, we were able to define distinct modules of gene expression that occurred earlier during infection, versus later, which generally correlated with a type I versus type II IFN response, respectively. Due to the frequency of sampling of naïve subjects, we were able to collect 27 samples from time points in the 40 days prior to initial symptoms (the estimated "incubation period" for primary EBV infection), allowing us to determine the onset and duration of PBMC gene expression changes during natural infection.

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Immunology and Immunotherapy **Abstract 30**

MALARIA CO-INFECTIONS SELECTIVELY EDIT IMMUNITY TO EBV LEADING TO DIFFERENTIAL EVOLUTION OF LYTIC AND LATENT EPITOPE-SPECIFIC MEMORY CD8$^+$ T CELLS

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Abstract

Plasmodium falciparum malaria and EBV co-infections are risk factors for endemic Burkitt lymphoma (eBL), the most prevalent pediatric cancer in equatorial Africa. Malaria has been implicated in "suppressing" T cell immunity to EBV however, the precise nature of immune dysregulation that would allow EBV-associated lymphomagenesis has not as yet been determined. Our previous studies of healthy Kenyans demonstrated a deficiency of EBV-specific IFN-γ producing T cells in young children chronically infected with malaria, subsequent to harboring relatively high EBV viral loads. In this study, we used 14-parameter flow cytometry to examine the phenotypic characteristics of CD8 T cell populations (i.e. CD45RO, CCR7, CD27, C57, CD127, PD-1) specific for EBV-derived lytic (BMFL1 and BRLF1) and latent (LMP1, LMP2, EBNA3C) antigens in individuals with divergent malaria exposure. While typical patterns of CD8$^+$ T cell heterogeneity were preserved, malaria exposure was associated with higher levels of EBV-specific CD8$^+$ T cell senescence (CD57$^+$), exhaustion (PD-1$^+$), and a loss of homeostatic potential (CD127$^-$). This phenotypic editing affected only certain EBV specificities (*i.e.* lytic and EBNA3C epitopes) and were more prevalent for CD8$^+$ T cells displaying features of late differentiation (CD45R0$^+$, CD27$^-$, CCR7$^-$). In contrast, malaria exposure did not appear to skew bulk CD8$^+$ T cell phenotypes or T cell subsets specific for a CMV epitope. The evolution of this qualitative difference in CD8$^+$ T cell immunity to EBV may result in an inability to impede the efficiency of viral lytic reactivation and be a step in the etiologic pathway for eBL.

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Immunology and Immunotherapy **Abstract 31**

BROAD TARGETING OF THE CD4$^+$ T CELL RESPONSE TOWARDS THE EPSTEIN-BARR VIRUS (EBV) LYTIC CYCLE ANTIGENS

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Abstract

The primary CD8 response in IM patients shows a strict hierarchy of immunodominance among the EBV lytic cycle proteins (immediate early (IE)>early (E)>>late (L)). This reflects the efficiency of antigen presentation to CD8$^+$ T cells in lytically infected cells, which is increasingly impaired during the EBV lytic cycle through the expression and coordinated actions of several viral immune evasion proteins. Although some of the rare E and L antigen-specific CD8 responses seen IM patients do not appear to persist into long term memory, CD4$^+$ T cells specific for these antigens have been reported in healthy EBV carriers.
Here, we have conducted a systematic study of the immunodominance of the lytic cycle antigens for the memory CD4$^+$ T cell response, focussing on a representative panel of 8 proteins from the IE, E and L phases. Peripheral blood mononuclear cells from 15 healthy virus-immune donors were screened *ex vivo* by IFNγ ELISpot assay against panels of overlapping peptides. Quite unlike the CD8 response, the CD4 response is spread across antigens expressed at all stages of the lytic cycle. Individuals have multiple responses, often within the same protein, and their dominant response may be to an antigen expressed at any stage. Isolated CD4$^+$ T cell clones specific for IE, E and L antigens are restricted through a range of HLA class II alleles and, again in contrast to the CD8 response, all have similar functional avidities. We are now studying their ability to recognise EBV-infected B cells undergoing lytic replication.

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Immunology and Immunotherapy **Abstract 32**

CD8+ CYTOTOXIC T CELLS SPECIFIC FOR LATE LYTIC INFECTION PROTEINS ARE PRESENT IN RHESUS MACAQUES WITH PERSISTENT LYMPHOCRYPTOVIRUS INFECTION

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Abstract

CD8+ Cytotoxic T cells specific for EBV late lytic infection proteins are detected much less frequently than those specific for immediate early and early proteins in patients with infectious mononucleosis. The deficit of CTL against late proteins may be due to EBV-mediated down-regulation of MHC class I expression on the surface of cells during lytic infection. Since these EBV immune evasion genes are conserved in the lymphocryptovirus naturally infecting rhesus macaques, we asked if CTL specific for late proteins are also rare in rhLCV infected rhesus macaques.

The lytic protein-specific CTL repertoire from 15 naturally and experimentally rhLCV-infected macaques was identified. rhLCV-specific CTL lines were generated from PBMC by stimulation with the autologous LCL, and assayed against target cells expressing one of 20 different rhLCV lytic proteins (2IE, 10E, and 8L) each expressed by recombinant vaccinia viruses. CTL responses specific for lytic infection proteins could be detected in all 15 animals (average=2.7 proteins recognized). Late protein-specific CTL could be detected as frequently as those specific for early proteins.

Thus, the LCV immune evasion genes that down-regulate MHC class I expression in lytic infection do not prevent the priming and maintenance of CTL specific for late lytic proteins in persistent rhLCV infection. It remains to be determined whether LCV down-regulation of MHC class I expression is important for immune evasion during acute primary infection, shedding and transmission during persistent infection, or the ability to super-infect hosts with established LCV immunity.

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Immunology and Immunotherapy **Abstract 33**

INDUCTION OF EBV-SPECIFIC IMMUNE RESPONSES BY VIRUS-LIKE PARTICLES

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Abstract

Epstein-Barr-Virus (EBV) is an ubiquitous herpes virus that is associated with several types of tumors. Especially immunocompromised individuals, like patients undergoing transplantation or suffering from AIDS are at high risk for EBV-associated lymphomas. Our goal is the development of a safe DNA-free EBV-vaccine by using virus-like particles as a highly immunogenic delivery tool.

EBV-Virus-like particles are empty virus particles that are generated by a HEK293-based EBV-packaging cell line upon doxycyclin-mediated induction of the lytic cycle. EBV-antigens delivered by VLPs efficiently reactivate EBV-specific T cells *in vitro* when presented via MHC class II molecules. A key player in this process is the major viral glycoprotein gp350 that binds to CD21 on human B-cells and thus confers a strict B-cell tropism. VLPs are efficiently processed by B-cells and EBV-derived antigens are presented professionally. In vaccinated mice they elicit strong humoral as well as cell-mediated immune responses.

In summary we demonstrate that VLPs derived from a proprietary EBV-packaging cell line are promising candidates for the development of an efficient and safe EBV-vaccine.

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Immunology and Immunotherapy **Abstract 34**

EBV-SPECIFIC T CELL RECEPTOR GENE TRANSFER TO TARGET NASOPHARYNGEAL CARCINOMA

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Abstract

Infusing EBV-specific T cells is an effective prophylactic and therapeutic treatment for EBV post transplant lymphoproliferative disease. However, extending this approach to treat NPC necessitates targeting viral antigens that are subdominant targets for T cells. Therefore we have explored the use of T cell receptor (TCR) gene transfer to rapidly and reliably generate such T cell responses from all patients.

To ensure a widely applicable treatment we cloned the genes encoding the TCR from an LMP2-specific T cell that is restricted through HLA A*1101, an allele carried by >50% of the Chinese population. Genes encoding the TCR α and β chains were cloned into a single retroviral vector, separated by a self-cleaving 2A peptide to ensure equal expression. T cells were transduced with this retrovirus and within 3-5 days HLA:peptide pentamer staining detected the transferred TCR in 12-17% of CD8+ T cells and 7-12% of CD4+ T cells. TCR-transduced T cells expanded rapidly *in vitro* in response to antigen and showed high avidity for the target peptide (10^{-10}M) in assays of interferon-γ release.

To improve safety and efficacy, the TCR was modified by codon optimization and introduction of an additional disulphide-bond. This increased almost two-fold the proportion of T cells expressing the receptor. TCR-transduced CD4+ T cells produced multiple cytokines (including IL2) in response to LMP2 suggesting they could provide a helper function *in vivo* to aid persistence of CD8+ effectors. Both CD8+ and CD4+ transduced T cells lysed an A11+ NPC cell line expressing LMP2.

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Immunology and Immunotherapy **Abstract 35**

DEVELOPMENT OF CHIMERIC T CELLS AGAINST EBV-ASSOCIATED TUMOURS

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Abstract

In order to facilitate dissemination of cellular adoptive immunotherapy against EBV-associated tumours into the clinical (patient) setting, we have genetically engineered T cells to express antibody-like chimeric T cell receptors (cTCR; 'T bodies') that can mediate tumour rejection out with the constraints of MHC restriction.

Initially, we used phage display technology to identify antibody targets on the external loops of LMP1. This entailed running phage libraries against peptides derived from these 3 loops that allowed us to identify 20 reactive phage clones as assessed in an EIA context using specific and non-specific targets. Sequence analysis showed the clones to portray one of 2 major sequence patterns. The phage sequences were inserted into retroviral cTCR constructs and transfected into Jurkat cells as well as peripheral blood leukocytes (PBL) from healthy blood donors. Investigations using NFAT luciferase reporter assays in the Jurkat cells demonstrated T cell activation upon recognition of LMP1-positive tumour cell targets when compared with EBV-negative control cells. In parallel, solubilised phage-derived antibodies were shown to bind to LMP1-expressing tumour cells by immunohistochemical methods. Currently, further *in vitro* investigations involving 51Cr-release and ELISpot assays are underway, and we have embarked on *in vivo* experiments involving the cTCR PBL in a scid mouse PTLD model – the results of which will be presented.

Our preliminary data suggests that we have identified novel LMP1 antibody targets to which LMP1-specific T bodies can be re-directed to out with MHC constraints. Our aim is to translate our findings into the clinical (patient) setting.

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Immunology and Immunotherapy **Abstract 36**

ADVANCES IN T CELL THERAPY FOR EBV TYPE 2 LATENCY LYMPHOMAS

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Abstract

Challenges associated with the treatment of EBV-associated, latency type 2 malignancies in immunocompetent persons, range from poor antigen presentation to active inhibition of T cells by the tumor itself or by tumor infiltrating cells or tumor stroma.
EBV-LCL-activated T cells proliferated after infusion and had anti-tumor function, including two complete remissions in patients with multiply-relapsed Hodgkin's disease, but did not control tumors in patients with bulky disease. To improve T cell specificity for viral antigens expressed in type 2 malignancies, we activated and expanded T cells using dendritic cells and LCLs expressing LMP antigens from an adenovirus vector. In two phase 1 trials clinical trials, LMP-specific T cells proliferated after infusion and produced complete remissions in over 60% of patients with EBV-positive Hodgkin and non-Hodgkin lymphoma.
TGF-β is secreted by lymphomas, tumor stromal cells and T regulatory cells and is a major mediator of T cell inhibition. We have now enrolled three patients in a trial of LMP-specific T cells expressing a dominant-negative TGF-β type II receptor (DNR): We observed no toxicities, the second patient had a complete response and the third patient had a very good partial response and will receive a second dose of CTLs.
In conclusion, LMP-specific T cells demonstrate high specificity and low toxicity and offer a potent treatment for EBV-positive lymphomas that arise in immunocompetent persons. However, to be considered for primary therapy, these studies must be validated in phase 2 trials and strategies to reduce the time taken for T cell production are required.

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Session 6 abstracts 37-42

Virus Replication

Virus Replication **Abstract 37**

DYNAMIC CHROMATIN STRUCTURE AT EBV ORIGIN OF LYTIC REPLICATION AND EARLY PROMOTERS DURING EBV REPLICATION

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Abstract

During the switch between EBV latency and lytic replication, the transcription profile of the genome changes dramatically with activation of around 80 viral promoters. The viral protein Zta (BZLF1, ZEBRA, Z) acts as both a transcription factor and a replication factor, binding to specific Zta response elements (ZREs) in both promoters and the two origins of lytic replication (Orilyt-L and -R). Alteration of chromatin structure in the vicinity of the immediate early promoters Zp (Zta) and Rp (Rta) is known to play a key role in gene expression and viral reactivation.

We investigated the chromatin structure at OriLyt-L, OriLyt-R and the promoters for BZLF1 (Zp), BMRF1 and EBV DNA polymerase using chromatin immuno-precipitation assays (ChIP). Surprisingly, we found significant binding of acetylated Histone H3 at both the origins and promoters in the absence of lytic replication, providing evidence for “poised” chromatin at these regions. Changes to chromatin structure marked by acetylation of Histone 4 is accompanied the induction of lytic cycle, both at the origins and viral promoters. Activation of the DNA-damage response and phosphorylation of EBV-bound H2AX accompanying viral replication is also observed.

Upon induction of lytic replication, we observed that Zta forms a tight footprint over regions containing ZREs at OriLyt L and R and its own promoter (Zp). At any point in time, Zta does not interact with all of the ZREs in the regions analysed. We are investigating what specifically stabilizes the interaction of Zta with Zp and Orilyt in the late stage of lytic cycle.

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Virus Replication **Abstract 38**

THE CELLULAR STRESS SENSOR, OCT-1, COOPERATES WITH THE IMMEDIATE-EARLY PROTEIN, BRLF1, TO INDUCE EBV LYTIC REACTIVATION

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Abstract

The EBV latent to lytic switch is an essential part of the viral life cycle and is regulated by two immediate-early viral proteins, BZLF1 (Z) and BRLF1 (R). Activation and repression of the immediate-early viral promoters is regulated by host cellular transcription factors. Here we demonstrate that the ubiquitously expressed cellular transcription factor, Oct-1, cooperates with R to induce lytic reactivation. We show that the Oct-1 and R proteins directly interact. Furthermore, we find that Oct-1 enhances R-mediated lytic reactivation in EBV-positive epithelial cells. In reporter gene assays, Oct-1 enhances R-mediated activation of both the BZLF1 immediate-early gene promoter and the early lytic SM promoter. Importantly, we show that loss of endogenous Oct-1 expression decreases constitutive lytic viral protein expression in both EBV-positive B-cells and epithelial cells. Taken together, our results suggest that Oct-1 is a key positive regulator of lytic viral reactivation in both B-cells and epithelial cells. Interestingly, as Oct-1 transcriptional function is known to be regulated by various forms of cellular stress, the propensity of EBV to reactivate during cellular stress may be at least partially mediated through stress-induced alterations in Oct-1 activity.

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Virus Replication **Abstract 39**

EPSTEIN-BARR VIRUS LF2 PROTEIN REGULATES VIRAL REPLICATION BY ALTERING RTA SUBCELLULAR LOCALIZATION

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Abstract

The switch from Epstein-Barr virus (EBV) latent infection to lytic replication is governed by two viral transactivators, Zta and Rta. We had previously reported that the EBV protein LF2 binds Rta, inhibits Rta promoter activation, and blocks EBV replication in cells. In addition, LF2 induces SUMO2/3 modification of Rta. We now show that this modification occurs at four lysines within the Rta activation domain and that sumoylation of Rta is not essential for its repression. Co-expression studies demonstrate that Rta is sequestered to the extra-nuclear cytoskeleton in the presence of LF2. We mapped the LF2 binding site to the Rta accessory activation domain and show that LF2 binding is critical for Rta relocalization and repression. The core of this binding site comprising 27 aa confers LF2-mediated relocalization and repression onto the artificial transcription factor GAL4-VP16. Mutational analysis of LF2 provides further evidence that Rta redistribution is essential for repression. Rta localization changes during replication of the LF2 positive P3HR1 genome, but not during replication of the LF2 negative B95-8 genome. BLRF2 protein expression was decreased and peaked later in P3HR1 cells compared with B95-8 cells, consistent with reduced Rta activity. By contrast, BMRF1 expression, regulated primarily by Zta, did not differ significantly between the two cell lines. Our results support a model in which LF2 regulates EBV replication by binding to Rta and redistributing it out of the nucleus.

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Virus Replication **Abstract 40**

EBV RTA INHIBITS PROTEIN KINASE CK2

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Abstract

Rta, a multifunctional protein with important roles in transcriptional activation and lytic replication of EB virus, must be regulated during the viral life cycle. We demonstrate that phosphorylation of Rta is temporally regulated. We identify CK2 as a kinase that phosphorylates Rta *in vitro;* Ser 337 is the major phosphorylation site. Full-length Rta R(1-605) and a C-terminal truncation mutant R(1-550) are weakly phosphorylated, but mutants R(1-350) and R(1-450) are robustly phosphorylated *in vitro* by CK2. This result suggested the presence of a CK2 phosphorylation inhibitory region (CPIR) between aa 450 and aa 550. Wild-type Rta and R(1-550) can trans-inhibit phosphorylation of R(1-350) and R(1-450) and unrelated substrates by CK2. Titration of inhibitor and substrate proteins suggest that inhibition of CK2 phosphorylation results from direct interaction of Rta with CK2. A sequence of amino acids in the CPIR of Rta is similar to the negative regulatory region of CK2β. The sequence PDEE is conserved. Alanine substitution mutants demonstrated the importance of these residues of Rta for inhibition of CK2 activity. Besides being essential for cell survival, CK2 also phosphorylates and modulates activity of EBV lytic proteins including ZEBRA and EB2 (BMLF1). Viruses exploit CK2 to facilitate their survival, by stimulating, redistributing or modulating substrate specificity. Our demonstration that EBV Rta can inhibit phosphorylation by CK2 *in vitro* suggests that, besides having a direct role in activation of genes in the lytic cascade, Rta can also modulate the function of cellular and viral proteins by regulating their phosphorylation by CK2.

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Virus Replication **Abstract 41**

EB2, THE EBV VIRAL MRNA EXPORT PROTEIN INTERACTS WITH SRP20, A FACTOR IMPLICATED IN CELLULAR MRNA SPLICING AND EXPORT

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Abstract

The Epstein-Barr Virus (EBV) early nuclear protein, EB2 (also called Mta, SM and BMLF1), allows the nuclear export of a subset of early and late viral RNAs derived from intronless genes and its presence is essential for the production of infectious particles. Moreover, we recently found that EB2 strongly increases the translation of its target mRNAs. However, the mechanisms by which EB2 fulfils these various functions are not yet completely understood. By using a yeast two-hybrid screen, we have now identified the cellular factor, SRp20, as a cellular partner for EB2. We confirmed this interaction by means of interaction studies (co-immunoprecipitation assays and GST-pull down) and we have characterized the interaction domain in both proteins. In order to understand the importance of this interaction in terms of EB2 function, we first tested the effect of a specific siRNA directed against SRp20 on EB2-mediated mRNA export. Interestingly, we found that the export by EB2 of a luciferase reporter messenger generated from an intronless construct, was strongly diminished in the absence of SRp20. We then tested the effect of EB2 on a β-globin reporter construct in the presence of overexpressed SRp20. Overexpression of SRp20 alone is known to promote aberrant mRNA splicing in this model. In the presence of EB2, we found that overexpression of SRp20 strongly increases EB2-dependent accumulation of unspliced β-globin mRNA in the cytoplasm. Interaction of EB2 with SRp20 thus appears to be crucial for its capacity to efficiently export unspliced mRNA.

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Virus Replication **Abstract 42**

EPSTEIN-BARR VIRUS SM PROTEIN MODULATES RNA SPLICING BY INTERACTING WITH SPLICING FACTOR SRP20

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Abstract

SM is a nuclear EBV protein essential for EBV replication. SM binds to cellular and viral RNAs regulating their expression. STAT1, a central mediator of IFN signaling, is expressed in two isoforms, STAT1α and STAT1β. STAT1β is an alternatively spliced form lacking transactivating domains that can act in a dominant negative manner to inhibit STAT1 signaling. SM affects STAT1 splicing and disproportionately increases STAT1 β levels. SR proteins influence alternative splicing regulation and an association between SRp20 and SM was discovered.

Hypothesis: EBV SM protein regulates STAT1 splicing by direct interactions with the alternative splicing factor SRp20.

SM complexes from mammalian cells isolated by affinity purification were analyzed by MS. An SM-SRp20 interaction was further confirmed by co-immunoprecipitation and the SM binding region was mapped to specific SRp20 sites. SM induces alternative splicing of STAT1 at a novel 5' splice site resulting in an increased STAT1β/α ratio. SRp20 increased SM-mediated STAT1 alternative splicing and knockdown of SRp20 protein decreased this activity in STAT1 minigene splicing assays. SRp20 itself is inactive in modulating STAT1 splicing but mutant SRp20 lacking the RS domain inhibited SM-mediated alternative splice site selection.

EBV SM interacts with SRp20 and modulates cellular gene expression by regulating alternative splicing. SM, which lacks RS domains typical of splicing factors, may recruit SRp20 to pre-mRNA and co-opt SRp20 function to direct alternative splicing. Viruses may thus regulate gene expression by affecting splice-site selection, increasing the complexity of the cellular "splicing code" to evade immune surveillance, facilitating virus survival and replication.

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Session 7 abstracts 43-49

Latency II

Latency II **Abstract 43**

MOLECULAR MECHANISM OF EPIGENETIC GENE SILENCING BY EBNA-3A

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Abstract

EBV immortalized B cells proliferate via the concerted action of viral nuclear antigens (EBNAs) and latent membrane proteins (LMPs). A subset of these viral proteins is rate limiting to activate primary B cells and to establish a stable homeostasis of growth promoting and anti-apoptotic activities. EBNA-3A deficient viral mutants can infect primary B cells and give rise to permanently growing immortalized B cell cultures which proliferate at reduced rates and show elevated levels of apoptosis. EBNA-3A positive and negative cells express a characteristic expression profile of cellular target genes. 296 differentially regulated genes were significantly enriched for genes involved in apoptosis or cell cycle progression like p16/INK4A, or might contribute to the viral life cycle in the infected host. In order to elucidate the molecular mechanism by which EBNA-3A represses cellular target gene expression we characterized the epigenetic modifications of a selected target gene locus encoding CXCL10, a chemoattractant for NK and T cells. EBNA-3A is sufficient to mediate repression of CXCL10 in the absence of other EBV-encoded proteins, but effective repression requires CBF1. Chromatin immunoprecipitation experiments indicate that EBNA-3A abrogates recruitment of RNA Polymerase II to the CXCL10 promoter and provokes deacetylation of histone H3 and H4, demethylation of H3K4, recruitment of Polycomb Group members and trimethylation of H3K27. Epigenetic analysis EBNA-3A target genes will permit a more detailed understanding of EBNA-3A´s contribution to viral pathogenesis and provides an important experimental approach to study the molecular mechanism of transcriptional repression by EBNA-3A.

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Latency II **Abstract 44**

EPSTEIN-BARR VIRUS NUCLEAR PROTEIN EBNA3C CONTRIBUTES TO THE GROWTH OF EBV-TRANSFORMED B CELLS THROUGH INHIBITING INK4A/ARF EXPRESSION

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Abstract

Epstein-Barr virus (EBV) nuclear antigen 3C (EBNA3C) is essential for EBV-induced conversion of primary human B cells into indefinitely proliferating lymphoblastoid cell lines (LCLs). EBNA3C inactivation in LCLs that harbor EBVs expressing conditionally-active EBNA3C resulted in the induction of $p16^{INK4A}$ and $p14^{ARF}$, which was accompanied by growth arrest of the LCLs. The induction of $p16^{INK4A}$ and $p14^{ARF}$ occurred at the transcriptional levels. EBNA3C inactivation did not change CpG methylation status of $p16^{INK4A}$ promoter. On the other hand, EBNA3C inactivation increased levels of histone H3 acetylation and H3K4 tri-methylation in the p16 locus, while it reduced level of H3K27 tri-methylation in the same region, indicating the association between EBNA3C activity and the chromatin status of p16 locus. Transfection of plasmids containing either shRNA targeting $p16^{INK4A}$ or shRNA targeting $p14^{ARF}$ partially rescued the LCL cells from the growth arrest induced by EBNA3C inactivation, and transfection of both shRNAs additively rescued, indicating that both $p16^{INK4A}$ and $p14^{ARF}$ play a critical role in the growth arrest of LCL cells induced by EBNA3C inactivation. Thus, the data suggest that EBNA3C contributes to the growth maintenance of LCLs through inhibiting the expression of $p16^{INK4A}$ and $p14^{ARF}$ from the INK4A/ARF locus.

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Latency II **Abstract 45**

THE CELL-CYCLE CONTROL GENE RGC-32 IS UPREGULATED BY EBNA 3C AND SELECTIVELY TRANSLATED IN LATENCY III CELLS

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Abstract:

EBV EBNA 3C is essential for B-cell transformation and promotes inappropriate progression through the G1/S, G2/M and M-phase cell-cycle checkpoints through mechanisms yet to be fully elucidated. Using mRNA expression arrays we now demonstrate that the novel cell-cycle regulator, RGC-32, is upregulated in EBNA 3C positive B-cell-lines. Overexpression of RGC-32 alone is sufficient to disrupt the G2/M checkpoint following DNA damage in B-cells and RGC-32 activates CDK1 *in vitro,* indicating that inappropriate CDK1 activation through RGC-32 upregulation may represent part of the mechanism through which EBNA 3C disrupts the block at G2/M. Accordingly, we demonstrate that RGC-32 protein is selectively expressed in EBV infected cells displaying the full panel of latent gene expression (Latency III). Moreover, initial RGC-32 knockdown experiments indicate that RGC-32 expression is required for the continued proliferation of lymphoblastoid cell-lines. Interestingly, RGC-32 mRNA expression is high in latency I cells where RUNX1 is expressed and we demonstrate that RUNX1 activates the RGC-32 promoter in both exogenous and endogenous assays. Despite this, RGC-32 protein expression is restricted to latency III cells as a result of apparent tight control at the level of mRNA translation. Our results identify a novel mechanism via which EBNA-3C may be able to disrupt the G2/M checkpoint and implicate RGC-32 as a key regulator of the proliferation of EBV immortalised cells.

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Latency II **Abstract 46**

EBNA-3A REPRESSES CDKN2A AND CDKN2B EXPRESSION THROUGH INTERACTION WITH MIZ-1

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Abstract

The Epstein-Barr virus (EBV) nuclear antigen 3A (EBNA-3A) is one of the essential latent proteins necessary for primary B-cell transformation. The exact role of EBNA-3A is not yet completely understood. To better understand the function of the protein, we searched for EBNA3A interacting partners using a yeast two-hybrid screen. One of the proteins identified in this screen is the Myc-interacting zinc finger protein-1 (Miz-1), a transcription factor initialy characterized as a binding partner of c-Myc. Miz-1 has a cell growth arrest activity via inhibition of cell-cycle progression and has been shown to activate transcription of target genes including *CDKN1A* and *CDKN2B*. We first confirmed the interaction between EBNA-3A and Miz-1 by co-immunoprecipitation assays in HeLa cells transiently transfected with EBNA-3A and Miz-1 expression vectors. Then we established the physiological relevance of the interaction by showing that EBNA-3A and Miz-1 could be co-immunoprecipitated from a lymphoblastoid cell line (LCL) expressing endogenous EBNA-3A and Miz-1. We next investigated the effect of EBNA-3A on Miz-1-mediated activation of *CDKN2A and CDKN2B*. HeLa cells were transfected with luciferase reporter constructs containing the human *CDKN2A* or *CDKN2B* promoters, in the presence of Miz-1 alone or Miz-1 plus EBNA-3A. Interestingly, we found that expression of EBNA-3A inhibits Miz-1 dependant activation of both *CDKN2A* and *CDKN2B* promoters in a dose-dependant manner. The interaction between EBNA-3A and Miz-1 is thus likely to play an essential role in EBV dependant transformation of primary B cells. The mechanisms of inhibition of Miz-1 dependant activation by EBNA-3A will be discussed.

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Latency II **Abstract 47**

BNLF2A AND BNLF2B ARE EXPRESSED AS LATENT GENES IN EBV-INFECTED B LYMPHOCYTES AND EPITHELIAL CELLS

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Abstract :

Around 10 EBV latent genes are known and many of them play roles for development and maintenance of EBV-associated malignancies. The BNLF2a gene, which locates on *Eco*RI Dhet region of EBV genome, is regarded as an early lytic gene and prevents EBV-infected cells from CD8+ T cell recognition by decreasing surface MHC class I expression. At 9bp downstream of the BNLF2a gene, the BNLF2b gene is mapped. Although both BNLF2a and BNLF2b are translated from the same mRNA, there is no report on biological significance of BNLF2b. Here, we show that both BNLF2a and BNLF2b are expressed in latently EBV-infected cells, both B lymphocytes and epithelial cells. Cytological staining using HaloTag system or rabbit antibodies against BNLF2a and BNLF2b peptides, respectively, shows that BNLF2a localizes at endoplasmic reticulum and BNLF2b at nucleus. BNLF2a and BNLF2b are expressed on both IB4 and Namalwa cells, which keep latent infection tightly. A BNLF2a and BNLF2b double knockout (BNLF2-KO) EBV is generated by substituting both genes with a hygromycin resistant gene using Akata cell system. Growth rate of BNLF2-KO EBV-transformed lymphocytes is slower than that of wild EBV-transformed lymphocytes, suggesting that BNLF2a and/or BNLF2b play some roles on EBV-associated malignancies.

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Latency II **Abstract 48**

LMP1 INDUCED NFKB PROMOTES GLUCOSE METABOLISM VIA GLUT1 MEMBRANE TARGETING

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Abstract :

Multiple oncogenes increase glucose metabolism and thus support macromolecule synthesis necessary for rapidly dividing cells. As EBV transformed lymphoblastoid cell lines exhibit a high glycolytic rate without genomic mutations, we investigated the effect of the EBV oncoprotein LMP1 on glucose metabolism.
LMP1 expression in BL41 increased glucose import by promoting translocation of the glucose transporter, GLUT1, from intracellular vesicles to the plasma membrane. LMP1 expressing BL41 cells exhibited increased glucose import and glycolytic activity. IKKb inhibitors blocked LMP1 effects on GLUT1 localization and glycolysis. Furthermore, NFkB inhibition in LCLs reduced GLUT1 at the plasma membrane, glucose import and lactate production. CpG stimulation, which also activates NFkB through TLR9, similarly induced GLUT1 localization to the plasma membrane in BL41.
The Pi3K/AKT-pathway is known to regulate GLUT1 localization in lymphoid cells. LMP1 induced AKT activity in BLs and NFkB inhibitors reduced AKT activity in both BL41 and LCLs.
Prolonged NFkB inhibition in LCLs induced starvation, shown by the accumulation of autophagosomes. Simultaneous inhibition of NFkB and autophagy resulted in rapid LCL death.
These data indicate that EBV LMP1 mediated NFkB activation functions as an essential signal for full AKT activity in LCLs and thereby regulates LCL proliferation through nutrient availability. Moreover, our data suggest that NFkB may play a similar role in normal lymphocyte growth and other NFkB dependent viral cancers including as KSHV peripheral effusion lymphoma and HTLV1 acute T cell leukemia.

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Latency II **Abstract 49**

THE EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN-1 (EBNA1) ACTIVATES ALTERNATIVE LENGTHENING OF TELOMERE VIA INDUCTION OF OXIDATIVE STRESS

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Abstract

Cell immortalization and malignant transformation are dependent on the activation of mechanisms for maintenance of telomere homeostasis. We found that the Epstein-Barr virus (EBV) nuclear antigen (EBNA)-1, that is regularly expressed in all EBV associated malignancies, activates Alternative Lengthening of Telomere (ALT) via induction of oxidative stress. Expression of EBNA-1 was associated with heterogeneity of telomere signals, induction of ALT-associated promyelocytic leukemia nuclear bodies (APBs), displacement of the shelterin protein TRF2 and accumulation of Telomere Dysfunction-Induced Foci (TIFs) containing phosphorylated histone H2AX and the DNA damage response protein 53BP1. APBs were induced in parallel with EBNA-1 in a conditional transfectant of the EBV negative B-lymphoma BJAB and during EBV-induced immortalization of normal B-cell. Treatment with scavengers of reactive oxygen species prevented the induction of TIFs and APBs, and promoted the accumulation of TRF2 at telomeres. These findings highlight a novel mechanism by which EBNA-1 may contribute to the immortalization of EBV infected cells and promote malignant transformation.

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Session 8 abstracts 50-57

Epithelial cells, Infection, Carcinoma

Epithelial Cells, Infection, Carcinoma **Abstract 50**

IMPORTANT BUT DIFFERENTIAL ROLES FOR ACTIN IN TRAFFICKING OF EBV IN B CELLS AND EPITHELIAL CELLS

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Abstract

EBV infects B cells via fusion with an endocytic vesicle but fuses directly at the surface of an epithelial cell, consistent with a subsequently different fate. QPCR analysis indicated that the amount of virus entering B cells increased for up to 4 h and then persisted. Infection of epithelial cells engineered to express CR2, or CR2 lacking a cytoplasmic tail, was equally efficient, but internalized virus increased for only 1 h and then declined by ~50% to a plateau at 4 h. Approximately 50% of that remaining reached the nucleus. In CR2-negative cells, where infection was less efficient, ~90% of virus was lost. Leupeptin and PI3K inhibitors increased survival of virus, consistent with loss due to autophagy. RT-QPCR analysis of gene expression at 4 h indicated that microtubule disruption reduced, but did not eliminate transport in both cell types and effects on infection were reversible. Actin inhibitors reduced the amount of virus that entered B cells, consistent with a block in endocytosis. In contrast, they increased the amount entering epithelial cells, consistent with disruption of an actin cortical barrier, and completely inhibited subsequent transport. Epithelial transport was also blocked by inhibitors of p38/MAPK, ROCK and RhoA, participants in networks controlling actomyosin contractility. Experiments are being repeated with hTERT-immortalized oral keratinocytes, but suggest a critical, mechanistically different role for actin reorganization in B cells and epithelial cells. In epithelial cells this may be initiated by BMRF2 or gH and integrins, but is probably not nucleated by interaction of CR2 and formins.

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Epithelial Cells, Infection, Carcinoma **Abstract 51**

EPSTEIN-BARR VIRUS INFECTION OF POLARISED EPITHELIAL CELL LAYERS BY B CELL-MEDIATED TRANSFER INFECTION: COMPONENTS OF THE VIROLOGICAL SYNAPSES.

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Abstract

Epstein Barr virus (EBV) exhibits a distinct tropism for both B cells and epithelial cells as demonstrated by a strong association with B cell (Burkitt's lymphoma, Hodgkin's lymphoma) and epithelial cell malignancies (nasopharyngeal carcinoma, gastric carcinoma). Direct infection of B cells is initiated by the interaction of the major viral glycoprotein gp350 with CD21 on the B cell surface. Fusion is triggered by the interaction of the glycoprotein, gp42 with HLA class II, and is thereafter mediated by the core fusion complex, gHgLgp42. In contrast, direct infection of CD21-negative epithelial cells is very inefficient. However, EBV can efficiently access the epithelium by first binding to the resting B cell and using this surface as a transfer vehicle.

EBV binding to CD21 on resting B cells induces activation of the B cell adhesion molecules. These molecules co-cap with the virus on the cell surface and induce firm binding to their cognate receptors on the epithelial cell. However, adhesion molecules are differentially expressed on the apical and basolateral surfaces of polarised epithelial cells, and physiologically B cells are most likely to interact with the basolateral surface. Infection of polarised primary tonsillar epithelial cells by EBV is mediated by transfer from memory B cells at the basolateral surface. B cell adhesion is achieved by CD11b and CD48 interaction with the heparan sulphate moieties of CD44v3, with the involvement of LEEP-CAM. Thereafter, infection is achieved by EBV interaction with integrins specific for epithelial cells at the basolateral surface.

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Epithelial Cells, Infection, Carcinoma **Abstract 52**

EBV-POSITIVE NPC TUMOURS CONTAIN CELLS DISPLAYING A STEM CELL PHENOTYPE AND SHOW DEREGULATED EXPRESSION OF THE HEDGEHOG SIGNALLING PATHWAY

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Abstract

As part of a study whose primary purpose was to identify differences in cellular gene expression between NPC tumours and normal nasopharyngeal epithelium, we identified over-expression of a number of epithelial stem cell/CSC markers (CD44, the α6 and β1 integrins, ΔNp63, BMI1, EZH2 and BCL2) in NPC tumours. Immunohistochemical staining confirmed stronger and more uniform staining for these markers in tumours compared to normal epithelium, where expression is confined to the basal cell layer. RT-PCR analysis revealed enrichment of a number of stem cell/CSC markers (CD133, Lrig1, BMI1, EZH2 and p75NTR) in the EBV-positive NPC and gastric epithelial cell lines, C666-1 and YCCEL.1, compared to normal epithelial cells and other oral cancer-derived cell lines. The same study identified deregulated expression of key signalling pathways implicated in stem cell/CSC maintenance. Expression of a number of Shh target genes was shown to be altered in NPC tumours relative to normal epithelium. Preliminary IHC staining confirmed many of the array predictions, with perturbation in the expression of a selected number of Shh target genes in NPC tumour cells compared to normal epithelium. Expression of the Shh targets PTCH and GLI1 was also shown to be upregulated in C666-1 cells compared to normal oral epithelial cells and other oral cell lines. These findings are supported by our observations showing that GLI1 luciferase reporter activity is higher in C666-1 cells than in EBV negative oral keratinocyte cell lines. These data provide a novel demonstration of the potential importance of the Hh signalling pathway in NPC.

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Epithelial Cells, Infection, Carcinoma **Abstract 53**

EPSTEIN-BARR VIRUS-ENCODED LMP2A INDUCES AN EPITHELIAL–MESENCHYMAL TRANSITION AND INCREASES THE NUMBER OF SIDE POPULATION STEM-LIKE CANCER CELLS IN NASOPHARYNGEAL CARCINOMA

Qing-Li Kong, Li-Juan Hu, Jing-Yan Cao, Li-Hua Xu, Yi Liang, Xing Zhang, Man-Zhi Li, Yun-Fei Xia, Li-Bing Song, Yi-Xin Zeng and Mu-Sheng Zeng

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Abstract

LMP2A, the Epstein-Barr virus encoded latent protein, has been reported to play roles in oncogenic processes. Here, we report that by immunostaining LMP2A is overexpressed in 57.6% of the nasopharyngeal carcinoma tumors sampled and is mainly localized at the tumor invasive front. We found also in NPC cells that the exogenous expression of LMP2A greatly increases their invasive/migratory ability, induces epithelial–mesenchymal transition (EMT)-like cellular marker alterations, and stimulates stem cell side populations and the expression of stem cell markers. In addition, LMP2A enhances the transforming ability of cancer cells in both colony formation and soft agar assays, as well as the self-renewal ability of stem-like cancer cell in a spherical culture assay. Additionally, LMP2A increases the number of cancer initiating cells in a xenograft tumor formation assay. More importantly, the endogenous expression of LMP2A positively correlates with the expression of ABCG2 in NPC samples. Finally, we demonstrate that Akt inhibitor (V) greatly decreases the size of the stem cell side populations in LMP2A-expressing cells. Taken together, our data indicate that LMP2A induces EMT and stem-like cell self-renewal in NPC, suggesting a novel mechanism by which Epstein-Barr virus induces the initiation, metastasis and recurrence of NPC.

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Epithelial Cells, Infection, Carcinoma **Abstract 54**

ABERRANT NOTCH3 EXPRESSION IN EBV-ASSOCIATED NASOPHARYNGEAL CARCINOMA

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Abstract

Nasopharyngeal carcinoma (NPC) is an EBV-associated epithelial malignancy which prevalent in Southeast Asia and Southern China. Our recent study showed that constitutive activation of NOTCH pathway is consistently detected in both NPC tumor lines and primary tumors. Upregulation of NOTCH ligands (JAG1 or DLL4) and effector (HEY1) was found in almost all EBV positive tumor lines and primary tumors. Importantly, overexpression and activation of NOTCH3 receptor occurred in 6/6 (100%) of NPC tumor lines and 18/26 (70%) of primary tumors. Knockdown of NICD3 expression by siRNA led to significantly reduction of RBP-Jk promoter activity and expression of the downstream effectors in the EBV-positive NPC cell line, C666-1. By microarray analysis, a number of targets (e.g. *CCND1*, *C-MYC*, and *BCL-XL*) involved in cell proliferation and survival were identified in the NICD3-silenced NPC cells. Our functional study demonstrated NICD3 inactivation down-regulated the proliferation of C666-1 cells, both on the viability and DNA synthesis. Increase apoptotic cells were found in the NICD3 siRNA-treated NPC cells. Our data support the hypothesis that NOTCH signaling pathway plays a crucial role in NPC through regulating apoptosis. Furthermore, we found that the drug resistance of C666-1 towards cisplatin was significantly decreased after Notch3 SiRNA treatment. Increased tumor apoptosis were detected in the NPC cells treated with both cisplatin and Notch3 SiRNA when compared with that treated with cisplatin only. Since NOTCH signaling is a highly conserved pathway important for human cancers, NICD3 may serve as a potential therapeutic target for treating patient suffering from this EBV-associated cancer.

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Epithelial Cells, Infection, Carcinoma **Abstract 55**

FUNCTIONAL INVESTIGATION OF TUMOR AND ANGIOGENESIS SUPPRESSIVE CANDIDATE TUMOR SUPPRESSOR, *CYSTEINE-RICH INTESTINE PROTEIN 2* IN NASOPHARYNGEAL CARCINOMA

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Abstract

A novel candidate tumor suppressor gene (TSG), *Cysteine-rich intestine protein 2* (*CRIP2*), was identified in NPC. This gene has previously been reported to be expressed in the heart endothelium; its possible relationship with tumor development is unknown. Therefore, its contribution and functional role in NPC was investigated. ***Methods/Principal findings:*** *CRIP2* is down-regulated in 5/7 NPC cell lines and 42/60 (70%) patient biopsies. *CRIP2* re-expression suppresses colony formation *in vitro* and tumor growth *in vivo.* Functional studies such as invasion, HUVEC tube formation, and *in vivo* matrigel plug angiogenesis assays were used to investigate *CRIP2* gene function. Expression of *CRIP2* inhibits angiogenesis both *in vitro* and *in vivo*. Using an angiogenesis protein array, several angiogenesis-related proteins were found to be down-regulated by *CRIP2* re-expression. Analysis of conditioned media and gene expression in *CRIP2*-expressing clones validated protein array analysis results. ***Conclusions and Significance*:** An interesting candidate TSG, *CRIP2*, was identified. Functional studies confirm that *CRIP2* can suppress tumor growth *in vivo* and inhibit angiogenesis. Angiogenesis is important for cancer development; inhibition of angiogenesis by *CRIP2*, which down-regulates the angiogenesis and VEGF signaling pathway proteins further confirms its importance in tumor development in NPC. *CRIP2* is a potential candidate for future NPC therapeutic treatment.

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Epithelial Cells, Infection, Carcinoma **Abstract 56**

FUNCTIONAL EPIGENOMICS IDENTIFIES UBE2L6 AS A TUMOR SUPPRESSOR GENE IN HUMAN NASOPHARYNGEAL CARCINOMA

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Abstract

Nasopharyngeal carcinoma (NPC) is a serious health problem in southern China. Besides the significant association with latent Epstein-Barr Virus infection, epigenetic inactivation of tumor suppressor genes (TSG) and other genes play significant roles in its carcinogenesis. Exploring novel genes that are targets of epigenetic regulation in NPC may provide further insights into NPC and may reveal novel targets for diagnostic and therapeutic strategies. To identify novel candidate TSGs in NPC, we performed a genome-wide screening for genes inactivated by promoter hypermethylation. After analyzing the changes in global gene expression profiles in two NPC cell lines before and after combined treatment with the a demethylating agent 5-aza-dC and the histone deacetylase inhibitor TSA, we selected 497 genes, which harbored a CpG island in the 5' region, and were downregulated by promoter hypermethylation in NPC. UBE2L6, an E2 enzyme participating both in ISGylation and ubiquitination of cellular targets, was selected for validation and further characterization. Our results demonstrated that UBE2L6 was frequently downregulated in NPC cell lines and primary tumors. Promoter hypermethylation UBE2L6 was detected in 100% of NPC cell lines and 47.5% of NPC primary tumors. UBE2L6 expression could be restored in NPC cell lines by 5-aza-dC treatment alone. Moreover, ectopic expression of UBE2L6 significantly suppressed the proliferation and colony formation of NPC cells. Thus, UBE2L6 is a functional candidate TSG in human NPC. Our present study lay the foundation for a comprehensive understanding of the full extent of epigenetic changes in NPC, and pave the road for discovering novel TSGs.

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Epithelial Cells, Infection, Carcinoma **Abstract 57**

EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 IS NOT ASSOCIATED WITH VESSEL DENSITY NOR WITH HYPOXIA INDUCIBLE FACTOR 1 ALPHA EXPRESSION IN NASOPHARYNGEAL CARCINOMA TISSUE

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Abstract

HIF-1A and the neo-angiogenic factors induced as a result of hypoxia-inducible factor (HIF) transcriptional activation may contribute to tumorigenesis by inducing vessel formation that in turn provides oxygen and nutrients promoting tumor expansion. *In vitro* studies of nasopharyngeal carcinoma (NPC), an aggressive malignancy that is nearly always infected by Epstein–Barr virus, show HIF-1A is upregulated by viral latent membrane protein 1 (LMP1). The current study used immunohistochemistry to examine the extent to which HIF-1A and LMP1 are co-expressed in naturally infected NPC tissues. *Methods*: Analytic procedures were optimized for sensitive localization of HIF-1A and LMP1 in fixed tissue sections using immunohistochemistry with sensitive fluorescent and signal amplification technologies. Vessel density was quantified by CD31 immunohistochemistry. *Results*: LMP1 was expressed focally in all 18 NPCs examined, including 7/8 *in situ* lesions. There was no consistent co-localization with HIF-1A which was usually only weakly expressed in a subset of neoplastic cells. Neither LMP1 nor HIF-1A expression correlated with vessel density, and degree of vascularization varied widely among cases. *Conclusion*: Advanced immunohistochemical technologies reveal that LMP1 is expressed more commonly than previously reported in NPC. There is no consistent relationship between LMP1 and either HIF-1A expression or degree of microvasculature. The biologic basis for the wide variation in vessel density deserves further investigation.

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Session 9 abstracts 58-61

Therapy

Therapy **Abstract 58**

A XENOTRANSPLANT MODEL OF CHRONIC ACTIVE EPSTEIN-BARR VIRUS (EBV) INFECTION BY USE OF NOG MICE

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Abstract

In chronic active EBV infection (CAEBV), an intractable disease with poor prognosis, EBV infects T and NK cells and causes their monoclonal or oligoclonal proliferation. No model animal of CAEBV has been developed so far. CAEBV can be divided into four types, CD4, CD8, γδT and NK types, depending on the target cells of EBV infection in respective patients. By transplanting NOD/Shi-*scid*/IL-2Rγ^{null} (NOG) mice with CAEBV patient-derived PBMCs containing EBV-infected T or NK cells, we obtained the following results. 1) EBV-infected T or NK cells proliferated in NOG mice and infiltrated into the liver, spleen, kidney, lung, small intestine, and other organs following transplantation of PBMCs of CAEBV patients of all CD4, CD8, γδT, and NK types. 2) The TCR Vβ repertoire analysis showed that the dominant EBV-infected T-cell clone was identical between a CAEBV patient and the NOG mouse model derived from the patient. 3) Peripheral blood of these model mice contained high levels of human cytokines including IL-8, RANTES and IFN-γ. 4) EBV-infected cells in the NOG mouse showed the latency II program of EBV gene explassion. 5) Removal of CD4$^+$ cells from PBMCs obtained from CAEBV patients resulted in failure of engraftment. These results indicate that the NOG mouse model of CAEBV recapitulates major pathologic features of CAEBV and may be useful in elucidating the pathogenesis of CAEBV and developing its treatment. It was also suggested that CD4$^+$ cells play an important role in the proliferation of EBV-infected T or NK cells.

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Therapy **Abstract 59**

EBV-POSITIVE DLBCL OF THE ELDERLY IS A DISTINCT CLINICO-BIOLOGICAL ENTITY WITH POOR OUTCOME IN CHOP-R TREATED PATIENTS, WITH PROPERTIES LIKELY AMENABLE TO ANTI-EBV TARGETING.

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Abstract.

Diffuse Large B-cell Lymphoma is the most common aggressive lymphoma. WHO classify Epstein-Barr virus-positive DLBCL of the elderly (EBV+DLBCL) as a provisional new entity. Data on EBV+DLBCL's response to the gold-standard chemo-immunotherapy regimen "CHOP-R" is extremely limited. We analysed a consecutive unselected cohort over a 6 year period. In 121 immunocompetent DLBCL patients, 9.1% were EBV+, exclusively in those aged >50 years. In the >50's receiving CHOP-R, EBV+DLBCL demonstrated inferior event-free (*P*=0.0002) and overall survival (*P*=0.027) versus EBV-DLBCL, with EBV-positivity an adverse risk by multivariate analysis (*P*=0.017). Critically, the absolute incidence of EBV+DLBCL is similar to EBV+ Hodgkin Lymphoma, but with far inferior outcome. Its adverse prognosis indicates new approaches are warranted. We performed a detailed biomolecular analysis to identify potential new anti-viral targeted strategies. Based on EBNA2 staining, EBV+DLBCL was previously believed to be predominantly EBV-latency type II (expressing only immuno-subdominant EBNA1, LMP1 and LMP2 EBV-genes). Unexpectedly we found a variant type III EBV-latency profile, expressing the immuno-dominant EBNA3A, but with down-regulation of EBNA2. The major EBV-latency promoters Cp and Wp were hyper-methylated. Genetic diversity was observed in EBNA1 and LMP1. Polymorphisms in regions encoding for EBNA3A CD8+ T-cell epitopes were not seen. EBNA3A-specific CD8+ T-cells could be expanded *in-vitro* from pre-therapy peripheral blood. The NFκB-signalling pathway was activated, and appeared proportional to LMP1 expression. The outcome of EBV+DLBCL is poor despite rituximab. Its latency, methylation and NFκB-signalling properties shed new light on the potential role of EBV in pathogenesis, and provide opportunities for new targeted therapeutic strategies.

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Therapy **Abstract 60**

THERAPEUTIC VACCINATION TO TREAT EPSTEIN-BARR VIRUS-POSITIVE MALIGNANCY: PHASE I CLINICAL TRIALS.

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Abstract

We have developed a therapeutic vaccine to treat patients with Epstein-Barr virus (EBV)-positive cancer. Such malignancies include almost all undifferentiated nasopharyngeal carcinoma (NPC) and 40% Hodgkin's lymphoma cases. In malignant cells, EBV antigen expression is restricted to EBNA1, LMP2 and LMP1 whereas immunodominant targets for MHC class I restricted T cell responses are down-regulated.

Our vaccine encodes a fusion gene comprising EBNA1 C-terminal 363-641 spliced to complete LMP2. This includes multiple MHC class I restricted epitopes in LMP2 known to be recognised by low abundance circulating cytotoxic T lymphocytes in some healthy donors and cancer patients, and also several MHC class II restricted epitopes in EBNA1.

To determine vaccine immunogenicity, a UK-based trial is recruiting patients with any EBV+ cancer outside the transplant setting, following one program of primary chemotherapy or chemoradiotherapy, in remission or for whom no other standard therapy exists. In parallel, in China a trial with the same design is recruiting NPC patients following first line therapy. These trials escalate from 5x10^7 to 5x10^8 pfu virus given intradermally three times at three week intervals. To date 15 and 18 patients have been treated in the UK and China respectively. The main toxicities are injection site reactions and systemic 'flu like symptoms, nevertheless the vaccine is well tolerated even at maximum dosage. Immune responses characterised to date indicate amplification of CD8+ T cell responses to LMP2 as well as CD8+ and CD4+ T cell responses to EBNA1 in a high proportion of vaccinated patients.

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Therapy **Abstract 61**

CLINICAL TRIAL DESIGN IN EBV-SPECIFIC IMMUNOTHERAPY FOR NASOPHARYNGEAL CARCINOMA

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Abstract

Translating the success of EBV-specific immunotherapy observed in PTLD to Nasopharyngeal Carcinoma (NPC) presents important differences in clinical study design. In most NPC patients with measurable disease, standard therapies cannot be ethically withheld during the months required for T cell manufacturing. Thus, T cell administration must be carefully planned to distinguish the impact of immunotherapy from continuing effects of indicated intercurrent chemotherapy (IC). We recruited ten patients with recurrent, EBV-positive NPC for a phase I study to evaluate the feasibility of documenting disease progression after IC before administering immunotherapy.

Most patients had disseminated disease (n=9) and salvage chemotherapy prior to enrollment (n=9, average=2.5 courses). T cell products were successfully manufactured from 9 patients (average=15.6 weeks), and 8 patients received IC during this period. Disease progression did not occur before immunotherapy in 2 patients (1 without and 1 with IC; average=15 weeks after manufacturing completed). Disease progression after IC was documented in 6 patients before immunotherapy (average=5.9 weeks). One patient became ineligible for immunotherapy from progressive CNS disease.

We demonstrate the feasibility of combining compassionate IC with a requirement for documented disease progression prior to immunotherapy. We observed a low response rate (1CR) definitively linked to immunotherapy, in one subject with relatively stable disease that did not require IC. Immunotherapy may be more effective in NPC patients with less aggressive disease or less chemotherapy experience. However, evaluating immunotherapy responses in these patients may be more difficult due to more frequent and sustained responses to IC.

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Session 10 abstracts 62-68

Lymphoma

Lymphoma **Abstract 62**

NONINVASIVE IDENTIFICATION OF EBV-INFECTED LYMPHOCYTE SUBTYPES IN EBV-ASSOCIATED T/NK LYMPHOPROLIFERATIVE DISEASES

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Abstract

EBV infects various types of lymphocyte and is associated with both B-cell-origin lymphoma and T or natural killer cell lymphoproliferative diseases (T/NK LPD). To diagnose EBV-associated LPD and to explore its pathogenesis, EBV-infected cells must be identified. Recently, we established a novel assay for quantifying and simultaneously identifying EBV-infected cells using the flow cytometric detection of fluorescent *in situ* hybridization (FISH). With a fluorescein-conjugated peptide nucleic acid probe that specifically hybridizes to EBV-encoded small RNA (EBER), both nuclear EBER and surface lymphocyte antigens can be stained. We analyzed peripheral blood from 24 patients who were clinically suspected of having EBV-associated T/NK LPD. In the FISH assay, 0.11–67% of the peripheral blood lymphocytes were positive for EBER in these patients. The number of EBER-positive cells was correlated with the EBV-DNA load determined using quantitative PCR. In 5 out of 7 patients with hydroa vacciniforme-like lymphoma, which is an EBV-positive cutaneous T cell lymphoma, the EBER-positive cells were $CD3^+$ $CD4^-$ $CD8^-$ $TCR\gamma\delta^+$ T cells. Interestingly, in a 25-year-old male with systemic EBV-positive T cell LPD, two lymphocyte lineages were positive for EBER: $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells. Therefore, this noninvasive assay is a direct, reliable method for quantifying and characterizing EBV-infected lymphocytes, and can be used not only to diagnose but also to clarify the pathogenesis and to expand the spectrum of EBV-associated diseases.

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Lymphoma **Abstract 63**

DIFFERENTIAL REGULATION OF BLIMP1 ISOFORMS BY THE EPSTEIN-BARR VIRUS; IMPLICATIONS FOR VIRUS PERSISTENCE AND THE PATHOGENESIS OF EBV-ASSOCIATED LYMPHOMAS

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Scientific category: Lymphomas

Abstract

The Epstein-Barr virus (EBV) is associated with several types of B cell lymphoma, which include Burkitt's lymphoma (BL) and Hodgkin's lymphoma (HL). An important pathogenic event in these cancers is the suppression of virus replication which would otherwise result in tumour cell apoptosis. Because the induction of virus replication in EBV-infected non-malignant B cells is intimately linked to their differentiation to plasma cells, we have asked if the physiological signals which drive normal B cell differentiation are absent in EBV-infected lymphoma cells. We have focussed on BLIMP1, a transcription factor which exists as two major isoforms; BLIMP1α, which is required for normal plasma cell differentiation, and BLIMP1β which has impaired ability to repress gene transcription and is highly expressed in myeloma cell lines. We have shown that BLIMP1α expression: is low in EBV-infected BL and HL cells; can be down-regulated by EBV infection of primary B cells; and, when introduced ectopically into EBV-infected primary B cells or BL cells, leads to induction of the virus replicative cycle. We have also shown that EBV infection up-regulates BLIMP1β expression, an effect that is associated with hypo-methylation of the BLIMP1β-specific promoter. Taken together our results support an important role for the differential regulation of the BLIMP1 isoforms in the pathogenesis of EBV-associated lymphomas.

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Lymphoma **Abstract 64**

C-MYC IMPAIRS AN INTERFERON INDUCING ACTIVITY IN EBV POSITIVE BURKITT LYMPHOMA CELLS.

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Abstract

EBV-positive cells harbor an interferon inducing activity that is recognized by cellular receptors which are not yet identified. The potential candidates are the cytosolic RNA receptors namely the RIG-I-like receptors as well as the membrane anchored Toll-like receptors that recognize viral RNA or DNA, like TLR-3, -7, -8, -9. Any of these receptors might be activated by viral components leading to type I interferon response. However, in EBV-positive Burkitt lymphoma cells the type I interferon response and its signaling is impaired by deregulated high c-Myc expression due to a chromosomal translocation. Our model cell-line P493-6 expressing c-Myc in a tetracycline dependent fashion showed that interferon is produced and secreted 24-48h after switching off c-Myc expression. In this model system we've identified several interferon signaling components and cellular receptors which are suppressed by high expression of c-Myc. Thus c-MYC masks an interferon-inducing activity in these cells. This could be verified in Akata cells established from an EBV-positive Burkitt lymphoma by suppressing the c-Myc expression level. Our data provide evidence that c-Myc is the key transcriptional down-modulator of viral pattern recognition and interferon signaling in BL-cells implying that immune escape of tumor cells is not only a matter of in vivo selection but may be additionally promoted by activation of a cellular oncogene.

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Lymphoma **Abstract 65**

A C-MYC INDUCED GENE EXPRESSION SIGNATURE IN HUMAN GERMINAL CENTER B CELLS PREDICTS SUBTYPES OF AGGRESSIVE NON-HODGKIN LYMPHOMA

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Abstract:

In a recent series of investigations a molecular definition of Burkitt's lymphoma (BL) was developed. Despite an abundant number of cell line investigations and murine models, systems are still missing to investigate the effects of c-Myc in the transformation process of human germinal center B (GC B) cells. Therefore we addressed the questions: How does an aberrant c-Myc expression in GC B cells changes the gene expression profile? Do theses changes permit to distinguish between different Non-Hodgkin lymphoma (NHL)? Human tonsillar $CD10^+$ GC B cells were transfected with a c-Myc expression plasmid or empty vector. Gene expression profiling from 11 independent tonsils was performed. To identify genes affected by c-Myc a bioinformatics approach was used to search for genes entrained by c-Myc in GC B cells and in NHL samples. A c-Myc expression index was defined. Gene set enrichment analysis showed a strong enrichment of c-Myc target genes within the c-Myc index but also a characteristical depletion of NF-kB pathway elements. When analyzing two individual NHL cohorts, this index stratifies NHL patients into major subgroups, intermediate, non-mBL and mBL. The index was highest among patients with a MYC-simple karyotype, intermediate with a MYC-complex karyotype and low for those with a MYC-negative karyotype. The presence of high c-Myc index in a non-mBL is significantly associated with a shorter overall survival. Our data demonstrate the use of human GC B cells to remodel the mode of protoonogene action and provide new insides into the role of c-Myc in different lymphomas.

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Lymphoma **Abstract 66**

INVESTIGATING THE ROLE OF VIRAL AND CELLULAR BCL2 PROTEINS IN BURKITT LYMPHOMA USING THE EμMYC TRANSGENIC MOUSE MODEL

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Abstract

Endemic Burkitt lymphoma (eBL) represents a classical example of a tumour whose pathogenesis is associated with both a cellular change, namely translocation of the c-myc gene into the immunoglobulin locus and a transforming virus, EBV. Understanding the complementation between viral and cellular factors, both in tumour initiation and maintenance, remains an important objective. The identification of a subset of BL tumours expressing the viral (v)Bcl2 homolog, BHRF1, as part of a Wp-restricted latency has strengthened the argument that protection from c-myc-induced apoptosis is key during tumour pathogenesis.

Here we utilise the EμMyc transgenic mouse model of BL to investigate the role of viral and cellular Bcl2 family proteins in lymphomagenesis. Firstly we ask if expression of BHRF1 can accelerate tumour progression in the same manner as Bcl2 over-expression through retroviral transduction of EmMyc transgenic fetal liver cells with mouse retroviral vectors expressing BHRF1 or Bcl2 and reconstitution of irradiated recipients. Secondly we address the question of which cellular Bcl2 family protein is important for the sustained growth of EμMyc tumours using the Cre-LoxP system to conditionally delete Mcl1 or Bclxl in EμMyc tumours *in vivo*. In each case tumour progression was monitored by blood cell count, *in vivo* whole body imaging, analysis of tumour latency and *ex vivo* analysis of tumour cells.

Our results suggest that BHRF1 can accelerate EμMyc tumour onset in a similar manner, but not as efficiently, to Bcl2. Furthermore our preliminary data suggests that Mcl1 is key for the sustained growth of EμMyc lymphomas *in vivo*.

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Lymphoma **Abstract 67**

EBNA3B IS A TUMOUR SUPPRESSOR

Robert E White [1], Patrick C Rämer [2], Kikkeri Naresh [3], Sonja Meixlsperger [1], Ernest Turro [4], Laurie Pinaud [1], Cliona Rooney [5], Barbara Savoldo [5], Christian Münz [2] and Martin J Allday [1].

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Abstract

LCLs were established from the B cells of healthy donors using EBNA3B-knockout (KO), revertant and B95.8-BAC (WT) EBV. Exon microarray analysis identified approximately 400 genes whose expression involves EBNA3B. Many of the differentially regulated genes encode proteins expressed at the cell surface, suggesting alterations in cell:cell interactions and possibly migration or homing properties *in vivo*. Moreover, microarray analysis indicated – and western blotting and qPCR confirmed – that a short form of the transcription factor FoxP1 was expressed in the EBNA3BKO LCLs, but not WT EBV-infected LCLs. This 64kDa protein is associated with poor prognosis for diffuse large B-cell lymphoma (DLBCL).
Nod-scid-$\gamma_c^{-/-}$ mice reconstituted with components of the human immune system were infected with similar doses of WT, EBNA3BKO or revertant B95.8-BAC-derived virus. A month later mice exhibited splenomegaly with large tumour masses in the EBNA3BKO infected mice, but not the WT or revertant infected animals. Histology and immunocytochemistry revealed the EBNA3BKO-infected mice had developed monomorphic B-cell tumours (>90% Ki-67+ve) – described as DLBCL-like – destroying the architecture of the spleen. In contrast, spleens from the WT and revertant groups were not so enlarged and had markedly less proliferative polymorphous infiltrates (30-60% Ki-67+ve). These included immunoblasts and plasma cells, consistent with plasmacytoid differentiation, and – in contrast to the 3BKO tumours – substantial T-cell infiltration. The phenotype of the recombinant EBNA3B-KO viruses identifies EBNA3B as a tumour suppressor and is consistent with the identification of two PTLD that failed to express EBNA3B progressing to aggressive lymphomas.

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Lymphoma **Abstract 68**

ENHANCED TUMORIGENESIS OF EBNA3B DEFICIENT EBV IN-VIVO.

Patrick C Rämer [1], Robert E White [2], Sonja Meixlsperger [1], Kikkeri Naresh [2], Laurie Pinaud [2], Cliona Rooney [3], Barbara Salvodo [3], Martin J Allday [2] and Christian Münz [1]

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Abstract

In order to evaluate the role of EBNA3B in vivo, Nod-scid-$\gamma_c^{-/-}$ mice with reconstituted components of the human immune system were infected with low doses of wt EBV (wtBAC), EBNA3B deficient EBV (EBNA3BKO) or the respective revertant virus (EBNA3Brev). Infection with EBNA3BKO lead to a significant increase in spleen size and tumor formation compared to wtBAC and EBNA3Brev infection. The observed tumors display hallmarks of diffuse large B cell lymphomas. Moreover, infection with EBNA3BKO resulted in an enhanced expansion of the T cell compartment of the animals, primarily $CD8^+$ T cells, but these failed to infiltrate into the tumour

The differences in immune activation could not be attributed to changes in costimulatory capacity of wtBAC, EBNA3BKO or EBNA3Brev infected cells. LCLs established from infected animals showed similar expression of costimulatory and MHC molecules and similar overall immunostimulatory potential, assessed by alloreactive mixed lymphocyte reactions. However, EBNA3BKO infected LCLs secrete less CXCL10 in the steady-state as well as upon IFN-Y stimulation, consistent with reduced levels of CXCL10 mRNA observed in exon-microarray analysis of 3BKO LCLs generated in-vitro. This could result in diminished T cell recruitment to the tumor microenvironment. Finally, the EBNA3BKO infected LCLs show a stronger proliferative capacity and less spontaneous apoptosis compared to wtBAC or EBNA3Brev infected cells.

In summary, infection of humanized mice with EBNA3BKO EBV leads to enhanced tumorigenesis, in part caused by changes in chemokine expression, proliferation and apoptosis resistance of the transformed B cells, which overcome an elevated immune control to cause lymphomas in vivo.

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Session 11 abstracts 69-72

Infection and Reactivation

Infection and Reactivation **Abstract 69**

PRIMARY EBV INFECTION IN EARLY INFANCY RESULTS IN POORLY CONTROLLED EBV VIRAL LOAD OVER TIME

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Abstract

Guy de-Thé hypothesized that infection of infants with EBV early in life could result in an infection that was poorly controlled by the host and thus increased the risk for Burkitt's lymphoma. To test this hypothesis, infants were enrolled from two sites in Kenya: Kisumu District where malaria transmission is stable and risk for BL is high and Nandi District where malaria transmission is unstable and the risk for BL is low. Blood samples were taken from 1 month through 2 years of age. EBV viral load was determined by Q-PCR and EBV antibodies were measured using a Luminex based bead suspension array assay. We observed a significantly earlier age of primary EBV infection in children from Kisumu compared to Nandi, with the mean age of 7.2 mo (+/- 0.23 SEM) in Kisumu vs 8.5 mo (+/- 0.26 SEM). We also found a significantly higher level of *P. falciparum* parasitemia in the Kisumu children ($p<0.0001$). To analyze how different predictors affected the outcome of EBV viral load over time in our infant cohort, we did multi-level (mixed modeling) using a Tobit statistical analysis. Our model revealed significant contributions of the prediction of EBV viral load based on subjects' site of residence, age at first EBV infection, and malaria load. In sum, we found that children from a high-risk region for BL are infected earlier in life with EBV than children from a low-risk BL region and that the infection is poorly controlled and correlated with malaria exposure.

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Infection and Reactivation **Abstract 70**

HERPESVIRUS KINASES ACTIVATE TIP60: A CONSERVED PATHOGEN-HOST INTERACTION PROMOTING VIRUS REPLICATION

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Abstract

Viruses have evolved elegant strategies to appropriate key host regulators to facilitate their replication and spread. We used a protein phosphorylation microarray-based strategy to identify common factors that are targeted by protein kinases from the human alpha, beta and gamma herpesvirus families. We identified 273, 178, 290, and 294 substrates of EBV-BGLF4, KSHV-ORF36, HCMV-UL97, and HSV1-UL13, respectively. Of the 644 non-redundant substrates identified by the four kinases, 110 are shared by at least three kinases. Gene Ontology analysis of the 110 substrates revealed the DNA-damage response to be the key pathway targeted by the conserved herpesvirus proteins kinases. We selected members of this category for further validation. The histone acetyltransferase TIP60, an upstream regulator of DNA damage response, was found to be essential for efficient herpesvirus replication. TIP60 was required for H2AX phosphorylation that occurs during EBV lytic replication. TIP60 also participates in chromatin remodeling. ChIP analysis of EBV promoters showed that TIP60 was specifically recruited to the RTA, LMP1 and BHLF1 promoters and shRNA knockdown of TIP60 indicated a requirement of TIP60 for efficient expression of these genes. The data demonstrate the value of the phosphorylation microarray approach for the identification of key cellular pathways whose essential role in the viral life cycle make them attractive as targets for broadly effective anti-viral therapies.

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Infection and Reactivation **Abstract 71**

SCREENING OF CELLULAR FACTORS THAT ENHANCE REACTIVATION FROM THE EPSTEIN-BARR VIRUS LATENCY

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Abstract

Reactivation of the Epstein-Barr virus (EBV) from latency is dependent on expression of the viral BZLF1 protein. The BZLF1 promoter (Zp) normally exhibits only low basal activity but is activated in response to chemical or biological inducers. Here, we screened cDNA library for factors that can activate the Zp by using reporter assay system. So far, we have scrutinized more than 20,000 clones and obtained 11 positive hits. MEF2B, Klf4, a member of Sp1/Klf family transcription factors, and some bZip transcription factors were identified to independently activate Zp. Isolation of those factors as Zp activators serves as strong evidence that our screening system is effective, because MEF2D, Sp1 and CREB/ATF/AP-1 transcriptional factors have already been reported to bind with the promoter sequence upon activation of the promoter. Several other genes were newly found to enhance the promoter (those factors will be disclosed in the presentation), and at least one of them was confirmed to play very significant role. These findings provide more articulate outline of how BZLF1 expression is induced in EBV reactivation from latency.

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Infection and Reactivation **Abstract 72**

THE EPSTEIN-BARR VIRUS ENHANCES INTERLEUKIN-8 DEPENDENT EXPRESSION OF MATRIX METALLOPROTEASES AND VASCULAR ENDOTHELIAL GROWTH FACTORS IN CARCINOMAS INDUCED IN SCID MICE

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Abstract

Epstein–Barr virus (EBV) is associated with different carcinomas, such as nasopharyngeal carcinoma and a proportion of gastric carcinomas. We investigated the potential impact of EBV-infection in epithelial cells : EBV infection of breast transformed epithelial cells was associated with the over-expression of genes involved in angiogenesis, tumor invasion and migration, such as vascular endothelial growth factors (VEGF), cyclooxygenase [COX]-2), matrix metalloproteinases (MMP) MMP2, MMP9, MMP14, extracellular matrix metalloproteinase inducer (EMMPRIN) and interleukin 8 (IL-8). In addition, as shown by migration and invasion assays, the conditioned medium of latently EBV-infected cells stimulated migration and invasion of non-infected cells. This stimulation was abrogated by addition of neutralizing anti-IL8 antibodies suggesting that EBV-induced IL8 production had a role in the invasion process.

When EBV-infected cells were injected in SCID mice, copies of human genome was found in liver, bone and lung of the mice; this number is statistically higher than when non-infected cells were used. Injection of neutralizing anti-IL-8 antibodies abolished this effect.

Taken together, these data suggest that EBV may contribute to tumor progression through the secretion of factors, especially IL-8, that could favor a local microenvironment that enhances the metastatic potential of surrounding non-infected tumor cells.

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Posters P1-P29

Latent Infection

Latent Infection **Abstract P1**

EBV LMP1 C-TERMINAL ACTIVATING REGION-3 CONTRIBUTES TO ITS ONCOGENIC PROPERTIES VIA ITS INTERACTION WITH THE SUMO-CONJUGATING ENZYME UBC9.

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Abstract

EBV latent membrane protein (LMP)-1, the principal viral oncoprotein and a member of the TNFR superfamily, regulates multiple signal transduction events via its C-terminal activating regions (CTAR) -1 and -2. LMP1-induced activation often involves the induction of protein post-translational modifications, including phosphorylation and ubiquitination. Protein sumoylation, another post-translational modification, regulates protein function at various levels and is associated with tumorigenesis, but it has not been studied in the context of LMP1 expression. Because many of the signaling pathways induced by LMP1 are also regulated downstream by sumoylation, we hypothesized that LMP1 is involved in sumoylation processes during EBV latency. By immunoprecipitation experiments, we show that LMP1 interacts with Ubc9, the only reported SUMO-conjugating enzyme. Requirements for the LMP1-Ubc9 interaction include enzymatically active Ubc9. However, LMP1 CTAR1 and CTAR2 are not required for this interaction. Instead, CTAR3 (specifically LMP1 amino acids 275-307) is necessary for the LMP1-Ubc9 interaction, and reconstitution of a JAK-interacting motif found in CTAR3 partially restores this interaction. Biologic consequences of the LMP1 CTAR3-Ubc9 interaction reveal effects on mitosis, cell survival (specifically apoptosis), and migration, suggesting a regulatory function for LMP1 CTAR3. Ubc9 has been proposed as a new target for anti-cancer therapies, and our findings suggest a possible means to target EBV-associated malignancies. These data identify a new function for CTAR3 and suggest a possible mechanism (induction of protein sumoylation that affects cellular behaviors) by which LMP1 acts as an oncoprotein.

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Latent Infection **Abstract P2**

E2F1, ARID3A/BRIGHT AND OCT-2 FACTORS BIND TO THE EPSTEIN-BARR VIRUS C PROMOTER, EBNA1 AND *ORIPI*, POSSIBLY FACILITATING LONG-DISTANCE PROMOTER-ENHANCER INTERACTIONS

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Abstract

The latent origin of plasmid replication (*oriP*) functions as an EBNA-1 dependent enhancer of Epstein-Barr virus C promoter (Cp) activity. This enhancer-protein complex is essential for activation of the Cp in lymphoblastoid cells expressing the viral growth program. To further study the molecular details of the activation process, we have used DNA-affinity purification in combination with high resolution mass spectrometry on a LTQ-FT instrument to identify proteins binding to the C-promoter and the *oriP*. Of the 30 transcription factors identified as putative Cp interactors, we selected Bright/ARID3A, E2F1 and Oct-2 for further characterisation. The interactions were verified by co-immunoprecipitation, and in vivo binding of the proteins was determined using Chromatin Immunoprecipitation (ChIP). The binding sites of all three proteins were mapped to a short segment of Cp in close proximity of each other. This region was previously shown to be required for transcriptional activation of the Cp. By Proximity Ligation Assays the interactions between endogenous EBNA1 on the one hand and E2F1, ARID3A and Oct-2, respectively, on the other, were investigated *in situ* in EBV-positive lymphoblastoid cell lines (WW1-LCL and B95.8-LCL) and negative control cells (DG75). The results demonstrated that there is a close interaction in the nucleus between EBNA1 and E2F1, ARID3A and Oct-2 *in vivo* in EBV-positive cells. DNA affinity purifications with a bait comprising the *OriPI*-region followed by immuno-blot analysis, revealed that the Bright/ARID3A, E2F1 and Oct-2 proteins also bound to this sequence, implying that the factors might contribute to promoter-enhancer interactions.

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Latent Infection **Abstract P3**

DETAILED CHARACTERIZATION OF THE EBNA3 INTERACTION WITH RBP-JK/CSL

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Abstract

The EBV nuclear proteins EBNA3A, EBNA3B, EBNA3C are tightly associated with the cellular DNA binding protein RBP-Jk/CSL that targets EBNA2 to promoters. The interaction of EBNA2, EBNA3A and EBNA3C with RBP-Jk/CSL are essential for continued proliferation of LCLs. How the N terminal “homology domains” of the EBNA3s proteins mediate interaction with RBP-Jk/CSL is not well understood. In particular, conserved residues among the EBNA3 proteins do not match the “WΦP” motif that mediates EBNA2 and Notch interactions with the RBP-Jk/CSL beta-trefoil domain. As a prelude to structural studies of the EBNA3 homology domains, we mapped the minimal boundaries within the EBNA3 homology domain required for interaction with RBP-Jk/CSL using bacterial GST-pulldown assays. Surprisingly the minimal EBNA3C interacting domain was much smaller than expected and included a WTP motif unique to EBNA3C. Mutation of this motif (WTP -> STP) disrupted binding of the minimal construct in GST-pulldown assays, but did not affect EBNA3C association with RBP-Jk/CSL in mammalian cells, suggesting this motif is not responsible for the EBNA3C homology domain’s binding activity. Further, the WTP motif was not essential for repression of EBNA2 in reporter assays or maintenance of LCL growth in EBNA3C-HT LCL complementation assays. In a complementary assay, RBP-Jk/CSL domains were tested for their ability to interact with EBNA3 proteins. This revealed that both EBNA3A and EBNA3C target the RBP-Jk/CSL N-terminal domain. These results confirm that the EBNA3 proteins differ in their mode of interaction with RBP-JK/CSL and suggest that they may not simply compete with EBNA2 for RBP-Jk/CSL binding.

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Latent Infection **Abstract P4**

SYK TYROSINE KINASE IN NASOPHARYNGEAL CARCINOMA TUMOR PROGRESSION

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Abstract

Spleen tyrosine kinase (Syk) is a nonreceptor tyrosine kinase. It is a mediator of Ig-receptor activation in B-lymphocytes, while in other cell types Syk modulates proliferation, phagocytosis, and migration via integrin mediated signalling. Currently the role of Syk in tumourgenesis in human cancers is still controversial. Reduced Syk expression correlates with tumor metastasis and poor prognosis in breast cancer, hepatocellular carcinoma and gastric carcinoma. In contrast, overexpression of Syk has been found in T-cell lymphomas and is associated with recurrence and poor prognosis of head and neck cancers.
Results: We have earlier shown that Epstein-Barr virus (EBV) encoded LMP2a induces migration in epithelial cell lines by binding to Syk, and that Syk knock down led to enhanced migration. Therefore we postulated that Syk might act as a migration/tumor suppressor gene in epithelial cells. We further showed that Syk binds to integrin β4, which carries a potential ITAM-like motif. An in vitro synthesized peptide representing this motif could bind phosphorylated Syk. Thus we propose that Syk-controlled migration operates partially via a $\alpha 6\beta 4$ pathway which may be subverted in invasive carcinoma cells. We are now studying co-localization of Syk with LMP2 and integrin β4, within different transformed cell lines
Conclusions: Epithelial cell migration is modulated by Syk and integrin expression, which also physically interact, and show some colocalization *in vivo*.

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Latent Infection **Abstract P5**

HIF1-ALPHA PROTEIN IS FUNCTIONAL AND STABILIZED IN LYMPHOBLASTOID CELLS

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Abstract

The Hypoxia-inducible factor alpha (HIF1-α) is a transcription factor that activates the responsive genes under low oxygen conditions. HIF1-α binds to DNA as heterodimer with HIF1-β. HIF1-β, or aryl hydrocarbon receptor nuclear translocator (ARNT), is present in the nucleus constantly.
At the normal conditions HIF1-α is degraded very fast on the proteasome through the binding to VHL protein which functions as ubiquitin E3 ligase for HIF1-α.It was shown earlier that latent membrane protein LMP1 induces HIF1-α and can stabilize it in nasopharengyal carcinoma cell lines. The level and activity of HIF1-α in lymphoblastoid cells was not studied to date.
We have compared peripheral blood resting B-cells with mitogen-activated and Epstein Barr virus (EBV) infected cells, and established lymphoblastoid cell lines (LCLs) as well.
We have found that HIF1-α protein is stabilized in lymphoblastoid cells, in contrast to resting and mitogen-activated B-cells. It can be due to the binding of HIF1-α with EBV-encoded proteins. We have shown that HIF-α may bind EBNA-3 and EBNA-5 under certain conditions. The responsive genes that are activated by the HIF1-α transcription factor are responsible for homeostasis and angiogenesis. HIF1-α has other responsive genes, which are pro-apoptotic, like HGTD-P, BNip3, RTP801 and Noxa. All these proapoptotic genes can be expressed in B cells. Little is known about HIF1-α regulation and function in LCLs. We have performed Real-time PCR, to analyze the expression of genes regulated by HIF1-α at the different conditions like hypoxia and proteasome machinery inhibition.

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Latent Infection **Abstract P6**

EBV REPRESSES TRANSFORMING GROWTH FACTOR-BETA (TGFB)-SIGNALLING AND MAY RESULT IN ENABLING OF BMP SIGNALLING

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Abstract

Transforming growth factor-beta (TGFb)-signalling is important in immune regulation and is defective in many cancers. TGFb is usually anti-proliferative and can induce apoptosis in B cells; it acts via TGFb receptors (TGFbRs) 1 and 2 leading to phosphorylation and activation of SMADs 2 and 3. Bone morphogenic proteins (BMPs) are a related family of cytokines, which act via BMP receptors and phosphorylation of SMADs 1, 5 and 8. TGFbR3 normally functions as a co-receptor for TGFbR2; however, it has been shown that BMPs can act directly on TGFbR3 to phosphorylate SMAD1.

A global exon microarray screen in our laboratory suggested that TGFbRs 2 and 3 are down-regulated and up-regulated respectively by EBV in BL31 cells. Using quantitative reverse-transcriptase PCR (qRT-PCR) we have shown that TGFbR2 mRNA is down-regulated by EBV, with functional loss of TGFb-signalling demonstrated using western blotting for phospho-SMAD2 after treatment with exogenous TGFb1. Using cells infected with gene knockout viruses, we have shown that EBNA2, EBNA3B and EBNA3C cooperate in this down-regulation. We have also shown that EBV up-regulates TGFbR3 and SMAD1, with repression of both seen in EBNA3B knockout LCLs relative to wild-type (B95.8-BAC infected) LCLs.

EBV blocks TGFb-signalling through SMAD2, with EBNAs2, 3B and 3C all contributing. EBV also up-regulates both TGFbR3 and SMAD1; this may promote a switch from TGFb- to BMP-signalling; this, and the mechanisms of regulation at the TGFbR2 and SMAD1 promoters, are being investigated.

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Latent Infection **Abstract P7**

ROLES OF EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 5 IN CHAPERONE-MEDIATED PROTEIN FOLDING AND PROTEASOME PROTEIN DEGRADATION

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Abstract

The Epstein-Barr virus nuclear antigen 5 (EBNA5) is expressed very early in acute primary EBV infection and is important for efficient immortalisation of human B cells. We previously reported that EBNA5 acts as a repressor of reporter protein activity in transient transfection systems and interfere with reporter pre-mRNA processing. Here, we investigated the mechanism underlying the repression by expressing EBNA5 and exogenous reporter proteins in EBV-negative B-lymphoma cells and analysing the EBNA5 effects on activity, solubility, and localization of the reporters luciferase and CAT. Quantitative RT-PCR analysis revealed that EBNA5 did not diminish the amount of luciferase poly(A) mRNA in the cytoplasm. Instesad, EBNA5 reduced reporter activity and this was correlated with reduced levels of soluble and accumulation of insoluble reporter proteins. Insolubilisation was accompanied by reporter protein translocation from the cytoplasm to the nucleoplasm, the nucleoli and to nuclear foci. The repressive and translocating functions of EBNA5 were mapped to the conserved region 3 of the EBNA5 W_1W_2 repeat domain. We previously identified interactions between EBNA5, heat shock protein-70 (Hsp70), and several (co-)chaperones including Bcl2-associated athanogen 2 (BAG2). Here, using *in situ* proximity ligation analysis we showed a close interaction between EBNA5 and BAG2. Furthermore, we demonstrated that transfected EBNA5 and luciferase co-localized with endogenous Hsp70 and BAG2 in nucleoli. Co-expression of Hsp70 and EBNA5 prevented both the accumulation of insoluble luciferase and the reduction of soluble luciferase. Our results suggested that EBNA5 recruits Hsp70 clients to the nucleoli and regulate protein folding and proteasomal protein degradation by modulating the function of Hsp70 chaperone-cochaperone complexes.

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Latent Infection **Abstract P8**

THE ROLE OF EPSTEIN-BARR VIRUS LATENCY I GENE PRODUCTS IN THE APOPTOSIS PHENOTYPE OF ENDEMIC BURKITT LYMPHOMA CELL LINES

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Abstract

Endemic Burkitt Lymphoma is a common childhood malignancy in Sub-Saharan Africa and it was in these aggressive tumours that Epstein-Barr virus was first identified. *In vivo* eBL cells are usually found to express a very limited subset of gene products known as the Latency I programme. We have generated a series of spontaneous EBV-loss clones derived from four well characterised Latency I cell lines. When challenged with apoptosis-inducing stimuli, EBV-loss cells were consistently more sensitive than their EBV-positive counterparts. Importantly, re-infecting EBV-loss clones with recombinant EBV to establish a Latency I infection restored apoptosis protection

To determine whether a Latency I infection influences the cellular gene expression profile of BL we performed microarray analysis to compare EBV-positive and loss clones. Irrespective of EBV status, we found that all clones retained the previously characterised molecular BL signature. Furthermore, no consistent differences in cellular gene expression were found between EBV-positive and loss clones across all four cellular backgrounds. This raises the possibility that BL may arise from divergent tumourigenic pathways with EBV playing subtly different roles in different eBL tumours

Using stable drug inducible vectors and lentiviruses, work is currently underway to determine which Latency I associated viral gene product is responsible for protecting BL cells from apoptosis. We have already shown that expression of EBNA1, even at supra-physiologic levels, in EBV-loss cells is insufficient to restore protection. We are now focusing our attention on the non-coding EBER RNAs and the micro-RNAs expressed from the BamHI rightward transcripts

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Latent Infection **Abstract P9**

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE ROLE OF TRAF3 IN EPSTEIN-BARR VIRUS MEDIATED CELLULAR TRANSFORMATION

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Abstract

The latent-infection membrane protein 1 (LMP1) of Epstein-Barr virus (EBV) is an oncoprotein which is essential for B lymphocyte growth transformation by EBV and closely associated with most EBV-associated malignancies. LMP1 mimics constitutively activated tumor necrosis factor receptor molecules to promote the activation of NF-kappaB and MAP kinase pathways. A prominent role in LMP1 signaling is played by members of the TRAF family of molecules. We have investigated the role of TRAF3 in LMP1 signaling and B cell transformation by EBV. It has been previously demonstrated that TRAF3 inhibits NF-kappaB activation by the CTAR1/TES1 domain of LMP1 but the mechanism of its action remains unclear. Additional studies have shown that TRAF3 mediates the inhibition of non-canonical NF-kappaB activation pathway by interacting with the NIK kinase and promoting its degradation. Unlike CD40 activation, LMP1 does not induce degradation of TRAF3. We have demonstrated that LMP1 can compete with NIK for binding to TRAF3, thus providing a mechanistic basis for the induction of non-canonical NF-kappaB activation pathway by LMP1. Downregulation of TRAF3 by RNA interference compromised severely the survival of EBV-transformed B cells, indicating a potentially essential role for TRAF3 in EBV-mediated transformation of human B cells. Finally, a yeast two-hybrid approach identified novel TRAF3-interacting proteins implicated in the regulation of apoptosis, which are currently under investigation with respect to their involvement in TRAF3-associated functions. Our findings will be discussed in the context of an expanded model of the involvement of TRAF3 in EBV-mediated B cell transformation.

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Latent Infection **Abstract P10**

EBV INFECTION OF IGD+CD27- NAÏVE B CELLS IN VITRO CAN INDUCE IMMUNOGLOBULIN GENE MUTATION IN THE ABSENCE OF IMMUNOGLOBULIN CLASS-SWITCHING

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Abstract

EBV infection is characterised by lifelong persistence within memory B cells, although how this is achieved is still unclear. We have investigated whether EBV infection of highly-purified IgD+ CD27- naïve B cells in vitro can induce changes in either B cell immunoglobulin variable gene (IgVH) sequences or immunophenotype. While clonal naïve LCLs established by limiting dilution consistently upregulated activation-induced cytidine deaminase and retained an IgM+ IgD+ phenotype, 21% of such LCL clones carried mutated IgVH sequences. To monitor the dynamics of EBV-infected naïve B cell cultures, we screened IgVH sequences amplified from bulk preparations at different time points post-infection. Starting from an initial polyclonal population, such cultures quickly showed emergence of distinct B cell clones and became either mono- or biclonal by 12 weeks post-infection. Importantly, in two of four experiments, the dominant clones carried mutated IgVH sequences which appeared to have originated from genotypically naive B cells. We argue that these results are not due to preferential outgrowth of rare contaminating memory cells in our naïve B cell preparations since we found no significant difference in transformation efficiency between sorted naïve, switched memory (IgD-CD27+) and non-switched memory (IgD+CD27+) B cells from several donors. Furthermore cultures of naive B cell blasts stimulated with CD40L/IL-4 for 9 weeks remained polyclonal with no evidence of SHM. Our data therefore suggest that EBV infection can induce significant levels of SHM in a proportion of naive B cells in vitro and may have important implications for clonal evolution and EBV persistence in vivo.

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Latent Infection **Abstract P11**

GADD34 UP-REGULATES EBNA2 MEDIATED TRANSCRIPTIONS OF EBV PROMOTERS ARE IMPLICATED IN CELL MAINTENANCE UPON CELLULAR STRESS

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Abstract

Gadd34 is a growth arrest and DNA damage-inducible gene of which the expression was originally discovered to be induced when cells undergo growth arrest and apoptosis in response to DNA damage, or by nutrient deprivation and negative growth signals. Nevertheless, Gadd34 was shown to interact with cellular transcription factors, such as HRX and BFCOL1, suggesting Gadd34 may play a role in transcription regulation. In this study, we investigated the role of Gadd34 in EBNA2 mediated transcription in EBV (-) BJAB lymphoma cells using specific EBV promoter reporter plasmids. In multiple experiments, ectopically expressed Gadd34 potentiated 5-8 fold co-activation above the intrinsic activity of LMP1-luc or Cp-luc activated by EBNA2. Further more, we demonstrated that the C-terminus ICP34.5 homologue, amino acids (a.a.) 536-674, is sufficient to perform the full up-regulating effects on EBNA2 mediated transcription while the N-terminal a.a. 1-400 can only result partial co-activation (~40% of wild type) with EBNA2. Although Gadd34 is a cytoplasmic protein, our results revealed EBNA2 and Gadd34 are colocalized in the nucleus and the translocation of Gadd34 from the cytoplasm to the nucleus is shuttled by EBNA2. Depletion of endogenous Gadd34 expression by introducing Gadd34 specific shRNAs into IB4 lymphoblastoid cells resulted a dramatically increasing rate of apoptotic cells under cellular stress induced by treatment of methyl methanesulfonate (MMS). Taken together, we demonstrate the first evidence showing that Gadd34 has a specific role in modulation of EBNA2 mediated transcription in order to protect the cellular maintenance from damaged conditions caused by cellular stress.

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Latent Infection **Abstract P12**

EBV-DRIVEN B CELL PROLIFERATION IS SYNERGISTICALLY INCREASED BY TOLL-LIKE RECEPTOR AGONISTS

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Abstract

EBV drives proliferation of human primary B cells, a process involved in diseases such as infectious mononucleosis and post-transplant lymphoproliferative disease. Apart from EBV, Toll-like receptor (TLR) agonists are among the strongest inducers of B cell proliferation. We quantitatively investigated how TLR agonists, notably DNA with unmethylated CpG dinucleotides (CpG DNA) which triggers TLR9, influence EBV-driven B cell proliferation and expression of effector molecules. CpG DNA synergistically increased EBV-driven proliferation and transformation, T-cell costimulatory molecules, and early production of interleukin-6. CpG DNA alone activated only memory B cells, but CpG DNA enhanced EBV-mediated transformation both of memory and naive B cells. Ligands for TLR2 or TLR7/8, or whole bacteria, had a weaker but still superadditive effect on B cell transformation. Additionally, CpG DNA facilitated the release of transforming virus by established EBV-infected lymphoblastoid cell lines. Thus, the proliferation of EBV-infected B cells and their capability to interact with immune effector cells may be directly influenced by components of other microbes present at the site of infection. Thereby, the microbial milieu may influence the clinical course of primary EBV infection or the initiation of EBV-associated malignancies.

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Latent Infection **Abstract P13**

KSHV INFECTION ENHANCES ENDOTHELIAL CELL MOTILITY

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Abstract

Kaposi's sarcoma (KS) is a histologically complex endothelial cell (EC) tumour caused by the lymphotropic gammaherpesvirus, Kaposi's sarcoma-associated herpesvirus. Abnormal vascular structures lined by KSHV-infected spindle cells are characteristic of KS but the mechanism underlying this pathological angiogenesis is unknown.

Angiogenesis requires EC migration. Therefore, to study the impact of infection on human umbilical vein endothelial cell (HUVEC) motility we used transwell-filter-transmigration and wound-recovery assays, initiated between 1 and 10-days post-infection. KSHV infection transformed cell morphology from cobblestone to spindle shape. Chronic infection was predominantly latent and increased to 80% by day 10 despite less than 3.5% horizontal transmission.

52% of infected HUVEC transmigrated the filters in 48 hours. Within the same cultures, transmigration of non-infected cells was less (35%; p=0.026) and equivalent to untreated controls suggesting either a direct effect of infection upon EC motility, or a soluble mediator acts selectively upon infected cells to enhance migration. Infection also accelerated wound-recovery by day 7.

Changes in basement membrane composition or expression of matrix-binding integrin adhesion molecules might influence motility. We observed 53% (p=0.021) reduction in basement membrane laminin and 2.4-fold (p=0.015) upregulation of surface α6-integrin upon KSHV infection. The relevance of these phenotypes to enhanced motility is under investigation.

Besides angiogenesis, KSHV-induced increases in EC motility might facilitate other facets of KS pathogenesis, such as infected cell dissemination, recruitment from the circulation and virus persistence. Understanding the mechanisms of KSHV-enhanced EC motility might identify novel ways to intervene in the lifecycle and pathogenesis of KSHV and related viruses.

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Latent Infection **Abstract P14**

IDENTIFICATION OF ΔNP63 AS A MODULATOR OF LMP1 EXPRESSION IN EBV-INFECTED EPITHELIAL CELLS.

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Abstract

Epstein–Barr virus (EBV) is a ubiquitous human herpesvirus implicated in the development of both lymphoid and epithelial tumours. Whereas expression of the LMP1 protein is variable in NPC, LMP2A is more frequently expressed, albeit at low levels. Previous studies from our group have shown that epithelial cell lines infected with an LMP2A-deleted EBV (LMP2A-rEBV) express low levels of LMP1, suggesting that LMP2A negatively regulates LMP1 expression in epithelial cells. We now show that LMP1 is also subject to regulation by the p53 family member ΔNp63 and propose that whilst ΔNp63 is necessary for LMP1 induction, it requires NFκB as a cofactor for optimal activation. Our data suggest that LMP2A modulates LMP1 expression through mechanism(s) involving the activation of Notch and inhibition of NFκB. As ΔNp63 and Notch reciprocally regulate squamous epithelial cell growth and differentiation, our findings show that the balance between these two transcription factors influences latent viral gene expression and has implications for EBV-mediated epithelial cell growth transformation.

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Latent Infection **Abstract P15**

THE IMPACT OF VIRAL REPETITIVE SEQUENCES ON THE BIOLOGICAL PROPERTIES OF EPSTEIN-BARR VIRUS RECOMBINANTS

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Abstract

Epstein-Barr virus (EBV) recombinants derived from a prototype strain B95-8 have been widely used in the field of EBV virology. However, the integrity of repetitive sequences of such recombinant EBV genomes has not been verified in details. We and others previously found that the FR (Family of Repeats) sequence of B95-8 strain EBV contains a 128-bp perfect palindrome, which is subject to frequent deletion when the sequence is subcloned into *E. coli*-based plasmid vectors. We here demonstrate that the FR sequences of bacterial artificial chromosome (BAC)-cloned EBV genomes (EBV-BAC) are also subject to partial deletion, which possibly affects the biological properties of the resultant recombinant viruses. Thus, we paid special attention to keep the integrity of the FR sequence and obtained novel EBV-BAC clones (B95-8 strain). Two of such BAC clones with either 11 copies or 6 copies of BamHI W repeat, having either 187-kb or 172-kb genome sizes, were stably introduced into HEK293 cells. Cells producing high-infection-titer recombinant viruses were repeatedly derived by introducing the 172-kb BAC clone, which presumably reflected the adequate genome size for packaging. The B-cell transforming titer of the obtained recombinant EBV with the full length FR was comparable to that of the parental B95-8 strain EBV. The FR sequences of EBV episomes in the established lymphoblastoid cell lines were very stable, making a striking contrast to their instability during propagation in *E. coli*. These results indicate that the integrity of repetitive sequences should be taken into consideration when characterizing various EBV recombinants.

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Latent Infection **Abstract P16**

EBV-ENCODED EBNA-5 BINDS TO MDM2 AND MDMX AND PREVENTS P53-INDUCED GROWTH ARREST AND APOPTOSIS

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Abstract

Recently, we have found that EBNA-5 may protect LCLs from p53-dependent G_1 arrest and apoptosis through its binding to MDM2, the E3 ubiquitin ligase that promotes polyubiquitination and subsequent degradation of p53. We have found that EBNA-5 forms a complex with MDM2 and MDMX. To explore the functional consequences of MDM-2–EBNA-5 binding, p53 polyubiquitination and degradation assays were performed *in vitro*. EBNA-5 was found to inhibit MDM2-dependent polyubiquitination (but not monoubiquitination) of p53. The degradation of p53 by 26S proteasomes was also inhibited by EBNA-5. This may explain the high p53 level in LCLs. Noteworthy p53 is not transcriptionally active in LCL. Treatment of freshly EBV-infected B-cells with DNA damaging agent (mitomycin C) led to increase of p53 protein without parallel upregulation of p21, GADD45, and VDR. In contrast, the above mentioned proteins were upregulated at mRNA and protein levels in anti-CD40+IL-4 activated B-cells upon treatment with mitomycin C. It could be explained by the fact that p53 in the trimolecular protein complex with MDM2 and EBNA-5 did not bind to the *P21* promoter.
We found that MDMX in LCLs is a nuclear protein, despite the fact that MDM2 shows heterogeneous staining. This could be due to its binding to EBNA-5, which was shown by GST pull downs, immunoprecipitations and SPR. MDMX also can form tri-molecular complexes with EBNA-5 as a bridging molecule and p53. Biological significance of the binding between MDMX and EBNA-5 is currently under investigation.

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Latent Infection **Abstract P17**

LYMPHOKINE INDUCED MODIFICATION OF EBV GENE EXPRESSION IN EBV CARRIER B LYMPHOCYTES

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Abstract

The EBV genome can be carried by several cell types. The details of its interaction with the host cell are best known for B lymphocytes. Immunoblastic proliferation is driven by a set of virally encoded proteins. Viral expression is determined by the differentiation state of the cell. It can be modified by changes of the cell phenotype and by the action of lymphokines.
Examples: B-CLL cells infected in vitro express a restricted set of viral genes that does not induce proliferation (EBNAs but no LMP-1). Exposure of the Type I Burkitt lymphoma line Rael to CD40L induced EBNA-2 and LMP-1 concomitantly with a change of the cell phenotype.
Exposure of Type I and Type III cells to IL-21 changed EBV expression to type II pattern, EBNA-1 and LMP-1. In Type I BL lines it induced LMP-1, in Type III BL lines and in LCLs it downregulated the C-promoter-initiated message. At the same time it upregulated LMP-1 through activation of STAT-3. Thus lymphokines produced in the microenvironment can determine the fate of the EBV infected B lymphocyte by modifying the expression of virally encoded genes.

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Latent Infection **Abstract P18**

BALF0/1- JUST ANOTHER BRHF?

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Abstract

The viral Bcl-2 homologue (v-Bcl-2) BHRF1 can suppress apoptosis in B cells and delay terminal differentiation of SCC12F epithelial cells. BALF1 was originally reported as EBV's second anti-apoptotic v-Bcl-2, but subsequent studies have been contradictory. The EBV gene, unlike its primate virus analogues, has the potential to encode two proteins, BALF0 and BALF1, differing at their amino termini. This intriguing observation led us to hypothesise that the different isoforms may have different functions.

We produced plasmids that express BALF0 and BALF1 alone or combination and investigated their abilities to suppress apoptosis in Ramos BL cells and delay SCC12F terminal differentiation. Although we achieved high-level transient expression, it was very difficult to establish stably transfected clones - those that did grow had very low levels of the transfected gene expression. In apoptosis assays, there was no evidence of a protective effect, indeed viabilities were consistently lower in cells BALF1/BALF0 transfectants than in vector controls. It was not possible to determine if there were any clear differences between the two isoforms.

In differentiation assays the control transfectants produced rafts with a clear suprabasal layer several cells thick and a thin terminally differentiated layer, whilst the BALF1/0 transfectants produced very thin structures with no discernable suprabasal layer and uniform staining for a marker of terminal differentiation.

Our results suggest that BALF0/1 is not just another BHRF1 and indeed that it may be a novel pro-apoptotic, pro-differentiation v-Bcl-2 whose main function may be to counter the activity of BHRF1.

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Latent Infection **Abstract P19**

RECONSITITUTION OF EBV-GENOME LOAD AND T CELL PHENOTYPES AFTER BONE MARROW TRANSPLANTATION

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Abstract

Bone marrow transplanted (BMT) patients suffer a high risk of developing Epstein-Barr virus (EBV)-associated post-transplantation lymphoproliferative disease (PTLD) and lymphomas. Although high EBV genome-load correlates to risk for PTLD in patient groups, it is insufficient as a clinical predictor in individual patients. In an ongoing collaborative study we are therefore monitoring details of immune reconstitution together with EBV genome load and clinical data. In in vitro experiments, we are exploring mechanisms behind the increase of EBV-genome load and its role in lymphomagenesis.

Patients (18 children and 90 adults) are followed with regular sampling after transplantation. Immune reconstitution is studied with multicolor FACS-analysis of T-cells. EBV-genome load, monitored by a sensitive real time PCR method, showed two major developments. While one group developed an apparent good control of EBV, another group showed oscillations of high EBV- genome load for one year post-BMT. The EBV-load is correlated to T-cell phenotyping of using TCR-binding tetrameric fluorescent MHC-class specific peptides. Thus we can quantify EBV-specific mature, immature and peripheral memory T cells of CD4, CD8 or CD4-CD8-negative types.

Subversion of the immune control in post-BMT patients allows the expansion of EBV-carrying B-lymphocytes. However, this requires a SWITCH from resting (G0 latency I) cells to proliferating latency III cells, which maybe the precursors of EBV-carrying lymphoma cells. These switches are studied at the transcriptional, cell phenotype as well as systems biology level. Based on an integrative, complex systems approach to gene regulatory networks we explore whether cancer cells are special cases of "cell attractors", so called "cancer cell attractors". We argue that cancer cells become reprogrammed and trapped in abnormal attractors in state space. We use an EBV cell line model to analyze the nature of normal and cancer cell attractors. Rare "outlier" cells may result in a high risk for lymphoma transition from latency III cells.

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Latent Infection **Abstract P20**

ASSOCIATION OF HLA-DQ B*03 ALLELES WITH RESISTANCE TO EBV INFECTION IN HEALTHY INDIVIDUALS

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Abstract

EBV initiates entry into B cells using two sets of receptor-ligand interactions. EBV gp350/220 binds CD21, and EBV gp42 interacts with its co-receptor HLA class II molecule. Previous work has shown that gp42 binds to all three HLA class II isotypes (DR, DP, and DQ). However, in vitro studies with cloned cDNAs of HLA alleles suggested that there is a co-receptor usage restriction by EBV within the highly polymorphic HLA-DQ locus. HLA-DQ β*02, but not DQ β*03, mediated EBV entry in vitro (Haan and Longnecker, 2000). To determine if this HLA class II restriction occurs during human infection with EBV, we performed high resolution HLA genotyping from 27 EBV seronegative and 97 EBV seropositive blood bank donors and analyzed the relationship between HLA-DQ β*02 alleles, DQ β*03 alleles, and EBV serologic status in these individuals. The prevalence of HLA-DQ β*02 alleles in EBV seronegative individuals (44%) was similar to that of EBV seropositive persons (38%). In contrast, the presence of HLA-DQ β*03 alleles in EBV seronegative individuals (59.3%) was higher than in EBV seropositive persons (46.4%). Furthermore, within the EBV seronegative population, most (66.7%) individuals with HLA-DQ β*02 alleles had DQ β*02/β*03 haplotypes, compared with 32.3% for EBV seropositive individuals. Finally, we identified four EBV seropositive individuals who had DQ β*03/β*03 haplotypes. These results indicate that while HLA-DQ β*03 is associated with resistance to EBV infection in humans, the effect is not absolute, and therefore additional determinants are important for EBV entry.

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Latent Infection **Abstract P21**

CTCF, COHESIN, AND POLYMERASE OCCUPANCY OF THE EBV GENOME

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Abstract

Chromatin structure of the episomal EBV genome is organized by loops that colocalize distant DNA. Instead of reading regulatory elements in the one dimension of a linear sequence, long-range spatial connectivity generates more complex combinations of functional elements in the three dimensions of a nucleus. The molecular architecture of CTCF, an essential mediator of loop formation, suggests that this factor acts as a scaffold for the assembly of other proteins on chromosomes. We have profiled the occupancy of CTCF, cohesin, and RNA Polymerase II on the episomal genome of the Epstein-Barr Virus in a cell culture model of latent infection. CTCF colocalizes with cohesin but not RNA Polymerase. CTCF and cohesin distribute ubiquitously throughout the genome, with most binding sites outside the boundaries of the latency control region. In addition to tracking with known transcripts, RNA Polymerase appears paused at two positions, one of which lies within the latent origin of replication. The occupancy profile of each protein suggests context-dependent functions. We can now propose a model in which combinatorial recruitment of different functionalities by CTCF generates chromatin loops with distinct mechanisms of transcriptional regulation.

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Latent Infection **Abstract P22**

COMPARISON OF LMP1 AND CD40 SIGNALS ON THE B CELL PHENOTYPE; RELEVANCE TO THE PATHOGENESIS OF HODGKIN'S LYMPHOMA.

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Abstract

Hodgkin and Reed-Sternberg (HRS) cells are derived from germinal centre B cells. However, they have lost the majority of their B cell phenotype. In EBV positive cases of Hodgkin's lymphoma this loss can be mediated by the EBV latent membrane protein 1 (LMP1). Although LMP1 has been shown to have transforming potential in B cells, this EBV gene shows strong functional homology to CD40, a receptor normally expressed on B cells. We have sought to identify differences between LMP1 and CD40 signalling, which might explain the lymphomagenic potential of LMP1 in B cells.

We examined the effect of CD40, LMP1 and an LMP1CD40 chimera (which provides a constitutive CD40 signal) on the B cell phenotype. For this we have used a previously established method for transient transfection of lymphoma cell lines. The downstream effects of LMP1 and CD40 signalling were examined by looking for alterations in the expression of some selected B cell markers, including CD79a, CD79b, IgM and BLNK. We have found similarities in all three signals; however, we have identified some differences in the downstream targets of LMP1 and CD40. We are currently investigating the effect of LMP1 and LMP1CD40 expression in primary germinal centre B cells.

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Latent Infection **Abstract P23**

EBNA3C CAN INDUCE MITOTIC KINASE ACTIVITY IN NON-MITOTIC CELLS AND IS ITSELF A TARGET OF MPM-2 PHOSPHORYLATION

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Abstract

MPM-2 is a monoclonal antibody that recognises a phospho-epitope on multiple cellular proteins during mitosis and is routinely used as a marker for cells in mitosis. Using various delivery systems we have shown that EBNA3C (but not EBNA3A or 3B) can induce the MPM-2 epitope in non-mitotic interphase cells. It was confirmed that in the EBNA3C-expressing cells the MPM-2 antibody recognises phosphatase-sensitive epitopes distributed in the nuclei and precisely co-localising in a punctate pattern with EBNA3C. A series of N- and C-terminal EBNA3C deletion mutants used in transient transfection and inducible EBNA3C assays revealed two regions of the protein ($aa^{249-300}$ & $aa^{914-923}$) that are necessary to activate the kinase(s). Furthermore, probing western blots of EBNA3C with MPM-2 revealed that EBNA3C not only induces the kinase activity, but also acts as a substrate, with the phosphorylated residue(s) probably mapping to a region of 10 amino acids ($aa^{914-923}$). The phosphorylated form of EBNA3C appears more tightly associated with chromatin. Although EBNA3C can be co-immunoprecipitated with the mitotic kinase Plk-1 and binds to Plk-1 in GST-pull-down assays, the MPM-2-phenotype was not abrogated by addition of a specific inhibitor of Plk-1. We therefore conclude that another cellular kinase(s) must be involved. We suggest that the capacity of EBNA3C to activate a mitotic kinase (or kinases) may play a role in its ability to disrupt the mitotic spindle checkpoint and perhaps induce genomic instability.

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Latent Infection **Abstract P24**

EBV MEDIATED DOWN-REGULATION OF CANDIDATE TUMOUR SUPPRESSOR GENES DURING B CELL TRANSFORMATION

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Abstract

Infection of resting B cells *in vitro* with EBV results in growth transformation to produce a lymphoblastoid cell line (LCL). Changes in cellular gene expression are likely to be essential for the observed outgrowth of EBV transformed B cells (LCLs) capable of continuous proliferation in culture. To identify cellular genes involved in EBV mediated cellular transformation, we compared the gene expression profiles of EBV infected B cells and B cells induced to transiently proliferate in culture with CD40L and IL4. The comparison between resting, proliferating and infected B cells allowed us to characterise the gene expression changes that occurred after EBV infection as either proliferation or transformation associated. This comparison revealed 61 genes that were differentially expressed between EBV infected and mitogen stimulated B cells at 7 days after infection or mitogen stimulation. Initially, we have focused on the small subset of genes that are selectively down-regulated by EBV; of which 3 out of 6 were well defined candidate tumour suppressor genes. Using a lentiviral expression vector, we have investigated the impact of re-expression of these TSGs on the transformed phenotype of LCLs.

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Latent Infection **Abstract P25**

THE ROLE OF THE HERPES VIRAL PROTEINS LMP2A, K1 AND K15 DURING THE ONCOGENIC TRANSFORMATION OF PRIMARY B CELLS

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Abstract

Epstein-Barr virus (EBV) is associated with several different B cell lymphomas, e.g. Hodgkin lymphoma (HL). Hodgkin and Reed-Sternberg cells, the genuine tumor cells in HL, are probably derived from pro-apoptotic germinal center (GC) B cells, which fail to express a functional B cell receptor (BCR). Recent experiments showed that EBV's Latent Membrane Protein 2A (LMP2A) can rescue BCR negative (BCR(-)) GC B cells from apoptosis by mimicking the function of the missing BCR.
To determine the influence of LMP2A *versus* BCR signaling shortly after infection of B cells with EBV, we designed two inducible mutant EBVs. Therefore, LMP2A was replaced by (i) an LMP2A:mCD69 chimera which can be specifically activated by crosslinking with an anti-mCD69 antibody and (ii) a murine immunoglobulin (mIgM) which can be activated by its antigen 4-hydroxy-3-nitrophenyl acetyl (NP). In infection studies of BCR(-) B cells we want to determine if the signaling strength of LMP2A and the presence of antigen has an influence on the transformation of B cells and the induction of the lytic cycle.
The two gene products K1 and K15 of Karposi's Sarcoma Associated Herpes Virus (KSHV) partially resemble LMP2A in structure and function. To date, it is unclear whether they can also mimic BCR's function. To test this, mutant EBVs with *k1* or *k15* replacing *LMP2A* were generated. Infection of BCR(-) B cells suggests, that K1 and K15 can rescue these cells from apoptosis, in a manner similar to LMP2A.

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Latent Infection **Abstract P26**

GLOBAL EXPRESSION ANALYSIS OF EARLY PROLIFERATING EBV-INFECTED PRIMARY HUMAN B CELLS REVEALS SEQUENTIAL ACTIVATION OF ONCOGENIC GROWTH AND SURVIVAL PROGRAMS

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Abstract

In order to better understand early changes induced by EBV infection in primary human B cells, we utilized CFSE staining and FACS sorting to isolate the earliest proliferating cells for genome wide expression analysis. We compared mRNA levels in these cells to their uninfected primary B cell and monoclonal LCL counterparts. Since cell cycle entry is asynchronous early after infection and many cells fail to proliferate, our sorting approach removed such confounding heterogeneity from this sample. Gene ontology analysis of our microarray data corroborated ongoing studies by our group indicating that early infected cells proliferate more rapidly than LCLs and display evidence of an activated DNA damage response. Genes up-regulated in early proliferation were most enriched for E2F and NF-Y promoter binding sites. Furthermore, c-Myc mRNA and its activity, as inferred from its expression signature, were also highly induced early. Importantly, analysis of changes from early proliferating cells to final LCL outgrowth revealed attenuation of these proliferation-associated gene sets and c-Myc mRNA and activity.

Most surprisingly, the major induction in gene expression from early proliferation through LCL was NF-κB activation as evidenced by promoter motif usage and canonical target induction including TRAF1, ICAM1, and NFKBIA. These results, validated using qRT-PCR, indicate that EBV-driven growth transformation proceeds through a novel biphasic strategy where a potentially oncogenic c-Myc proliferation signal is used initially followed by an attenuation of proliferation rate and increased NFkB mediated survival signaling. The significance of these results in the context of known EBV biology will be discussed.

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Latent Infection **Abstract P27**

ATTENUATION OF NF-KB ACTIVATION FROM THE VIRAL TNF-RECEPTOR MIMIC LMP1 THROUGH ITS SELECTIVE SORTING INTO CD63-ENRICHED EXOSOMES

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Abstract

Epstein Barr Virus (EBV) is an ubiquitous y-herpesvirus that encodes a viral oncogene Latent membrane protein 1 (LMP1) expressed in many EBV-associated tumors. LMP1 is a viral homologue of human CD40, a member of the TNF-receptor superfamily that constitutively activates NF-kB required for growth of EBV-transformed B cells (LCL). The mechanism(s) that prevent LMP1 overstimulation in infected B cells from healthy carriers are poorly understood.

We report here that a significant portion of intact LMP1 protein escapes degradation through selective sorting into tetraspanin-enriched intraluminal vesicles (ILV) of multivesicular endosomes (MVE) and is secreted through exosomes. LMP1 sorting into exosomes is independent on mono-ubiquitylation or its raft-anchoring domain. Rather biochemical analysis, immuno-electron microscopy and confocal imaging indicate that endogenous LMP1 interacts intracellularly with CD63 in microdomains distinct from conventional rafts. Furthermore, induction of LMP1 expression initiates aggregation and redistribution of the endosomal tetraspanin CD63 in peripheral vesicles in both B and epithelial cell backgrounds. Reporter assays show that knockdown of CD63 in HEK293 cells by siRNA does not affect LMP1-induced NF-kB activation, however LMP1 mutants that fail to aggregate with CD63 in peripheral vesicles are retained in golgi structures and precluded from secretion through exosomes thereby causing NF-kB overstimulation. Collectively, these observations provide new mechanistic insights how intracellular trafficking of LMP1 defines NF-kB signaling and present an alternative explanation of how latently EBV-infected proliferating B cells are able to protect themselves from constitutive NF-kB overstimulation by LMP1. The shared trafficking of LMP1 with CD63 through the late-endocytic pathway to exosomes is consistent with the notion that LMP1 has additional role(s) outside infected cells.

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Latent Infection **Abstract P28**

NUCLEOLIN IS INVOLVED IN EBNA1 MEDIATED TRANSCRIPTION ACTIVATION FROM EBV EPISOMES

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Abstract

EBNA1 is found ubiquitously in all three types of EBV latently infected cells. This consistent expression of EBNA1 results from its pivotal roles in the maintenance and transcription activation of the EBV episomal genome, which depend on a cisacting element, oriP. To further investigate how cellular factors are involved in EBNA1 mediated transcription, the EBNA1 associated cellular factors that were co-immunoprecipitated with the flag epitope-tagged EBNA1 (FEBNA1) from BJAB lymphoblasts in which FEBNA1 was stably expressed were determined using LCMS- MS. Nucleolin was identified as one of the EBNA1 associated cellular proteins and the interaction of two proteins was confirmed by co-immunoprecipitation and immunofluorescent analyses. Our results indicated the N-terminal amino residues 1-100 of EBNA1 are essential for binding to nucleolin, whereas the RNA recognition motif (RRM) of nucleolin is critical for EBNA1 binding. Nucleolin is an abundant protein that presents in both nucleolus and nuclear plasma, and its multifunctional profiles have been implicated in ribosome biosynthesis, chromatin structure, viral genome replication, and transcription regulation. Our studies revealed that transiently expressed nucleolin substantially augmented about 2.5 fold activation above the intrinsic activity reached by an EBNA1-dependent oriP reporter plasmid. In addition, the oriP-reporter activity driven by EBNA1 was completely abolished when endogenous expression of nucleolin was knocked down by introducing nucleolin specific shRNAs into BJAB cells, suggesting nucleolin is indispensable for EBNA1 mediated transcription activation from EBV episomes. Taken together, our data suggested that nucleolin plays a critical role in the life cycle of EBV infection.

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Latent Infection **Abstract P29**

ROLE OF EBNA-6 BINDING MRPS18-2 PROTEIN IN IMMORTALIZATION OF PRIMARY FIBROBLASTS

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Abstract

Epstein-Barr virus (EBV) like other DNA tumor viruses induces an S-phase in the natural host cell, the human B-lymphocyte. This is linked with blast transformation. We found that EBNA-6 binds to a mitochondrial ribosomal protein, MRPS18-2, and targets it to the nucleus. We found that S18-2 binds to Rb protein specifically. EBNA6 may play a major role in the entry of EBV infected B-cells into the S phase, by binding to and raising the level of nuclear S18-2 protein. This would inhibit pRb binding to E2F1 competitively and lift the block preventing S-phase entry.

Recently we have found that an overexpression of the human S18-2 protein leads to immortalization of the rat primary fibroblasts (REFs). Immortalized by S18-2 cells (18IM) loose contact inhibition and become capable of anchorage-independent growth. They are not tumorigenic, however. Immortalization accompanied by the change of differentiation markers both *in vitro* and *in vivo*. 18IM cells expressed embryonic stem cell markers, such as SSEA-1 and Sox2 *in vitro* that were not present on the control REFs. In contrast, the mesodermal markers vimentin, smooth muscle actin, and Fut4, characteristic of fibroblastic differentiation, were downregulated. 18IM cells expressed new markers in confluent culture and in mice, such as ecto- and endoderm specific pan-keratin, ectoderm specific beta-III-tubulin, and mesoderm specific MHC class II. They could also differentiate into Oil red O stainable fat cells.

We have found, using microarray techniques, that 18IM cells and REFs differ dramatically in gene expression, despite minor changes of the genome methylation pattern.

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Posters P30-P52

Virus Replication

Virus Replication **Abstract P30**

POST TRANSLATIONAL MODIFICATION OF ZTA (*BZLF1*, ZEBRA, Z) PROTEIN

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Abstract

The EBV protein Zta (*BZLF1,* ZEBRA, Z) plays a pivotal role in controlling the expression of viral genes during (i) the early stages of immortalization and (ii) during viral replication. Zta also functions as the origin binding protein at Ori Lyt.

Multiple forms of Zta protein are observed in the cells. One candidate for mediating post translational modification of Zta is the viral protein kinase, encoded by *BGLF4*. The viral protein kinase plays an important role in the replication of EBV phosphorylating a number of viral and cellular targets including Zta. The viral protein kinase interacts with Zta (*BZLF1,* ZEBRA, Z) forming a tight complex when Zta is phosphorylated on amino acid S209.

In order to explore the potential role of this phosphorylation on other post translational modifications, we have developed Zta mutants that are either compromised for phosphorylation by viral protein kinase or else are mimics of phosphorylated Zta. The impact of these mutations on the generation of Zta post translation modification is under investigation.

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Virus Replication **Abstract P31**

IN SILICO ANALYSIS OF THE SUBSTRATE SPECIFICITY OF BPLF1 AND HOMOLOGS

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Abstract

The large tegument proteins of herpesviruses contain conserved N-terminal domains shown to act as cysteine proteases of unknown function. We have found that the Epstein-Barr virus (EBV) encoded member of the family, BPLF1, is a deneddylase that binds to cullins and inactivates cullin-ring ligase (CRLs). Expression of BPLF1 during the productive virus cycle induces an S-phase-like cellular environment that is required for efficient virus replication. Homologs encoded by other herpesviruses share these functions. A bioinformatics approach was used to investigate the structural basis for the deneddylase activity and interaction with cullins. Starting from the crystal structure of the M48 protein encoded by MCMV, homology models were generated for the catalytic N-termus of all members of this viral enzyme family. The models were than used for rigid body docking of ubiquitin and NEDD8, followed by energy minimization and refinement steps via molecular dynamic simulations in order to define high scoring models of interaction. Models with comparable energy of binding to ubiquitin and NEDD8 were found for all homologues and the predicted contact residues were conserved, suggesting that there are no structural constraints that would hinder their activity as deneddylases. A similar methodology based on sequence alignment and molecular modelling was used to identify conserved surface residues of BPLF1 that could be important for its interaction with the cullins and/or their adaptors. Conserved charged residues were found in an helical structure facing away from the catalitic groove. This solvent exposed domain appears to be a likely candidate for binding to cullins.

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Virus Replication **Abstract P32**

EBV RTA-MEDIATED CELL CYCLE ARREST ENABLES PERMISSIVE REPLICATION OF EBV AND KSHV IN 293 CELLS

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Abstract

One of the viral latent-lytic molecular switches, functionally termed replication and transcription activator (RTA), is evolutionarily conserved in the genomes of gammaherpesviridae. In previously studies, doxycycline-inducible EBV RTA molecule, formally known as Rta/BRLF1, was shown to potently induce an irreversible G1 arrest followed by cellular senescence in 293 cells. Here, we demonstrate that in these cells, Rta-induced growth arrest provided a suitable milieu for permissive replication of EBV and Kaposi's sarcoma-associated herpesvirus (KSHV), respectively. It was observed that before the onset of latent EBV or KSHV switching to the lytic phase, Rta simultaneously augmented the expressions of senescence inducers (e.g. p21CDKN1A) and inhibited the expressions of proliferation drivers (e.g. MYC). Although the time course of viral lytic replication was relatively slow (≈ 168 h) compared to other well established systems, homogenous anoikis-like cell detachment accompanied by cell death was detected at later times of Rta-mediated viral lytic cycle progression, reminiscent of permissive replication described in bacterophage and other herpesviruses. The robustness of Rta-mediated EBV or KSHV lytic reproduction was verified by the detection of 2 × 107 viral particles / ml in the culture medium of doxycycline-treated cells and close to 1% of the virions were infectious when titrated on fresh 293. Taken together, we propose that the combination of 293 cell and EBV Rta may provide a novel *in vitro* system for modeling a complete lytic cycle of either EBV or KSHV in a non-chemical induction way.

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Virus Replication **Abstract P33**

INHIBITION OF EPSTEIN-BARR VIRUS LYTIC CYCLE BY SULFORAPHANE

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Abstract

Epstein-Barr virus (EBV), a member of herpesviridas, is a famous human tumor viruse. Sulforaphane (SFN) is a plant-extracted anticarcinogenic isothiocyanate and has been shown to possess many cancer chemopreventive activities. Recently, after screening several compounds, we found SFN has potential to inhibit EBV reactivation. In this study, we tried to determine whether SFN inhibit EBV to go into lytic cycle. We used nasopharyngeal carcinoma (NPC) cell lines (NA and HA) to examine the relationship between SFN and EBV. In the results of cytotoxicity, cytotoxic concentration 50% (CC_{50}) of SFN on NA and HA were 32.78 μM and 11.17 μM, respectively. Then, we treated various amounts of SFN on NA and HA cells, however, it can not activate EBV into lytic cycle. Furthermore, after 40 ng/ml TPA plus 3mM SB induction in 24 hours, we found SFN treatment (>10 μM) decreased expression of EBV lytic proteins, Zta, Rta, EAD, and DNase by western blot detection. We also found it can reduce the EAD expression percentage determined by immunofluorescence and flow cytometry. Taken together, these results suggested that SFN can inhibit EBV lytic cascade in NPC cells.

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Virus Replication **Abstract P34**

EFFECTS OF THE UNFOLDED PROTEIN RESPONSE ON THE EBV LIFE CYCLE IN B-LYMPHOBLASTOID CELL LINES.

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Abstract

EBV-immortalized B-lymphoblastoid cell lines (LCLs) resemble some lymphomas that arise in immunosuppressed patients. LCLs are used as models for cellular transformation and as antigen-presenting cells in immunological assays. LCLs vary in cell surface phenotype and gene expression profile, but it is not known how this heterogeneity influences the EBV life cycle. We have investigated the connections between cellular and viral phenotypes in LCLs, categorizing them as either low in EBV copy number or fluctuating within a high range. High-copy status is accompanied by higher lytic viral gene expression and lower latent gene expression except LMP2A. High or low lytic permissivity (likelihood that a cell will trip over into producing lytic switch proteins) was a stable property in continuously growing LCLs and was not affected by abrogating lytic DNA replication. We identified genes overexpressed in non-permissive LCLs including transcription factors involved in maintaining B cell linage (EBF1, PAX5, TCF3). Genes associated with the unfolded protein response (CALR, DNAJB11, ARMET) were overexpressed in permissive LCLs, as was the UPR protein Grp94. Thapsigargin treatment was used to investigate the effect of UPR induction on permissive and non-permissive LCLs to determine if there were differences in the speed and/or degree of lytic reactivation under conditions of ER stress. UPR induction reliably induces the lytic switch protein ZEBRA, but this did not necessarily lead to lytic viral replication.

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Virus Replication **Abstract P35**

VALPROIC ACID BLOCKS INDUCTION OF THE EBV LYTIC CYCLE IN A BURKITT LYMPHOMA CELL LINE

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Abstract

Different stimuli reactivate EBV into the lytic cycle in Burkitt lymphoma cells. In HH514-16 cells, lytic reactivation occurs in response to HDAC inhibitors, sodium butyrate (NaB) and trichostatin A (TSA), and the DNA methyltransferase inhibitor azacytidine (Aza). Valproic acid (VPA), a short-chain fatty acid HDAC inhibitor of the same class as NaB, failed to induce the EBV lytic cycle in these cells. VPA blocked induction of EBV early lytic proteins, ZEBRA and EA-D, in response to NaB, TSA, or Aza. Nonetheless VPA behaved as an HDAC inhibitor causing increased acetylation of histone H3. Induction of BZLF1 mRNA in response to NaB, TSA, and Aza was inhibited by VPA; thus the block is upstream of BZLF1 mRNA expression. MS-275 and Apicidin, representing two additional classes of HDAC inhibitors, also reactivated EBV in HH514-16 cells; this activity was inhibited by VPA. Affymetrix human genome arrays revealed that global changes in cellular gene expression in response to VPA, NaB, or TSA were highly similar. Using qRT-PCR we found that treatment of HH514-16 cells with NaB, TSA, or VPA lead to similar increases in the levels and similar kinetics of specific cellular transcripts. When combined with NaB or TSA, VPA did not inhibit activation of cellular genes. Therefore, VPA does not generally block transcription or exert toxic effects. Since different HDAC inhibitors are used in medical treatment, it will be important to understand whether they activate or repress the EBV lytic cycle in patients.

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Virus Replication **Abstract P36**

GENETIC EVIDENCE FOR A ROLE FOR RTA IN LYTIC REPLICATION OF EPSTEIN-BARR VIRUS

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Abstract

Expression of the Rta protein is essential to switch EBV infected cells from a latent to lytic state. While the role of Rta as a transcription activator is well established, involvement of Rta in lytic DNA replication is poorly understood. In transient replication assays, expression of Rta was dispensable for amplification of an origin-containing plasmid (Fixman, 1995). However, the importance of Rta in activating DNA synthesis from the endogenous viral genome has not been studied. In the present study, we used ZEBRA mutants to develop assays to assess the requirement for Rta in activation of viral DNA synthesis from the endogenous origin of lytic replication. We found that expression of the six virally encoded components of the EBV lytic replication machinery together with a mutant form of ZEBRA that is competent to bind to oriLyt but unable to activate transcription of replication genes was insufficient to activate replication. Addition of Rta to this mixture of replication factors activated viral replication and late gene expression. Deletion mutagenesis indicated the importance of the C-terminal 10 amino acids of Rta for its function in replication. A chimeric protein of Rta (1-595) fused to the heterologous transactivation domain of the herpes simplex virus protein, VP16, synergized with ZEBRA to activate transcription of *bmrf1* but failed to support viral replication. Our findings indicate that Rta plays an independent role in DNA replication and characterize a region of Rta that is necessary for this function.

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Virus Replication **Abstract P37**

A DENEDDYLASE ENCODED IN THE N-TERMINUS OF THE TEGUMENT PROTEIN BPLF1 PROMOTES VIRUS REPLICATION BY REGULATING THE ACTIVITY OF CELLULAR CULLIN-RING-LIGASES.

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Abstract

Pathogenic viruses have evolved elaborated strategies for manipulating the host's Ubiquitin Proteasome System (UBP), including the production of functional homologues of cellular enzymes. We have shown that a conserved cysteine protease encoded in the N-terminal domain of the major tegument protein of *herpersviridae* acts *in vivo* as a NEDD8-specific protease that targets cullin-ring E3 ligases (CRLs). Expression of BPLF1 alone in HeLa cells or in the context of the productive virus cycle in Akata-BX1 cells induces accumulation of the licensing factor Cdt1, deregulates cell cycle progression and promotes the establishment of a persistent pseudo S-phase that is accompanied by re-replication of cellular DNA. This pseudo-S-phase environment appears to be required for efficient replication of EBV DNA since shRNA mediated inhibition of BPLF1 in Akata-BX1 cells entering the productive cycle did not affect the progression of the virus cycle but severely impaired the yield of viral DNA. Cellular DNA re-replication and viral DNA synthesis was restored by overexpression of Cdt1 indicating that stabilization of the cellular licensing factor plays a critical role in this phenotype. BPLF1 homologues share its deneddylase activity and capacity to promote cellular DNA re-replication suggesting that these viral enzymes play a key function in the life cycle of herpesviruses by inducing a replication-permissive S-phase-like cellular environment.

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Virus Replication **Abstract P38**

UBINUCLEIN, A NACOS PROTEIN, NEGATIVELY REGULATES THE EBV PRODUCTIVE CYCLE IN EPITHELIAL CELLS

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Abstract

The Epstein-Barr Virus (EBV) productive cycle is initiated by the expression of the viral trans-activator EB1 (also called ZEBRA, Zta and BZLF1), which belongs to the basic-leucine zipper transcription factor family. We previously identified the cellular NACos (Nuclear and Adherent junction Complex components) protein Ubinuclein (Ubn-1) as a partner for EB1 but the function of this complex has not been studied. Here, we have evaluated the consequences of this interaction on the EBV productive cycle and find that Ubn-1 over-expression represses the EBV productive cycle whereas Ubn-1 down regulation by shRNA, increases virus production. By ChIP assay, we show that Ubn-1 blocks EB1-DNA interaction. We also show that in epithelial cells, re-localization and sequestration of Ubn-1 to the tight junctions of non-dividing cells allows a stronger activation of the productive cycle. We propose a model in which Ubn-1 acts as a modulator of the EBV productive cycle: in proliferating epithelial cells, Ubn-1 is nuclear and inhibits activation of the productive cycle whereas in differentiated cells, Ubn-1 is sequestrated to tight-junctions, allowing EB1 to function in the nucleus.

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Virus Replication **Abstract P39**

THE EBV BRRF1 GENE PRODUCT, NA, CAN INDUCE THE LATENT TO LYTIC SWITCH IN EBV-INFECTED EPITHELIAL CELL LINES VIA A TRAF2/P53 DEPENDENT MECHANISM.

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Abstract

The EBV BRRF1 lytic gene product (Na) is encoded within the same immediate-early region as the BZLF1 (Z) and BRLF1(R) gene products, and Na can activate the Z promoter in some cell lines. However, the role of Na during viral reactivation in various EBV-infected cell types is unclear. Here we show that over-expression of Na alone is sufficient to induce lytic gene expression in several different latently infected epithelial cell lines (including Hone-Akata, CNE2-Akata, and AGS-Akata), while the combination of Na and p53 is required for induction of lytic gene expression in C666-1 NPC cells. Consistent with its ability to interact with TRAF2 in a yeast two-hybrid assay (PNAS 104:7606-7611), we demonstrate that Na interacts with TRAF2 in cells. Furthermore, we show that TRAF2 is required for Na induction of lytic gene expression. Additionally, we find that Na induces JNK activation in a TRAF2-dependent manner, and that a JNK inhibitor abolishes the ability of Na to disrupt viral latency. Although Na and the tumor suppressor protein p53 cooperate to induce lytic gene expression in epithelial cells, we show that the major form of the p53 family member, p63, found in NPC cells (deltaN63) inhibits the ability of Na to disrupt viral latency. Together these data suggest that Na plays an important role in regulating the switch between latent and lytic infection in epithelial cells, and that the relative amounts of various p53 family members present in the cell may affect the latent to lytic switch.

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Virus Replication **Abstract P40**

STRUCTURE AND ACTIVITY OF THE EBV NUCLEASE BGLF5

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Abstract

The EBV nuclease, also called alkaline exonuclease, is a lytic protein conserved in the herpesvirus family and part of the replication machinery. Still, its function is still not fully understood. In γ-herpesviruses it has a double role: it appears to resolve branched replication intermediates during the rolling-circle replication of the viral genome and plays a role in host-shutoff due to mRNA degradation. This latter activity appears as well to be crucial for the regulation of expression levels of EBV proteins.
We determined the structure of this enzyme by X-ray crystallography. The EBV nuclease belongs to the D-(D/E)XK nuclease superfamily. The central domain is shared with the exonuclease of bacteriophage λ. We could show that besides the already described exonuclease activities on ssDNA and dsDNA and a weak endonuclease activity there is an exonuclease activity on RNA in presence of Mn^{2+} explaining the host-shutoff function.
We are interested in the structural base for these different activities. Our current work concentrates on the development of a fluorescent assay for the enzymatic activity and structural studies on the interaction with non-cleavable oligonucleotides. Using a phosphorothioate modified DNA oligomer, we observed an interaction in gelshift assays which we characterized further using biophysical techniques. Kinetic and structural data point to a cooperative role of the nuclease in substrate degradation.

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Virus Replication **Abstract P41**

MOLECULAR BASIS FOR THE FUNCTIONAL INACTIVATION OF CULLIN E3 LIGASES BY THE EBV TEGUMENT PROTEIN BPLF1

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Abstract

The N-terminal domain of the EBV tegument protein BPLF1 enhances viral replication by promoting the accumulation of the cellular licensing factor Cdt1. BPLF1 interacts with the cullin scaffold and inactivates cullin-ring ligases (CRLs) by acting as a deneddylase. The molecular basis for this interaction was investigated using purified components in *in vitro* binding assays and the interacting domain of BPLF1 was identified by combining in silico and biochemical approaches. Pull-down assays performed with eukaryotic expressed or purified bacterial expressed His-tagged BPLF1, and GST-tagged Cull4A and Cull4A subdomains suggest that the interaction does not require cellular adaptors and involves a region of Cull4A that is close to the RBX1 E2 adaptor binding domain and the site of neddylation. Based on sequence alignment of the N-terminal domains of BPLF1 and homologues encoded by other members of the herpesvirus family, and on molecular modeling of BPLF1 based on the crystal structure of homologue encoded by MCMV, a conserved helical domain containing multiple negatively charged residues facing away from the catalytic groove was identified as a candidate binding site on BPLF1. The relevance of the predicted residues for binding to Cull4A was confirmed by mutation analysis and co-immunoprecipitation assays. These finding provide a molecular rationale for the selective capacity of BPLF1 to modulate the activity of cellular CRLs that regulate DNA synthesis and cell cycle progression.

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Virus Replication **Abstract P42**

MODULATION ON AUTOPHAGY DURING EPSTEIN-BARR VIRUS REACTIVATION BY TGF-BETA1; POSSIBLE ROLE IN LYMPHOMAGENESIS

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Abstract

The Epstein-Barr virus (EBV) is a persistent gamma herpesvirus, which is associated with malignancies. The requirement of lytic gene expression for outgrowth of lymphoproliferations in a SCID mouse model, suggests the importance of reactivation for EBV pathogenesis. Transforming Growth Factor beta 1 (TGF-β1) induces EBV reactivation. During TGF-β1-mediated EBV reactivation, an autophagy inhibitor, PI3K/Akt, was activated, and an autophagy inducer, the interferon-inducible protein kinase activated by double-stranded RNA (PKR), was inactivated. This prompted us to investigate the effect of TGF-β1-mediated viral reactivation on the autophagy process. The autophagic marker microtubule-associated protein1 light chain 3 (LC3 II protein) was monitored by western-blot and visualization of the autophagosomes was performed by the use of the autofluorescent drug monodansyl-cadaverine (MDC). Autophagy markers were decreased after treatment by TGF-β1 of EBV-infected Burkitt's lymphoma (BL) cell lines and not in EBV negative BL cells showing an EBV-dependent inhibition of autophagy during TGF-β1 mediated EBV reactivation. The autophagic pathway is a cellular defence process involving the bulk degradation of cellular contents by autophagosomes/lysosomes during starvation or viral infection and autophagy is postulated to play a role in antiviral innate immunity. Inhibition of autophagy might prevent cellular antiviral defence.

Moreover, although TGF-β1 has anti-proliferative and pro-apoptotic effects on EBV negative BL cells, those effects are much milder on EBV-infected BL cells. EBV infection might then counteract adverse effect of TGF-β1, preventing premature cell death of the host cell during virus particle production.

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Virus Replication **Abstract P43**

KINETICS OF EPSTEIN-BARR VIRUS (EBV) LATENT AND LYTIC PROTEIN EXPRESSION IN EBV-POSITIVE BURKITT, EPITHELIAL AND LYMPHOBLASTOID CELLS UPON INDUCTION OF LYTIC CYCLE

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Abstract

Epstein Barr virus (EBV) has latent and lytic infection cycles. We hypothesize that a subset of EBV latent proteins may be expressed and have functional roles in lytic cycle. We examined by Western blotting the expression of three latent proteins (EBNA1, EBNA2 and LMP1) and three lytic proteins (Zta, VCA-p18 and gp350/220) in Akata (Burkitt), AGS-BX1 (gastric carcinoma) and B95-8 (lymphoblastoid) cells at defined intervals between zero and 72 hours after lytic cycle activation with anti-IgG, suberoylanilide hydroxamic acid and TPA/sodium butyrate, respectively. EBNA1 was stably expressed in lytic cycle at the same level as that of latent cycle in all cell lines. EBNA2 was neither expressed in latent nor lytic phases of AGS-BX1 whilst it was expressed in Akata from 24 hours but downregulated in B95-8 from 48 hours post-induction. LMP1 was expressed in Akata from 16 hours and upregulated in B95-8 also from 16 hours but was never expressed in AGS-BX1. Zta was induced in Akata at 2 hours reaching maximal level at 24 hours whilst it was upregulated or induced in AGS-BX1 and B95-8 at 8 hours reaching maximal level at 48-72 hours post-induction. VCA-p18 and gp350/220 were induced at 8 hours in both Akata and AGS-BX1 rising to maximal level at 72 hours post-induction. These data suggest that EBNA2 and LMP1 may have function in lytic cycle of B but not epithelial cells. Simultaneous activation of Zta, VCA-p18 and gp350/220 in AGS-BX1 cells indicates different regulation of lytic cycle between epithelial and B cells.

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Virus Replication **Abstract P44**

PRIMARY INFECTION WITH THE EPSTEIN-BARR VIRUS AND RISK OF MULTIPLE SCLEROSIS

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Abstract

To determine whether multiple sclerosis (MS) risk increases following primary infection with the Epstein-Barr virus (EBV), we conducted a nested case-control study including 305 individuals who developed MS and 610 matched controls selected among the over 8 million active-duty military personnel with serum stored in the Department of Defense Serum Repository. Time of EBV infection was determined by measuring antibody titers in serial serum samples collected before MS onset among cases, and on matched dates among controls. Ten (3.3%) cases and 32 (5.2%) controls were initially EBV negative. All of the 10 EBV-negative cases became EBV positive before MS onset; in contrast, only 35.7 % (10) of the 28 controls with follow-up samples seroconverted (exact p value = 0.0008). We conclude that MS risk is extremely low among individuals not infected with EBV, but it increases sharply in the same individuals following EBV infection.

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Virus Replication **Abstract P45**

EFFECTS OF CELLULAR INTERFERON-STIMULATED GENE PRODUCTS (ISGS) ON EBV REPLICATION

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Abstract

Interferons lead to the induction of over four hundred cellular genes that regulate myriad cellular functions. Many of the gene products (ISGs) are synthesized in response to viral replication and are an important component of the innate immune response to invading pathogens. We recently demonstrated that several cytoplasmic ISGs are produced in large amounts in response to Epstein-Barr virus (EBV) replication.

Hypothesis: ISGs produced by the cell in response to EBV infection are likely to act as inhibitors of virus replication.

To study the effect of ISG54 and MxA on EBV replication, we engineered derivatives of Burkitt lymphoma cells that inducibly allow robust EBV replication and also produce each ISG individually when treated with doxycycline, allowing us to examine the effect of individual ISGs on EBV replication at the molecular level.

ISG54 and MxA both inhibited EBV replication when expressed at physiological levels. Interestingly, both MxA and ISG54 inhibited EBV replication, which is a nuclear process, despite their cytoplasmic localization. Further, ISG54 appeared to specifically inhibit synthesis of an early EBV protein, while sparing other early EBV proteins and without globally inhibiting cellular protein synthesis.

Cellular ISGs may inhibit herpesvirus replication by novel mechanisms. MxA inhibits RNA virus replication by sequestering viral proteins in oligomeric cytoplasmic structures. Whether a similar mechanism operates on nuclear replication of EBV remains to be determined. ISG54 also appears to have specific effects on EBV replication. The mechanism of this effect will be explored by examining the profile of its effects on EBV gene expression.

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Virus Replication **Abstract P46**

LYTIC REACTIVATION OF EPSTEIN-BARR VIRUS IS BLIMP1-DEPENDENT IN EPITHELIAL CELLS BUT NOT IN B-CELLS

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Abstract

Lytic reactivation of EBV in B lymphocytes is promoted by B-cell maturation protein 1 (Blimp1)-induced plasma cell differentiation and is triggered by expression of BZLF1. EBV also replicates in differentiated epithelial cells of oral hairy leukoplakia (OHL) and it has been shown that terminal differentiation of squamous epithelial cells also requires Blimp1. Using immunohistochemistry, we demonstrate co-expression of Blimp1 and BZLF1 in OHL. Specifically, double labelling revealed that BZLF1 expression was restricted to $Blimp1^+$ epithelial cells. Assays employing the firefly luciferase ORF controlled by the BZLF1 promoter, Zp, demonstrated induction of Zp activity upon enforced expression of Blimp1 in 293T cells and in HeLa epithelial cells. As Blimp1 acts as a transcriptional repressor, we hypothesised that this effect might be due to down-regulation of ZEB1, a cellular transcription factor which inhibits lytic activation by binding to Zp. However, Blimp1 expression did not affect the expression levels of ZEB1 mRNA or protein. Next, we analysed the expression of Blimp1 and BZLF1 in infectious mononucleosis tonsils. Unexpectedly, this revealed that only approximately 50% of BZLF1+ lymphoid cells co-expressed Blimp1. In summary, we show that EBV replication in epithelial cells is strictly dependent on Blimp1-induced terminal differentiation, although the effect of Blimp1 on BZLF1 expression is likely to be indirect. By contrast, BZLF1 expression in B-cells is observed in $Blimp1^-$ as well as in $Blimp1^+$ cells, suggesting that in this cellular environment other factors may substitute for Blimp1 in the induction of EBV replication.

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Virus Replication **Abstract P47**

TGF-B1-MEDIATED REACTIVATION OF THE EPSTEIN-BARR VIRUS UP-REGULATES DIFFERENT ANTI-APOPTOTIC PATHWAYS

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Abstract

The development of Epstein-Barr virus (EBV)-associated diseases is dependent on the balance between latency and reactivation. Entry into the viral lytic cycle is triggered by expression of Z Epstein-Barr virus Replication Activator (ZEBRA). It has been shown that iNOS activity is involved in maintaining EBV latency through down-regulation of ZEBRA expression. The entry into the viral lytic cycle can be mediated by Transforming growth factor beta 1 (TGF-β1) through the ERK1/2 signalling pathway. These results prompted us to study NO effect on Erk1/2 signalling pathway. ZEBRA expression as well as Erk1/2 phosphorylation were inhibited by the NO donor treatment, suggesting that inhibition of ZEBRA expression by NO was mediated by this MAPK pathway; moreover, nitric production was diminished drastically upon TGF-ß1 treatment. This reduction was depended on NF-kB since it was abolished by different NF-kB inhibitors, BAY-11-7082 and IKK inhibitor V. This result was confirmed by examining the effect of transfection of a siRNA of relA in DG75 cells. Thus, these data provide evidence that NF-κB participate to TGF-β1 mediated-Erk1/2 pathway by a mechanism implicating down-regulation of iNOS protein. The signal proceed then, from Erk1/2 to activation of PI3K/Akt. Activation of the later enables Smad3, a mediator of TGF-β1 signalling, to be acetylated by direct interaction with the co-activator CREB-binding protein (CBP), and then regulate TGF-β1-induced ZEBRA expression. Taken together, our results provide new findings for TGF-β1-induced EBV reactivation, and demonstrated that canonical (Smad3) as well as non-canonical pathways, NF-κB, ERK1/2 and PI3-K/Akt, in concert, contribute to TGF-β1-mediated EBV reactivation.

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Virus Replication **Abstract P48**

MUTANT ZEBRA PROTEINS WITH ARGININE TO GLUTAMATE SUBSTITUTIONS IN THE DNA-RECOGNITION DOMAIN INDUCE FORMATION OF NUCLEAR AGGRESOMES AND INHIBIT LYTIC REPLICATION OF EPSTEIN-BARR VIRUS

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Abstract

We have studied changes in intra-nuclear localization resulting from single amino acid mutations within the basic DNA-recognition region of ZEBRA, the Epstein Barr virus (EBV)-encoded bZIP protein that is essential for lytic viral replication (Heston et al. 2006, Park et al., 2008). Wild-type (WT) ZEBRA is diffusely distributed within the nucleus. Several early and late ZEBRA mutants display a speckled appearance. Here we show that three non-DNA binding mutants, Z(R183E), Z(R179E) and Z(R187E), localized to large intranuclear rings, to discrete aggregates, and to the cytoplasm. Other non-DNA-binding mutants Z(N182K), Z(N182E), and Z(S186E), did not exhibit this appearance, so the failure to bind DNA alone is not responsible for the phenotype. The 3-dimensional shape of the ring-shaped structures was a sphere. ZEBRA was located on the periphery of these spheres. The interior of the spheres contained PML and HSP70 proteins, two invariant markers of nuclear aggresomes. The nuclear aggresomes recruited specific cellular (SC35) and viral (WT ZEBRA, Rta, BMLF1) proteins, but did not recruit other cellular (nucleolin) or viral (EA-D) proteins. Co-transfection of increasing concentrations of Z(R183E) and Z(R179E) with WT ZEBRA inhibited late lytic viral protein expression and lytic viral DNA amplification. Nuclear aggresomes induced by ZEBRA mutants with arginine to glutamate substitutions are similar in appearance and function to those observed in Huntington's and other neurodegenerative diseases.

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Virus Replication **Abstract P49**

SALUBRINAL REVERSES ER-STRESS INDUCED ACTIVATION OF EBV LYTIC GENES

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Abstract

EBV lytic genes are silent in latency III infection (in vitro and in vivo), as well as in latency I infection of memory B-cells. EBV lytic gene activation is required for production of progeny virions in oropharynx during acute EBV infection, as well as for reactivation from latency III in immunosuppressed patients. EBV lytic gene expression occurs in nasopharyngeal carcinoma, EBV positive gastric carcinoma, chronic active EBV infection, and oral hairy leukoplakia. A burst of lytic activity is also thought to occur during initial EBV infection as a viral pre-amplification step. Therefore control of the lytic gene program is likely critical for initial EBV infection, transmission and conversion to malignancy. Pharmacologic conversion of lymphomas such as PTLD from a latency III, to a lytic (including thymidine kinase) pattern of gene expression, may be a strategy for sensitizing these lymphomas to acyclovir. The host proteins and the physiologic cues governing latent to lytic switch are largely unknown. Plasma cells may be more permissive EBV lytic replication (Laichalk LL, Thorley-Lawson DA J. Virol. 2005 79 (2)). Plasma cells also rely, for successful differentiation from post-germinal B-lymphocytes, on the transcription factor XBP1. XBP1 and PKD have also been shown to cooperate in the transactivation of the lytic ZTA promoter. We therefore hypothesized that the ER stress and unfolded protein response pathways might be involved in EBV lytic gene activation. Treatment of latency III lymphoblastoid cell lines with the ER stressor, thapsigargin, resulted in the induction of EBV immediate early (BZLF1, BRLF1) early (BMRF1/EA-D) and late (gp350) genes. These genes were more robustly induced in B-cells lacking the latency III antigen, EBNA3C. Paradoxically, EBNA3C expression caused continued eIF2a S51phosphorylation, a condition which should result in translational arrest. Similarly, salubrinal, a chemical which causes eIF2a S51 phosphorylation, protects against ER-stress inducted apoptosis, and reduces HSV-1 lytic replication was able to reverse thapsigargin induced induction of EBV lytic genes. Thapsigargin treatment did not alter levels of ZEB1 or ZEB2 transcription factors (implicated in control of latency via ZTA repression), or alter levels of BCL6 or Pu.1 mRNAs, genes involved in plasma cell differentiation.

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Virus Replication **Abstract P50**

EBV TRANSCELLULAR TRANSCYTOSIS THROUGH POLARIZED ORAL EPITHELIAL CELLS

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Abstract

EBV is an orally transmitted virus; however, the mechanism of viral transmission through oropharyngeal epithelia is not well understood. In this study, we have investigated transcytosis of EBV in polarized human tongue and tonsil epithelial cells. Polarized cells were exposed to EBV B95-8 at their apical or basolateral surfaces, and after 4 h transepithelial migration of virions was detected by confocal microscopy and Q-PCR assays. To determine the roles of clathrin- and caveolin-mediated endocytosis and macropinocytosis in EBV transcytosis, polarized cells were preincubated with inhibitors of each pathway. We found that EBV transcytosis in oral epithelial cells may occur both from apical to basolateral membranes and from basolateral to apical membranes. However, the mechanism of apical to basolateral transcytosis was different from that of basolateral to apical viral transcytosis. Apical to basolateral transcytosis was mediated by macropinocytosis, whereas basolateral to apical transcytosis was initiated by caveolin/caveosomal endocytosis. Inhibition of protein kinases in polarized cells led to a reduction in both apical to basolateral and basolateral to apical transcytosis, suggesting that EBV transcytosis may be regulated by the activation of specific signaling pathways through the interaction of viral glycoproteins with cell-surface receptors. Altogether, these data suggest that EBV might transmigrate across the oropharyngeal mucosal epithelium by both apical to basolateral and basolateral to apical transcytosis – the former of which may contribute to initial penetration and systemic infection by the virus, and the latter of which may contribute to the secretion of viral progeny from the epithelium into saliva in EBV-infected individuals.

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Virus Replication **Abstract P51**

THE DEUBIQUITINATING ACTIVITY OF EBV BPLF1 ALTERS EARLY GENE EXPRESSION AND VIRAL INFECTIVITY OF EPSTEIN-BARR VIRUS

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Abstract

The N-terminal region of the large tegument protein BPLF1 contains both deubiquitinating and deneddylase activity which is conserved across the *Herpesviridae* family. Mutation of an active site cysteine (C61) abolishes the deubiquitinase activity. We have recently shown that enzymatically active BPLF1 targets the large subunit of the EBV ribonucleotide reductase and results in reduced RR activity. We are now investigating the effects of BPLF1 DUB activity on the lytic cycle by over-expression and with the use of an EBV DUB activity knockout virus under development. Over-expression of active DUB N-terminal region 1-246 during EBV lytic cycle induction of 293EBV+ cells resulted in reduced yield of infectious virus whereas over-expression of the inactive BPLF1 (C61S) fragment did not. Induction of 293EBV+ cells together with over-expression of BPLF1 1-246 also reduced EA-D expression. Further, mutant EBV genomes were constructed containing a C61S point mutation in the full-length BPLF1 sequence by recombination with an EBV WT bacmid. Stable 293 and 293T cell lines containing the full length C61S mutant as well as cell lines containing revertant viral genomes expressed similar levels of the early lytic cycle protein EA-D. Thus the recombined genomes are capable of lytic reactivation by induction with BZLF1. Now under further study is the effect of BPLF1 DUB activity on mutant virus yield and infectivity. Thus the late maturation protein BPLF1 reduces ribonucleotide reductase activity and over-expression of its N-terminal fragment reduced yield of infectious virus.

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Virus Replication **Abstract P52**

EPSTEIN-BARR VIRUS DNASE BGLF5 INDUCES GENOMIC INSTABILITY THROUGH DUAL PATHWAYS

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Abstract

EBV DNase (BGLF5) is an alkaline nuclease and has been suggested to be important in the viral life cycle. However, its effect on host cells remains unknown. Serological and histopathological studies implied that EBV DNase seems to be correlated with carcinogenesis. Therefore, we tried to investigate the effect of EBV DNase on epithelial cells. At first, we found that expression of EBV DNase induces increased formation of micronucleus (MN), an indicator of genomic instability, in human epithelial cells. We demonstrated, using immunofluorescence and flow cytometry, that EBV DNase induces γH2AX formation in a dose-dependent manner. We also demonstrated that EBV DNase could cause DNA damage detected by comet assay. However, the expression of a DNase-null plasmid did not show similar effects. Furthermore, using a host cell reactivation assay, we found that EBV DNase expression repressed repair in various epithelial cells. Taken together, we propose that EBV DNase induces genomic instability in epithelial cells, which may be through induction of DNA damage and cooperate with repression of DNA repair, and may contribute consequently to the carcinogenesis of human epithelial ce

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Posters P53-P73

Genetics, Epigenetics, Non-coding RNA

Genetics, Epigenetics, Non-coding RNA **Abstract P53**

QUANTITATIVE STUDIES OF EPSTEIN-BARR VIRUS-ENCODED MIRNAS PROVIDE NOVEL INSIGHTS INTO THEIR REGULATION

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Abstract

MicroRNAs (miRNAs) are an important class of molecules that regulate the expression of target genes post-transcriptionally. EBV encodes at least 40 miRNAs located in the BHRF1 and BamHIA regions of the virus genome, but little is known about the expression of these miRNAs in different forms of EBV infection or their possible roles in the pathogenesis of EBV-associated tumours. Using newly-developed real-time PCR assays, we quantified the levels of mirBHRF1 and mirBART miRNAs in (a) B cell lines with well-defined Latency I, Wp-restricted and Latency III patterns of viral latent gene expression, (b) Akata cells induced into lytic cycle and (c) primary B cells infected with EBV in vitro. BHRF1 and BART mRNAs were measured in parallel to examine any correlation between the abundance of transcripts containing the pre-miRNA sequences and mature miRNA species. As previously reported, the full complement of mirBHRF1 miRNAs was only expressed in Latency III, while mirBART miRNAs were detectable at variable levels in all forms of latency. Furthermore the pattern of mature miRNA expression correlated with expression of the corresponding mRNA transcripts in different forms of latency. Strikingly, however, levels of mirBART miRNAs were relatively unchanged during lytic cycle despite dramatic increases in BART transcription. Likewise there was a lack of correlation between induction of mRNA transcripts and the corresponding mirBHRF1 and mirBART miRNAs early after infection of primary B cells. In summary, our data provide novel insights into the regulation of EBV miRNA biogenesis which may have important implications for EBV's role in tumourigenesis.

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Genetics, Epigenetics, Non-coding RNA **Abstract P54**

CPG-METHYLATION REGULATES A CLASS OF EPSTEIN-BARR VIRUS' PROMOTERS

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Abstract

DNA methylation is the major modification of eukaryotic genomes and plays an essential role in mammalian gene regulation. In general, cytosine-phosphatidyl-guanosine (CpG)-methylated promoters are transcriptionally repressed and nuclear proteins such as MECP2, MBD1, MBD2, and MBD4 bind CpG-methylated DNA and contribute to epigenetic silencing. Methylation of viral DNA also regulates gene expression of Epstein-Barr virus (EBV), which is a model of herpes virus latency. In latently infected human B cells, the viral DNA is CpG-methylated, the majority of viral genes is repressed and virus synthesis is therefore abrogated. EBV's *BZLF1* encodes a transcription factor of the AP-1 family and is the master gene to overcome viral gene repression. In a genome-wide screen, we now identify and characterize those viral genes, which Zta regulates. Among them are genes essential for EBV's lytic phase, which paradoxically depend on strictly CpG-methylated promoters for their Zta-induced expression. We identified novel DNA recognition motifs, termed meZRE (methyl-Zta-responsive element), which Zta selectively binds in order to 'read' DNA in a methylation-and sequence-dependent manner unlike any other known protein. Zta is a homodimer but its binding characteristics to meZREs suggest a sequential, non-palindromic and bipartite DNA recognition element, which confers superior DNA binding compared to CpG-free ZREs. Our findings indicate that Zta has evolved to transactivate cytosine-methylated, hence repressed, silent promoters as a rule, which is a novel means to overcome epigenetic silencing.

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Genetics, Epigenetics, Non-coding RNA **Abstract P55**

EBER1 INTERACTION WITH RIBOSOMAL PROTEIN L22 AND POLYSOME EXPRESSION PROFILING

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Abstract

The interaction of EBER1 with ribosomal protein L22, a component of the large ribosomal subunit was confirmed. RpL22 is not essential for translation, suggesting a regulatory role in translation or an extra-ribosomal function for the protein. Interaction of EBER1 with rpL22 results in delocalisation of rpL22 from nucleoli and the cytoplasm into the nucleoplasm in the presence of EBV or EBER1 in HEK 293, AGS and HoneI cells. As a consequence, EBV infection leads to a partial depletion of rpL22 from ribosomes in 293 cells, possibly allowing EBER1 to affect host cell translation.

LCLs transformed with recombinant virus from EBV BACs containing wt, ΔEBER1 and EBER1-revertant EBV were used to identify effects of EBER1 RNA on host cell gene regulation. To analyse EBER1 mediated regulation of host cell translation, LCLs representing each of the wt, mutant and revertant EBVs were fractionated on sucrose gradients and RNA associated with polyribosomes used in microarray profiling. Bioinformatic analysis of the data identified several genes whose association with polyribosomes is significantly altered with EBER1 expression and rescued in the EBER1-revertant background. In combination with host cell gene expression profiles of cytoplasmic RNA isolated from LCLs transformed with wt, ΔEBER1 and EBER EBER-revertant EBV, these data will allow us to further understand the role of EBER1 in EBV infection.

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Genetics, Epigenetics, Non-coding RNA **Abstract P56**

CHARACTERIZATION OF EPSTEIN-BARR VIRUS (EBV) GENETIC VARIANTS IN HEALTHY CONTROLS FROM TAIWAN AND UGANDA

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Abstract

Background: The full extent of Epstein-Barr virus (EBV) genetic diversity may be greater than previously recognized, in part due to the lack of systematic characterization across multiple gene regions. In this pilot study, we sequenced and characterized EBV in 20 normal samples using high-throughput technology. **Methods:** 454 pyrosequencing was used to sequence roughly 10% of the EBV genome (corresponding to B95-8 genome coordinates 99387-110481, 167259-171730 which include LMP-1, EBNA-1, and BZLF-1 open reading frames (ORFs)) from 20 saliva samples from healthy Taiwanese and Ugandans and 1 B95-8 control sample. Phylogenetic trees based on consensus EBV sequences were constructed. Variable regions were identified and mapped to the reference strain B95-8. Correlations among the variable positions were determined using the Kappa statistic. **Results:** Phylogenetic analyses showed that the tree structure based on single gene regions differed from those based on the entire sequenced regions. There were 601 variable positions out of 15,256 total positions (3.9%). The frequency of variable positions in the LMP-1 coding region (9.1%) was higher than that of EBNA1 (2.6%) and BZLF1 (3.5%). Ninety-two percent (550) of the 601 variable positions were correlated with at least one other position based on a Kappa statistic > 0.8. **Conclusions:** These results demonstrate that while linkage fixation is observed for EBV, a more complete characterization of EBV variability can be obtained by sequencing extended regions than single ORFs. These results will guide efforts to apply high-throughput multiplex genotyping assays for studies assessing the association of EBV genotypes and risk of EBV-associated tumors.

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Genetics, Epigenetics, Non-coding RNA **Abstract P57**

POTENTIAL HOST TARGETS OF EPIGENETIC REPROGRAMMING BY EPSTEIN BARR VIRUS

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Abstract

Methylation of promoters at CpG motifs is an important epigenetic silencing mechanism. Zta (ZEBRA, BZLF1), the product of the immediate early gene *BZLF1*, is required to activate viral transcription and lytic replication through sequence specific Zta Response Elements (ZREs). Zta shows enhanced binding to methylated CpG-containing ZREs in both the viral (Rp, Nap) and host (*Egr1*) genome environments. This suggests EBV has the ability to overturn epigenetic silencing in cellular genes.

We analysed the entire human genome to identify other host genes that may be regulated in a similar manner. The promoter region of each gene (defined as -500 to +1) was subjected to a pattern match search to identify those that contained the core sequence of a methylation dependent ZRE (RpZRE3). This identified 274 genes, 17 of which were tested by EMSA and interacted with Zta when they were methylated, supporting the theory. The methylation status of these genes *in vivo* was questioned using methylome data from 3 different cell types.

One of the 17 sites, in the promoter region of *XPC*, was shown to bind in the unmethylated state. Upon further investigation, a novel ZRE was discovered in the flanking region. The sequence matches that of a characterized AP1 site in the promoter of the host gene *ENK*.

The presence of ZREs in host genes identifies host targets for EBV and suggests that a cellular transcription factor may interact with methylated CpG ZREs.

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Genetics, Epigenetics, Non-coding RNA **Abstract P58**

NEW PREDICTION TOOL TO IDENTIFY METHYLATION-DEPENDENT ZTA RESPONSE ELEMENTS (ZRES)

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Abstract

Zta (ZEBRA, BZLF1), the product of the immediate early gene *BZLF1*, is required to activate viral transcription and lytic replication through sequence specific ZREs. These sites have been identified in both viral and human gene promoters. A sub-set of these sites contain a CpG motif. The EBV genome undergoes a biphasic cycle of methylation; it is unmethylated within the virion, immediately after infection of B-lymphocytes and following viral replication, but becomes increasingly methylated during viral latency. Zta interacts with these sites in a methylation-dependent manner, which allows Zta to overturn the epigenetic silencing of the latent viral genome and prevents full lytic cycle during immortalization.

It is difficult to predict the location of methylation sensitive and insensitive ZREs, as substantial sequence diversity occurs between characterized sites. To address this, we developed a highly accurate prediction tool by generating a Position Weight Matrix (PWM) to identify CpG-containing ZREs. This tool, in combination with a pattern match search, was used to generate a global map of potential ZREs within the EBV genome. This revealed 516 potential binding sites for Zta, including 293 methylation-dependent sites. The analysis identified novel epigenetic reversal sites; viral promoters containing appropriate ZREs to allow Zta binding and associated gene expression, despite methylation status. In addition, 11 promoters that contain *only* methylation-dependent ZREs were found; all are methylated in latency and upregulated during lytic cycle. The inability of Zta to interact with these promoters when unmethylated may act as a safeguard to prevent full viral replication immediately following infection.

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Genetics, Epigenetics, Non-coding RNA **Abstract P59**

EBNA3 COOPERATION IN WIDESPREAD POLYCOMB-MEDIATED REPRESSION

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Abstract

Our gene expression microarray analysis has started to reveal the extent and complexity of EBNA3-mediated gene control: the regulation of more than 1000 genes by EBV requires EBNA3 expression and a considerable number of these genes appear to be regulated by combinations of the EBNA3 proteins. For instance, the regulation of two genes (*BIM* and $p16^{INK4A}$) is mediated by the cooperation of EBNA3A and EBNA3C through the establishment of the polycomb-associated repressive chromatin marker histone H3 lysine 27 trimethylation (H3K27me3). Therefore, we have asked whether any further genes highlighted by the microarrays are regulated by polycomb-mediated methylation of H3K27. We have found that many genes are indeed regulated in this fashion and that there is considerable cooperation between all three EBNA3 proteins. For example, the microarray analysis indicated that repression of *RASGRP1* expression requires EBNA3A, EBNA3B and EBNA3C. This was confirmed by qRT-PCR and ChIP analyses. Repression of this and other examples involves the establishment of H3K27me3 on cellular promoters, generally with a concomitant loss of histone H3 lysine 9 acetylation (H3K9ac) but little change to histone H3 lysine 4 trimethylation (H3K4me3), both markers of transcriptional activity. This is consistent with the chromatin structure of bivalent promoters commonly found in embryonic stem (ES) cells. We have now undertaken global H3K27me3 analysis for polycomb-regulated cellular genes during infection of Burkitt's lymphoma cells with wild type and independent EBNA3 virus mutants, utilising chromatin immunoprecipitation linked to promoter array analysis (ChIP-chip), the results of which will be presented.

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Genetics, Epigenetics, Non-coding RNA **Abstract P60**

EXPLORING THE ROLE OF EPSTEIN BARR-VIRUS ENCODED RNA1 (EBER-1) IN INNATE AND ADAPTIVE IMMUNE RESPONSE USING TRANSGENIC MOUSE MODELS

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Abstract

EBER-1 is a non poly adenylated, non coding, double stranded small RNA encoded by the EBV genome. It is abundantly transcribed in many EBV associated tumors. It is known that the EBERs act as pathogen associated molecular pattern (PAMP) molecules and elicit innate immune responses (particularly the interferon (IFN) response) in the host by associating with the pathogen recognition receptors (PRR) such as RIG-1. However, whether this impacts the adaptive immune response has been largely unaddressed. In the present studies the action of EBER-1 has been explored in an *in vivo* system. Briefly, transgenic mouse lines were generated expressing EBER-1 in lymphoid cells. Cohorts of transgenic mice and controls were injected with polyI:C (as an IFN inducing adjuvant) and NIP-BSA as antigen by intravenous or intraperitoneal routes. Specific Immunoglobulin responses to antigen were then measured by ELISA. In order to explore the IFN response, serum IFN levels were measured in the polyI:C injected and control cohorts of mice by ELISA. Additionally, expression of proteins involved in the interferon response like PKR, eIF2 alpha, OAS are being examined by western blotting from the tissues collected at different time intervals. We find that EBER1 expressing mice respond to polyI:C treatment with enhanced level of IFN production. Despite this, our preliminary observations suggest that EBER-1 mice might be impaired in their adaptive immune response and these data will be presented.

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Genetics, Epigenetics, Non-coding RNA **Abstract P61**

COMPARATIVE ANALYSIS OF EPSTEIN-BARR VIRUS (EBV) LATENT GENE AND MICRORNA SEQUENCES AMONG AKATA, B95.8, AG876 AND GD1 EBV GENOMES

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Abstract

Akata cell line is an Epstein-Barr virus (EBV)-producing line established from a Japanese patient with EBV-positive Burkitt lymphoma. It is extensively used in EBV-related studies in the published literature. Yet the existing DNA sequences of the Akata EBV genome in NCBI Entrez and other public genome databases are very scanty and do not include the polymorphic latent gene regions. In this study, we PCR-amplified and sequenced all latent genes and microRNA regions of Akata EBV genome. The Akata EBV DNA sequences were then compared with those of corresponding EBV genetic loci in prototype cell lines, B95.8 (type 1 EBV) and AG876 (type 2 EBV), and in GD1 (type 1 EBV DNA sequence of a Chinese patient with nasopharyngeal carcinoma). Type-specific sequence divergence was observed at the EBNA2, EBNA3A, -3B and -3C loci of Akata EBV with >95% homology to those of B95.8 and GD1 but only 86% homology to that of AG876. Thirty-bp deletion and four 11-amino acid repeat sequences were present in the C-terminus of LMP1 gene of Akata EBV. LMP1, -2A and -2B loci, which do not correlate with type 1 or type 2 status, showed significant genetic diversity between Akata EBV and the prototype sequences. MicroRNA regions, miR-BART cluster 2 and miR-BHRF1, have >99% homology among Akata, AG876 and GD1 genomes. The single nucleotide change (A>T, 146466) at the hinge region of miR-BART7 did not affect the microRNA folding (as predicted by MFold software). The data supported the idea that EBV microRNAs are highly conserved throughout evolution. Genetic variations of LMP1, LMP2A, LMP2B, EBNA1, EBER and miR-BART cluster 1 among Akata and the prototype EBV genomes will be further analyzed for potential functional polymorphisms.

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Genetics, Epigenetics, Non-coding RNA **Abstract P62**

MIR34 PLAYS AN UNEXPECTED ROLE IN EBV-INDUCED B CELL GROWTH TRANSFORMATION

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Abstract

microRNAs are small non coding RNAs that post-transcriptionally regulate gene expression to control a variety of processes including development, cell cycle, and immunity. Their role in EBV transformation and lymphomas is currently not well understood. Using an Exiqon v10.0 miRNA microarray, we identified a number of cellular miRNAs that were over- or under-expressed comparing resting CD19+ B cells to EBV-infected, proliferating B cells and immortalized LCLs. Most of the described oncogenic miRNAs including miR-155, miR-21, and miR-146 were induced by EBV and most tumor suppressive miRNAs such as miR-29, miR-16, and let-7 family members were repressed. However, we focused on miR-34a, whose expression was strongly induced by EBV despite its well characterized role as a p53-responsive tumor suppressor. We found that p53 was not essential for miR-34a expression in LCLs, but rather LMP1 mediated NFκB activation was sufficient for miR-34a induction. In order to understand the functional role of this miRNA in EBV transformation we constructed a miRNA sponge targeting the miR-34 seed family. miR-34 family suppression in LCLs surprisingly compromised cell proliferation and homotypic aggregation. The miR-34 targets responsible for this phenotype are currently under investigation and will be discussed. In summary, the global analysis of cellular miRNA expression during the process of EBV mediated B cell transformation identified the unique and unexpected role of a potentially tumor suppressive miRNA, miR34a, in B cell growth and aggregation.

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Genetics, Epigenetics, Non-coding RNA **Abstract P63**

MICRORNA PROFILING OF EBV ASSOCIATED DIFFUSE LARGE B-CELL LYMPHOMA AND NK/T-CELL LYMPHOMA

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Abstract

The Epstein-Barr Virus can be found in ~15 % of diffuse large B-cell lymphoma (DLBCL) and in nearly all NK/T-cell lymphoma. EBV encodes for 25 viral miRNAs but also changes the cellular miRNA profile. MiRNAs are small non-coding RNAs of about 19-24 nt length which act as posttranscriptional gene regulators. The miRNA profiles of DLBCLs and NK/T-cell lymphoma were generated by deep sequencing and selected miRNAs were validated by qRT-PCR in the tumour samples and by Northern blotting in tumour cell lines. All known EBV miRNAs with the exception of those from the BHRF1 cluster were present in both tumour entities. The viral miRNAs represented only about 2 % of all miRNAs expressed in both tumour types and the question as to their impact on the tumour development arises.
Comparing EBV-positive with EBV-negative DLBCLs, 9 miRNAs were up-regulated and 7 were down-regulated with a more than 1.5 fold regulation and an expression of at least 0.05 %. The NK/T-cell lymphoma showed 15 induced and 16 repressed miRNAs in the EBV-positive tumours when miRNAs showed a more than 2-fold regulation and an expression of at least 0.1 % in both of the libraries. To confirm predicted miRNA-target interactions, luciferase reporter assays and western blot analysis were used. Selected miRNAs could be shown to have an effect on their target *wt* 3'UTRs in luciferase assays but not after the mutation of the corresponding seed sequence. Endogenous target protein levels were also downregulated by overexpressing the corresponding miRNA in cell lines.

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Genetics, Epigenetics, Non-coding RNA **Abstract P64**

EPSTEIN-BARR VIRUS MICRORNAS ARE DIFFERENTIALLY EXPRESSED DURING EBV INFECTION AND EBV-DRIVEN DIFFERENTIATION OF NAÏVE B-CELLS

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Abstract

Primary EBV-infection is characterized by in an atypical Germinal Centre (GC) reaction, in which B-cells differentiate through a well-characterized latency gene expression program. The expression kinetics of B-cell cellular genes and viral EBV-encoded miRNAs during primary infection remain to be established.

Following an Initiation phase of 3 days post-infection, up-regulation of host genes known to be critical in the GC-reaction occurred in two distinct phases, an Early-GC-like phase at 4-7 days and a Late-GC-like phase at 2-3 weeks. This was followed by a switch to Post-GC-like gene expression between 4-6 weeks. Significant somatic hypermutation was only observed at six weeks.

miRNAs function dose-dependently with two-fold differences in their levels having significant biological consequences. Also, they may potentially act together in a concerted fashion. Examination of the expression of 31 EBV-miRNAs over this time course revealed four groups of EBV-miRNAs each exhibiting coordinated expression, which changed in a manner coincidental with the phases observed by gene expression. Although a number of EBV-miRNAs were expressed at low levels early post-infection, the majority exhibited a spike at the beginning of the Late-GC-like phase. This was followed by a general up-regulation during the post-GC-like phase. These phases in EBV-miRNA expression were also coincidental with differential BART and BHRF1 promoter usage and alternate splicing.

This is the first comprehensive profiling of EBV-miRNAs during the infection process. We demonstrate that differential expression of EBV-miRNAs in a temporal manner is correlated with phenotypic changes during infection, implicating these regulatory molecules in the EBV-driven differentiation process.

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Genetics, Epigenetics, Non-coding RNA **Abstract P65**

CLONING OF THE EPSTEIN-BARR VIRUS-RELATED RHESUS LYMPHOCRYPTOVIRUS (RHLCV) AS A BACTERIAL ARTIFICIAL CHROMOSOME (BAC): A LOSS OF FUNCTION MUTATION FOR THE RHBARF1 IMMUNE EVASION GENE

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Abstract

The first molecular clone for the EBV-related lymphocryptovirus naturally infecting rhesus macaques (clone16 rhLCV) carries a truncated rhBARF1, a putative immune evasion gene because EBV BARF1 can block CSF-1 cytokine signaling. Previous results showed that clone16 rhLCV was defective for both acute and persistent infection in experimentally infected macaques. In order to determine whether the phenotype in vivo was due to the rhBARF1 mutation, we repaired the rhBARF1 ORF to create a wild-type (WT) rhLCV BAC and tested whether the WT molecular clone was comparable in vitro to the naturally derived LCL8664 rhLCV.

Quantitative assays showed that the WT rhLCV had B cell immortalizing and virus replication properties comparable to LCL8664 rhLCV, whereas the clone16 rhLCV replicated slightly better, but immortalized slightly less efficiently than the naturally derived rhLCV. rhBARF1 could inhibit CSF-1 signaling comparable to EBV BARF1, but the truncated rhBARF1 from clone16 rhLCV was defective for both cell secretion and CSF-1 blocking ability.

These in vitro experiments show that WT rhLCV derived from the repaired clone16 rhLCV BAC is comparable to naturally derived rhLCV and that the truncated rhBARF1 in clone16 rhLCV is unable to block CSF-1 signaling. Thus, the WT rhLCV BAC provides a molecular prototype for rhLCV and an important control for experimental rhesus macaque infections with the clone16 rhLCV.

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Genetics, Epigenetics, Non-coding RNA **Abstract P66**

EBNA-LP ENHANCES EBNA-2 TRANSCRIPTIONAL EFFECTS ON CELLULAR PROMOTERS BY REDUCING NCOR AND RBP-JK/CSL PROMOTER OCCUPANCY

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Abstract

EBNA-LP and EBNA-2 are critical for EBV-dependent immortalization of B cells. EBNA-2 was shown to activate transcription from both viral and cellular promoters and EBNA-LP enhances EBNA-2 on viral promoters. Very little is known about EBNA-LP's ability to enhance EBNA-2 effects on cellular promoters. Here we present data showing that EBNA-LP can in fact enhance EBNA-2 effects on some cellular promoters. Moreover, we obtained evidence suggesting that EBNA-LP effects are at least in part due to a decrease in NCoR repressor occupancy at promoters, measured by Chromatin immunoprecipitation (ChIP) followed by quantitative PCR (qPCR). Interestingly, while RBP-jK/CSL promoter occupancy was increased in EBNA-2 expressing cells, measured by ChIP-qPCR, it was significantly decreased in EBNA-LP expressing cells and in EBNA-2 and EBNA-LP-expressing cells. Our results suggest a mechanism consistent with the dismissal of the RBP-jK/CSL-NCoR repressive complex from promoters, indicating a much more dynamic role for RBP-jK/CSL in transcription regulation than previously anticipated.

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Genetics, Epigenetics, Non-coding RNA **Abstract P67**

EPIGENETIC ALTERATIONS AS A MARK FOR PAST EPSTEIN-BARR VIRUS INFECTION

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Abstract

Although EBV is a known tumor virus, the association of the virus with certain malignancies is often erratic. To study the incomplete association of EBV in tumorigenesis, we established a transient model of infection to determine if any cellular alterations remained upon viral loss. Carcinoma cell lines were infected with recombinant EBV, selected with G418 and selection pressure removed to allow loss of virus. Analysis of global gene expression comparing EBV-negative, transiently-infected clones to uninfected controls identified multiple genes downregulated known to be methylated in cancer. Among these genes was E-cadherin, a tumor suppressor shown to be DNA methylated upon LMP1 expression and hypermethylated in EBV-positive gastric and nasopharyngeal carcinomas. Treatment with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine or the histone deacetylase inhibitor trichostatin A partially restored E-cadherin transcript levels to that of uninfected controls implicating DNA methylation and/or repressive chromatin in the suppression. Transient EBV infection was shown not to alter DNA methylation of the E-cadherin promoter. However, chromatin immunoprecipitation analysis implicated formation of repressive chromatin in suppressing E-cadherin expression. Increases in abundance of H3K9 di- and tri-methylation at the 5' end of the E-cadherin gene along with decreases in H3, H4, and H3K9 acetylation were observed in our transiently-infected clone. SuV39H1, a histone-lysine methyltransferase, showed increased expression levels in our EBV-infected cells identifying a potential effector for formation of repressive chromatin. These results suggest that EBV can stably modify gene expression in a heritable fashion in formerly infected cells, while its own contribution to the oncogenic process is masked.

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Genetics, Epigenetics, Non-coding RNA **Abstract P68**

THE B-CELL SPECIFIC PROTEIN, OCT-2, PREVENTS LYTIC EBV REACTIVATION BY INHIBITING THE TRANSCRIPTIONAL FUNCTION OF THE VIRAL IMMEDIATE-EARLY PROTEIN, BZLF1

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Abstract

EBV is normally latent in B-cells, but lytic in epithelial cells. However, cellular factors which promote viral latency specifically in B-cells have not been identified. The EBV latent to lytic switch is mediated by the BZLF1 (Z) and BRLF1 (R) viral immediate-early proteins; Z preferentially activates the methylated form of some viral promoters. Here we demonstrate that the B-cell specific transcription factor, Oct-2, negatively regulates EBV reactivation by inhibiting Z activation of methylated viral promoters. We show that Oct-2 (but not the closely related protein, Oct-1) inhibits Z-mediated lytic viral reactivation in latently infected EBV-positive epithelial cells. In addition, loss of endogenous Oct-2 from EBV-positive B-cells results in an increase in constitutive EBV lytic protein expression. We find Oct-2 inhibits Z-mediated, but not R-mediated, activation of early lytic viral promoters. Interestingly, the inhibitory effect of Oct-2 on Z transactivation is much more striking on methylated promoters versus unmethylated promoters. Oct-2 and Z proteins directly interact *in vivo*, and preliminary data suggest that Oct-2 inhibits Z binding to certain promoters. These results suggest that the propensity of EBV to enter the latent form of infection in B cells may be at least partially mediated through the B-cell specific transcription factor, Oct-2. Furthermore, since differentiation of B cells into plasma cells results in decreased Oct-2 expression, the tendency of EBV to reactivate during plasma cell differentiation may reflect not only the acquisition of positively regulating transcription factors such as XBP-1, but also the loss of Oct-2.

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Genetics, Epigenetics, Non-coding RNA **Abstract P69**

THE IDENTIFICATION OF APOPTOTIC AND PROLIFERATIVE TARGETS OF EBV-ENCODED MICRORNAS

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Abstract

The small regulatory RNA molecules, microRNAs (miRNAs), play major roles in developmental processes and their aberrant expression is indicative of diseases such as cancer. Epstein Barr virus (EBV) encodes more than 40 miRNAs within its genome. These are differentially expressed between different EBV-associated malignancies, suggesting a role in the development of such diseases. To date, there is limited knowledge regarding the functions of EBV-miRNAs but the pro-apoptotic and immunoregulatory targets currently identified suggest that EBV-miRNAs may play a role in pathogenesis.

Apoptosis and proliferation are crucial mechanisms during B-cell differentiation and dysregulation of these processes is strongly associated with tumourigenesis. EBV has been demonstrated to manipulate these processes via several mechanisms during the establishment of latent infection. We reasoned that EBV-miRNAs may be used by the virus to modulate apoptosis and proliferation.

Using bioinformatic approaches we identified a range of potential EBV-miRNA targets, and from this list selected candidates involved in apoptotic and proliferative processes. Using luciferase assays we have demonstrated the ability of EBV-miRNAs to silence the expression of two genes involved in cellular growth and survival. Both genes play important roles in B cell development: the first is a transcription factor that regulates B-cell proliferation and differentiation while the second plays a major role in BCR-mediated apoptosis. Current studies are focused on assessing the functional impact on the cell of silencing of these genes in models of EBV infection and transformation of B-cells.

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Genetics, Epigenetics, Non-coding RNA **Abstract P70**

EBV-SPECIFIC METHYLATION OF HUMAN GENES IN GASTRIC CANCER CELLS

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Abstract

EBV is found in 10% of all gastric adenocarcinomas but its role in tumor development and maintenance remains unclear. To study EBV-mediated dysregulation of cellular factors involved in carcinogenesis, gene expression patterns were examined in EBV-negative and EBV-positive AGS gastric epithelial cells using a low density microarray, reverse transcription PCR, and methylation-specific DNA sequencing. In array studies, nearly half of the 96 human genes tested were dysregulated by EBV, representing 15 different cancer-related signal transduction pathways. Reverse transcription PCR confirmed significant impact on factors having diverse functions such as cell cycle regulation (*IGFBP3*, *CDKN2A, CCND1, HSP70, ID2, ID4),* DNA repair *(BRCA1, TFF1*), cell adhesion (*ICAM1*), inflammation (*COX*2), and angiogenesis (*HIF1A).* Demethylation using by 5-aza-2'-deoxycytidine reversed the EBV-mediated dysregulation for all 11 genes listed here. For some promoter sequences, CpG island methylation and demethylation occurred in an EBV-specific pattern as shown by bisulfite DNA sequencing. Our results demonstrate EBV's ability to alter human gene expression in ways that could impact the unique pathobiology of EBV-associated cancers. Furthermore, the frequency and reversability of methylation-related transcriptional alterations suggest that demethylating agents have therapeutic potential for managing EBV-related cancers.

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Genetics, Epigenetics, Non-coding RNA **Abstract P71**

EPIGENETIC REPRESSION OF *P16^{INK4A}* BY LATENT EPSTEIN-BARR VIRUS REQUIRES THE INTERACTION OF EBNA3A AND EBNA3C WITH CTBP

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Abstract

Cyclin-dependent kinase inhibitor p16^{INK4A} is an important tumour suppressor and inducer of cellular senescence often inactivated during the development of cancer by promoter DNA methylation. We investigated the mechanism by which EBV latency-associated nuclear antigens EBNA3C and EBNA3A repress p16^{INK4A} expression.

Using lymphoblastoid cell lines (LCLs) expressing a conditional EBNA3C, we demonstrate that EBNA3C inactivation resets the epigenetic status of *p16^{INK4A}* to permit transcriptional activation: the polycomb-associated repressive H3K27me3 histone modification is substantially reduced, while the activation-related mark H3K4me3 is modestly increased. Activation of EBNA3C reverses the distribution of these epigenetic marks, represses *p16^{INK4A}* transcription and allows proliferation. LCLs lacking EBNA3A express relatively high levels of p16^{INK4A} and have a similar pattern of histone modifications on *p16^{INK4A}* as produced by the inactivation of EBNA3C. Since binding to the co-repressor of transcription CtBP was linked to the oncogenic activity of EBNA3A and EBNA3C, we established LCLs with viruses encoding EBNA3A- and/or EBNA3C-mutants that no longer bind CtBP. These novel LCLs revealed that the epigenetic repression of *p16^{INK4A}* requires the interaction of both EBNA3A and EBNA3C with CtBP.

Epigenetic repression of *p16^{INK4A}* by latent EBV will not only overcome senescence in infected B cells, but may also facilitate *p16^{INK4A}* DNA methylation during lymphomagenesis.

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Genetics, Epigenetics, Non-coding RNA **Abstract P72**

DIFFERENTIAL REGULATION OF MIR-21 AND MIR-146A BY EPSTEIN-BARR VIRUS ENCODED EBNA2

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Abstract

The discovery of microRNA (miR) represents a novel paradigm in RNA based regulation of gene expression and their dysregulation has become a hallmark of many a tumor. In virally associated cancers, the host-pathogen interaction could involve alteration in miR expression. Epstein-Barr virus (EBV) encoded EBNA2 is indispensable for the capacity of the virus to transform B cells in vitro. Here, we studied how EBNA2 affects cellular miRs. Extensive miR profiling of the virus infected and EBNA2 transfected B lymphoma cells revealed that oncomiR miR-21 is positively regulated by this viral protein. Conversely, Burkitt lymphoma (BL) cell lines infected with P3HR1 strain lacking EBNA2 did not show any increase in miR-21. The primary miR-21 transcripts were unaltered by EBNA2 and this suggests that the increase in mature miR-21 is due to higher post-transcriptional processing. Intererstingly, miR-146a, whose expression is known to be induced by LMP1 was severely downregulated by EBNA2. Consequently, EBNA2 expressing cells expressed higher type I interferon. Taken together, the present data suggest that EBNA2 might contribute to EBV induced B cell transformation by altering miR expression and in particular by increasing oncomiR like miR-21 and by affecting the antiviral responses of the innate immune system through downregulation of its key regulator miR-146a.

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Genetics, Epigenetics, Non-coding RNA **Abstract P73**

Abstract P73 was retracted

Posters P74-P87

Signal transduction

Signal transduction **Abstract P74**

DIFFERENTIAL WEIGHT AND FUNCTION OF RELA AND RELB NF-KB COMPLEXES IN BOTH EBV-TRANSFORMED B-CELLS AND ACTIVATED B-CELL-LIKE DIFFUSE LARGE B-CELL LYMPHOMAS

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Abstract

The nuclear factor KB (NF-KB) is constitutively activated in various B-cell lymphomas, including Epstein-Barr Virus (EBV) associated lymphoproliferative disorders with expression of the latent membrane protein 1 (LMP1), the main EBV oncogene, and activated B-cell-like diffuse large B-cell lymphomas (ABC-DLBCL). Two NF-KB activation pathways have been described, so called classical and alternative, leading to nuclear translocation of dimers containing RelA or c-Rel and RelB. We first studied the composition of NF-KB DNA binding complexes induced by LMP1 alone or in the context of EBV-latency III immortalized B-cells. We then studied the functional effects of RelA, RelB and p100. We further analysed RelA and RelB DNA binding activity in nuclei isolated from biopsies of patients with ABC-DLBCLs. LMP1 induced only NF-KB complexes of the classical pathway. RelA and RelB were in competition for p50. Despite activation of the alternative pathway, p100 was not at the origin of RelB/p52 dimers but mainly exerted a repressive activity against RelB/p50 dimers. P50 rather than p52 was preferentially associated with RelB. RelA but not RelB dimers were associated with both survival and proliferation of EBV-immortalized B-cells. A RelA DNA binding activity was found in 100% of ABC-DLBCLs. Both RelB and RelA complexes were found in 22% of these cases. RelB was never found alone. We concluded that activation of RelA containing NF-KB complexes is the main oncogenic pathway of LMP1 in EBV-transformed B-cells and is very likely to be the main oncogenic target in ABC-DLBCLs.

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Signal transduction **Abstract P75**

EBNA3C INHIBIT CHECKPOINT KINASES AND P73 COLLABORATIVE PATHWAY MEDIATED APOPTOSIS IN P53 MUTATED EBV POSITIVE CELLS

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Abstract

The establishment of latent infection by EBV requires the expression of a unique repertoire of genes. The product of one of these viral genes, the EBV nuclear antigen 3C (EBNA3C), is essential for the growth transformation of primary B lymphocytes in vitro and can regulate the transcription of a number of viral and cellular genes along with inhibit the host induced p53 dependent apoptosis. But many of the EBV infected cells were either inactive mutant p53 or have no p53. Therefore , we investigated the probable role of p73, a p53 analogue in EBV positive cells. To our surprise, the a signalling revealed the role of EBNA3C inhibiting the p73-dependent transactivation of pro-apoptotic protein activation also . We next aimed at examining the upstream molecules regulating this EBNA3C mediated p73 inhibition. The relation of EBNA3C in checkpoint kinases mainly Chk2 to bypass the of cell cycle checkpoints was already established. We demonstrate here that EBNA3C actually controls the inhibition of p73, through checkpoints kinases. However, in presence of wild type p53, EBNA3C plays no significant role on p73. Thus, checkpoint kinases and p73 collaborative pathway counteracted by EBNA3C to make the p53 null or mutated lymphoma cells less prone to apoptosis

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Signal transduction **Abstract P76**

THE AT-HOOK MOTIF IS A NOVEL PORTABLE STABILIZATION SIGNAL THAT INHIBITS PROTEASOMAL DEGRADATION BY MEDIATING STRONG BINDING TO DNA

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Abstract

The rate of turnover of proteasomal substrates is regulated by the combined effect of degradation and stabilization signals delivered by portable domains that can transfer the phenotype to a new host protein. The Gly-Ala repeat (GAr) of the Epstein-Barr virus (EBV) nuclear natigen-1 is the first recognized examples of stabilization signal that inhibits proteasomal processing. However, EBNA-1 lacking the GAr is still long-lived, suggesting that protein turnover is further regulated. We found that, in the absence of the GAr, EBNA1 is stable in the nucleus but is rapidly degraded in the cytoplasm upon deletion of the nuclear localization signal (NLS). EBNA1 binds to cellular DNA via two Gly-Arg repeat (GRr) domains that resemble the AT-hook motif of non-histone chromatin proteins. Deletion of both the GAr and GRr was required for proteasomal-dependent degradation of nuclear EBNA1. Using as model a short-lived proteasomal substrate we show that the EBNA1 GRr and the AT-hook motifs of High Mobility Group A (HMGA) proteins serve as transferable cis-acting inhibitors of proteasomal processing.

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Signal transduction **Abstract P77**

EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 1 (EBNA1) MODULATES THE TGFβ SIGNALLING PATHWAY BY INDUCING CHANGES IN SMAD PHOSPHORYLATION IN CARCINOMA CELL LINES

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Abstract

Epstein-Barr virus (EBV) infection is a consistent feature of the undifferentiated form of nasopharyngeal carcinoma (NPC). The EBV-encoded EBNA1 protein is expressed in all virus-associated malignancies where it not only plays essential roles in the maintenance, replication and transcription of the EBV genome, but also influences the transcription of a variety of cellular genes in *in vitro* models. Aberration of the TGFβ signalling pathway is a common trait in tumour progression, and EBNA1 has been shown to abrogate TGFβ signalling in carcinoma cells through a reduction in the phosphorylated form of Smad2 and an increase in Smad2 protein turnover. Here we provide evidence that EBNA1 may downregulate Smad phosphorylation at the C-terminal region through increasing expression of the Smad-specific phosphatase, PPM1A, and in addition may promote degradation of the type I TGFβ receptor (TβRI). Conversely, we demonstrate that EBNA1 enhances Smad2 linker phosphorylation, which is associated with TβRI-independent pro-tumourigenic effects, such as the development of epithelial-mesenchymal transition (EMT), that enhance tumour invasion and metastasis. These data therefore suggest a model whereby EBNA1 selectively influences Smad phosphorylation to desensitise carcinoma cells against the growth inhibitory stimuli of TGFβ, whilst maintaining and perhaps even potentiating those TGFβ-mediated effects that promote tumour invasion and metastasis.

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Signal transduction **Abstract P78**

IS THE COOPERATION BETWEEN C-MYC AND EBV LATENCY III PROGRAM IN CELL PROLIFERATION DEPENDING ON NF-KB?

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Abstract

The Epstein-Barr virus (EBV) is involved in a variety of human malignancies that include Burkitt lymphoma (BL) and immunodeficiency-related B-cell lymphomas. In fact, the EBV-latency III program is directly responsible for immortalization of B cells *in vitro*. LMP1, the main viral oncogene, acts as a functional homolog of the tumor necrosis factor receptor (CD40) and leads to the overactivation of the NF-κB transcription factor which is implicated in proliferation and cell survival. The proto-oncogene c-Myc, another transcription factor implicated in cell cycle progression and in regulation of proliferation, is a direct target of EBV since EBNA2 is able to enhance *c-myc* gene expression and is also the oncogene responsible for BLs. Our previous work showed that c-Myc and NF-κB are the two master transcriptionnal systems activated in EBV-immortalized B-cells, raising the question of the cooperation between these two transcription factors. To study the cooperation between c-Myc and the EBV-latency III program in cell proliferation, we used a conditional cell line (P493-6) in which the estrogen-dependant EBNA2 viral protein and a tetracycline-regulable *c-myc* gene can be induced independently of each other. Proliferation of the P493-6 cells was increased in presence of both proliferation programs (EBV-latency III and c-Myc). However, induction of c-Myc probably decreased EBNA2 activity, as assessed by a decrease in CD23 expression, and consequently LMP1 expression with NF-κB activity and expression of NF-κB target genes. Now, we have to determine the exact place of NF-κB in this cooperation between c-Myc and EBV-latency III program for cell growth.

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Signal transduction **Abstract P79**

THE EFFECT OF THE KSHV VIRF4 PROTEIN ON THE INTERFERON STIMULATED RESPONSE ELEMENT (ISRE) AND THE INTERFERON B PROMOTER

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Abstract

Background: Kaposi's sarcoma-associated herpes virus (KSHV) encodes multiple immune modulatory proteins. They include four interferon regulatory factor (IRF) homologues sharing some sequence identity with cellular IRFs and are therefore known as viral IRFs (vIRFs)-1-4. Since no reports have described the potential role of the lytic cycle vIRF-4 protein in regulating type I interferon (IFN) responses, we investigated this possibility. We examined the effect the vIRF4 protein has on both the early (activation of the IFNβ promoter) and late (activation of the ISRE promoter) IFN response.

Methods: vIRF-4 was cloned into an expression vector as pvIRF-4-HACR3 and 293 cells were transiently transfected with this vector. Examination of the early IFN response was achieved by transactivation of the IFNβ promoter reporter plasmid (p125-luc) and its activation by transfection of poly (I:C). The late IFN response was analysed with the aid of the IFN-responsive reporter plasmid (pISRE-luc) that was activated by treatment with recombinant IFN-α2b. Reporter gene activtity was assessed by dual luciferase assays (DLA). The pRLSV40 plasmid, constitutively expressing *Renilla* luciferase, provided the internal control for normalising firefly luciferase levels.

Results & Conclusion: vIRF4 inhibited DLA activity in a dose-dependent manner in transiently transfected 293 cells. Inhibition was over 60%. In contrast, no inhibition of an *IFN* promoter activated by polyI:C was demonstrated. These data suggest vIRF-4 specifically inhibits IFNα–driven transactivation of IFN-responsive promoters. Thus, one role for vIRF-4 in the context of KSHV infection may be to modulate the type I IFN response, contributing to virus dissemination and production.

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Signal transduction **Abstract P80**

PATHOGENESIS AND THERAPY OF EBV-ASSOCIATED HEMOPHAGOCYTIC SYNDROME

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Abstract

Epstein-Barr virus (EBV) is associated with a fatal hemophagocytic syndrome (HPS) or hematophagic lymphohistiocytosis (HLH) in young children, characterized by prolonged fever, hepatosplenomegaly, cytopenia and coagulopathy. Histopathologically, macrophage activation with phagocytosis of red cells, lymphocytes, or platelets are characteristic. In the past 30 years, series of studies from our laboratory in Taiwan and other groups, mainly from Japan, have documented that EBV infects T cells (CD8, CD4) or rarely NK cells in HLH, instead of the classically believd B cells in infectious mononucleosis. The infection of T cells by EBV will lead to activation of EBV-infected T cells and secretion of pro-inflammatory cytokines (IL-6, interferon-gamma, TNF-alpha, etc.) which subsequently results in macrophage activation and proinflammatory cytokine secretion. We further demonstrated that anti-red cells autoantibodies production is closely related to the phagocytosis of red cells in a HVP rabbit model. EBV LMP-1 is the responsible viral protein to upregulate cytokines via the TRAFs/NFkB signaling pathway. Interestingly, this signaling process is mediated through ATF5 to suppress the XLP gene of *SAP/SH2D1A*, leading to T cell activation and thereby providing a common mechanism for the pathogenesis of HPS in XLP and HLH. Because of the poor prognosis or high co-morbility of current therapy, we started to apply one of the NFkB inhibitor, the agonist of peroxisome proliferator activated receptor (PPAR), to treat patients of HLH. In the rabbit model, PPAR agonist—rosiglitazone can significantly ameliorate the clinical parameters, cytokine levels, and most importantly, the inhibition of viral loads in tissues or serum. Preliminary data revealed that rosiglitazone can significantly improve the survival of 4 reactivation cases of HLH in Vietnamese children. A clinical trial on the treatment of HLH is now ongoing in the Children Hospital No. 1 of Ho-Chi-Min City, Vietnam. Our series of studies clarify the mechanism of HLH and provide a novel therapy for HLH.

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Signal transduction **Abstract P81**

EPSTEIN-BARR VIRUS BGLF4 KINASE INTERFERES WITH CELL CYCLE PROGRESSION

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Abstract

To benefit virus replication, EBV adopts various strategies to create an unsynchronized cell cycle status. For example, an S-phase-like cellular environment is coupled with some prophase-like events in EBV replicating cells. Previously, we found that EBV BGLF4 kinase induces unscheduled chromosome condensation through the activation of condensin and topoisomerase II, coupling with the reorganization of nuclear lamina to facilitate the egress of nucleocapsid from nucleus. Because BGLF4 functions through a Cdc2 mimicking pathway to induce prophase-like cell morphology, here we set out to investigate the regulatory effects of BGLF4 on cell cycle phenotype. Transiently transfected HeLa cells expressing GFP-BGLF4 were sorted into two groups, according to the expression levels of BGLF4. An-S phase accumulation was found in cells with higher BGLF4 expression, whereas such effect was not obvious in cells with lower BGLF4 expression. In a tetracycline inducible 293 T-REx cell background, we demonstrated the expression of BGLF4 induces cell growth retardation in different clones, accordant to BGLF4 expression levels. Cell cycle progression analysis of 293T-Rex-BGLF4 cells after double thymidine synchronization suggested BGLF4 interferes with S-phase progression. Remarkably, abnormal chromosome structure and micronucleus formation were observed in BGLF4 expressing cells, suggesting that in addition to modulating cellular environment for viral DNA replication, BGLF4 may induce host chromosome instability.

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Signal transduction **Abstract P82**

UPREGULATION OF ID1 BY EPSTEIN-BARR VIRUS-ENCODED LMP1 CONFERS RESISTANCE TO TGFB-MEDIATED GROWTH INHIBITION

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Abstract

The Epstein-Barr virus-encoded LMP1 protein is commonly expressed in nasopharyngeal carcinoma. LMP1 is a prime candidate for driving tumourigenesis given its ability to activate multiple signalling pathways and to alter the expression and activity of variety of downstream targets. Resistance to TGFβ-mediated cytostasis is one of the growth transforming effects of LMP1. Of the downstream targets manipulated by LMP1, the induction of Id1 and inactivation of Foxo3a appear to be particularly relevant to LMP1-mediated effects. Id1 is implicated in cell transformation and plays a role in cell proliferation, whilst Foxo3a controls cell integrity and homeostasis by regulating apoptosis. In this study, we demonstrate that the ability of LMP1 to induce the phosphorylation and inactivation of Foxo3a is linked to the upregulation of Id1 and that this is mediated through LMP1-activated PI3K/Akt and ERK-MAPK signalling. Furthermore, we show that the induction of Id1 is essential for the transforming function of LMP1. Over-expression of Id1 increases cell proliferation, attenuates TGFβ-SMAD-mediated transcription and renders cells refractory to TGFβ-mediated cytostasis. Id1 silencing in LMP1-expressing epithelial cells abolishes the inhibitory effect of LMP1 on TGFβ-mediated cell growth arrest and reduces the ability of LMP1 to attenuate SMAD transcriptional activity. In response to TGFβ stimulation, LMP1 does not abolish SMAD phosphorylation but inhibits p21 protein expression. Stimulation of LMP1-expressing cells by TGFβ induces Id1. LMP1 suppresses the transcriptional repressor ATF3, possibly leading to the TGFβ-induced Id1 upregulation. These data provide novel information regarding the mechanisms by which LMP1 suppresses TGFβ-induced cytostasis.

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Signal transduction **Abstract P83**

TGF-B-INDUCED REACTIVATION OF EBV INVOLVES ALLEVIATING ZEB1-MEDIATED REPRESSION OF THE BZLF1 GENE

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Abstract

A central question is how physiologically relevant agents reactivate virus present in latently infected cells into lytic replication. Incubation of EBV-infected B-cells with transforming growth factor-β (TGF-β) mimics a physiological pathway by which such an induction can occur. We examined the mechanism by which TGF-β activates transcription from the EBV *BZLF1* latent-lytic switch gene promoter, Zp, in Burkitt's lymphoma type I latency MutuI cells. Co-culture of MutuI cells with EBV-negative Jurkat T-cells, which secrete TGF-β, led to induction of EBV lytic-gene expression. Incubation of MutuI cells with either SB431542, a specific inhibitor of the TGF-β pathway, or a TGF-β neutralizing antibody eliminated TGF-β-induced reactivation of EBV. Exogenous expression of the TGF-β intracellular signaling proteins Smad2 and Smad4 in a transient transfection assay was sufficient to activate transcription from Zp. We identified a novel Smad-binding element, named SBE2, overlapping the primary ZEB1-binding ZV element of Zp. Mutation of SBE2 reduced Smad-mediated transcription from Zp. Furthermore, Smad4 was found to compete with ZEB1 both for binding to Zp in an electrophoretic-mobility-shift assay and for activating transcription from Zp in a transient transfection assay. Data from a chromatin immunoprecipitation assay confirmed that Smad4 newly bound the Zp region of the EBV genome following incubation of MutuI cells with TGF-β. Thus, we conclude that TGF-β primarily induces latently infected cells into lytic replication by alleviating ZEB1-mediated repression of the *BZLF1* gene, doing so via its canonical Smad signaling pathway.

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Signal transduction **Abstract P84**

EPSTEIN-BARR VIRUS (EBV) AND RESISTANCE TO P53-DEPENDENT APOPTOSIS

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Abstract

P53 inactivation is often observed in Burkitt's lymphoma (BL) cells, due either to mutations in p53 gene or to overexpression of the p53-negative regulator, MDM2. BL is closely associated with Epstein-Barr virus (EBV) infection although not all cases are EBV(+). Three patterns of EBV latency have been defined, based on the differential production of the viral proteins. Most cases of BL express the latency I program (only EBNA1 protein produced), but some express the latency III (all viral proteins produced). We have investigated the consequences of p53 activation on the cell fate of EBV(-) and EBV(+) B cells. We show that nutlin-3, a potent antagonist of MDM2, activates the p53 pathway in all BL cell lines harboring wild-type p53. However, nutlin-3 strongly induced apoptosis in EBV(-) or latency I EBV(+) cells, whereas latency III EBV(+) cells were much more resistant. This resistance to apoptosis was also observed in latency III EBV(+) lymphoblastoid cell lines (LCL). In EBV(-) cells, relocalization and activation of Bax in the mitochondria following nutlin-treatment is involved in the apoptotic process. In latency III EBV(+) cells, Bcl-2 is overexpressed, interacts with Bax and prevents its activation. We also investigated the effects of nutlin-3 in combination with various chemotherapeutic drugs. We show that sublethal doses of nutlin-3 sensitizes EBV(-) or latency I EBV(+) cells to apoptosis induced by drugs, but protects latency III EBV(+) cells. $P21^{WAF1}$ which is overexpressed in the latter, is involved in this protective effect, as siRNA-mediated inhibition of $p21^{WAF1}$ restored sensitivity to drugs.

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Signal transduction **Abstract P85**

CHOLESTEROL DEPLETION BY METHYL-BETA-CYCLODEXTRIN HAS NO EFFECT ON LMP-1 HOMO-OLIGOMERIZATION, NFKB ACTIVATION, OR CO-MIGRATION WITH DETERGENT-RESISTANT MEMBRANES

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Abstract

The viral oncoprotein latent membrane protein-1 (LMP-1) is a major contributor to EBV-associated tumor development. LMP-1's activity correlates with its co-migration with detergent resistant-membranes (DRMs) in isopycnic gradients, which is often described as lipid raft-association. However, variations in DRM-isolation techniques can drastically change the lipid and protein constituents found in DRMs, and this heterogeneity makes it difficult to precisely demarcate classically-defined lipid rafts from other, novel membrane microdomains. It remains unclear whether LMP-1 activity is specifically dependent upon localization in classical lipid rafts and/or alternative membrane microdomains. The inhibition of cholesterol synthesis by simvastatin is reported to decrease the amount of LMP-1 in DRMs from EBV-transformed B cells, however performing the same treatment on nasopharyngeal carcinoma cell lines has no effect on LMP-1's distribution in DRMs. Here, we characterize the behavior of LMP-1 after treatment with methyl-beta-cyclodextrin (MBCD), a drug that efficiently depletes cholesterol at the cell surface. We report that cholesterol depletion with MBCD disrupts the ability of classical lipid raft elements such as Lyn kinase and the ganglioside GM1, but not LMP-1, to comigrate with DRMs on isopycnic gradients. Importantly, neither LMP-1's ability to stimulate NFkB signaling nor it's ability to homo-oligomerize requires classical lipid rafts. These results suggest that LMP-1 may not be a component of classical, cholesterol-dependent lipid rafts and point to the need for re-examination of LMP-1's relationship with, and dependence upon, membrane microdomains.

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Signal transduction **Abstract P86**

COMPREHENSIVE DETECTION ASSAY OF PHYSIOLOGICAL INTERACTION PARTNERS FOR THE EPSTEIN-BARR VIRUS LATENT MEMBRANE PROTEIN 1 BY BIMOLECULAR FLUORESCENCE COMPLEMENTATION SYSTEM COUPLED WITH ENHANCED RETROVIRUS MUTAGENESIS

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Abstract

Epstein-Barr virus (EBV) Latent membrane protein 1 (LMP1) is critical for B cell growth transformation and the survival in EBV infection. It is well known about the LMP1 C-terminus cytoplasmic region is associated with TNF receptor-associated factors including TRAF3 and its interaction induces NF-kB activation. However, the entire molecular mode of LMP1-induced B cell growth transformation is not fully understood. To elucidate the mechanism we established the system for the visualization of LMP1 interaction with itself or host cellular proteins. This system is based on the Bimolecular fluorescence complementation (BiFC) assay in which N-terminus monomeric green fluorescence protein (GFP) fused to LMP1 (LMP1-NGFP) is co-expressed with C-terminus GFP fused to an interacting partner for LMP1. When the subsequent fusion protein linked to LMP1 (LMP1-CGFP) was expressed together with LMP1-NGFP the complex was created as functional GFP following restored fluorescence. We also found LMP1-LMP2a complex as well as LMP2a homo-multimerization formed in cells suggesting that LMP1 might interact directly with LMP2a. These data also suggest that this system would be of help to identify unknown LMP-interacting host proteins and to find out small compounds which inhibits LMP function by disrupting the interface of protein-protein interaction. Furthermore we show a comprehensive assay for identifying the LMP1-binding partners derived from host cells. Human B lymphoma cell line expressing LMP1-CGFP is tagged with NGFP gene encoded by Enhanced retrovirus mutagenesis (ERM) vector which act as exon-trap. We would discuss the EBV pathogenesis leading from the molecular basis by which LMP1 takes over host signaling pathways.

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Signal transduction **Abstract P87**

EXPRESSION OF CELLULAR IMMEDIATE-EARLY GROWTH RESPONSE AND NUCLEAR ORPHAN RECEPTOR GENES PRECEDES EXPRESSION OF EPSTEIN-BARR VIRUS *BZLF1* AND *BRLF1* FOLLOWING CROSS-LINKING OF THE B CELL ANTIGEN RECEPTOR IN THE AKATA BURKITT LYMPHOMA CELL LINE

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Abstract

Our previous studies showed that the EBV lytic cycle activator genes, *bzlf1* and *brlf1*, do not behave with immediate-early (IE) kinetics when their expression is induced by HDAC inhibitors and Protein Kinase C agonists (Ye, 2007). Here we demonstrate in the Akata cell line, where lytic cycle activation occurs very rapidly, that *de novo* protein synthesis is also required for induction of *bzlf1* and *brlf1* expression by anti-IgG. New protein synthesis is required up to 1.25 hrs after application of anti-IgG; *bzlf1* and *brlf1* mRNAs can be detected 1.5 hrs after anti-IgG. Five cellular immediate-early IE genes were shown to be expressed by 1 hr after addition of anti-IgG and preceded expression of *bzlf1* and *brlf1*. These include early growth response genes (*egr1*, *egr2*, *egr3*) and nuclear orphan receptors (*nr4a1* and *nr4a3*). These genes were activated by anti-IgG treatment of Akata cells with and without the EBV genome; therefore their expression was not dependent on expression of any EBV gene product. Expression of EGR1, ZEBRA and Rta proteins was inhibited by Bisindolylmaleimide X, a selective inhibitor of PKC. The findings suggest a model in which cross-linking of the B-cell receptor activates a signal transduction cascade that induces expression of cellular IE genes, such as early growth response genes, whose products, in turn, regulate *bzlf1* and *brlf1* expression.

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Posters P88-P109

Lymphoma

Lymphoma **Abstract P88**

CAN SERUM IL6 LEVELS ASSIST IN THE ASSESSMENT OF PATIENTS AT RISK OF EPSTEIN-BARR POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER?

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Abstract

Background: Epstein-Barr Virus (EBV) related post-transplant lymphoproliferative disorder (PTLD) can be devastating for transplant recipients. Although limited by specificity, PCR-based measurement of viral DNA load is used to monitor patients for PTLD. Given the pathogenesis of PTLD, we postulated that markers of B-lymphocyte growth, such as serum IL6 levels, are likely to be elevated in PTLD and thus may be of value in diagnosing PTLD.
Objective: To examine the utility of serum IL6 assays as adjuncts to EBV load monitoring for PTLD among pediatric organ transplant recipients.
Methods: Patients were monitored with viral load (VL) and serum IL6 testing. Results were compared between PTLD cases and non-cases at < 3, 3-6 and > 6 months after transplantation.
Results: Median IL6 levels in PTLD cases were 15.5 (2.0-87.1) and 23.3 (2.1-276) pg/ml versus 3.25 (0.92-114) and 3.5 (0.75-199.25) pg/ml in non-cases at 3-6 and > 6 months, respectively ($p = 0.006$ and 0.005). At > 6 months, IL6 levels correlated with VL and PTLD occurrence [Spearman's Coefficients = 0.40; $p = 0.001$ and 0.32; $p = 0.003$]. The sensitivity and specificity of high VL as a test of PTLD was 76.3% and 92.5%, while the negative predictive value was 94.9% and positive predictive value (PPV), 68.4%. Elevated IL6 plus high VL increased the PPV and specificity to 80% and 96.2%, respectively and improved the Receiver Operating Characteristic Curve.
Conclusion: Serum IL6 levels can improve the clinician's ability to predict PTLD, in the setting of elevated EBV viral loads following organ transplantation.

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Lymphoma **Abstract P89**

SEROPOSITIVITY FOR HUMAN CYTOMEGALOVIRUS IS ASSOCIATED WITH AN INCREASED RISK OF BOTH EBV-NEGATIVE AND EBV-POSITIVE CLASSICAL HODGKIN LYMPHOMA

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Abstract

Classical Hodgkin Lymphoma (cHL) is associated with Epstein-Barr Virus (EBV) in a proportion of cases. Among adults, risk of EBV-associated cHL is associated with age and HLA-A genotype suggesting that cell mediated immunity to EBV plays a critical role in disease development. In CMV seropositive individuals much of the T-cell response is CMV-restricted and EBV-specific responses are impaired. We therefore investigated the hypothesis that CMV infection increases risk of EBV-associated cHL, particularly among older adults. Serum samples from 549 cHL cases and 396 controls were analysed using the Abbot CMV IgG chemiluminescent microparticle immunoassay. Overall, 51.1% of subjects were CMV seropositive and seropositivity increased with age. After adjusting for effects of age and sex, cHL was independently associated with CMV seropositivity (odds ratio (OR) cases versus controls=1.4, 95% confidence interval (CI) 1.05-1.86.) Significant differences were observed for both EBV-associated cHL (OR EBV-positive cases versus controls=1.59 95% CI 1.06-2.46) and EBV-negative cHL (OR 1.41, 95% CI 1.02-1.95.) There was no significant difference in CMV seropositivity between EBV-positive and negative cHL cases. Following stratification by age, case:control differences were significant only in adults >50 years (OR 1.76, 95% CI 1.08- 2.85); ORs were greatest for EBV-positive cHL cases versus controls (OR 2.37, 95% CI 1.16-4.82.) The data support the hypothesis that reduced immune surveillance due to CMV infection leads to an increased risk of cHL, particularly in older adults; however, this increased risk is not restricted to EBV-associated cHL.

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Lymphoma **Abstract P90**

IMMUNO-EVASION BY PARACRINE EFFECT OF LYTIC INFECTION IN EBV-INDUCED LYMPHOPROLIFERATION

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Abstract

EBV establishes a lifelong latency in B-lymphocytes following infection. Although in immune-competent individuals, EBV remains in a silent state, under immunodeficient conditions such as HIV infection and posttransplantation, EBV-infected B-lymphocytes proliferates giving rise to lymphoproliferative disease and lymphoma. Like in immune-deficient hosts, EBV transforms human B-lymphocytes into indefinitely growing lymphoblastoid cells in vitro. Although transformed lymphocytes harbor the entire EBV genome as episome, they remain in latent infection, expressing only a fraction of EBV genes. Lytic infection is spontaneously induced in a scarce fraction of EBV-transformed cells. Here, we report that a lytic infection occurring scarcely contributes to EBV-induced transformation by paracrine mechanism.

The BZLF1 plays a key role in switching EBV infection from latent to lytic form. Previously, we reported that BZLF1-KO EBV could transform primary B-lymphocytes as efficiently as wild-type EBV. Here we demonstrate that BZLF1-KO EBV transforms B-lymphocytes with approximately 10-fold lower efficiency than wild EBV, when unfractionated peripheral blood mononuclear cells (PBMCs) are used as target cells. On the other hand, BZLF1-KO EBV transforms B-lymphocytes as efficiently as wild EBV, when T-lymphocytes-depleted PBMCs are used as target cells. BZLF1 induces IL-13, which is known to suppress T-cell response, thus enabling proliferation of EBV-transformed lymphocytes in the presence of T-lymphocytes.

This is the first report showing that the lytic gene product promotes virus-induced proliferation via paracrine mechanism. The present finding should provide a good insight on the mechanism of oncogenesis by KSHV, which codes many viral homologues of cytokine genes as lytic genes.

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Lymphoma **Abstract P91**

EXPRESSION OF MICRORNAS IN NASAL NATURAL KILLER/T-CELL LYMPHOMA CELL LINES

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Abstract

Nasal natural killer (NK)/T-cell lymphoma has distinct clinical and histologic features and is characterized by a poor prognosis and progressive necrotic lesions with tumor and inflammatory cell infiltrations in the nasal cavity, nasopharynx, and palate. With respect to etiologic factors, we first showed the presence of EBV DNA, EBV oncogenic proteins, and the clonotypic EBV genome in this lymphoma, suggesting that EBV plays a role in its genesis. In late years, carcinogenesis due to the abnormality expression of microRNA has been reported for various cancers as one of the mechanism of the carcinogenesis. On the other hand, EBV's microRNA also affects carcinogenesis and its latent infection. In this study, we examined the human and EBV's microRNAs expressed by SNK-6, SNK-1 and SNT-8 cells, which were established from nasal NK/T-cell lymphomas. To determine which microRNAs are expressed specifically in nasal NK/T-cell lymphoma, we compared the microRNA expression patterns among SNK-6, SNK-1, SNT-8, and NK-92 cells. NK-92 cells were established from a patient with non-nasal NK-cell lymphoma (non-Hodgkin's lymphoma with large granular lymphocytes). We also used peripheral blood NK cells as a control. We found some microRNAs expressed specifically in nasal NK/T cell lymphomas. We report the meanings of specifically expressed microRNAs in nasal NK/T-cell lymphoma.

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Lymphoma **Abstract P92**

EPSTEIN BARR VIRUS LATENT MEMBRANE PROTEIN 1 IN HODGKIN LYMPHOMA

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Abstract

Epstein Barr virus is now known to be firmly associated with Hodgkin lymphoma (HL). The working hypothesis of the project is that LMP1 expression in lymphocytes will lead to the activation of the MAPK and PI3K pathways which are associated with the pathogenesis of HL. Plasmid harbouring LMP1 gene was transfected into EBV-negative HL cells. A sample of lysate protein was subjected to SDS-PAGE before blotting onto membrane to detect the phosphorylated status of major proteins involved in MAPK and PI3K pathways. Immunohistochemistry was performed on HL archival tissues. In this study, ERK2 has a higher level of activation compared to ERK1 in the LMP1 transfected cells, less so in stable established EBV-positive cells, but not in naturally EBV-positive L591 cells. Both JNK2 and JNK3 have higher activation than JNK1 in the LMP1 expressing cells. Activation of c-Jun is observed to be positively correlated with JNK in CD30-positive classical HL. On the other hand, ATF-2 is seen to be activated by p38 MAPK. It is also found that LMP1 phosphorylates Akt on Thr450 and Ser473 in the KMH2-EBV cells, but to a lesser phosphorylation level on Ser473 in the LMP1 transfected L428 cells. GSK-3, a downstream kinase of Akt is found to be activated in KMH2-EBV. The tumour suppressor protein, p16 is believed to down-regulate cyclin D1 but not c-Myc. The results of analysis demonstrate that EBV LMP1 plays an important role in activation of ERK, JNK, p38 MAPK and Akt pathways to maintain its survivability in Hodgkin tumours.

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Lymphoma **Abstract P93**

VIRALLY INDUCED CELLULAR MIR-155 PLAYS A KEY ROLE IN B CELL IMMORTALIZATION BY EBV

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Abstract

EBV infection of primary B cells in culture causes transformation into indefinitely dividing lymphoproliferative cell lines (LCLs). This process serves as a model system of lymphomagenesis, especially for diffuse large B-cell lymphoma (DLBCL) and AIDS associated lymphomas. Upon infection, EBV up-regulates a number of cellular microRNAs, with the biggest effect seen on miR-155. As early as 70 hours after infection, miR-155 levels were increased over 30 times compared to resting B cells and, in established LCLs, miR-155 makes up approximately half of the total miRNA population. The extent of up-regulation of miR-155 by EBV and its high level of expression in LCLs suggest a role for miR-155 in the transformation of primary B cells by EBV as well as the maintenance of LCLs, especially given previous data demonstrating that miR-155 over-expression is etiologically linked to B cell transformation. We used a miR-155-specific sponge to achieve the functional knock down of miR-155 in LCLs. In competitive growth assays, miR-155 deficient LCLs were strongly impaired for growth compared to control-transduced cells. The failure of miR-155 deficient LCLs to compete is due, at least in part, to increased apoptosis as well as their arrest in the G1 phase of the cell cycle. We are currently testing the consequences of miR-155 over-expression on the efficiency of transformation by EBV and performing experiments to better characterize the observed phenotype and elucidate the underlying mechanisms.

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Lymphoma **Abstract P94**

LMP1 GENE VARIANTS IN PEDIATRIC EPSTEIN-BARR VIRUS ACUTE AND PERSISTENT INFECTION

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Abstract

In Argentina Epstein Barr virus (EBV) infection occurs at an early age and may occasionally develop infectious mononucleosis (IM). EBV is also related with lymphoma development. Viral variants display polymorphisms in LMP1 gene. Objective: To study viral type and variants distribution among children with IM at diagnosis (T0), a month (T1) and three months (T2) and compare them with EBV+ pediatric lymphomas. Methods: Oral secretions (OS) and peripheral blood mononuclear cells (PBMC) from 11 VCA IgM+ children with IM and biopsies from 19 EBV+ lymphomas were included. Two PCR strategies were performed: EBNA3C for genotyping and LMP1 C-ter region. PCR products were directly sequenced. Results: Among IM EBV1 was predominant. LMP1 variants were wt and del30 with a repeat number varying from 4 ½ to 6 ½. Co-existence of two EBV variants was observed in PBMC and OS of 1 patient. All other patients maintained the same genotype and variants during follow up. Among tumors, both EBV genotypes showed similar incidence. LMP1 variants included wt and del30 with a number of repeats that varied between 3 ½ and 6 ½. Del30 variants were associated with a higher number of repeats. Conclusions: del30 and high repeat variants were found in IM and lymphomas, but were not malignancy related as previously described. Variants at T0 were sustained all through follow up both in OS and PBMC in all but one patient. This is the first study to address distribution and compartmentalization in pediatric patients with IM and its convalescence.

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Lymphoma **Abstract P95**

MECHANISMS OF VIRUS-INDUCED GENOMIC INSTABILITY IN EBV ONCOGENESIS

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Abstract

Epidemiological and molecular evidence links Epstein-Barr virus (EBV) carriage to the pathogenesis of a variety of human malignancies of lymphoid and epithelial cell origin but the mechanisms by which the virus promotes tumor development are not well understood. Burkitt's lymphoma (BL), a tumor occurring in both EBV positive and negative forms, provides a convenient model for analysis of the relative contribution of genetic change and viral products that are expressed in the malignant cells. We have shown that EBV carriage is associated with a statistically significant increase of non-clonal chromosomal aberrations, including dicentric chromosomes, chromosome fragments, double minutes and chromosome gaps, that are caused by ongoing DNA damage and defective DNA repair. Using a panel of transfected sublines of the B-lymphoma line BJAB expressing the viral genes associated with latent infection, we demonstrate that the EBV nuclear antigens EBNA-1 and EBNA-3C and the membrane protein LMP-1 independently promote genomic instability, as detected by non-clonal chromosomal aberrations, DNA breaks and phosphorylation of histone H2AX. EBNA-1 plays a direct role in the generation of DNA damage via induction of reactive oxygen species (ROS), while DNA repair is inhibited in LMP-1 expressing cells through down-regulation of the DNA damage sensing kinase ATM, reduced phosphorylation of its downstream targets Chk2 and inactivation of the G_2 checkpoint. The propagation of damaged DNA is promoted in EBNA-3C expressing cells by inactivation of the mitotic spindle checkpoint, which correlates with transcriptional downregulation of BubR1. Thus, multiple cellular functions involved in the maintenance of genome integrity appear to be independently targeted by EBV, pointing to the induction of genomic instability as critical event in viral oncogenesis.

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Lymphoma **Abstract P96**

CONTRIBUTION OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) TO THE PATHOGENESIS OF EBV-ASSOCIATED LYMPHOMAS

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Abstract

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that is implicated in the pathogenesis of several inflammatory diseases, and more recently in cancer. We have studied the expression of MIF, its regulation, and its phenotypic impact in EBV-transformed B cells and in lymphoma cell lines. We have shown that when compared to human germinal centre B cells, MIF is over-expressed in the malignant HRS cells of Hodgkin's lymphoma where it is accompanied by the expression of both MIF co-receptors, CD74 and CD44. MIF is also over-expressed in HL-derived cell lines and in primary B cells *in vitro* transformed by EBV. Elevated levels of MIF in the supernatant of these cells was also associated with the phosphorylation of ERK1 and ELK1, previously described as downstream signaling targets of MIF. In EBV-transformed cells, inhibition of the catalytic activity of MIF reduces proliferation, suggesting that MIF induction is important for the maintenance of established LCL and of EBV-associated lymphoma cells.

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Lymphoma **Abstract P97**

RAPID AND SUSTAINED CONTROL OF EBV PTLD AFTER TRANSFER OF PEPTIDE-SELECTED T CELLS

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Abstract

EBV-associated post-transplant lymphoproliferative disease (PTLD) is a potentially life-threatening complication after allogeneic hematopoietic stem cell transplantation (HSCT). PTLD can be prevented or cured by adoptive transfer of EBV-specific T cells from the donor. However, methods to produce EBV-specific T cells for therapy have been time-consuming. Therefore, we developed a rapid protocol for their isolation by overnight stimulation of donor blood cells with peptides derived from 11 EBV antigens, interferon-gamma surface capture, and immunomagnetic separation. Six patients with PTLD received one transfusion of EBV-specific T cells. No response was seen in three patients with late-stage disease and multi-organ dysfunction. In three patients who received T cells at an earlier stage of disease, we observed complete and stable remission of PTLD. Two patients have remained free from EBV-associated disease for more than two years. T cell reconstitution was dominated by CD8+ T cells specific for EBV immediate-early and early antigens. These T cells rapidly expanded after therapy, reached levels over 20% of all peripheral blood lymphocytes, and remained present throughout the observation period. Latent antigen-specific T cells were also reconstituted, but at lower levels. Thus, a rapid and sustained reconstitution of a protective EBV-specific T cell memory was observed after the infusion of small numbers of directly isolated EBV-specific T cells.

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Lymphoma **Abstract P98**

POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) IN ADULT RENAL TRANSPLANT RECIPIENTS. SINGLE CENTRE REVIEW OF PRESENTATIONS AND OUTCOMES.

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Abstract

Cases of PTLD occurring in adult renal transplant recipients in a single UK centre were identified from pathology databases. Medical, histological and radiological reports were reviewed.
From a total of 4012 recipients, 71 cases of PTLD presenting over 30 years were identified. This is the largest reported UK based PTLD series.
Overall PTLD incidence was 1.8%. Mean age at diagnosis was 47 years and 72% male. Mean time from transplantation to diagnosis was 78 months with 82% presenting late (>1year). Disease presentations typically involved B symptoms (47%), neck (37%) and abdomen (27%). Lung, CNS and graft involving disease was uncommon. Histological diagnosis included 5 with early disease, 11 polymorphic, 49 monomorphic and 5 Hodgkin like. 77% cases were tissue EBV positive and 85% EBV whole-blood PCR positive at diagnosis. Patient survival was 68% at 1 year and 60% at 5 years. 40% non survivors died within 1 month of diagnosis. 81% of those alive at 1 year survived to 10 years. Poor prognosis was found in those with raised LDH at diagnosis (p=0.01). Death censored graft survival was excellent at 84% at 10 years. No significant change in serum creatinine 6 months pre to 1 year post diagnosis was detected.
Conclusion:
Development of markers to identify high risk patients at diagnosis could guide early management and reduce mortality. In view of the excellent late graft survival, earlier, aggressive immunosuppressive reduction or withdrawal may be warranted, perhaps with greater allograft loss, to try and improve patient survival.

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Lymphoma **Abstract P99**

EXPRESSION OF CHEMOKINES IN NASAL NATURAL KILLER/T-CELL LYMPHOMA

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Abstract

Purpose: Nasal natural killer (NK)/T-cell lymphoma is associated with Epstein-Barr virus and has poor prognosis because of local invasion and/or multiple dissemination. Recently, the role of chemokines in tumor proliferation and invasion has been shown. In this study, we examined the chemokines expressed by nasal NK/T-cell lymphoma cell lines.
Methods: Chemokine protein array was used to examine specific chemokines produced by nasal NK/T-cell lymphoma cell lines. The expression of chemokines and chemokine receptors was investigated by ELISA and flow cytometry, respectively.
Results: In chemokine protein array, TARC, MDC, IL-8, and MCP-1 were specifically produced by nasal NK/T-cell lymphoma cell lines compared with non-nasal NK-cell line. ELISA analysis confirmed that nasal NK/T-cell lymphoma cell lines produced these chemokines in a time-dependent manner. CCR4, the receptor of TARC and MDC, was expressed on nasal NK/T-cell lymphoma cell lines but CXCR1/2 and CCR2, the receptors of IL-8 and MCP-1, were not expressed.
Conclusions: These results suggest that TARC and MDC may play an important role in nasal NK/T-cell lymphoma cell lines possibly via an autocrine mechanism.

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Lymphoma **Abstract P100**

LYMPHOMA DEVELOPMENT IN XLP PATIENTS CAN BE ATTRIBUTED TO ABSENCE OF THE PROAPOPTOTIC SAP

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Abstract

XLP patients have a 200 times higher risk for lymphoma development than the general population. This was attributed to their impaired immunosurveillance. SAP protein is either absent or nonfunctional in the XLP condition and it was shown to be involved in signal transduction pathways of T and NK cells. However, we have shown that SAP is a direct target of p53 and it has pro-apoptotic function. Therefore it can be assumed that in the absence of a functional SAP DNA damaged lymphoid cells might escape death and can accumulate further mutations leading to lymphoma development.
While SAP is regularly expressed in T and NK cells, it was demonstrated only in a rare subset of B lymphocytes. We have suggestive evidence for the involvement of SAP in the development of Burkitt lymphoma (BL). We found a difference in SAP expression of EBV carrying and EBV negative Burkitt lymphoma lines. In the former 7/9 were SAP positive, while none of the 9 EBV negative lines expressed SAP. We propose that the contribution of SAP to apoptosis is important for the fate of the rare, Ig-c-myc translocation carrying GC B cells (the cells of origin of BL). The EBV carrying ones could survive because EBV inhibits the pro-apoptotic function of SAP. However, EBV negative precursors of BL could grow into lymphomas only if they are SAP negative.
Importantly, high proportion of the XLP associated lymphomas were classified as BLs and some were shown to carry Ig/Myc translocations.

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Lymphoma **Abstract P101**

HIGH FREQUENCY OF ATYPICAL SEROLOGICAL PATTERNS IN KENYAN INFANTS WITH PRIMARY EBV INFECTION

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Abstract

It has been hypothesized that infection of infants by EBV, while the immune system is immature, may be an important pre-disposing factor for endemic Burkitt's lymphoma. To investigate the immune response to primary EBV infection in infants, detailed serological studies were conducted in a longitudinal infant cohort from two sites in Kenya. Blood samples were taken from 1 month through 2 years of age. Luminex & ELISA were used to measure levels of IgM, IgG and IgA to VCA, EBNA1, EAd and Zta. EBV DNA levels were measured by Q-PCR. Independent of site 21% of 152 infants had atypical serological patterns, including infants with an incomplete seroconversion at first, followed by normal seroconversion at later age, and infants with multiple peaks in VCA IgM and EBV DNA, and slow increase in VCA-IgG and EBNA1-IgG. A classical seroconversion pattern was observed in 51% of infants studied starting with detection of EBV DNA and VCA-IgM, concurrent with or followed by VCA-IgG and sometimes IgG EAd, with EBNA1-IgG appearing later. Interestingly, in 28% of infants, a classical serological pattern preceded the appearance of DNA. In contrast to primary EBV infection in adults with IM, most infants also developed a high VCA-IgA and EBNA1-IgA response. In summary, these data demonstrate a very diverse pattern of EBV serology in young infants, suggesting an important influence of age on the immune response to EBV. Studies are ongoing to further understand this response, including antibody function and repertoire.

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Lymphoma **Abstract P102**

EXPRESSION AND FUNCTION OF LFA-1 AND ICAM-1 IN NASAL NK/T CELL LYMPHOMA CELLS

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Abstract

Nasal NK/T-cell lymphoma is characterized by progressive necrotic lesion in nasal cavity. The prognosis is very poor with progressive infiltration in local lesion and distant organs. We have already reported that Epstein-Barr virus (EBV) was present in the lymphoma cells, and increased the expressions of IL-9, IL-10 and IP-10, resulting in affecting the lymphoma progression. However, the characteristics have not been fully understood yet.

LFA-1 (Leukocyte Function-associated Antigen-1) on NK-cells reportedly enhanced activation and proliferation after binding of ICAM-1 (Intercellular Adhesion Molecule-1) on target cells during cell killing. We examined the expression and function in EBV positive nasal NK/T-cell lymphoma cell line (SNK6) and EBV negative NK-cell line (KHYG-1). Moreover, because we have already reported existence of soluble ICAM-1 in serum form nasal NK/T-cell lymphoma patients, soluble ICAM-1 was also measured in culture supernatant of SNK6 cells.

Flow cytometric analysis revealed that LFA-1 and ICAM-1 were expressed on both SNK6 and KHYG-1 cells. ELISA analysis revealed that soluble ICAM-1 was detected in culture supernatant of SNK6 cells, but not in supernatant of KHYG-1. Moreover, MTS assay showed that proliferation potency of the SNK6 cells decreased by LFA-1 blocking antibody. Immunohistochemical analysis on paraffin sections of biopsy samples revealed that LFA-1 was expressed on tumor cells. According to our results, SNK6 cells may combine by using LFA-1 and ICAM-1 and enhanced proliferation in each other.

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Lymphoma **Abstract P103**

AN INVESTIGATION OF LMP2A IN HUMAN GERMINAL CENTRE B CELLS

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Abstract

Although previous studies have suggested that the latent membrane protein 2 (LMP2) could be important for the EBV-induced transformation of germinal centre (GC) B cells, the contribution of this viral gene to the pathogenesis of EBV-associated lymphomas, such as Hodgkin's lymphoma (HL), remains unknown. It has been shown previously in cell lines and in murine models that LMP2a can mimic B cell receptor (BCR) functions, but so far the effects of LMP2a and BCR signalling have not been investigated in primary human GC B cells, the putative progenitor cells of cHL. We developed a transfection based method that enables the study of gene expression and signalling mediated by LMP2a in purified GC B cells. Consistent with previous reports on cell lines and in transgenic mice, we observed that LMP2A induced a global down-regulation of B cell lineage genes, including B cell signalling components, as well as affecting the expression of genes involved in antigen presentation. Many of the LMP2 target genes were also modulated in GC B cells by BCR cross-linking, demonstrating the overlapping functions of LMP2a and BCR in this cellular context. Remarkably, B cell specific genes regulated by LMP2a showed an overlap with LMP1 targets in GC B cells. These observations support the hypothesis that EBV latent proteins contribute to transformation by bypassing critical checkpoints in B cell development. It also emphasises the importance of using primary human GC B cells when investigating the contribution of viral and cellular genes to the pathogenesis of GC-derived lymphomas.

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Lymphoma **Abstract P104**

EPSTEIN-BARR VIRUS-ENCODED BCL-2 HOMOLOGUE FUNCTIONS AS A SURVIVAL FACTOR IN WP-RESTRICTED BURKITT LYMPHOMA CELL LINE P3HR-1

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Abstract

EBV-positive BL tumors display two latent forms of infection. One is referred to as latency I infection, in which EBV expresses only the virus genome maintenance protein EBNA1. The other is referred to as Wp-restricted latency and was recently identified in a subset of BL tumors. In Wp-restricted BLs, EBV expresses EBNA1, EBNA3A, EBNA3B, EBNA3C, a truncated form of EBNA-LP, and the viral Bcl-2 homologue BHRF1, all of which are driven by the BamHI W promoter (Wp). To investigate the role of EBV in Wp-restricted BL, we conditionally expressed a dominant-negative EBNA1 (dnEBNA1) mutant which interrupts the virus genome maintenance function of EBNA1 in the P3HR-1 BL cell line. Induction of dnEBNA1 expression caused loss of the EBV genome and resulted in apoptosis of P3HR-1 cells in the absence of exogenous apoptosis inducers, indicating that P3HR-1 cells cannot survive without EBV. Stable transfection of the BHRF1 gene into P3HR-1 cells rescued the cells from the apoptosis induced by dnEBNA1 expression, whereas stable transfection of truncated EBNA-LP, EBNA3A, or EBNA3C did not. Moreover, knockdown of BHRF1 expression in P3HR-1 cells resulted in increased cell death. These results indicate that EBV is essential for the survival of P3HR-1 cells and that BHRF1 functions as a survival factor. Our work suggests the critical requirement for BHRF1 in the maintenance of Wp-restricted BL cells. Therefore, removal of the EBV genome from cells by inhibiting EBNA1 function could be an effective and attractive therapeutic approach for the treatment of Wp-restricted BL tumors.

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Lymphoma **Abstract P105**

THE EFFECT OF IL4 AND IL21 ON EBV POSITIVE DIFFUSE LARGE B-CELL LYMPHOMA CELL LINES

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Abstract

Diffuse Large B-cell lymphomas (DLBCLs), the most common group of malignant lymphomas, account for 30% of adult non-Hodgkin lymphomas (NHLs). Epstein-Barr virus (EBV) positive DLBCL of the elderly is a new subtype of DLBCL.
IL4 and IL21 are homologous cytokines produced by activated CD4+T cells and NKT cells which can induce (Latent Membrane Protein 1) LMP1 expression in EBV positive non DLBCL lymphoma lines.
To investigate the role of cytokines I.E. IL4 and IL21 in the modulation of EBV gene expression in EBV positive DLBCL cell lines and to explore the role of EBV in EBV positive DLBCL cell lines, we characterized five DLBCL cell lines by checking the EBV gene and cellular gene expression. Cytokine treatment experiments were performed to check the modulation of EBV gene and cellular gene expression, chemokine receptor expression, cell migration, apoptosis and proliferation. After IL21 treatment, EBNA2 and EBNA1 expression was down-regulated while LMP1 was up-regulated in the characterized EBV positive DLBCL line, i.e. Farage. Both IL4 and IL21 treatment induced changes in chemokine receptor expression and cell migration. EBV was essential in cell proliferation, apoptosis and chemotactic responses in the characterized EBV positive DLBCLs. IL4 and IL21 can modulate EBV gene and cellular expression in EBV positive DLBCL cell lines.

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Lymphoma **Abstract P106**

INTERFERON REGULATORY FACTOR-4 (IRF-4) TARGETS IRF-5 FOR REGULATION OF EBV TRANSFORMATION

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Abstract

Epstein-Barr virus (EBV) infection is an important cause of lymphomas in AIDS patients, especially in central nervous system lymphoma (CNS lymphoma). Interferon regulatory factor-4 (IRF-4) is a member of the IRF family with oncogenic potential. We have established previously that IRF-4 is essential for EBV transformation of human B cells in vitro, and possibly involved in CNS lymphomas in vivo (Xu et al., 2008; J Virol.82:6251-8). The mechanism of IRF-4 in the regulation of EBV transformation has been investigated in this study. We find that knockdown of IRF-4 leads to high expression of IRF-5, a pro-apoptotic member in IRF family. Over-expression of IRF-4 represses the expression of IRF-5. In addition, knockdown of physiological levels of IRF-5 enhances cell growth and rescues IRF-4-knockdown-mediated growth inhibition in EBV-transformed cells. IRF-5-overexpression alone is sufficient to induce cellular growth inhibition and apoptosis. Therefore, IRF-4 targets IRF-5 to regulate cellular growth in EBV-transformed cells. Because IRF-5 is also associated with EBV transformation and represses LMP-1 expression, it is apparent that EBV uses the expression ratio of IRF4/IRF-5 to control the infected cell to proliferation or apoptosis during EBV transformation. This dynamic equilibrium maybe used for EBV's own benefits in vivo in various microenvironments. This work expands our understanding of EBV transformation processes and the pathogenesis of EBV-associated tumors.

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Lymphoma **Abstract P107**

EXPRESSION OF CD70 IN NASAL NATURAL KILLER/T-CELL LYMPHOMA

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Abstract

Extranodal , nasal NK/T-cell lymphomas are regularly Epstein-Bar virus (EBV)-positive, with a type II latency pattern and has distinct clinical and histological features. In this study, we examined the genes expressed specifically by SNK-6, SNK-1 and SNT-8 cells, which were established from nasal NK/T-cell lymphomas by cDNA array. These three cell lines were EBV-positive lines. CD70 was specifically expressed in SNK-6 and SNT-8 by cDNA array analysis. CD70 is the only known ligand for its receptor CD27, and both belong to the tumor necrosis factor receptor superfamily 7. In normal cells CD70 is expressed on lymphocytes, mainly restricted to activated B cells and T cells. The functions of CD70 are not known well. By CD27-CD70 interaction, CD27 implicate in induction of apoptosis or cell differentiation, proliferation and survival in CD27 positive cells. CD70 can also operate as receptor and might contribute to progression of B-cell malignancies.
We analyzed CD70 and its receptor CD27 mRNA expression by RT-PCR in nasal NK/T-cell lymphomas and the other lymphomas. CD70 mRNA was expressed in nasal NK/T-cell lymphoma but CD27 mRNA was not expressed. We also analyzed the protein expression of CD70 and CD27 by western blots and flowcytometry methods, CD70 protein was expressed in nasal NK/T-cell lymphoma and its surface but CD27 protein was not expressed. Then, we analyzed the expression of CD70 in clinical specimens by immunohistochemical stainings. We report the meanings of CD70 expression in nasal NK/T-cell lymphoma.

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Lymphoma **Abstract P108**

TLR9 TRIGGERING IN BURKITT'S LYMPHOMA CELL LINES SUPPRESSES THE EBV *BZLF1* TRANSCRIPTION VIA HISTONE MODIFICATION

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Abstract

Chronic co-infection with Epstein-Barr virus (EBV) and the malaria pathogen *Plasmodium falciparum* is strongly associated with endemic Burkitt's lymphoma in equatorial Africa. The interaction and contribution of both pathogens in the oncogenic process are poorly understood. Earlier, we showed that innate immune activation with a synthetic Toll-like receptor 9 (TLR9) ligand suppresses the initiation of EBV lytic replication in primary human B-cells. Here, we investigate the mechanism involved in the suppression of EBV lytic gene expression in Burkitt's lymphoma cell lines. We demonstrate that suppression of EBV lytic gene expression is dependent on functional TLR9 and MyD88 signaling but independent of downstream signaling elements including PI3K, MAPKs, and NF-kB. We identify histone modifications triggered by TLR9 activation to negatively regulate the promoter of EBV's master regulatory lytic gene *BZLF1*. Finally, we show that *P. falciparum's* hemozoin, a natural TLR9 ligand, suppresses EBV lytic gene activation in a dose-dependent fashion. Thus, we provide molecular evidence for a possible interaction between EBV and *P. falciparum* at the B-cell level. The repression of EBV lytic reactivation and the resulting reinforcement of latent EBV infection in B-cells by chronic TLR signaling might unleash EBV's unique oncogenic potential.

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Lymphoma **Abstract P109**

DUAL FUNCTIONS OF INTERFERON REGULATORY FACTORS 7C IN EBV-MEDIATED TRANSFORMATION OF HUMAN B LYMPHOCYTES

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Abstract

Epstein-Barr virus (EBV) infection is associated with several human malignancies. Interferon (IFN) regulatory factor 7 (IRF-7) has several splicing variants, and at least the major splicing variant (IRF-7A) has oncogenic potential and is associated with EBV transformation processes. IRF-7C is an alternative splicing variant with only the DNA-binding domain of IRF-7. In this report, we prove the existence of IRF-7C protein and RNA in certain cells under physiological conditions, and find that high levels of IRF-7C are associated with EBV transformation of primary B cells in vitro as well as EBV type III latency. Latent membrane protein 1 (LMP-1) stimulates IRF-7C expression. IRF-7C has oncogenic potential and partially restores the growth properties of EBV-transformed cells under a growth-inhibition condition. A tumor array experiment has identified six primary tumor specimens with high levels of IRF-7C protein-all are lymphomas. Furthermore, IRF-7C is closely associated with other IRF-7 splicing variants. IRF-7C inhibits the function of IRF-7 in transcriptional regulation of IFN genes. These data suggest that EBV may use splicing variants of IRF-7 for its transformation in two strategies: to use oncogenic properties of various IRF-7 splicing variants, but use one of its splicing variants (IRF-7C) to block the IFN-induction function of IRF-7 that is detrimental for viral transformation. The work provides a novel relation of host/virus interactions, and expands our knowledge about IRFs in EBV transformation.

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Posters P110-P130

Nasopharyngeal carcinoma and gastric cancer

Nasopharyngeal carcinoma and gastric cancer **Abstract P110**

CHARACTERIZATION OF EPSTEIN-BARR VIRUS GP350/220 GENE VARIANTS IN VIRUS ISOLATES FROM GASTRIC CARCINOMA AND NASOPHARYNGEAL CARCINOMA

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Abstract

The gp350/220, transcribed from the BamHI-L fragment of EBV, is the dominant protein in the extracellular virus envelope and critical for EBV binding to B lymphocytes. EBV gp350/220 is an extensively glycosylated polypeptide (907 residues). In the N-terminal region, three distinct domain (4–153,165–305 and 317–426) form an "L"-shaped arrangement in which located three peptide binding CR2. The C-terminal region comprises extra-cellular, transmembranous (859-888), and intracellular domains (889-907). A long repeat region (508–641) was located in the splicing region of gp350/gp220. To characterize the variations of the gp350/220 and to explore the association between gp350/220 variations and EBV associated diseases, we analyzed the gp350/220 gene in totally 157 EBV-positive samples, including 41 EBV-associated gastric carcinoma (EBVaGC), 81 nasopharyngeal carcinoma (NPC) biopsies and 35 throat washing (TW) samples from healthy donors (all of the samples were collected from Northern China, a non-endemic area of NPC). The N-terminal region and C-terminal transmembranous and intracellular domains were highly conserved. Most mutations and deletions located at the repeat region and C-terminal extra-cellular domain. In the N-terminal region, samples were classified into two group named types a/b , which have a close corresponding relationship with types A/B (also referred to as type 1/type 2) and the C-terminal region is lack of this divergence. Strong conservation in the peptides binding to B cells and the CTL epitopes confirm its function against EBV infection. In addition, no evidence suggested that particular gp350/220 types were preferentially associated with EBVaGC or NPC in this study.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P111**

CLINICOPATHOLOGIC CHARACTERISTICS AND EPSTEIN-BARR VIRUS GENOME POLYMORPHISMS OF EPSTEIN-BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA IN GASTRIC REMNANT CARCINOMA IN GUANGZHOU, SOUTHERN CHINA, AN ENDEMIC AREA OF NASOPHARYNGEAL CARCINOMA

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Abstract

To investigate the clinicopathologic characteristics, Epstein-Barr virus (EBV) latency pattern and genome polymorphisms of EBV-associated gastric carcinoma (EBVaGC) in gastric remnant carcinoma (GRC) in Guangzhou, where nasopharyngeal carcinoma (NPC) is endemic, an *in situ* hybridization assay of EBV-encoded small RNA-1 (EBER-1) was used to identify the presence of EBV in 33 consecutive GRC cases. EBV-encoded proteins EBNA1, EBNA2, LMP1, LMP2A and ZEBRA were detected by immunohistochemistry. EBV genome polymorphism was also analyzed by PCR and DNA sequencing. Of the 33 cases, 9 EBV-positive cases (27.3%) were identified. All cases were male and with the histology of diffuse-type carcinoma. The tumor cells expressed EBNA1 (88.9%) and LMP2A (66.7%) but not LMP1, EBNA2 and ZEBRA. Thus, the EBV latency pattern was type I. In the EBV genome polymorphism analysis, type A strain, prototype F, type I, mut-W1/I1, XhoI- and del-LMP1 variants were predominant among EBVaGC patients, accounting for 8 (88.9%), 8 (88.9%), 9 (100%), 7 (77.8%), 5 (55.6%) and 8 (88.9%) cases, respectively. The proportion of EBVaGC in GRC was significantly higher than that in conventional gastric carcinoma (CGC), while the clinicopathologic characteristics, EBV latency pattern and genome polymorphisms of EBVaGC in GRC were similar to those in CGC in Guangzhou (J Med Virol. 2010;82(4):658-67.), suggesting that EBV may play an important casual role in the carcinogenesis of GRC, and EBV may act with other factors such as injuries of gastric mucosa and changes of the microenvironment within the stomach in the development of GRC.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P112**

N-METHYL-*N*'-NITRO-*N*-NITROSOGUANIDINE (MNNG) INDUCES AND COOPERATES WITH TPA/SB TO ENHANCE EPSTEIN-BARR VIRUS REACTIVATION AND GENOME INSTABILITY IN NASOPHARYNGEAL CARCINOMA CELLS

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Abstract

Epstein-Barr virus infects almost 90% of human population and has been associated with many human cancers including Burkitt's lymphoma and nasopharyngeal carcinoma (NPC). *N*-nitroso compounds are chemical carcinogens in preserved foodstuffs and have been implicated as risk factors contributing to the development of NPC. Here, we demonstrate that reactivation of EBV was increased with increasing concentrations of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), a mutagenic and carcinogenic nitrosamide. Although MNNG at a single non-toxic concentration (0.1mg/ml) did not induce discernible reactivation of EBV, repeated treatment with this concentration of MNNG significantly induced viral reactivation. Furthermore, low dose MNNG (0.1mg/ml) had a synergistic effect with 12-*O*-tetradecanoylphorbol-1, 3-acetate (TPA) and sodium butyrate (SB) (10 ng/ml and 0.75 mM, respectively) on EBV reactivation.

Although MNNG does not activate the EBV immediate early gene promoters, Rp and Zp directly, it enhances the transcriptional activity of Rta on Rp and Zp. Using siZta to block EBV reactivation, the reactivation of EBV and concomitant induction of genome instability were diminished in a dose-dependent manner, indicating that reactivation of EBV is critical for enhancing genome instability. Taken together, these data provide evidence for the cooperation between chemical carcinogens and EBV infection in the enhancement of genome instability. Although the development of NPC seems to be very complicated and the precise roles played in carcinogenesis by *N*-nitroso compounds in foods and EBV remain controversial, our findings, based on the studies with MNNG, offer a possible mechanism by which *N*-nitroso compounds induces reactivation of EBV and contribute to malignant progression by enhancing genome instability in NPC cells.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P113**

SUBEROYLANILIDE HYDROXAMIC ACID MEDIATES ENHANCED APOPTOSIS IN NASOPHARYNGEAL CARCINOMA THROUGH INDUCTION OF EPSTEIN-BARR VIRUS LYTIC CYCLE AND ACTIVATION OF CASPASE-3

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Abstract

Epstein-Barr virus (EBV) persists in tightly latent form in nasopharyngeal carcinoma (NPC) evading immune surveillance. Induction of EBV lytic cycle will lead to expression of a much larger number of viral proteins which may serve as potential therapeutic targets for the cancer. We previously showed that suberoylanilide hydroxamic acid (SAHA), a FDA-approved histone deacetylase inhibitor, could strongly induce viral lytic cycle in EBV-positive gastric carcinoma cells. In this study, we tested SAHA for its ability to induce EBV lytic cycle in NPC cell lines including HONE1-EBV, HK1-EBV, HA and NA. Following treatment with 5-10 μM SAHA, increased replication of EBV DNA (8-70 folds), expression of immediate early (Zta and Rta), early (BMRF1) and late (gp350/220) viral lytic proteins (up to 60% of cells expressing BMRF1) and production of infectious viral particles were observed in all four NPC cell lines. Furthermore, enhanced killing of EBV-positive NPC cells, compared with that of EBV-negative counterparts, was demonstrated by MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Higher percentage of EBV-positive than EBV-negative NPC cells expressing annexin V suggested that enhanced killing of EBV-positive NPC cells was related to apoptosis. Increased expression of cleaved poly(ADP-ribose) polymerase (PARP) and cleaved caspase-3 in EBV-positive compared with that in EBV-negative NPC cells indicated that enhanced apoptosis was mediated by activation of caspase-3. In conclusion, SAHA is a potent inducing agent of EBV lytic cycle and can mediate enhanced apoptosis of NPC cells.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P114**

CELL LINE C666-1 AS A MODEL FOR INVESTIGATING DYSREGULATION OF CELLULAR SIGNALLING PATHWAYS IN NASOPHARYNGEAL CARCINOMA

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Abstract

Carcinogenesis commonly involves extensive genetic changes and perturbation of cellular gene expression profiles in tumour cells. Using microarray analysis of RNA extracted from microdissected epithelial cells from a series of frozen biopsies of nasopharyngeal carcinoma (NPC), normal nasopharynx and tonsil, we identified alterations in key signalling pathways that regulate epithelial cell growth and differentiation, including the Wnt/β-catenin, Hedgehog and TGF-beta pathways. The cell line C666-1 is the only NPC-derived line that consistently maintains the EBV genome in culture and its overall gene expression profile is very similar to that of authentic NPC tumours. Similarly, tonsillar epithelial cells are a good model for normal nasopharyngeal epithelial cells. For these reasons C666-1 was chosen as our model system for further investigation of the TGF-β pathway *in vitro*. Using RT-PCR and Q-PCR we validated array-predicted changes in the expression levels of a number of TGF-β pathway-related genes in C666-1 cells compared with early passage, primary tonsillar epithelial cells. *In vitro* investigation of TGF-β signalling in C666-1 cells revealed that the TGFBR2 receptor is absent in this cell line. Thus activin A, but not TGF-β1, induced Smad-responsive reporter activity in a dose-dependent manner. In addition, activin A but not TGF-β1, induced phosphorylation of Smad2 and upregulated the TGF-β-responsive genes, SERPINE1 (PAI1) and CDKN1A (p21) in C666-1. However activin did not appear to affect cell growth control. These findings suggest that activin-mediated cell growth inhibition is defective in these cells.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P115**

NASOPHARYNGEAL CARCINOMA (NPC) ENHANCER, NOLC1, IS REQUIRED FOR TP53 TO REGULATE MDM2 EXPRESSION

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Abstract

Nasopharyngeal carcinoma (NPC) is one of the common cancers among Chinese living in South China, Singapore, and Taiwan. In order to elucidate the global gene expression in NPC tumorigenicity, we used the PCR-selected cDNA subtractive hybridization and the microarray analysis of genome-wide parallel analysis to investigate the differential gene expression between NPC cell lines and normal nasomucosal cells in primary culture. One up-regulated gene NOLC1 (nucleolar and coiled-body phosphoprotein 1, also called hNOPP140) was identified. Western blot and Q-RT-PCR analyses showed that the NOLC1 gene is expressed in abundance in a majority of NPC cells lines and is very weak or undetectable in normal epithelial cells. In NOLC1-shRNA transfected NPC cells, the scratch test for cell migration ability, and cell invasion ability were all inhibited. Q-RT-PCR analysis of the expression levels of genes related to tumor growth in NOLC1-shRNA-NPC transfectants revealed marked suppression of mdm2, MMP9 and VEGF expression, and up-expression of TNF-α, BAX and CASP1 genes. However, NOLC1 did not alter TP53 and p21 WAF/CIPI expression. Severe combined immunodeficiency mice bearing NPC xenografts derived from NOLC1-siRNA-transfectant were found to have 82% lower levels of tumor growth than that of control mice. The co-transfection and chromatin immunoprecipitation experiments suggest that TP53 regulated MDM2 expression requires the NOLC1 co-activation. The EBV-infected NPC cell lines with wild type TP53 showed no significant alteration of NOLC1 gene expression. It is concluded that NOLC1 may play a role as a tumor enhancer in NPC pathogenesis; in addition, TP53 requires NOLC1 to co-activate TP53-regulated downstream genes.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P116**

EBV-ENCODED BAMHI-A RIGHTWARD FRAME-1 (BARF1) GENE SEQUENCE ANALYSIS IN INDONESIAN NASOPHARYNGEAL CARCINOMA

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Abstract

BARF1 is a carcinoma-specific EBV-encoded oncogene. We describe the BARF1 sequence diversity in nasopharyngeal carcinoma (NPC), other EBV-related diseases and Indonesian EBV carriers in relation to EBV genotype, viral load and serology markers. Nasopharyngeal brushings from 56 NPC cases, blood/tissue from 15 other EBV-related disorders and spontaneous B-lymphocyte cell lines (LCL) from 5 Indonesian healthy individuals were analysed by PCR-direct sequencing. Most NPC isolates revealed specific BARF1 nucleotide changes compared to the prototype B95-8 virus. At the protein level they resulted in 3 main substitutions (V29A, W72G, H130R), which are not considered to cause gross tertiary structure alterations in the hexameric BARF1 protein. At least one codon conversion was detected in 80.3% of NPC samples compared to 33.3% of non NPC samples ($p< 0.001$) and 40.0% of healthy LCLs ($p= 0.074$). NPC isolates showed more frequent mutation than non NPC samples. EBV genotyping revealed most isolates as type 1. The viral load of NPC and non NPC samples was high, but only in non NPC group it related to BARF1 variant. Serology on NPC sera using IgA/EBNA-1 ELISA, IgA/VCA-p18 ELISA, and immunoblot score showed no relation with BARF1 mutation ($p= 0.802$, 0.382, and 0.058). NPC patients had variable antibody reactivity against purified hexameric NPC-derived BARF1 irrespective of the endogenous BARF1 sequence. The sequence variation of BARF1 observed in Indonesian NPC patients and normals may reflect a natural selection of EBV strains unlikely to be predisposing to carcinogenesis. The conserved nature of BARF1 may reflect an important role in EBV persistence.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P117**

MULTIPLE DNA METHYLATION AS SELECTIVE INDEPENDENT MARKER FOR EARLY DETECTION OF NASOPHARYNGEAL CARCINOMA IN A HIGH RISK POPULATION

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Abstract

Undifferentiated nasopharyngeal carcinoma (NPC) is strongly related to EBV infection, allowing aberrant antibodies against EBV and viral DNA load to be a screening tool in high risk populations. Methylation analysis in the promoter of tumor suppressor genes (TSGs) may serve as complementary marker in identifying early cases. This study determined methylation status of multiple TSGs and evaluated whether it may improve early detection. Nasopharyngeal brushing were taken from 53 NPC patients, 22 high risk patients, and 25 normal EBV carriers. Corresponding NPC-paraffin tissue was also included. DNA was bisulfite-modified preceding amplification using methylation-specific PCR. Ten tumor suppressor genes were studied. Most of NPC-paraffin and -brushing DNA (85.7%) revealed a concordant result so that methylation detection was determined using either one of both sampling. NPC group showed high frequency of methylated genes (DAPK1 79.2%, CDH13 77.4%, DLC1 76.9%, RASSF1A 75.5%, CADM1 69.8%, p16 66.0%, WIF1 61.2%, CHFR 58.5%, RIZ1 56.6% and RASSF2A 29.2%). High risk individuals, demonstrating elevated reactivity of IgA against EBV and viral load, showed high frequency of methylated genes of CDH13 (72.7%), DAPK1 (40.9%), DLC1 (40.9%) and CADM1 (36.4%), low methylation of p16 (22.7%) and WIF1 (18.2%) and undetectable methylation of RASSF1A, CHFR, RIZ1 and RASSF2A. Healthy individuals showed similar pattern as high risk population. Using a panel of markers (RASSF1A, p16, WIF1, CHFR and RIZ1) the methylation detection in NPC cases was improved. This multiple promoter methylation is proposed as selected marker in distinguishing NPC from non NPC individuals for early screening in combination with EBV-based markers.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P118**

A NEW EBV SEROLOGIC SCREENING PROGRAM FOR CONTROLLING NPC IN A HIGH INCIDENCE AREA, ZHONGSHAN CITY

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Abstract

Objective. To evaluate the efficacy of a new EBV serologic screening program for early NPC detection;
Methods. To screen the serum levels of EBV antibody, EBNA1 IgA and VCA IgA, among 15,000 adults age 30 to 59 years old residing in a community in Zhongshan City (population ~65,000); To stratify the subjects of 15,000 adults into high, moderate and normal NPC risk groups by regression analysis of the levels of the EBV antibody, which were defined as having regression coefficients of <0.65, 0.65-0.98 and >0.98, respectively. To conduct clinical examination of subjects in the high risk group, endoscopy and biopsy were performed subsequently. **Results.** Of 6798 subjects screened by EB virus serology in the period of August 2009 and March 2010, 174 were identified as high risk population. The clinical examination conducted subsequently, 31 suspected cases were confirmed. Eleven out of and 31suspected cases were confirmed to be nasopharyngeal carcinoma histopathologically. Based on clinical and MRI assessment, three of the 11 cases were of Stage I and 8 Stage II（UICC 2002）. The detection rate of NPC is 162 per 100,000 population. **Conclusion.** EB virus serologic screening plus risk assessment could effectively identify the high risk population of suffering nasopharyngeal carcinoma and detect NPC cases, particularly those at early stages.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P119**

LMP2A PROMOTES TUMOUR GROWTH IN A XENOGRAFT MODEL OF NASOPHARYNGEAL CARCINOMA VIA DEREGULATION OF MIRNAS

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Abstract

The Epstein-Barr virus Latent Membrane Protein 2A (LMP2A) is an integral membrane protein of Epstein-Barr virus (EBV) which is commonly expressed in nasopharyngeal carcinoma (NPC). To study the role of LMP2A in NPC, the EBV negative NPC cells, HK1, was stably transduced with LMP2A. HK1/LMP2A cells showed increased phophorylation of Akt indicating the activation of the Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/Akt) pathway and decreased apoptosis on treatment with cis-platinum. When tumourigenecity of HK1/LMP2A cells was evaluated by subcutaneous xenografting into nude mice, it was found that HK1/LMP2A cells lead to significantly larger tumours as compared to the controls. To determine the underlying mechanisms, microarray analysis and microRNA profiling for the xenograft tumours were performed. Gene ontology (GO) analysis showed that the GO terms cell cycle, chromosome segregation and DNA repair were significantly overrepresented among the genes upregulated in HK1/LMP2A tumours as compared to controls. Of these, overexpression of *Cdk1/cdc2*, *TYMS* and *LUM* in HK1/LMP2A tumours were validated by real time PCR. In addition, upregulation of miR-18a and downregulation of miR-30a, miR-143 and miR-145 were observed in HK1/LMP2A tumours as compared to the controls. With bioinformatics analysis that combined the gene expression data, miRNA profiling data and TargetScan prediction, 83 putative targets of miR-30a, miR-143 and miR-145 were identified. Under pathway analysis, these genes were shown to be involved in the regulation of cell cycle, DNA damage checkpoint and proliferation. Our work suggests that LMP2A promotes NPC tumour growth via deregulation of miRNAs.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P120**

ESTABLISHMENT OF DOXYCYCLINE-INDUCIBLE SYSTEM FOR EBV LYTIC CYCLE REACTIVATION IN NASOPHARYNGEAL CARCINOMA CELLS

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Abstract

EBV Rta/BRLF1 is an immediate-early transcription factor that controls the conversion of the latent viral genome into one undergoing lytic replication. In previous studies, ectopic expression of EBV Rta in NPC-TW01 and HONE1 cells potently triggered the cellular senescence process, as evidenced by increasingly detected $p21^{CDKN1A}$, $p27^{CDKN1B}$, and senescence associated β-galactosidase (SA-β-Gal) staining. Here, to maximize the homogeneity and percentage of NPC cells expressing EBV Rta, we report a newly established line, designated as TW01TetER, by using ViraPower™ T-REx™ System (Invitrogen). Specifically, in response to doxycycline treatment, EBV Rta is conditionally synthesized from a recombinant lentiviral unit stably transduced into the host genome of TW01TetER cells. Preliminary results showed that, similar to previously described 293TetER cells, EBV Rta exerted to arrest the growth of TW01TetER via down-regulation of MYC. The changes of other cell cycle regulators in doxycycline-treated TW01TetER cells are intensively under investigation. Furthermore, we will transfer EBV bacmid p2089 into TW01TetER cells. Reactivation, lytic cycle progression, and reproduction of infectious EBV virions by doxycycline-induced EBV Rta in the TW01TetER /p2089 cells will be systematically examined.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P121**

SYNERGISTIC EFFECTS OF HYPOXIA CONDITION AND EPSTEIN BARR VIRUS LATENT MEMBRANE PROTEIN1 ENHANCE EXPRESSION OF SIAH1, AND HIF1-ALFA IN NASOPHARYNGEAL CARCINOMA PATIENTS

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Abstract

We previously have shown the evidence a principal oncoprotein of Epstein Barr virus (EBV), latent membrane protein 1(LMP1) stabilizes hypoxia inducible factor 1-alfa (HIF1-alfa) via stabilization by Seven in absentia homolog 1 (Siah1) in nasopharyngeal epithelial cell lines. However whether these factors express in clinical nasopharyngeal tissues is still unknown. Eighty-one biopsy samples from nasopharyngeal carcinoma (NPC) patients, we investigated expressions of LMP1, HIF1-alfa, and Siah1 by immunohistchemical analysis. In the analysis, we found that: (i) the expression of Siah1 was correlated with LMP1 strongly ($r = 0.805$, $p < 0.001$). (ii) The expression of LMP1, Siah1, and HIF1-alfa showed correlation with T classification. (iii) Only HIF1-alfa expression showed correlation with lymph node metastasis. Since only HIF1-alfa looks most important factor, we hypothesis not only LMP1 inducible stimulation but hypoxia condition itself also affects HIF1-alfa expressions in NPC tissues. To prove this hypothesis, we investigated with LMP1 expressed nasopharyngeal cell lines with or without hypoxia stimulation with hypoxia chamber. Synergistic effect of hypoxic conditions and LMP1 expression increased their HIF1-alfa expression. The crosstalk of hypoxia and LMP1 expression may be a key of understanding tumor invasion and metastasis of NPC.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P122**

EBV ZTA INDUCES IMMUNE MODULATORS FROM NASOPHARYNGEAL CARCINOMA CELLS

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Abstract

Epidemiologic studies have suggested a link between EBV reactivation and nasopharyngeal carcinoma (NPC), an epithelial malignancy featured by intensive leukocyte infiltration and strong local immunosuppression. How EBV lytic proteins affect local immune response in NPC remains unclear. Our previous study has shown that an EBV lytic transactivator Zta can induce a potent chemokine interleukin-8 (IL-8) in NPC cells. In this study we further search for other Zta-induced immune modulators in the cancer cells by using cDNA microarray and cytokine antibody array. We first reveal that Zta upregulates expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) in NPC cells. Zta binds to and transactivates the GM-CSF promoter, elevating the mRNA level and secretion of this cytokine. Zta also induces expression of cyclooxygenase-2 (COX-2) and increases secretion of its downstream product prostaglandin E_2 (PGE_2) from NPC cells. Knockdown of COX-2 or treatment with a COX-2 inhibitor blocks Zta-mediated induction of PGE_2. Interestingly, culture supernatants of the NPC cells expressing Zta or lytically infected with EBV enhance production of interleukin-10 (IL-10) from human monocytes and macrophages. Further functional study shows that GM-CSF and PGE_2 synergistically promote secretion of IL-10 from monocytes, while PGE_2 alone sufficiently increases IL-10 secretion from macrophages. Our results suggest that, through Zta-induced immune modulators, EBV-infected NPC cells may direct infiltrating myeloid immune cells to secrete the immunosuppressive cytokine IL-10, facilitating a tumor microenvironment that favors evasion from anti-cancer or anti-viral immunity.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P123**

THE EFFECTS OF LYTIC REACTIVATION ON DISTINCTIVE EBV-INFECTED NASOPHARYNGEAL (NP) EPITHELIAL CELLS UNDER THE INFLUENCE OF LYTIC-INDUCING AGENTS

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Abstract

Epstein-Barr virus (EBV) is commonly known to establish latent infection in various cell types. However, lytic transition of EBV can be initiated by upregulating *BZLF1* leading to the production of infectious viral particles. In this study, lytic-infections of EBV-infected cells with distinctive characteristics under various treatments were investigated. The cell lines involved are HONE1-EBV (undifferentiated NPC), HK1-EBV (differentiated NPC), and NP460hTert-EBV (telomerase-immortalized NP epithelial cell). These cell lines were treated with Tetradecanoyl Phorbol Acetate (TPA), Tumour Necrosis Factor-alpha (TNF-α), Tumour Growth Factor-beta (TGF-β) and *BZLF1* plasmid transfection. HONE1-EBV cells exhibited basal transcriptions of *BZLF1* and *BMRF1*, and were capable of producing infectious virions prior to any treatments. The transcription levels of *BZLF1*, *BMRF1* and *gp350/220* of HONE1-EBV and HK1-EBV cells were found to be increased upon TPA treatment. Moreover, exogeneous *BZLF1* transfection demonstrated a more effective lytic infection than TPA treatment. Conversely, TPA treatment failed to reactivate the EBV of NP460-EBV cells to execute a complete lytic-infection. All three cell lines were not effective upon TNF-α and TGF-β treatment. Interestingly, *BMRF1* was found to be upregulated regardless of the expression of *BZLF1* while latent gene promoter (*Qp*) transcription was suppressed in TNF-alpha treated HK1-EBV cells. In summary, HONE1-EBV produces EBV spontaneously while HK1-EBV cells requires lytic-inducing agent to produce infectious EBV virions. The lytic cyle of NP460hTert-EBV cells is normally abortive in nature. Therefore, lytic cycle reactivation of EBV in EBV-infected cells is cell-type dependent and the involvement of nasopharyngeal epithelial cellular context is essential in the EBV lytic infection.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P124**

T CELL HOMING TO NASOPHARYNGEAL CARCINOMA

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Abstract

Undifferentiated nasopharyngeal carcinoma (NPC) is a very good candidate disease for treatment with adoptive T cell transfer. Uniformly Epstein-Barr virus (EBV)+, these tumours reportedly have functional antigen processing and presentation machinery and express viral proteins that contain known cytolytic T cell target epitopes. This raises the possibility that boosting relevant EBV-specific T cell immunity in NPC patients might defeat the tumour. The persistent nature of EBV infection may also encourage the long-term survival of therapeutically administered virus-specific cells.

However, efficient delivery of tumour-specific T cells from the circulation to solid tumour tissue is a clear requirement for effective cellular therapy, yet the mechanisms by which T cells gain entry to NPC tumours have not yet been determined.

The malignant cells of NPC are usually associated with a substantial lymphoid infiltrate mainly consisting of T cells. Using unmanipulated diagnostic biopsy samples, we have characterised chemokine receptor expression (CR) on tumour-infiltrating T cells, and established whether specific chemokine ligands are detectable at the NPC tumour site. We found that functional CXCR6 and CCR5 were expressed on tumour infiltrating T cells, consistent with the presence of specific ligands for these receptors at the tumour site. Furthermore, our data suggests that effector and regulatory cells may use shared homing mechanisms to gain entry to NPC tumours.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P125**

EPSTEIN–BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA AMONG PATIENTS WITH PERNICIOUS ANEMIA

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Abstract

Background & Aims Pernicious anemia, a condition with achlorhydria, is strongly associated with gastric carcinoma. Approximately 9% of gastric carcinomas worldwide are associated with Epstein-Barr virus (EBV), making it the most frequent EBV-associated malignancy. We speculated whether gastric acidity reduces the likelihood of EBV infection of the neoplastic gastric epithelium. If so, a larger proportion of EBV-associated gastric carcinoma would be expected in patients with pernicious anemia compared with controls.
Methods Using the Danish population-based registries we performed a nationwide case-control study comparing gastric carcinoma patients with pernicious anemia (PA-GC) with those without pernicious anemia (nonPA-GC), frequency matched 1:2. Tumor tissues were reclassified by expert histopathologists blinded to pernicious anemia- and EBV status.
Results In total, 186 samples (55 PA-GC and 131 nonPA-GC) were identified. EBV-associated gastric carcinoma (EBV-GC) was more common among PA-GC compared with nonPA-GC, adjusted odds ratio (OR) = 2.94 (95% confidence interval (CI) 0.99; 8.67), p=0.05. Gastric carcinomas with signet-ring cell morphology were significantly less common in patients with PA-GC compared with nonPA-GC ($OR_{\text{signet-ring cell vs. tubular}}$ =0.05, CI 0.01;0.24). Overall, 10-year survival was similar when comparing PA-GC with nonPA-GC, and EBV-GC with GC without EBV-infection (nonEBV-GC), respectively.
Conclusions We found suggestive evidence that EBV-associated gastric carcinomas are more common among gastric carcinoma patients with pernicious anemia compared with those without.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P126**

EPSTEIN-BARR VIRUS EBNA1 DISRUPTS PML NUCLEAR BODIES IN EBV-ASSOCIATED EPITHELIAL TUMOURS

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Abstract

EBNA1 is the only Epstein-Barr virus (EBV) protein expressed in all EBV-associated tumours and we have been examining its contribution to the EBV-induced epithelial tumours, nasopharyngeal carcinoma (NPC) and gastric carcinoma (GC). We have shown that EBNA1 disrupts nuclear bodies formed by the PML tumor suppressor in NPC by inducing the degradation of PML proteins and that this effect requires EBNA1 binding to the host ubiquitin specific protease, USP7. Recently, we have shown that USP7 itself negatively regulates PML bodies by a mechanism that is independent of its catalytic activity. We have now shown that EBNA1 induces PML loss in part by increasing the association of USP7 with PML bodies. In addition, we generated NPC cells with nuclear bodies formed from only one of the six PML isoforms normally found in PML bodies. We found that EBNA1 preferentially disrupted PML IV nuclear bodies indicating the importance of this isoform for EBNA1-PML interactions.

To further understand the functional significance of these observations, we studied EBNA1 effects on PML in gastric carcinoma. We find that PML bodies are greatly reduced in cells containing EBV or expressing only EBNA1 and that EBNA1-silencing in the EBV-infected cells results in very high levels of PML bodies and proteins and decreased cell proliferation. In keeping with these observations, immunohistochemistry of GC biopsies showed that EBV-positive GC biopsies had significantly lower PML staining compared to EBV-negative samples. The data suggest that EBNA1 triggers the degradation of PML in EBV-associated epithelial tumors thereby promoting their growth.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P127**

EBV INFECTION SUPPRESSED STARVATION-INDUCED APOPTOSIS IN NASOPHARYNGEAL EPITHELIAL CELLS

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Abstract

Tumor growth *in vivo* is known to be under ischemic conditions, i.e. inadequate supply of nutrients and oxygen due to insufficient supply of blood. Nutrient insufficiency or oxygen depiction has been speculated as the *in vivo* selection force for EBV infected epithelial cells to succeed in transformation. In concordance, we have observed that EBV-infected HK1 (nasopharyngeal epithelial cell line) did not have any growth advantage *in vitro*, but could be grown into bigger tumors than the non-EBV-infected counterparts when injected subcutaneously into nude mice. To examine whether EBV infection may confer resistance to nutrient depiction, the growth factor-supplemented media for EBV-infected and EBV-non-infected HK1 and NP460hTert (premalignant nasopharyngeal epithelial cell line) were replaced with serum-free RPMI and examined for differences in growth properties. Interestingly, both the HK1-EBV and NP460hTert-EBV cells were shown to have suppressed starvation-induced apoptosis, evidenced by the suppressed expression of apoptotic markers (e.g. cleaved-PARP, cleaved-capase-3) and sustained expression of survival marker (p-Akt), when compared to their non-infected counterparts. Besides, an upregulated expression of survivin during the prolonged starvation stress was found in EBV-infected NP460hTert cells but not the EBV-non-infected ones. We further examined whether any EBV encoded gene was upregulated under the starvation stress. Transcripts of LMP1, but not EBNA1 and LMP2A, were found to be increased after nutrient withdrawal using semi-quantitative RT-PCR assay. Western blot using anti-LMP1 antibodies has confirmed the upregulation of LMP1 in EBV-infected cells under starvation stress. In conclusion, EBV infection could confer resistance towards starvation-induced apoptosis, probably through the upregulation of LMP1.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P128**

NPC: LMP1 INDUCED UPREGULATION OF HLA I MACHINERY –A CONTRADICTION WITH IMMUNOESCAPE?

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Abstract

The nasopharyngeal carcinoma (NPC) is one of the most common cancer types in endemic regions such as parts of Southern China accounting for 20% of all adult cancers. Besides genetic and environmental risk factors, EBV was declared to be involved in the causation of NPC. LMP1 appears to be partially accountable for EBVs transforming potential and is therefore considered as a classical oncogene. LMP1 exerts immune modulatory functions such as overexpression of HLA I components, which appears paradoxical in the view of immunoescape. We could confirm this with epithelial cell lines (Rhek1, SCC12F, HeLa) stably expressing LMP1. Surprisingly we could not see a difference in the expression of HLA I components in NPC histological sections comparing LMP1 positive with negative cases. However we observed a significant upregulation of cmyc in LMP1 positive NPCs, which was shown to generate a nonimmunogenic phenotype in EBV infected B cells via the downregulation of HLA I components. Could c-myc overexpression also be beneficial for EBV in NPC by introducing an immuno escape mechanism? We could show that knocking down c-myc in Rhek1 cells increased immunogenic effects of LMP1 by increased expression of components of the HLA I machinery. This leads to the suggestion that LMP1 might abate its immunogenicity by activating c-myc and therefore affecting the immune response. In this context we aim to analyse c-mycs activity as a function of LMP1 expression.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P129**

IDENTIFICATION OF FOUR-JOINTED BOX 1 (FJX1) AS A POTENTIAL ONCOGENE IN NASOPHARYNGEAL CARCINOMA

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Abstract

Nasopharyngeal carcinoma (NPC) displays a distinct geographical pattern, being especially prevalent in Southern China and South East Asia. Using gene expression microarrays, we have identified genes that are de-regulated in primary NPC tissues relative to cancer-free controls. One gene, four-jointed box 1 (FJX1), was shown to be significantly up-regulated in NPC. FJX1 is a *Drosophila* orthologue of *four-jointed* gene, acts as a Golgi-resident kinase that phosphorylates specific cadherin domains and functions downstream of the Notch pathway. The overexpression of FJX1 mRNA was validated in primary tissue samples (n=14) and the level of FJX1 protein was significantly higher in NPC tissues (42%; 17 of 39) compared to non-malignant nasopharyngeal tissues, which consistently showed negative staining (n=11; p=0.01). Interestingly, our preliminary microarray data showed that *in vitro* infection of an EBV-negative NPC cell line with a recombinant EBV resulted in the upregulation of FJX1. Both ectopic expression and siRNA-mediated knockdown experiments in NPC cell lines showed that FJX1 promotes cell proliferation, and this result was supported by the observation that the cells with depleted FJX1show reduced mRNA level of cyclin D1 and cyclin E1. We also showed that FJX1 enhanced cell motility in wound healing assays and increased the number of colonies in anchorage-independent colony formation assays. The results of the present study demonstrate that FJX1 is expressed in a subset of NPC but not in normal surface epithelium and that FJX1 might function as an oncogene in NPC by promoting cell motility and proliferation through cell cycle regulation.

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Nasopharyngeal carcinoma and gastric cancer **Abstract P130**

FOLLISTATIN-LIKE1 IS A NOVEL TUMOR SUPPRESSOR GENE IN NASOPHARYNGEAL CARCINOMA FREQUENTLY SILENCED BY PROMOTER HYPERMETHYLATION

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Abstract

Aberrant DNA methylation of CpG islands in the promoter region is well established as a common epigenetic mechanism for the silencing of tumor suppressor gene (TSG) in human cancers. To address the global profile of genes silenced by promoter hypermethylation in nasopharyngeal carcinoma (NPC) , we analyzed the global expression changes of genes reactivated by the combined treatment with the demethylating drug 5-aza-2 deoxycytidine (5-aza-dC) and the HDAC inhibitor trichostatin A in two NPC cell lines(CNE2 and HONE1). By this analysis, FSTL1 was as a target of DNA methylation, because its transcription was upreguated 21 and 17 fold in CNE2 and HONE1 respectively after the treatment. Down-regulation of FSTL1 in NPC cells and primary tumors was confirmed by semi-quantitative RT-PCR in 7 NPC cell lines, 8 primary tumor biopsies and 8 normal nasopharyngeal epithelia. By methylation specific PCR, promoter hypermethylation of FSTL1 was detected in all of the 7 NPC cell lines, and 75.5% (21/27) of NPC primary tumors, but in none of the normal epithelia. Ectopic expression of FSTL1 in NPC cells inhibits cell colony formation, invasion and migration, which provided further evidence that FSTL1 is functional TSG in NPC. In conclusion, we identified FSTL1 as a novel tumor suppressor gene, which frequently inactivated by promoter hypermethylation in NPC.

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Posters P131-P161

Immunology

Immunology **Abstract P131**

EVOLUTION OF THE CD8$^+$ T CELL RESPONSE TO EBV

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Abstract

The CD8$^+$ T cell response to EBV lytic cycle proteins in infectious mononucleosis (IM) patients shows a distinct hierarchy where strong responses are made to immediate early proteins and progressively weaker responses to later expressed proteins. When the abundance of responses are tracked prospectively from an IM episode, not all specificities are maintained in memory. It has previously been observed that all HLA-A2-positive patients make a response to three EBV epitopes, YVLDHLIVV from the immediate early protein BRLF1, GLCTLVAML from the early protein BMLF1, and TLDYKPLSV from the delayed early protein BMRF1. While YVL- and GLC-specific cells persist long-term, the TLD response disappears. Cross sectional studies on acute IM patients and healthy carriers, carried out using IFN-γ ELISPOT assays, have allowed us to identify several other reactivities present in IM but not in long term carriage. These include two additional epitopes from BMRF1, restricted through HLA-B7 and HLA-B35, and an HLA-A2 restricted epitope from the late lytic protein BALF4. We have also used HLA class I–peptide multimers in prospective studies to confirm that these responses are progressively lost following IM. We are currently identifying new epitopes in delayed early and late antigens and working to identify factors potentially underlying response exhaustion. We propose that the CD8 response to EBV is directly primed by infected B cells, which explains why the response is heavily skewed towards the immediate early and early antigens whose epitopes are preferentially displayed on lytically-infected cells.

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Immunology **Abstract P132**

SURFACE EXPRESSION OF GALECTIN 9 BY EBV-INFECTED CELLS: DETECTION AND POTENTIAL ROLE IN HOST-TUMOR INTERACTIONS

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Abstract

Galectin 9 is a mammalian lectin which binds b-galactoside residues. Expression of its gene is induced by EBV-infection in some types of cell backgrounds (Carter et al., J. Virol. 76 : 10427-436, 2002). It has internal signaling activity initiated from lipid rafts (Tanikawa at al., J Bone and Mineral Research 23 : 278-286, 2008). It also behaves as a cytokine when it is secreted. Through its interactions with the Tim-3 receptor, it induces activation of monocytes and dendritic cells on one hand and apoptosis of mature Th1 lymphocytes on the other hand (Anderson et al., Science 318: 1141-1143, 2007). We have previously reported that malignant NPC cells secrete exosomes carrying galectin 9 and acting as agonists of Tim-3 (Klibi et al. Blood 113 : 1957-1966, 2009). In a different pathological context, exosomes carrying galectin-9 are suspected to enhance immune evasion of the hepatitis C-virus (Mengshol et al., PlosOne 5: e9504, 2010). However, so far it has not been clear whether galectin-9 is contained in the plasma membrane of producer cells and whether it is presented at the cell surface. To address this problem, we have used a novel rabbit polyclonal antibody raised against recombinant galectin 9. This antibody reacts with surface galectin-9 on intact live cells. LCL as well as NPC cells have intense surface expression of galectin 9. In contrast, galectin 9 is undetectable on Burkitt's lymphoma cells. One future aim is to investigate functional activity of membrane galectin 9.

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Immunology **Abstract P133**

T CELL IMMUNITY AGAINST EBNA1 IN JAPANESE POPULATIONS

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Abstract

Rare incidences of NK/T cell infection by EBV lead to the development of NK/T-cell lymphoproliferative disease (LPD), which is most prevalent in Southern Asia including Japan. EBNA1 is uniformly expressed in infected NK/T cells and thus constitutes a potential target for immunotherapeutic intervention. We screened a small panel of Japanese donors for EBNA1-specific T cell immunity; all donors tested had demonstrable CD8+ and/or CD4+ T cell responses to EBNA1. Interestingly, the dominant CD8 T cell reactivity was directed against the HLA-B*3501-restricted HPVGDADYFEY peptide, which sequence differs by a single amino acid from the HPVGEADYFEY epitope immunodominant in Caucasian populations. HPV-specific T cell clones derived from Japanese donors recognised HLA-matched B-lymphoblastoid cell lines (LCLs) transformed with CKL strain EBV, but showed little/no recognition of B95.8-transformed LCLs. Accordingly, >100-fold higher concentrations of HPVGEADYFEY versus HPVGDADYFEY were required to mediate half-maximal recognition in peptide titration assays. CD8+ T cell responses were detected against a second epitope that varies between CKL and B95.8 EBV strains (VPQCRITPL and IPQCRLTPL respectively, B*0702-restricted); T cell recognition was again strain-specific. All donors tested were HLA-DP*0501-positive and recognised the conserved epitope VFLQTHIFAEVLKDAIKDLV in the context of this allele. Importantly, EBNA1-specific T cell clones were able to recognise HLA-matched NK/T cell lines. These results suggest (i) a dominant role for the specificity of antigen processing in determining T cell epitope choice since the corresponding CD8 epitopes are selected from different EBV strains in diverse ethnic populations; (ii) EBNA1-specific T cells may have therapeutic potential against NK/T-cell LPD in Japanese populations.

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Immunology **Abstract P134**

PLASMACYTOID DENDRITIC CELLS: A NOVEL TARGET FOR EBV INFECTION

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Abstract

EBV has been associated with the development of several lymphomas and autoimmune diseases. Given the key role of plasmacytoid dendritic cells (pDC) in the establishment of an antiviral state against several viruses and in the induction of autoimmune diseases through the massive release of type I Interferons (IFN), we investigated the effect of EBV infection on pDC function. Healthy donor-isolated pDC produce high amounts of type I IFN after exposure to the B95.8-derived EBV strain by mostly TLR9, but also by TLR7. Furthermore, delivery of EBV-derived nucleic acids to TLR7- and TLR9-containing endosomes in pDC requires activation of the autophagic machinery. Next, by cytofluorimetric analysis, we find that EBV is able to induce the surface expression of the IFN-inducible activation marker CD38, but not that of the costimulatory molecule CD86 in pDC. In accordance with the lack of a full maturation status, EBV-treated pDC are incapable of producing the pro-inflammatory cytokine TNF-a and can not stimulate allogeneic T cells. Consistently with all these data, we show by confocal microscopy, that a GFP-tagged strain of EBV infects pDC *via* viral binding to MHC class II molecules and establish latency as seen both by quantitative PCR for LMP-2a and EBERs and by confocal microscopy for LMP-1.
Our findings demonstrate that EBV infection targets also pDC and raise the possibility that impaired pDC maturation could represent an immune-evasion strategy promoted by EBV. This mechanism might lead to dysregulated immune responses linked to EBV-associated autoimmune diseases.

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Immunology **Abstract P135**

EXPLOITATION OF THE IMMUNOGENICITY OF BARF1 TO DEVELOP IMPROVED PROTOCOLS FOR THE GENERATION OF EBV-SPECIFIC CTL LINES

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Abstract

The oncogenic BARF1 protein is frequently expressed by NPC cells and may act as tumor-associated antigen to improve the efficacy of current adoptive immunotherapy protocols. We have recently demonstrated strong CD4+ and CD8+ T cell responses to the BARF1 protein in NPC patients and validated 5 HLA-A*0201 epitopes. Considering that HLA-A*0201 is significantly under-represented in Italian NPC cases and taking into account the peculiar geographic distribution of this tumor, we have identified and validated 10 additional BARF1 CTL epitopes presented by HLA class I alleles common in South-East Asia. We analyzed by IFNg-ELISPOT 3 A*1101, 2 A*2402, and 5 B*5101 putative epitopes identified by immunoinformatic engines. All these peptides elicited low-to-moderate CD8+ T cell responses in all 10 EBV+ donors investigated. Notably, 6 of these peptides induced a three-to-four fold increase in the number of CD8+ T cell responses in 11/15 NPC patients. Moreover, peptide-specific CTL cultures have been generated and preliminary data indicate that these effectors efficiently recognize and kill BARF1-expressing tumor cells. Considering that conventional approaches to generate EBV-specific CTLs do not allow the production of CTL lines endowed with BARF1 specificities, we are developing protocols able to enrich in BARF1-specific effectors. We found that treatment of LCLs with suboptimal doses of lytic cycle inducers (TPA+Na-Butyrate or 5-azacytidine) markedly enhances BARF1 mRNA expression. Experiments are under way to assess whether the use of BARF1-expressing LCLs may be suitable to generate “ex vivo” autologous EBV CTLs enriched in BARF1-specific effectors. The results of these studies further strengthen the relevance of BARF1 as immunotherapeutic target and provide the rationale to develop improved protocols for the generation of EBV CTL lines with a broader specificity and a possible higher efficacy for the treatment of EBV-associated malignancies.

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Immunology **Abstract P136**

A NOVEL LATENT MEMBRANE 2 TRANSCRIPT EXPRESSED IN EPSTEIN-BARR VIRUS NK AND T CELL LYMPHOPROLIFERATIVE DISEASE ENCODES A TARGET FOR CELLULAR IMMUNOTHERAPY

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Abstract

Therapeutic targeting of virus-encoded proteins in Epstein-Barr Virus (EBV)-associated malignancies using cellular immunotherapies has proved successful for tumours expressing the full range of immunogenic viral antigens. However, the limited repertoire and immunogenicity of EBV-encoded proteins in malignancies such as Hodgkin lymphoma and extra-nodal NK/T lymphoma (ENKTL) has presented a greater challenge. The immunosubdominant latent membrane protein 2 (LMP2) is considered the optimal target in such 'latency II' tumours, although data relating to its expression and immunologic recognition in T and NK malignancies is limited.

We first addressed the validity of LMP2 as an immunotherapeutic target but were faced with an apparent paradox. LMP2-specific effector CD8+ T cells could recognise and kill EBV-positive NK and T cell tumour lines, notwithstanding an apparent absence of LMP2A protein and very low levels of conventional LMP2A and LMP2B mRNA. The specificity of the T cell recognition was further assured in the context of alternate HLA class I types and, notably, by use of a recombinant T cell receptor specific for HLA A*1101;SSCSSCPLSKV.

We went on to identify and characterize an alternate LMP2 mRNA by quantitative PCR and 5' rapid amplification of cDNA ends (RACE); confirming a novel transcript encoding a truncated protein. Presence of this novel LMP2 transcript in NK and T cell lymphoproliferative diseases - highly expressed in ENKTL cell lines - serves as an attractive target for cellular immunotherapy and raises new questions about the role of LMP2 in NK and T cell lymphoproliferations.

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Immunology **Abstract P137**

STRONG CORRELATION BETWEEN THE LEVELS OF EBNA-1-IgG AND THE SEVERE CLINICAL COURSE OF HEREDITARY ANGIOEDEMA

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Abstract

Hereditary angioedema (HAE) is a severe, life-threatening disease due to inherited deficiency of the C1-inhibitor (C1-INH), an important regulator of the plasma enzyme systems. HAE occurs in attacks due to subcutaneous swelling in extremities or submucous edemas in the gut or larynx. Both the frequencies and severity of these attacks that is the clinical course of the disease are characteristic for a single patient but can be markedly different even among members of families carrying the same mutations of the C1-INH gene. The cause(s) of these clinically most important differences has not been revealed. Since EBV infection is widespread in the population, it seemed worthwhile to study if the clinical course of HAE is related to the titer of EBNA-1-IgG. The study was performed in 116 HAE patients (49 males, 67 females, 35,7 $\pm$ 17.1 ys old, 16 children (<18 ys old)). EBNA-1 IgG titers were measured by the DIA-SORIN kit. Positive (>20 AU/ml) EBNA-1 titers was found in 101 (87%) patients, in 7/16 and 94/100 children and adults, respectively. A significant positive correlation (p=0.007) was found between the EBNA-1 IgG titers and the clinical activity score of HAE, while negative correlation (p=0.021) was found with the age of first symptoms. Age and sex–adjusted odds ratio of the EBNA-1 positive vs. negative patients to have a severe (1 to 3) activity score was 5.625 (1.408-22.476), p=0.015). These findings indicate that EBV infection may be associated with the severe clinical course of HAE.

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Immunology **Abstract P138**

DOWN-REGULATION OF MHC CLASS I BY THE EPSTEIN-BARR VIRUS G-PROTEIN COUPLED RECEPTOR BILF1

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Abstract

EBV persists for life despite the presence of virus-specific adaptive immunity, indicating that it has evolved tactics to counter the host immune response. During the viral lytic phase, EBV can evade eradication by CD8+ T lymphocytes by subverting the MHC class I antigen processing and presentation pathways. We have shown that the EBV G-protein coupled receptor (GPCR), BILF1, contributes to this immunoevasive strategy by down-regulating cell surface levels of MHC class I molecules and directing their lysosomal degradation (1), a novel function for a viral GPCR. In this way, it profoundly reduces the ability of cytotoxic T-lymphocytes to recognize cells displaying antigens derived from EBV proteins.
Here, we show that BILF1 decreases cell surface display of various HLA class I A and B alleles, and of HLA-E. The C-terminal tail of this viral GPCR appears to be crucial in mediating its effect - a BILF1 truncation mutant lacking this region is still expressed at the cell surface, but fails to bring about a reduction in surface MHC class I levels. This C-terminal region contains potential internalization motifs, which may be important in directing MHC class I molecules from the cell surface to lysosomes. Thus, by reducing MHC class I surface expression, BILF1 can hamper the recognition of virus-infected cells by cytotoxic T lymphocytes, thereby likely facilitating evasion from adaptive immunity.

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Immunology **Abstract P139**

EFFECT OF EBV AND IMMEDIATE-EARLY/EARLY PROTEINS ON PERIPHERAL DENDRITIC CELLS

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Abstract

This work is aimed at assessing the effect of EBV on isolated peripheral blood dendritic cells (mDCs and pDCs), and macrophages as well. Here, we compared the effect of viral particles (B95-8 strain), Early and Immediate-Early EBV proteins on the primary immune response. DCs and macrophages were generated from total blood from healthy donors and then maturated with LPS (mDCs and macrophages) and CpG (pDCs) and finally stimulated by EBV: (i) supernatant of the B95-8 cell line, (ii) the recombinant proteins BALF2 (Major DNA binding protein), the BGLF5 exonuclease, the BZLF1 IE-protein (also called ZEBRA). Regarding B95-8, its effects on HLA-DR is dependent on the virus titer (upregulation with low and moderate EBV titers, downregulation with high EBV titers). The effect of the virus on CD80, CD83, CD86, CD40, CCR-7, was also investigated, but the reactivity pattern is largely different from one donor to another. Both BALF2 and BGLF5 proteins dramatically reduced HLA-DR expression both in mDCs and macrophages. The BZLF1 IE protein had hardly any effect on HLA-DR expression, but strongly stimulated all of the others. BZLF1 effect on macrophage showed i) a strong downregulation of HLA-DR ii) accompanied with a highest expression of CCR7. We also investigated the impact of EBV on the pDCs and studied its functional effect regarding TLR expression. pDCs from human donors have been challenged with complete EBV particles or viral capsid: preliminary results showed an important display of immune response to these subset cells with a marked modulating expression of TLR9 and TNF-α production.

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Immunology **Abstract P140**

EBV IMMEDIATE-EARLY ANTIGEN-SPECIFIC T CELLS RECOGNIZE RECENTLY INFECTED B CELLS – IMPLICATIONS FOR IMMUNODOMINANCE AND THERAPY

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Abstract

CD8+ T cells specific for immediate-early (IE) proteins often dominate EBV-specific T cell responses in healthy carriers, during infectious mononucleosis, and after transplantation or immunotherapy. The sporadic expression of IE proteins BZLF1 and BRLF1 in latently infected B cells initiates lytic replication. Recent results showed that IE antigens are expressed within hours after EBV infection of B cells. At this early stage, these proteins do not initiate lytic cycle, but mediate efficient establishment of latent infection. We hypothesized that IE antigen-specific CD8+ T cells might be able to detect early stages of B cell infection. We co-cultivated IE antigen-specific CD8+ T cell clones with EBV-infected primary B cells in different phases of early infection and transformation. We found that CD8+ T cells specific for epitopes from BZLF1 and BRLF1 started to recognize B cells between 3 and 5 days after EBV infection. On average, specific CD8+ T cells started to detected IE proteins earlier after infection than latent/transforming proteins. The depletion of IE antigen-specific T cells from primary T cell populations distinctly reduced their potential to kill recently infected B cells. We conclude that IE antigen-specific T cells contribute to the control and elimination of B cells in the earliest phase of EBV infection. Therefore, they should be included in T cell therapy protocols of EBV disease after transplantation. Early presentation of IE antigens might contribute to the immunodominance of IE antigen-specific T cells.

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Immunology **Abstract P141**

A NOVEL WAY OF EBV TO UPREGULATE PRODUCTION OF HUMAN INTERLEUKIN-10

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Abstract

Since viral evasion from immune attack is important for establishment of EBV persistent infection, EBV has developed multiple strategies to impede presentation of viral antigens and to reduce the risk of being recognized by host immune system. In addition, EBV may actively inhibit functions of surrounding immune cells through induction of certain immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor-b. Previous studies have reported that several EBV gene products can directly upregulate IL-10 expression in lymphoid cells, but in our study IL-10 production can not be increased by latent or lytic EBV infection in nasopharyngeal carcinoma (NPC) cells. Interestingly, our data show that the conditioned medium of NPC cells with lytic EBV infection enhances IL-10 secretion from monocytes and macrophages. The same effect can also be achieved by conditioned medium of NPC cells ectopically expressing an EBV lytic transactivator Zta, suggesting that certain Zta-induced factors secreted from NPC cells augment IL-10 production of the myeloid cells. We further identify at least two Zta-induced mediators potentially involved. One is granulocyte-macrophage colony-stimulating factor (GM-CSF) and the other is prostaglandin E_2 (PGE_2). The enhancement of IL-10 production from monocytes can be diminished by neutralization of GM-CSF in the conditioned medium of Zta-expressing NPC cells, or by blocking of PGE_2 receptors on monocytes. Thus we propose a novel indirect way of EBV to upregulate human IL-10. Through the Zta-induced immune modulators, EBV lytic infection may promote IL-10 secretion from bystander myeloid cells and forward an immunosuppressive microenvironment.

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Immunology **Abstract P142**

TARGETING TO DEC-205 ENHANCES MHC PRESENTATION OF LATENT EPSTEIN BARR VIRUS ANTIGENS BY VIRUS-TRANSFORMED B CELLS

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Abstract

DEC-205 is a type I transmembrane multilectin receptor that has been explored for targeting of vaccine antigens to dendritic cells (DCs) to improve processing and presentation on MHC I and MHC II molecules. Antigen presentation was found to be increased when antigens were targeted to DEC-205 by conjugation to a monoclonal antibody specific for this receptor. As DEC-205 is predominantly expressed on DCs, all previous studies analysed handling of DEC-205-targeted antigen by this potent antigen presenting cell type, but not by other cell types. Here we show that Epstein Barr virus (EBV) transformed lymphoblastoid B cell lines (LCLs) not only express DEC-205 at high levels, but also efficiently present targeted EBV nuclear antigen 1 (EBNA1) and the EBV-latent membrane protein 1 (LMP1) to EBNA1- and LMP1-specific $CD4^+$ and $CD8^+$ T cell clones *in vitro*. With respect to antigen processing for MHC class I versus II presentation, targeting EBNA1 to DEC-205 on LCLs is more efficient in stimulating $CD4^+$ T cell than $CD8^+$ T cell recognition. These data suggest that DEC-205-targeted antigen gains access to MHC class II loading compartments and to a lesser extent to cross-presenting endosomes. We are currently comparing the targeting of antigens to DEC-205 on DCs and LCLs. These studies will further characterize antigen processing after DEC-205 targeting of antigens for the development of this strategy as a vaccination approach.

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Immunology **Abstract P143**

NUCLEAR LOCATION OF ENDOGENOUSLY EXPRESSED EBNA1 RESTRICTS PROCESSING BY MACRO-AUTOPHAGY AND THE RANGE OF CD4 EPITOPE DISPLAY

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Abstract

All EBV-infected cells express both MHC II molecules and the Epstein-Barr virus (EBV)-coded nuclear antigen EBNA1. Since EBNA1 contains multiple CD4+ T cell epitopes, EBV-infected cells could potentially be eliminated by EBNA1-specific CD4+ T cells. To investigate this further we studied three epitopes within EBNA1, measuring the ability of specific CD4+ T cell clones to recognise EBV-infected lymphoblastoid cell lines (LCLs). Two epitopes were weakly displayed on the LCL surface while the third was undetectable, a pattern of limited epitope presentation that was maintained even when nuclear expression of EBNA1 was induced to high supra-physiological levels. Inhibitor and si-RNA studies show that, of the two epitopes weakly presented under these conditions, one involved macroautophagy, the second involved antigen delivery to the MHC II pathway by another endogenous route. In contrast, when EBNA1 was expressed as a cytoplasmic protein, all three CD4 epitopes were processed and presented much more efficiently, and all involved macroautophagy. We conclude that EBNA1's nuclear location limits its accessibility to the macroautophagy pathway and, in consequence, limits the level and range of EBNA1 CD4 epitopes naturally displayed on the infected cell surface.

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Immunology **Abstract P144**

EPSTEIN-BARR VIRUS-SPECIFIC CD4+ T CELL RESPONSES DURING ACUTE AND CHRONIC PHASE OF INFECTION

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Abstract

EBV infection is controlled by T cells, indicated by an increased incidence of EBV-associated malignancies in patients with T cell dysfunction, and by successful treatment of EBV-associated PTLD by the infusion of polyclonal EBV-specific T cell lines.
To gain more insight into the EBV-specific CD4+T cell response, we analyzed virus-specific CD4+T cells during acute and persistent phase of infection *ex vivo*, and assessed the tumor-protective capacity of different virus-specific T cells in a SCID mouse model of PTLD *in vivo*.
Responses against latent cycle antigens and/or autoantigens were elevated in patients with IM and decreased during convalescence. By contrast, CD4+T cells reactive against structural antigens of the virus were found to be significantly increased in healthy virus carriers as compared to patients with IM, suggesting that these responses develop late in infection.
To assess their tumor-protective potential, various CD4+ T cell specificities were tested in a preclinical PTLD model. Tumor development was delayed when LCL-stimulated T cell lines with different proportions of CD4+T cells were adoptively transferred, demonstrating that this T cell population is capable of restricting tumor growth and is as effective as CD8+T cells. Unexpectedly, CD4+T cell clones specific for EBNA1, EBNA3B and BNRF1, enhanced rather than delayed tumor development whereas T cell clones specific for gp350 and EBNA3C led to a prolonged survival. These results indicate that not all virus-specific CD4+T cells may be therapeutically beneficial and that defining the relevant CD4+T cell specificities may facilitate the generation of T cell lines with improved clinical efficacy.

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Immunology **Abstract P145**

VACCINE STRATEGIES FOR THE IMMUNOTHERAPY OF NASOPHARYNGEAL CARCINOMA

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Abstract

The Epstein–Barr virus (EBV) is not only one of the most widespread human viruses, but it has also been linked to a range of neoplasms. These include various B-and T-cell non Hodgkin's lymphomas, Hodgkin's diseases and nasopharyngeal carcinoma (NPC).
In NPC, latent membrane proteins 1 and 2 (LMP1 and 2) offer the best opportunity for specific targeting since they are typically expressed and T cell determinants in each of these proteins have been defined. We have attempted to maximize the opportunity of incorporating every possible CD4 and CD8 determinants in a single formulation. We have achieved this by generating a scrambled protein incorporating random overlapping peptide sets from EBNA1, LMP1 and LMP2 which was then inserted into a replication-deficient strain of adenovirus (Ad-SAVINE).
Using this SAVINE vaccine, we have activated CTL responses from peripheral blood mononuclear cells of human healthy EBV carriers, as well as NPC patients.
This formulation could have a role in NPC immunotherapy in all ethnics groups since it has the potential to activate all possible CD4 and CD8 responses within EBNA-1 and LMP proteins.
These studies provide an important basis for the development of an LMP-based vaccine as an immunotherapeutic tool for the treatment of EBV-associated NPC.

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Immunology **Abstract P146**

IMPAIRMENT OF ACTIVATION INDUCED T CELL DEATH CONTRIBUTES TO THE SEVERE MONONUCLEOSIS IN THE PRIMARY EBV INFECTION OF XLP PATIENTS

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Abstract

Lack of the functional SAP protein due to deletion or mutation of its gene is the cause of the X linked lymphoproliferative disease (XLP). XLP is characterized by exceptional sensitivity to Epstein-Barr virus (EBV) infection. Primary infection leads to serious mononucleosis. This was attributed to the demonstrated impairment of cellular immunological functions and was explained by the function of SAP protein in signal transduction of the lymphocytes.
Earlier studies from our laboratory have discovered that SAP has pro-apoptotic function and its expression is regulated by p53. Our further studies suggest that impairment of T cell homeostasis, due to lack of the pro-apoptotic SAP function, contributes to the development of serious mononucleosis. Time kinetic studies of PHA activated T cells showed that, when the cells express high levels of SAP, the rate of proliferation start to decrease. In parallel to the increase of SAP expression in the T cells, p53 is also up-regulated. P53 expressed in these cells is phosphorylated (Ser-15) and p53 target genes (i.e. MDM2, p21) are induced, confirming that PHA activated T cells express activated p53. We are currently studying the possible involvement of p53 in the regulation of SAP expression during T cell activation. High level of SAP is necessary for activation induced cell death that is pivotal in the termination of the T cell response in mononucleosis.

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Immunology **Abstract P147**

EPSTEIN-BARR VIRUS, SERUM 25-HYDROXYVITAMIN D AND RISK OF MULTIPLE SCLEROSIS: NO EVIDENCE FOR INTERACTION

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Abstract

EBV infection and low serum 25-hydroxyvitamin D (25(OH)D) levels are associated with an increased risk of multiple sclerosis (MS). However, no studies have examined whether they are independent risk factors or whether the strong association with EBV is modified by sex or race. In this prospective, nested case-control study including 222 cases of MS occurring among active duty US military personnel, each case was matched to two controls on age, sex, race, branch of military service, and dates of serum sample collection. Up to three samples collected before symptom onset were available. We used conditional logistic regression to estimate the relative risks (RR) and 95% CIs and to evaluate statistical interactions. A 4-fold increase in average anti-EBNA complex IgG antibodies was associated with a strong increased risk of MS (RR=2.9, 95% CI: 2.2-4.0, p=4.5E-13). Adjusting for average serum 25(OH)D levels did not change this association. This association was not modified by sex (p for interaction=0.34) or race (p for interaction=0.30). Further, after adjusting for anti-EBNA complex IgG antibodies, the inverse association previously observed between 25(OH)D levels and MS risk among whites in our population remained (RR for the top vs. bottom quintile of 25(OH)D=0.40, 95% CI: 0.17-0.90, p=0.03). Additionally, there was no significant interaction between average anti-EBNA complex IgG titers and 25(OH)D levels on MS risk (p for interaction=0.67). Increased anti-EBNA complex IgG antibodies and low 25(OH)D serum levels appear to be independent risk factors for MS, and the association with anti-EBNA complex is not modified by sex or race.

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Immunology **Abstract P148**

ANALYSIS OF CD4+ AND CD8+ EPSTEIN-BARR VIRUS-SPECIFIC POLYFUNCTIONAL T CELL RESPONSES IN CHINESE LONG TERM CARRIERS BY A NOVEL 9-COLOUR FLOW CYTOMETRIC ASSAY

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Abstract

Recent evidence shows that polyfunctional CD4+ and CD8+ T cells capable of secreting multiple cytokines simultaneously are the protective immune correlates of different viral infections. Here, we developed a novel 9-color flow cytometric assay for analysis of virus-specific CD4+ and CD8+ T cell responses to EBV infection. Overlapping peptide pools of four EBV latent proteins (EBNA1, EBNA3A, EBNA3B and LMP2) and one lytic protein (BZLF1) were used to stimulate peripheral blood mononuclear cells (PBMCs) of ten healthy Chinese EBV-seropositive long term carriers. After 6-hour stimulation by the peptides, PBMCs were stained sequentially by aqua blue dye (for exclusion of dead cells) and antibodies to surface markers (CD3-APC-Cy7, CD4-PE-Texas Red and CD8-Pacific Blue), cytotoxic marker (CD107a-PE-Cy5) and cytokines (interferon-g [IFN-g]-FITC, interleukin-2 [IL-2]-APC, macrophage inhibitory protein 1-a [MIP1]-a-PE and tumour necrosis factor-a [TNF-a]-PE-Cy7), and analyzed by an optimized 9-colour flow cytometric assay using BD-FACS LSR-II. Positive (PBMCs stimulated by Staphylococcal Enterotoxin B), negative (unstimulated and unstained PBMCs) and biological (unstimulated but stained PBMCs) controls were included for each study subject. Polyfunctional CD4+ and CD8+ T cells (defined as those that secrete three or more cytokines) reactive to EBV latent and lytic peptides could be clearly demonstrated in the long term carriers: CD4+ T cells secrete three (IFN-g, TNF-a and MIP1-a) or all four cytokines whereas CD8+ T cells secrete three cytokines (IFN-g, TNF-a and MIP1-a) and have a cytotoxic function (CD107a expression). The new assay can be applied to the study of EBV-specific polyfunctional T cell responses in EBV-related clinical diseases.

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Immunology **Abstract P149**

CORRELATES OF DISEASE SEVERITY IN PRIMARY EPSTEIN-BARR VIRUS INFECTION- A PROSPECTIVE STUDY

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Abstract

Epstein-Barr virus (EBV) infection in early childhood is common and usually asymptomatic while infection in the second decade or later, often causes infectious mononucleosis (IM). We are performing a prospective study of EBV-naïve University of Minnesota freshman to determine factors that differentiate symptomatic and asymptomatic seroconversion. In 63 cases of natural infection so far, we observed a range of symptoms on a scale of 0-6, with approximately 10% asymptomatic (score 0), 7% being essentially bedridden (score 6) and the remaining experiencing some symptoms (score 1-5). We measured viral load, CD8 lymphocytosis, and the EBV specific lymphocyte response (measured by LCL-induced IFNγ production, and with HLA-peptide tetramers for CD8 T cells). In contrast to previously published data, we observed a positive correlation between viral load in the blood, the severity of illness, and CD8 lymphocytosis. Our data suggest high numbers of circulating EBV infected B cells drive the massive CD8 lymphocytosis associated with IM and that likely causes IM symptoms. It also suggests that targeting viral load may be an effective way to impede IM and EBV-related sequelae. Finally, using a tetramer enrichment method to isolate and characterize the rare EBV specific CD8 T cells in HLA-B7+ and HLA-A2 individuals prior to infection, we did not observe an increase in memory phenotype cells in adults compared to cord blood, arguing against a model where pre-existing memory CD8 T cells, which are cross-reactive to EBV, drive IM in adults versus children.

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Immunology **Abstract P150**

SPECIAL REQUIREMENT OF SAP IN IMMUNITY TO EPSTEIN BARR INFECTION

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Abstract

X-linked lymphoproliferative disease (XLP) is an inherited immunodeficiency characterised by extreme sensitivity to Epstein Barr Virus (EBV) infection, but not to other common viruses. In contrast to EBV infection of healthy individuals, exposure of XLP patients to EBV induces a vigorous and uncontrolled immune response. Despite such immune activation, XLP patients fail to control EBV infection. The gene mutated in XLP, *SH2D1A*, encodes the cytoplasmic SH2-domain containing adaptor protein SAP (SLAM-associated protein) that associates with the cytoplasmic domains of members of the SLAM family of cell surface receptors. We have taken a novel approach by studying female carriers of XLP to dissect the role of SAP in anti-EBV immunity and immunity to other common viruses. Random inactivation of the X-chromosome results in both SAP^+ and SAP^- T cells in female carriers. Analysis of virus-specific $CD8^+$ T cells against EBV, CMV and Influenza demonstrated a selective advantage for cells expressing SAP in anti-EBV immunity, but not in anti-CMV or anti-Influenza immunity. We isolated $CD8^+$ T cell clones specific for CMV or flu that were SAP sufficient or SAP deficient from these carriers in order to understand the reasons underlying the increased susceptibility to EBV, a B cell tropic virus, in XLP patients. We demonstrate that in the absence of SAP, even $CD8^+$ T cells specific for CMV or flu are incapable of responding to antigen presenting B cell targets but remain efficient at responding to non-B cell targets such as monocytes or fibroblasts. Our results demonstrate the molecular mechanisms involved in the increased susceptibility to EBV infection in XLP patients.

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Immunology **Abstract P151**

CO-EXPRESSION OF EBV GH AND GL ENHANCES GP42-MEDIATED EVASION FROM HLA CLASS II-RESTRICTED ANTIGEN PRESENTATION

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Abstract

The EBV lytic phase protein gp42 binds to HLA class II molecules and has a dual role in the viral life-cycle: gp42 acts as the co-receptor for viral entry into B-cells and hampers T-cell activation through sterically hindering T-cell receptor binding to HLA class II/peptide complexes. Cellular expression of gp42 does not cause a reduction in HLA class II surface display. Interestingly, HLA class II expression is downregulated on productively EBV-infected B-cells, pointing towards additional mechanisms operative in the full viral context.
During natural EBV infection, gp42 associates with two other viral glycoproteins, gH and gL. Formation of these trimeric complexes facilitates fusion of EBV with the B-cell membrane. To determine whether gHgL also contributes to immune evasion, we investigated biosynthesis and cell surface display of HLA class II in the presence of gp42, gHgL, or both. The gp42gHgL complex associates with HLA class II early in the biosynthetic pathway. Moreover, co-expression of gHgL increases levels of cell-associated soluble gp42. At the surface of cells stably expressing both gp42 and gHgL, additional shielding of MHC class II was detected, as assessed with different HLA class II-specific antibodies recognizing epitopes in proximity of the T-cell receptor binding area. Our data show that shielding of HLA class II by gp42 is enhanced by the gHgL complex, which is likely responsible for additional evasion from T-cell recognition. Acquisition of multiple viral gene products that target HLA class II antigen presentation illustrates that EBV is extremely well-adapted to remain invisible within the infected B-cell.

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Immunology **Abstract P152**

EPSTEIN-BARR VIRUS REACTIVATION DOES NOT AFFECT EARLY T CELL RECONSTITUTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Abstract

Epstein-Barr virus reactivation is a major complication after allogeneic stem cell transplantation (SCT). Reactivation of Epstein-Barr can lead to uncontrolled lymphoproliferation and subsequent post transplant lymphoproliferative disease due to the lack of T cell control. We hypothesise that development and severity of the EBV reactivation could be caused by a poor T cell reconstitution following SCT. On the other hand, viral reactivation itself could activate the immune system and enhance T cell reconstitution. To investigate whether viral reactivation leads to better T cell reconstitution or that better T cell reconstitution leads to lower viral reactivation, we prospectively followed 117 allogeneic SCT recipients. Epstein-Barr virus loads in plasma were determined as part of the diagnostic regime. During the first 3 months we measured absolute levels of $CD3^+$, $CD4^+$, and $CD8^+$ T cells weekly in whole blood by TRUcount (BD) and monthly until 6 months. Based on peak viral load we subdivided patients in 3 reactivation categories, no reactivation, low (load 50-1000 copies/ml) and high reactivation (>1000 copies/ml). Absolute numbers of T cells were correlated to the onset and severity of the EBV reactivation. The rate of T cell reconstitution was similar for all reactivation categories. Median levels of $CD8^+$ T cells did not differ at 1, 2 and 3 months post SCT between reactivation categories. In conclusion the development and severity of the Epstein-Barr virus reactivation does not drive the T cell reconstitution post SCT nor does poor T cell reconstitution play a role in the severity of the viral reactivation.

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Immunology **Abstract P153**

THE EFFECT OF THE EBV IMMUNE EVASION GENES ON CD8+ T CELL RECOGNITION OF TARGETS DURING EBV-LYTIC CYCLE REPLICATION

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Abstract

During lytic cycle replication Epstein-Barr virus (EBV) expresses at least three immune evasion genes, BNLF2a, BGLF5 and BILF1, which individually act to inhibit efficient processing and presentation of $CD8^+$ T cell epitopes. We have previously shown that BNLF2a is expressed transiently during early phase replication and its expression inhibits presentation of epitopes derived from immediate early, some early but not late proteins.
In the present study we used cells replicating recombinant-EBV viruses which lacked either BNLF2a, BGLF5 or BILF1. These were used to probe the recognition of antigens expressed in the different phases of the replication cycle, allowing us to determine the contribution of BGLF5 and BILF1 to inhibiting antigen presentation during the different phases of replication. Novel $CD8^+$ T cell specificities reactive to epitopes derived from the three phases of lytic replication were established and these incubated with cells replicating the different virus knock outs. Cells replicating viruses lacking the different immune evasion genes were more efficiently recognised by the $CD8^+$ T cells when compared to cells replicating wild type EBV. In particular, viruses lacking BNLF2a were more efficiently recognised by $CD8^+$ T cells specific for immediate early and early expressed antigens relative to those lacking BGLF5 and BILF1. Currently the impact of these latter two immune evasion proteins on recognition of epitopes derived from late expressed antigens is being assessed. We suggest that the concerted expression of these three immune evasion genes gives the most efficient protection from $CD8^+$ T cell recognition across all phases of gene expression.

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Immunology **Abstract P154**

EPSTEIN-BARR VIRUS BGLF5 ACTS AS AN RNASE TO CAUSE HOST SHUTOFF

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Abstract

Productive infection with EBV drastically impairs cellular protein synthesis. This host shutoff phenotype results from mRNA degradation induced upon expression of the early lytic phase protein BGLF5. Interestingly, BGLF5 is the EBV DNase, or alkaline exonuclease, which is present throughout the herpesvirus family. During viral replication, this DNase is essential for processing and packaging of the viral genome. In contrast to this widely conserved activity, host shutoff is only mediated by the alkaline exonucleases of gamma-herpesviruses, including EBV, KSHV, and MHV68. The reasons for this restricted activity are still poorly understood.

We observed that in the presence of Mn^{2+} recombinant BGLF5 exerts RNase activity in vitro, degrading both cellular and viral transcripts. Based on the crystal structure and other information, mutants of BGLF5 were generated and analyzed with respect to both DNase and RNase activities in vitro and in cells. On the one hand, a point mutation destroying DNase function also blocked RNase activity, implying that both nuclease activities share a catalytic site. On the other hand, other mutations were more selective affecting either DNA degradation or shutoff, pointing towards genetic separation of the two functions.

These findings are relevant for our understanding of immune evasion as we have shown that host shutoff results in a block in the synthesis of immunologically relevant molecules, reflected by for instance reduced HLA-restricted antigen presentation to T cells or signalling through Toll-like receptors on EBV-producing B cells. This could lead to immune escape prolonging the time for the generation of viral progeny.

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Immunology **Abstract P155**

TARGETED THERAPY WITH CD154+/GP350+ EXOSOMES RESULTS IN EFFICIENT REACTIVATION OF AUTOLOGOUS CLL- AND EBV-SPECIFIC T LYMPHOCYTES IN PATIENTS WITH B-CLL

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Abstract

Interaction of CD40 with its ligand (CD154) plays a critical role in immune activation. T-cells from patients with chronic lymphocytic leukemia of B-cell origin (B-CLL) express reduced levels or no CD154 and thus fail to activate CD40-bearing cells, including CLL-cells. As a consequence, these cells fail to express immune-relevant accessory surface molecules and escape T-cell recognition. Ectopic expression of CD154 on B-CLL cells is therefore regarded as a promising concept for the immunotherapy of B-CLL.

We demonstrate here that CD154-positive exosomes with a B-cell tropism provided by the Epstein-Barr Virus (EBV) protein gp350 can successfully transfer functional CD154 onto B-CLL cells and activate them to express immune accessory molecules. The activated B-CLL cells then became potent stimulators for allogenic and tumor-reactive autologous T-lymphocytes. In addition, transfer of EBV-peptides to B-CLL cells reactivated EBV-specific, MHC class II restricted CD4+ T cells and redirected the strong antiviral cellular immune response in patients to leukemic B-cells. Furthermore there should be the opportunitiy to induce also immune responses against other relevant viruses by delivering for example pp65 of CMV in addition to gp350.

The Co-transfer of functional CD154 and viral proteins can induce anti-leukemic and antiviral immune responses with therapeutic potential in patients with B-CLL and possibly other B-cell derived malignancies.

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Immunology **Abstract P156**

CD4+ T CELL EPITOPES DERIVED FROM EBNA1 AND LANA ARE POORLY PRESENTED BY THE KSHV ASSOCIATED MALIGNANCY PRIMARY EFFUSION LYMPHOMA

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Abstract

Primary Effusion Lymphoma (PEL) is a B cell malignancy associated with infection by the human γ-2 herpes virus Kaposi's sarcoma-associated herpes virus (KSHV). Often these cells are co-infected with Epstein-Barr virus (EBV) and they express subsets of genes from both viral genomes which potentially may be targeted by the immune response. Here we have identified CD4+ T cell responses to the KSHV and EBV genome maintenance proteins LANA and EBNA1, respectively, which are expressed in these malignancies. These T cells were used to probe endogenous presentation of LANA and EBNA1 epitopes by PELs. LANA and EBNA1-specific T cells incubated with HLA matched PEL lines did not recognise endogenously expressed LANA or EBNA1, nor PELs engineered to overexpress these proteins, nor PELs fed with exogenous LANA and EBNA1 protein. Only LANA and EBNA1 directed into the endosomal compartment of the PELs stimulated some response. By contrast, Epstein-Barr virus transformed B cells efficiently processed and presented these different overexpressed forms of the LANA and EBNA1 protein. We are currently investigating the role of other KSHV genes expressed in PELs which may regulate antigen processing and inhibit LANA and EBNA1-epitope presentation. We conclude that the class II antigen processing pathway of PELs is inefficient and provides an explanation for the poor control of these tumours in vivo.

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Immunology **Abstract P157**

EBV INVOLVEMENT IN THE DEVELOPMENT OF BRAIN LESIONS IN MULTIPLE SCLEROSIS

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Abstract

During the last 30 years Epstein-Barr virus (EBV) has been repeatedly associated with multiple sclerosis (MS), a chronic neuroinflammatory disease, through epidemiological and immunological studies. The possibility that EBV establishes a persistent infection in the CNS and reactivates periodically bolstering an immunopathological response has been explored by several groups with contrasting results. Using EBER in situ hybridization, immunohistochemical techniques with antibodies specific for EBV latent and lytic proteins, and RT-PCR techniques coupled with laser capture microdissection to detect EBV latent and lytic transcripts within brain immune infiltrates we have been able to detect EBV in all MS brain samples with an established B-cell/plasma cell infiltrate. We have shown that a high proportion of B cells infiltrating the MS brain are latently infected with EBV, that viral reactivation occurs in plasma cells in acute MS lesions and meningeal B-cell follicle-like structures, and that CD8+ T cells show signs of cytotoxicity toward EBV infected plasma cells. These findings support the possibility that an immunopathological response targeting intracerebral EBV deposits is the main cause of brain damage in MS. In contrast with our data, several earlier and more recent studies have failed to detect EBV in all or the majority of MS brain samples analyzed. In this presentation, we present several technical issues that may explain the differences between our results and those obtained by other groups. Elucidation of these issues is critical for understanding the elusive link between increased immune reactivity to EBV and brain pathology in MS.

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Immunology **Abstract P158**

LOCAL IMMUNITY OF TUMOR SITE AND APOPTOSIS OF TUMOR CELLS IN EPSTEIN-BARR VIRUS-ASSOCIATED GASTRIC CARCINOMA

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Abstract

To describe the immunophenotypic characterization of the tumor infiltrate in EBV-associated gastric carcinoma (EBVaGC) in both common gastric carcinoma (CGC) and gastric remnant carcinoma (GRC), we analyzed the distribution of TILs including CD3+ T cells, CD79+ B cells, CD56+ NK cells by immunohistochemistry in 46 EBVaGCs and 20 EBV-negative gastric carcinomas (EBVnGCs) in CGC, also in 8 EBVaGCs and 18 EBVnGCs in GRC. We discovered that the TILs were more abundant in EBVaGCs, which were predominantly CD8+ TILs. Then we studied the mechanisms of abundant CD8+ TILs infiltrate and their functions. The results indicated that the proliferating activity and chemiotaxis of IL-1β may contribute to the CD8+ TILs' recruitment in EBVaGCs, and CD8+ T cells expressed higher Granzyme B and IFN-γ which suggested more cytotoxicity to tumor cells.

To investigate whether the effective CD8+T cell immunity resulted in the higher apoptosis of tumor cells of EBVaGC, we determined the apoptotic index (AI) of tumor cells by TdT-mediated-dUTP nick and labeling in all above samples. Strangely, the tumor cells of EBVaGC had a lower AI. Then we analyzed the expression of IL-10 and distribution of FoxP3+ TILs using immunohistochemistry in the above samples to explain the contradiction. The result indicated that the upregulation expression of IL-10 in both TILs and tumor cells and more abundant FoxP3+ TILs may contribute to the lower apoptosis in EBVaGCs.

In conclusion, EBV infection may elicit both effective CD8+ TILs immune responses and immunosuppression at tumor site, and the local immunosuppression might be stronger considering the lower AI of tumor cells in EBVaGCs.

In addition, the EBVaGCs in GRC had the lower CD4/CD8 ratio than those in CGC, suggesting that the local immunity of EBVaGCs in GRC may be suppressed more intensely than those in CGC, which might be one of the mechanisms to explain the high incidence of EBVaGCs in GRC.

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Immunology **Abstract P159**

STRUCTURAL BASIS OF THE INTERACTION OF THE HUMAN CYTOKINE CSF1 AND THE EBV ONCOPROTEIN BARF1

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Abstract

BARF1 protein, the product of one of the 86 EBV ORFs, exhibits an oncogenic, mitogenic and immortalizing activity in infected host B-cells and epithelial cells. Intriguingly, BARF1 was shown to bind the human cytokine CSF1, a key growth factor for the development of the human hematopoietic and immune systems. Furthermore BARF1 inhibits secretion of IFN-α by mononuclear cells (Strockbine *et al.*, 1998), suggesting a role in immunomodulation. BARF1 protein expression is detected in endemic nasopharyngeal carcinoma (NPC) and some gastric cancers and it can be expressed in absence of the lytic cycle. We determined previously the crystal structure of BARF1, showing a hexameric structure of immunoglobulin domains (Tarbouriech *et al*., 2006).
In this contribution we report on the application of a combined biophysical approach to study the interaction of BARF1 with the human cytokine CSF1. We will present the crystal structure of the BARF1-CSF1 complex. The CSF1 dimer binds symmetrically at the interface of 2 N-terminal domains of BARF1. The interaction surface on CSF1 is distinct from the interface involved in the interactions between CSF1 and its human receptor c-fms. Biophysical techniques showed a sub-nanomolar dissociation constant with extremely slow dissociation kinetics.
These kinetics point to a role of BARF1 protein as scavenger for circulating CSF1 blocking its action on cells of the monocyte lineage and raises the question of the role of this interaction in EBV physiopathology.

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Immunology **Abstract P160**

REGULATION OF *CIS*-PRESENTATION OF CD8$^+$ T-CELL EPITOPES BY EBV-ENCODED LMP1 THROUGH SELF-AGGREGATION

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Abstract

The expansion of EBV-specific CD8$^+$ cytotoxic T-cell (CTL) to treat posttransplant lymphoproliferative disorder (PTLD) employs EBV-transformed lymphoblastoid cell lines (LCLs) as the antigenic source. Successful treatment of EBV-associated PTLD using CTL has been reported. Nasopharyngeal carcinoma (NPC) represents a type II latency malignancy, whereby antigen expression is restricted to latent membrane protein 1 (LMP1), LMP2, and EBV nuclear antigen (EBNA) 1.However, EBNA1, LMP1 and LMP2 are poorly immunogenic presumably due to poor processing and the subsequent limited amount of antigen available for presentation by MHC class I molecule. Thus, the predominance of T-cells specific for the EBNA3 antigens generated by LCL-mediated expansion is likely to limit the efficacy of this treatment for NPC.

Here we show that, although expression of LMP1 in human cells enhanced the *trans*-presentation of CD8$^+$ T-cell epitopes, *cis*-presentation of LMP1-derived epitopes was severely impaired. Although mutations within the C-terminal activator regions had no impact on the presentation of LMP1 epitopes, deletion of the first trans-membrane domain enhanced endogenous presentation of MHC class I-restricted LMP1 epitopes. In addition, intracellular localization analysis using fluorescent microscopy revealed that, although full-length LMP1 and its CTAR mutants formed large aggregates in the perinuclear region, the deletion mutant lost its ability to aggregate. These observations indicate a novel mechanism of immune evasion, aggregation of this protein (a critical requirement for *trans*-presentation) limits its accessibility to the MHC class I processing machinery.

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Immunology **Abstract P161**

EPSTEIN-BARR VIRUS USES A UNIQUE STRATEGY TO BLOCK PEPTIDE LOADING OF MHC CLASS I MOLECULES

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Abstract

EBV persist for life in infected individuals despite the presence of a vigorous anti-viral immune response. Especially during replication, a large number of viral proteins is synthesized, yielding numerous T-cell antigens, resulting from either normal protein turnover or occurring as Defective Ribosomal Products (DRiPs). Detection and elimination of virus-infected cells by cytotoxic T lymphocytes relies on the recognition of these virus-derived peptides in the context of MHC class I molecules at the cell surface. This ingenious detection system is extremely sensitive, as only a couple of MHC-peptide complexes are sufficient for CTL recognition.

The translocation of peptides from the cytoplasm into the ER by the Transporter associated with Antigen Processing (TAP) is a critical step in the MHC class I presentation pathway. BNLF2a, a tail-anchored protein encoded by EBV and related lymphocryptoviruses of Old World primates, effectively inhibits TAP function. Based on extensive structure-function studies, we have developed a model showing how this 60-amino acid residue protein might block TAP function. The hydrophobic C-terminal domain serves as a signal anchor, whereas the cytosolic hydrophilic N-terminal domain blocks both peptide and ATP-binding to TAP.

Thus, EBV BNLF2a inhibits TAP using a strategy that is completely different from that employed by other herpesviruses and by cowpoxvirus – that has recently been found to encode a TAP-inhibitor as well. The fact that TAP inhibitors are encoded by so many large DNA viruses, but are unrelated in sequence and mechanism of action, presents a striking example of functional convergent evolution.

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Posters P162-P175

Therapy and Diagnostics

Therapy and Diagnostics **Abstract P162**

DETECTION OF EBV GENOMES IN PLASMABLASTS/PLASMA CELLS AND NON-B CELLS IN THE BLOOD OF MOST PATIENTS WITH EBV LYMPHOPROLIFERATIVE DISORDERS USING IMMUNO-FISH

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Abstract

Epstein-Barr virus (EBV) is present in B cells in the blood of healthy individuals; few studies have looked for EBV in other cell types in blood from patients with lymphoproliferative disorders. We employ a new technique combining immunofluorescent cell surface staining and fluorescent in situ hybridization, Immuno-FISH, to quantify both EBV copy number per cell and cell types in blood from patients with high EBV DNA loads. In addition to $CD20^+$ B cells, EBV was present in plasmablast/plasma cells in the blood of 50% of patients, in monocytes or T cells in a small proportion of patients, and in "non-B, non-T, non-monocytes" in 69% of patients. The mean EBV copy number in B cells was significantly higher than in other subpopulations except for "non-B, non-T, non-monocytes." While we detected CD21, the EBV receptor for B cells, on EBV-infected B cells, we could not detect it on virus-infected T cells. There was no correlation between EBV load and virus copy number per cell. These findings expand the range of cell types infected in the blood. Determining the number of EBV genomes per cell and the type of cells infected in patients with high EBV loads may provide additional prognostic information for development of EBV lymphoproliferative diseases. Identification of EBV-positive, CD20-negative cells in blood from patients with lymphoproliferative diseases may have implications for rituximab therapy.

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Therapy and Diagnostics **Abstract P163**

TOWARDS THE STANDARDISATION OF NUCLEIC ACID AMPLIFICATION TECHNOLOGY-BASED ASSAYS FOR EPSTEIN-BARR VIRUS

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Abstract

Viral load measurements using nucleic acid amplification technology (NAT) are important for the diagnosis and management of Epstein-Barr virus (EBV) infections. However, the lack of traceability of results generated by different NAT assays makes it difficult to compare results and develop uniform treatment strategies. Indeed, variability in the performance of diagnostic NAT assays for EBV, as demonstrated through external quality assessment schemes and other studies, has highlighted a need for standardisation.

The World Health Organisation (WHO) establishes reference standards for biological substances used in the prevention, treatment or diagnosis of human disease. WHO International Standards are recognised as the highest order of reference for biological substances, and are arbitrarily assigned a potency in International Units (IU). Their primary purpose is to calibrate secondary reference reagents used in routine assays in terms of the IU, thereby providing a uniform reporting system and ensuring traceability of measurements independent of the method used.

In recognition of the need to standardise NAT-based assays used in the management of EBV infections, the WHO Expert Committee on Biological Standardisation has recently approved a proposal to develop the first WHO International Standard for EBV. The candidate standard comprises a whole virus preparation of the prototype laboratory strain B95-8 formulated in a universal buffer to standardise both DNA extraction and amplification steps. The results of a worldwide collaborative study to determine the suitability and potency of the candidate standard in a range of EBV NAT-based laboratory assays will be presented.

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Therapy and Diagnostics **Abstract P164**

IMMUNOLOGIC AND VIROLOGIC ANALYSES IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS WITH CHRONIC HIGH EPSTEIN-BARR VIRAL LOADS

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Abstract

Post-transplant lymphoproliferative disorder (PTLD) is a significant cause of morbidity and mortality after transplantation, and Epstein–Barr virus (EBV) DNA monitoring is now used for the early detection of EBV infection. Long-term monitoring programs have identified asymptomatic patients who subsequently developed and maintained very high EBV loads over long periods. The pathologic condition and clinical outcomes of this “chronically high EBV load carrier” state remain unclear. A total of 31 pediatric liver transplant recipients were examined. The median ages at transplant and during the post-transplant follow-up period were 1.7 and 4.3 years, respectively. The patients underwent weekly EBV DNA analysis and were subsequently divided into 11 chronically high EBV load carriers (EBV DNA > 5,000 copies/ml in whole blood for > 6 months) and 20 controls. Most of the chronically high EBV load carriers were seronegative at transplant, and half of them transiently displayed EBV-related symptoms. The median time to resolution of a chronically high EBV load state was 18 months, and no recipient developed late onset PTLD. EBV DNA was detected predominantly in $CD19^+$ cells. The plasma concentration of IL-10 and EBV-specific $CD8^+$ cell frequency did not differ significantly between the chronically high EBV load carriers and controls. Analysis of gene expression showed that EBER1, BARTs, and LMP2 were positive in peripheral blood mononuclear cells from chronically high EBV load carriers. EBV-infected cells in the blood of chronic high EBV recipients expressed a highly restricted set of latency genes, suggesting that the EBV-infected cells escaped from a T-cell response.

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Therapy and Diagnostics **Abstract P165**

RNA PROFILING OF EPSTEIN-BARR VIRUS INFECTED LYMPHOMAS AND CARCINOMAS BY MULTI-PRIMED QUANTITATIVE RT-PCR

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Abstract

Epstein-Barr virus (EBV) has tropism for lymphoid and epithelial cells as reflected in multiple EBV associated malignancies. EBV displays different latency patterns and careful analysis of EBV RNA profiles in lymphomas and carcinomas may improve understanding EBV's role in oncogenesis.

We developed a multi-primed quantitative RT-PCR simultaneously detecting transcripts for EBNA1 (Y3K and QK), EBNA2, LMP1, LMP2, BARTs, EBERs, BHRF1 (latent and lytic variant), BARF1, ZEBRA, TK, PK, VCAp18 and the housekeeping gene U1A. Calibration and quantification is performed with a pool of plasmids comprising all targets allowing exact determination of RNA molecules per cell.

This precise technique allowed us to characterise expression levels in EBV positive cell lines of lymphoid and epithelial origin. RNA profiles were used as reference for analysing patient material obtained from different EBV associated malignancies.

Cell lines showed expected RNA profiles accordingly to their latency program and presence of lytic cells. The viral housekeeping gene EBNA1 had steady expression enabling to use this gene as standard for quantification of the EBV content in patient material. In latency I only EBERs, BARTs and EBNA1 transcripts were detected. In most LCLs with some lytic cells early and late genes were detected. In latency III EBNA1 and 2, LMP1 and LMP2, BHRF1 latent variant and sporadic lytic transcripts were detected. In lytically induced Burkitt cells all viral RNA levels were increased except LMP2. This newly developed multi-primed quantitative RT-PCR allows in-depth normalised analysis of EBV gene expression in cells and tissues of patients with various EBV related diseases.

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Therapy and Diagnostics **Abstract P166**

A CASE OF ACUTE ENCEPHALOPATHY WITH BIPHASIC SEIZURES AND LATE REDUCED DIFFUSION(AESD) ASSOCIATED WITH PRIMARY EBV INFECTION

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Abstract

AESD, only reported in Japanese or East Asian infants is characterized status within 24 hours of fever, consciousness relatively to be improved and secondly clustered seizures after 4-6 days of the first seizure. Influenza virus and HHV-6 and 7 are known as causative agents but not EBV. MRI shows no change after first seizures but widespread reduced diffusion 3-9 days after the onset. This time we report the first case of AESD associated with EBV, detected EBV-DNA in the CSF by real time PCR. 1 year-old girl had clonic seizure with fever and went to hospital, diagnosed febrile seizure. Though consciousness recovered, gait and standing difficulties remained in following days. 3 days after the first seizure, second clustered clonic seizures occurred and admitted our hospital. MRI showed high-intensity area in the subcortical white matter of the bilateral frontal and occipital areas on DWI. M-PSL pulse therapy was prescribed for AESD. EBV-DNA was detected in the serum and CSF by real time PCR. EBV related antibodies showed primary EBV infection, such as positive anti-VCA-IgG antibody and negative anti-EBNA antibody, converted positive on following days. In addition, DIHS, approved by DSLT for PB having been used after the clustered seizures was recognized. It is known HHV-6 reactivation is related to DIHS, but EBV related DIHS might be caused by primary infection or reactivation. No typical symptoms of infectious mononucleosis were found in the course of AESD. In this case no neurological sequelae remain.

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Therapy and Diagnostics **Abstract P167**

COMBINING ANTI-TUMOR ACTIVITIES OF RAPAMYCIN WITH RAPAMYCIN-RESISTANT T CELLS

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Abstract

LMP-specific T cells have proved effective for the treatment of type 2 latency tumors that arise in immunocompetent individuals, producing complete remissions in over 60% of patients with EBV-positive lymphoma and over 30% of patients with nasopharyngeal carcinoma. To improve these results, T cells must be engineered to resist the multiple immune evasion strategies used by tumors to inhibit immune responses.

Rapamycin (rapa) is an inhibitor of mTor that regulates extracellular signals at the levels of mRNA translation, and has pivotal regulatory effects on cell cycle progression, cellular growth and proliferation, autophagy and angiogenesis. Recently, rapa has been shown to have potent inhibitory effects on many tumors and is in clinical trials as single and multi-agent chemotherapy. In addition to its direct anti-tumor activity, mTor inhibitors promote proinflammatory responses from macrophages and myeloid dendritic cells and reverse multiple tumor-intrinsic immune evasion mechanisms, effects that should enhance the activity of tumor-specific T cells. However, rapa also directly inhibits T cells so this potential benefit is wasted. A single point mutation in mTor (mTorRR) disrupts its rapa binding site, so we reasoned that expression of this mutant in tumor-specific T cells would provide resistance to rapa allowing a synergistic anti-tumor therapy.

We have expressed mTorRR in tumor-specific T cells from a piggyBac transposon and evaluated their ability to function in the presence of rapa and to combine with rapa to eliminate immunosuppressive tumors. The use of rapa-resistant, LMP-specific T cells could provide a means to improve tumor response rates, particularly in nasopharyngeal carcinoma

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Therapy and Diagnostics **Abstract P168**

EBNA1-SPECIFIC T-CELLS SHOULD BE INCLUDED IN ADOPTIVE IMMUNOTHERAPY FOR POST-TRANSPLANTATION LYMPHOPROLIFERATIVE DISORDERS

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Abstract

Iatrogenic immunosuppression resulting in impaired Epstein-Barr virus (EBV)-specific T-cell immunity is involved in the pathogenesis of EBV-positive Post-Transplant Lymphoproliferative Disorder (EBV^+ PTLD). Restoration of EBV-specific T-cell immunity by adoptive immunotherapy can induce remission. Despite some notable successes, further optimization is required. Clinical data suggests cognate CD4+ T-cell help enhances CD8+ T-cell surveillance, and that broadening antigen-specificity minimizes the risk of tumour escape. EBV-nuclear antigen-1 (EBNA1) is unique in being expressed in all cases of EBV^+ PTLD. Recent data demonstrates EBNA1 is not immunologically silent and can be exploited as a T-cell target. There is no data on EBNA1-specific T-cells in PTLD. Surface capture live cell-sorting of polyclonal EBNA1-specific T-cells, expanded *in-vitro* with 17-mer overlapping peptide pools, were capable of lysing endogenously expressed EBNA1. EBNA1-specific T-cells capable of proliferation, interferon-γ release and CD107a/b de-granulation were assayed in 14 EBV^+ PTLD diagnostic blood samples and 19 healthy controls. EBNA1-specific $CD4^+$ T-cells predominated and were expanded in 10/14 patients and 19/19 controls. Although human leukocyte antigen class I alleles influenced the magnitude of the response, EBNA1-specific $CD8^+$ effector T-cells were successfully generated in 9/14 EBV^+ PTLD patients and 16/19 controls. The majority of PTLD patients had a polymorphism in an EBNA1 epitope, and T-cell recognition was greatly enhanced when EBNA1 peptides from the appropriate viral strain were used rather than equivalent peptides from the prototype EBV-B95-8 laboratory strain. These results indicate that EBNA1-specific T-cells should be included in adoptive immunotherapy for PTLD. Furthermore, expansion protocols should utilize antigenic sequences from relevant EBV strains.

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Therapy and Diagnostics **Abstract P169**

BORTEZOMIB INDUCES APOPTOSIS IN T LYMPHOMA CELLS AND NATURAL KILLER LYMPHOMA CELLS INDEPENDENT OF EPSTEIN-BARR VIRUS INFECTION

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Abstract

Epstein-Barr virus (EBV), which infects not only B cells, but also T cells and natural killer (NK) cells, is associated with multiple lymphoid malignancies. Recently, the proteasome inhibitor bortezomib was reported to induce apoptosis of EBV-transformed B cells. We evaluated the killing effect of this proteasome inhibitor on EBV-associated T lymphoma cells and NK lymphoma cells. First, we found that bortezomib treatment decreased the viability of multiple T and NK cell lines. No significant difference was observed between EBV-positive and EBV-negative cell lines. The decreased viability in response to bortezomib treatment was abrogated by a pan-caspase inhibitor. The induction of apoptosis was confirmed by flow cytometric assessment of annexin V staining. Additionally, cleavage of caspases and polyadenosine diphosphate-ribose polymerase, increased expression of phosphorylated IκB, and decreased expression of inhibitor apoptotic proteins were detected by immunoblotting in bortezomib-treated cell lines. We found that bortezomib induced lytic infection in EBV-positive T cell lines, although the existence of EBV did not modulate the killing effect of bortezomib. Finally, we administered bortezomib to peripheral blood mononuclear cells from two patients with EBV-associated lymphoproliferative diseases. Bortezomib had a greater killing effect on EBV-infected cells. These results indicate that bortezomib killed T or NK lymphoma cells by inducing apoptosis, presumably via the suppression of NF-κB activation by inhibiting the degradation of phosphorylated IκB, regardless of the presence or absence of EBV.

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Therapy and Diagnostics **Abstract P170**

COMPARED EFFICIENCY OF AN ANTI-PROTEASE SIRNA AND ANTI-DNA POLYMERASE DRUGS ON THE INHIBITION OF EBV REPLICATION

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Abstract:

Background: Currently available anti-*herpesviridae* drugs are not licensed for treatment of EBV infection because of their mild efficiency. A small interfering RNA (siPR1) targeting EBV-protease (PR) has recently been described decreasing mRNA protease and virions production *in vitro* [9]. To assess the therapeutic potential of this siRNA, its ability to inhibit the viral replication was directly compared with those of anti-DNA polymerase drugs on the same epithelial cell culture.
Methods: Epithelial 293 cells stably transfected with the complete B95-8 EBV episome were treated in parallel either with siPR1 and/or anti-DNA polymerase drugs (ganciclovir, cidofovir and foscarnet). The reduction of PR mRNA was quantified by real-time RT-PCR in cells and the production of virions in the supernatant was evaluated by Raji cell superinfection and encapsidated EBV DNA quantification by real-time PCR.
Results: A significant decrease ($P<0.05$) of the mRNA protease was obtained with all antiviral compounds but was statically stronger ($P<0.01$) with ganciclovir-siPR1 associated. SiPR1 alone or associated to ganciclovir led to a strongest decrease of the infectious viral titre than all anti-DNA polymerase drugs (95.5% inhibition *versus* 68% for ganciclovir, $P<0.05$). The superiority of the association over the siPR1 alone, assessed with the encapsidated EBV-DNA quantification, was not confirmed by the infectious virions measurement.
Conclusion: The anti-EBV-protease siRNA used in this study exhibited a most important potency to decrease the production of new infectious virions than the conventional anti-*herpesviridae* drugs. Moreover the association of this siRNA with ganciclovir seems even more potent and opens interesting therapeutic perspectives

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Therapy and Diagnostics **Abstract P171**

INTERCEPTING LMP1 WITH DNAZYME TARGET NPC

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Abstract

Epstein-Barr virus (EBV)-encoded latent membrane protein 1 (LMP1) has been known to have oncogenic properties during latent infection in nasopharyngeal carcinoma (NPC). Genetic manipulation of the LMP1 expression may provide a novel strategy for the treatment of NPC. DNAzymes are synthetic, single stranded DNA catalysts that can be engineered to bind to and cleave the target mRNA of a disease-causing gene. By targeting the LMP1 mRNA, we successfully obtained a phosphorothioate-modified ‘‘10–23’’ DNAzyme namely DZ1, by screening a series of DNAzymes. DZ1 could significantly down-regulate the expression of LMP1 in NPC cells, inhibited cell proliferation, metastasis, and promoted apoptosis in NPC through interfering signaling pathways that was abnormally activated by LMP1, which include NF-κB, AP-1 and STAT3 signaling pathways. Furthermore the active DNAzyme enhanced radiosensitivity both in vitro and in vivo. To explore underling the DZ1 activity, we showed that the radiosensitivity was mediated via NF-κB/ATM pathway. Together, interfering LMP1 signaling pathway could be a promising strategy to target the malignant phenotypes of NPC.

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Therapy and Diagnostics **Abstract P172**

EBV-EA IGG RAPID TEST FOR NPC SCREENING

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Abstract

Nasopharyngeal carcinoma (NPC) is a very common cancer in China and Southeast Asia. In Indonesia, undifferentiated carcinoma (WHO type III) is the most common head and neck cancer and among the five most prevalent cancers overall, with 12.000 new cases diagnosed each year. Due to unspecific symptoms and the hidden localization of the primary tumor at the early stage, more than 80% of the patients were diagnosed at a late stage (III or IV), when they already have metastasis in cervical lymph nodes. At early stage NPC may reach complete remission by radiotherapy only, but late-stage disease requires combined chemo-radiotherapy, which unfortunately produces undesirable complications. Furthermore, the outcome of patients with advanced stage or relapsing disease is very poor. Therefore, screening for early-stage NPC is important and clinically relevant. For developing countries, such an approach should be economical, with simple but well standardized methods suited for mass screening.

This study produced a “native” EA antigen applied for a simple rapid test using immunochromatography method for NPC diagnosis detecting IgG-EA EBV. Sera from Indonesian NPC patients taken at primary diagnosis (n=102) were analyzed compared to regional healthy blood donors (n=16), yielding combined sensitivity/specificity values of 83.5%/100%. In combination with an established peptide-based IgA-EBV ELISA developed previously the sensitivity will increase close to 99.3%. This rapid test provides an affordable approach for NPC screening and will be applied in patients with chronic head and neck problem before used in mass screening for identification of early-stage NPC in high-risk regions.

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Therapy and Diagnostics **Abstract P173**

EBV-INFECTED NK-CELL LPD ARISING FROM DONOR NK CELLS AFTER UMBILICAL CORD BLOOD TRANSPLANTATION FOR THE TREATMENT OF CHRONIC ACTIVE EBV INFECTION (NK-CELL TYPE)

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Abstract

Background. Chronic active Epstein-Barr virus infection (CAEBV) is a prototype of EBV-associated T- and/or NK-cell lymphoproliferative diseases (EBV+ T/NK-cell LPD). Chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) is the only way for cure. In the situation of umbilical cord blood transplantation (UCBT), as donor cells are free from EBV, endogenous EBV can be eradicated from recipients (patients with CAEBV) by preconditioning and transplantation. However, such patients should be infected with third party-derived (exogenous) EBV afterward: that is called “secondary primary EBV infection.”
Case. A female, who had been diagnosed as NK cell-type CAEBV at the age of 9 years, had achieved complete remission by prednisolone, interferon gamma and IL-2 administration and splenectomy. However, she relapsed in 5 years after off therapy. She underwent UCBT in success at the age of 28, and anti-VCA IgG became almost negative. She suffered from B-cell LPD in her brain and retina 10 months after UCBT, and was treated with R-CHOP chemotherapy in success. However, she suffered form EBV+ NK-cell LPD 2 years after UCBT. EBV-infected NK cell was confirmed as donor origin. She achieved complete remission again after 3 courses of high-dose cytarabine-based chemotherapy, and is alive well without EBV reactivation.
Discussion. Although LPD after HSCT is mostly donor-derived B cell, donor T/NK cell can also be infected with EBV. Secondary primary (exogenous) infection may occur several months-years after UCBT. Anti-EBV antibody titers and EBV load in PB should be monitored in the routine medical checkup particularly after UCBT.

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Therapy and Diagnostics **Abstract P174**

T CELLS REDIRECTED AGAINST CD70 FOR THE IMMUNOTHERAPY OF EBV-POSITIVE LATENCY TYPE I, II, AND III MALIGNANCIES

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Abstract

CD70, a tumor necrosis family member, is aberrantly expressed on EBV-associated malignancies regardless of EBV latency type. While type III latency tumors can be readily targeted with EBV-specific T cells, targeting type II and I latency is more challenging since these malignancies express a limited number of subdominant EBV-antigens. Redirecting T-cells to cell surface antigens with chimeric antigen receptors (CARs) is an attractive strategy to overcome this limitation.

A CD70-CAR was constructed by fusing the full-length CD70 receptor (CD27) to the signaling domain of the T-cell receptor zeta chain. T-cells expressing CD70-CARs were generated by retroviral transduction. CD70-CAR T-cells recognized CD70+/EBV+ lymphoblastoid cell lines (LCLs), Burkitt's and NK/T-cell lymphomas, nasopharyngeal carcinoma cell lines, and primary tumor samples as judged by cytokine secretion as well as cytolytic activity. In contrast, normal B- and T-cells were not recognized. Co-IP studies revealed that the CD27 costimulatory domain of the CD70 receptor retains its ability to associate with TRAF2, costimulating T-cells and enhancing their effector function in comparison to T cells expressing CD70-CARs with a deleted TRAF2 binding site. Murine xenograft studies demonstrated significant *in vivo* antitumor activity of CD70-CAR T-cells.

We have successfully engineered a CD70-CAR that simultaneously redirects T-cell specificity to CD70 and provides a costimulatory signal to T-cells. CD70-specific T-cells but not control T-cells had antitumor activity ex vivo as well as in a murine xenograft model. Targeting CD70 using genetically modified T-cells may be an attractive new immunotherapeutic approach for the treatment of CD70+ malignancies with minimal 'off target' effects.

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Therapy and Diagnostics **Abstract P175**

THE ROLE OF GALECTIN-1 IN RITUXIMAB MEDIATED ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY IN PTLD.

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Abstract

EBV+ post-transplant lymphoproliferative disorder (PTLD) is in part a consequence of iatrogenic immunosuppression. However reduction in immunosuppression results in objective clinical response in only a minority of patients. A variety of immune evasion mechanisms in non-PTLD lymphoma patients have been described. We investigated whether these mechanisms were operational in the context of established PTLD. Real-time PCR and immunohistochemistry revealed intra-tumoural positivity for Galectin-1 but not the immune inhibitory molecules PDL1/2 and Galectin-9. Galectin-1 is a carbohydrate-binding lectin known to trigger the selective apoptosis of CD4+ Th1 cells and CD8+ CTL. We have previously demonstrated Galectin-1 impaired EBV-specific T-cell immunity in Hodgkin's Lymphoma. However, data on the role of Galectin-1 on NK-cell mediated ADCC is lacking. This is critical, as the main-stay of current PTLD management is the anti-CD20 monoclonal antibody rituximab, with the principal mechanism being antibody dependent cellular cytotoxicity (ADCC). Rituximab monotherapy induces remission in only 30% of patients. We developed a flow cytometry based ADCC assay against a CD20 positive lymphoma cell-line which enables functional and phenotypic analysis of NK-cells. Preliminary data with purified resting NK-cells indicates that Galectin-1 reduces Rituximab mediated ADCC. Furthermore, we have also established a method for expanding NK-cells that preserves their capacity to mediate rituximab mediated ADCC. These expanded cells contain seemingly diverse populations of NK-cells, as determined by staining with CD16, CD56, NKp44, NKp46 and CD27. The ADCC function and cytokine profile of these subsets, along with their sensitivity to Galectin-1 will also be determined.

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Author Index

INDEX (Author, page number)