Propagating Epstein-Barr Virus in Stratified Epithelium

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The Epstein-Barr Virus (EBV) – “The First Human Tumor Virus”

Enveloped γ-herpesvirus with 170 kb linear dsDNA genome
Originally discovered in 1964 in Burkitt lymphoma
Primarily infects B and epithelial cells
Approximately 95% prevalence in world’s adult population
EBV Primarily Infects B Cells and Epithelia

(Thorley-Lawson and Allday, 2008)
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EBV is Associated with Lymphocyte and Epithelial Malignancies

Several big questions remain in the field:
1. How does EBV infect epithelia and through what receptors?
2. How does productive replication proceed in epithelia?

<table>
<thead>
<tr>
<th>Disease</th>
<th>At risk population</th>
<th>EBV association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte origin</td>
<td>Adolescents/young adults from western societies/high socioeconomic groups</td>
<td>Majority. IM-like syndromes also occur in cytomegalovirus, HIV primary infection</td>
</tr>
<tr>
<td>Infectious mononucleosis (IM)</td>
<td>Male offspring of female carriers of XLPS mutation</td>
<td>Majority. A few non-EBV-associated lymphomas occur in children with the mutation</td>
</tr>
<tr>
<td>X-linked lymphoproliferative syndrome (XLPS)</td>
<td>Post-transplant lymphoproliferative disease</td>
<td>^90% &lt;100% ^50%</td>
</tr>
<tr>
<td>B lymphoproliferative disease (BLPD)</td>
<td>HIV infection—primary central nervous system lymphoma—peripheral lymphoma</td>
<td>97–100% ^25%</td>
</tr>
<tr>
<td>Burkitt’s lymphoma (BL)</td>
<td>African children—endemic BL</td>
<td>Overall ^65%</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>HIV infection—sporadic BL</td>
<td>Mixed cellularity type 80%</td>
</tr>
<tr>
<td>T/NK cell lymphoma</td>
<td>Chronic active EBV</td>
<td>Childhood ^80%</td>
</tr>
<tr>
<td>Primary effusion lymphoma</td>
<td>HIV infection</td>
<td>10–100%, depending on histological type</td>
</tr>
<tr>
<td>Epithelial cell origin</td>
<td>HIV infection</td>
<td></td>
</tr>
<tr>
<td>Oral hairy leukoplakia</td>
<td>Other Immunodeficiencies</td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>Chinese and Inuit races—high incidence</td>
<td>Non-keratinised 100%</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>Not identified</td>
<td>Keratinised 30–100%</td>
</tr>
</tbody>
</table>

(Macsween and Crawford, 2003)
EBV Productively Replicates in Raft Culture

Early Genes

Late Genes

Magnification = 10X
MHV-68 Encodes a Glycoprotein for Viral Spread

A Gamma-Herpesvirus Glycoprotein Complex Manipulates Actin to Promote Viral Spread

Michael B. Gill, Rachel Edgar, Janet S. May, Philip G. Stevenson*

Division of Virology, Department of Pathology, University of Cambridge, Cambridge, United Kingdom

Cos7 cells infected with MHV68
A (New) Glycoprotein Discovered: BDLF2

The BDLF2 protein of Epstein–Barr virus is a type II glycosylated envelope protein whose processing is dependent on coexpression with the BMRF2 protein.

Mindy Gore, Lindsey M. Hutt-Fletcher *

Previously believed to be a tegument protein*
Primary Hypothesis

- EBV glycoprotein BDLF2 plays an important role in intercellular trafficking during EBV infection
- BDLF2(BMRF2) may modulate the actin cytoskeleton of its host cell in order to facilitate infection of nearby susceptible cells.
BDLF2 is Expressed in Raft Culture

BDLF2
Nucleus
Zta

Ian Hayman
BX-1 and BDLF2 - Knockout Viruses

neoR
GFP

PuromycinR

Latency-Associated
Early Lytic
Late Lytic
Uncharacterized

Adapted from Kristen Yetming with permission
**Confirmation of Loss of BDLF2 in rEBV**

<table>
<thead>
<tr>
<th>BX-1</th>
<th>BX-1 (fAb)</th>
<th>ΔBDLF2</th>
<th>ΔBDLF2 (fAb)</th>
<th>EBV(-)</th>
<th>EBV(-) (fAb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDLF2</td>
<td><img src="image1" alt="BDLF2 BX-1" /></td>
<td><img src="image2" alt="BDLF2 ΔBDLF2" /></td>
<td><img src="image3" alt="BDLF2 ΔBDLF2 (fAb)" /></td>
<td><img src="image4" alt="BDLF2 EBV(-)" /></td>
<td><img src="image5" alt="BDLF2 EBV(-) (fAb)" /></td>
</tr>
<tr>
<td>Zta</td>
<td><img src="image6" alt="Zta BX-1" /></td>
<td><img src="image7" alt="Zta ΔBDLF2" /></td>
<td><img src="image8" alt="Zta ΔBDLF2 (fAb)" /></td>
<td><img src="image9" alt="Zta EBV(-)" /></td>
<td><img src="image10" alt="Zta EBV(-) (fAb)" /></td>
</tr>
<tr>
<td>EBNA1</td>
<td><img src="image11" alt="EBNA1 BX-1" /></td>
<td><img src="image12" alt="EBNA1 ΔBDLF2" /></td>
<td><img src="image13" alt="EBNA1 ΔBDLF2 (fAb)" /></td>
<td><img src="image14" alt="EBNA1 EBV(-)" /></td>
<td><img src="image15" alt="EBNA1 EBV(-) (fAb)" /></td>
</tr>
<tr>
<td>Lamin B</td>
<td><img src="image16" alt="Lamin BX-1" /></td>
<td><img src="image17" alt="Lamin ΔBDLF2" /></td>
<td><img src="image18" alt="Lamin ΔBDLF2 (fAb)" /></td>
<td><img src="image19" alt="Lamin EBV(-)" /></td>
<td><img src="image20" alt="Lamin EBV(-) (fAb)" /></td>
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</table>
BX-1 and ΔBDLF2 Akatas Produce Similar Titers of Virus

Genome Count from Akata Supernatants

Endonuclease Resistant Virions / mL

10^9
10^8
10^7
10^6

BX-1
ΔBDLF2
BX-1 and ΔBDLF2 Viruses Infect Similarly in Monolayer

**Genome Count from Akata Supernatants**

- BX-1
- ΔBDLF2

**Monolayer Infection with rEBVs**

- BX-1
- ΔBDLF2 infected

**Cell Line**

- Raji
- 293s
- AGS
Infection with ΔBDLF2 is Impaired in Raft Culture

<table>
<thead>
<tr>
<th></th>
<th>Hoechst</th>
<th>Zta</th>
<th>BDLF2</th>
<th>Merge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akata 21 (WT)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
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<td>BX-1</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
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<td>ΔBDLF2</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Uninfected</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
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Ian Hayman
Primary Conclusions Thus Far

- BDLF2 does not appear necessary for monolayer / suspension infection
- BX-1 and ∆BDLF2 Akata strains produce similar titers of virus
- EBV BDLF2 appears to play role in viral spread in differentiated epithelium
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Questions?