Epstein-Barr Virus (EBV) complications following HSCT: from viremia to post transplant lymphoproliferative disease (PTLD) and lymphoma

Laurie A. Milner, MD

Associate Professor,
Pediatric Hematology / Oncology / HSCT
Pathology and Laboratory Medicine
Medical Oncology and the Wilmot Cancer Center
Medical Director, Stem Cell Processing Laboratory
University of Rochester Medical Center

February 12, 2009

No conflicts of interest or financial disclosures
Diseases Associated with EBV

1. Mononucleosis
2. Burkitt Lymphoma
3. Nasopharyngeal carcinoma
4. Hemophagocytic lymphohistiocytosis (HLH)
5. Lymphoproliferative Syndromes, e.g. Juvenile Myelomonocytic Leukemia (JMML) Post-Transplant Lymphoproliferative Disease (PTLD)
1. γ Herpes virus

2. Once you’ve had it…..you have it “forever” (virus becomes latent in memory B cells)

3. Expansion of infected B cells is controlled by CD 8⁺ cytotoxic T cells (CTL) and NK/LAK cells
EBV Infection Post-HSCT

1. Viral Reactivation

2. T cell immunosuppression

3. Expansion of infected B cells
Clinical Manifestations of EBV Infection

1. Asymptomatic
2. Fever
3. Tonsillar enlargement
4. Lymphadenopathy
5. Hepatosplenomegaly
6. Bowel obstruction
7. Respiratory symptoms due to pulmonary infiltration or airway compression
8. “B” symptoms: sweats, weight loss
9. CNS symptoms
10. Cytopenias due to BM involvement
Spectrum of EBV infections in the immunocompetent host

- Asymptomatic
- Mononucleosis (Fever, LAD, HSM, Fatigue)
- Chronic EBV Infection
- Lymphoproliferative syndromes
  - HLH, Malignancies

Common

Rare
Spectrum of EBV infections in the immunocompromised host

“Reactivation”
- Viremia
  - HLH
  - PTLD
    - Malignancies

asymptomatic
- Nonspecific (fever, rash, fatigue)
  - focal or disseminated
  - PTLD

LYMPHOMA
  - leukemias

Common

Uncommon
Natural history of EBV infections in the immunocompromised host

“Reactivation”

- Viremia
- HLH
- PTLD
- Malignancy

low copy number (viral genome)

- high copy number

- high copy number & cellular proliferation

spontaneous resolution

- rapid progression

- Fatal (~90% MR)
Development of EBV-associated PTLD requires:

1. EBV reactivation

2. T cell suppression

3. Presence of B cells
Factors that influence the development of PTLD

1. EBV reactivation
   - Serologic status of donor and recipient
   - Age: children > adults

2. T cell suppression
   - T cell depletion of the stem cell product
   - T cell directed immunosuppression of the patient
     - especially antibody therapy: ATG, OKT3, α-CD3

3. Presence of B cells
   - Stem cell product - “early” PTLD (< 1 year) almost always involves proliferation of donor B cells
   - Cell dose
# EBV infections post HSCT: therapeutic options

<table>
<thead>
<tr>
<th>Target</th>
<th>Agents</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Virus</td>
<td>Antiviral drugs</td>
<td>Mixed (not usually sufficient)</td>
</tr>
<tr>
<td></td>
<td>Ganciclovir</td>
<td></td>
</tr>
<tr>
<td>2. B cells</td>
<td>Rituximab (α-CD20 MoAb)</td>
<td>Very good (if used early)</td>
</tr>
<tr>
<td></td>
<td>chemotherapy</td>
<td>anecdotal responses</td>
</tr>
<tr>
<td>3. T cells</td>
<td>Discontinue immunosuppression</td>
<td>Mixed (limited by GVHD)</td>
</tr>
<tr>
<td></td>
<td>Donor Lymphocyte Infusions (DLI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EBV specific cytotoxic T cells (CTL)</td>
<td>Very good (if available)</td>
</tr>
</tbody>
</table>
Monitoring HSCT patients for EBV infection/PTLD

**Pre-transplant**
- helpful (postpone transplant)
- helpful to assess risk of “reactivation”
- may be helpful in specific cases

**Post-transplant**
- generally not helpful (non-specific, too late)
- Serologies (recipient and donor)
- NOT helpful

**Clinical symptoms**
- generally not helpful

**Quantitative PCR for viral DNA**
- Helpful (if done correctly & standardized)
Studies using Q-PCR to evaluate EBV viremia & PTLD have been limited by numerous factors

- Relatively low incidence of PTLD
  - unrecognized and unreported cases
  - few cases in any given study

- Single institution and retrospective analyses
  - variable treatment regimens & patient populations

- Changing technologies to detect viremia
  - lack of standardization and variable sensitivity
  - use of different patient samples & unit basis

- Evolving transplant regimens and the use of new immunosuppressive agents
  - especially the increasing use of reduced intensity (RIC) and nonmyeloablative (NMA) regimens
Despite the complicating factors, Q-PCR studies have led to important insights into the incidence and risk factors for EBV viremia & PTLD following HSCT.

The following slides represent combined data from multiple studies; they are intended to illustrate trends and consensus observations.

Variable patient populations, transplant regimens, and other factors unique to each study preclude direct comparisons in most cases.
## Incidence of EBV Viremia & PTLD post HSCT

<table>
<thead>
<tr>
<th>Type of HSCT</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloablative Matched sib</td>
<td>8-10%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>MUD</td>
<td>25-30%</td>
<td>4-6%</td>
</tr>
<tr>
<td>UCB</td>
<td>2-5%</td>
<td></td>
</tr>
<tr>
<td>Non-myeloablative or reduced intensity</td>
<td>30-50%</td>
<td>10-15%</td>
</tr>
</tbody>
</table>

- The increased incidence of EBV reactivation and PTLD in NMA/RIC transplants is likely due to the highly immunosuppressive regimens employed, particularly Fludarabine + ATG
Incidence of EBV PTLD post UCBT

- Low incidence of EBV reactivation & PTLD with UCBT reflects the lower numbers of mature lymphocytes & decreased probability of EBV in the product.

- However, UCB recipients treated with NMA/RIC regimens that include ATG are at significant risk for PTLD.

Brunstein. Blood. 2006

<table>
<thead>
<tr>
<th></th>
<th>Myeloablative</th>
<th>Non-myeloablative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/240 (2%)</td>
<td>6/95 (7%)</td>
</tr>
<tr>
<td>without ATG</td>
<td></td>
<td>1/65 (1.5%)</td>
</tr>
<tr>
<td>with ATG</td>
<td></td>
<td>5/30 (17%)</td>
</tr>
</tbody>
</table>
## Impact of T cell depletion on EBV Viremia & PTLD

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ex vivo treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmanipulated graft</td>
<td>25-30%</td>
<td>0-5%</td>
</tr>
<tr>
<td>T cell depleted graft</td>
<td>30-65%</td>
<td>10-20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>In vivo treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>in vivo T cell depletion</strong> (ATG, OKT3, $\alpha$-CD3)</td>
<td>50-65%</td>
<td>15-25%</td>
</tr>
<tr>
<td><strong>in vivo T &amp; B cell depletion</strong> (Campath)</td>
<td>15%</td>
<td>1-7%</td>
</tr>
</tbody>
</table>

- *Risk depends on method of depletion: highest risk with ATG, $\alpha$-T cell Abs; lower with Campath, CD34 selection.*
### Pediatric Patients

<table>
<thead>
<tr>
<th>Conditioning Regimen</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced Intensity (RIC)</td>
<td>35.4%</td>
<td>15.4%</td>
</tr>
<tr>
<td>Conventional (myeloablative)</td>
<td>8.8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*In vivo* T-cell depletion

<table>
<thead>
<tr>
<th>T-cell depletion</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG</td>
<td>34.9%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Campath</td>
<td>16.4%</td>
<td>6.8%</td>
</tr>
<tr>
<td>None</td>
<td>11.8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Disease**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1º Immunodeficiency</td>
<td>32.0%</td>
<td>12.0%</td>
</tr>
<tr>
<td>All Others</td>
<td>15.7%</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

**Acute GVHD**

<table>
<thead>
<tr>
<th>Acute GVHD</th>
<th>Viremia</th>
<th>PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>32.0%</td>
<td>16.0%</td>
</tr>
<tr>
<td>Absent</td>
<td>15.7%</td>
<td>2.4%</td>
</tr>
</tbody>
</table>
Changing the natural history of EBV infections in HSCT patients

“Reactivation”

Viremia

HLH

PTLD

Malignancy

low copy number (viral genome)

high copy number

monitoring

high copy number & cellular proliferation

preemptive or prompt therapy

spontaneous resolution

progression

fatal
Using Q-PCR to predict progression of viremia to PTLD

Viremia (>1000 gEq/ml) → 39% PPV, 100% NPV for PTLD

Wagner, et.al. Blood. 2004
Viremia (>4000 gEq/μg DNA) x 2 → 50% PPV, 100% NPV

Viremia >1000 gEq/ml plasma distinguished asymptomatic viremia from EBV disease, but not EBV disease from PTLD
The predictive value of Q-PCR can be increased by combining it with an assessment of T cell immunity

1. Absolute lymphocyte counts

2. EBV specific CTL– tetramer analysis

<table>
<thead>
<tr>
<th>QPCR</th>
<th>QPCR + EBV specific CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>40%</td>
<td>100% PPV</td>
</tr>
</tbody>
</table>

- Patients with very low T cell / EBV CTL numbers in the context of EBV viremia have an extremely high risk of developing PTLD
## Preemptive therapy for EBV infections post HSCT

**Van Esser, et.al. Blood. 2002**

**T Cell depleted Allo-HSCT**

<table>
<thead>
<tr>
<th></th>
<th>Preemptive TX</th>
<th>Historical controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viremia &gt;1000 gEq/ml</td>
<td>17/49 (35%)</td>
<td>26/85 (31%)</td>
</tr>
<tr>
<td>PTLD</td>
<td>3/17 (18%)</td>
<td>10/26 (38%)</td>
</tr>
<tr>
<td>PTLD mortality</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

**Cohen, et.al. Leuk&Lymph. 2007**

**RIC Allo-HSCT**

<table>
<thead>
<tr>
<th></th>
<th>Preemptive TX</th>
<th>Prompt TX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viremia &gt;1000 gEq/ml</td>
<td>26/52 (50%)</td>
<td>23/65 (35%)</td>
</tr>
<tr>
<td>PTLD</td>
<td>0</td>
<td>10/23 (43%)</td>
</tr>
<tr>
<td>PTLD mortality</td>
<td></td>
<td>2/10 (20%)</td>
</tr>
</tbody>
</table>
Preemptive vs prompt therapy for post HSCT EBV

Brunstein, et.al. Blood. 2006

UCBT

<table>
<thead>
<tr>
<th></th>
<th>Prompt TX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Rituximab x 4)</td>
</tr>
<tr>
<td>Viremia</td>
<td>15/335 (4.5%)</td>
</tr>
<tr>
<td>PTLD</td>
<td>11/15 (73%)</td>
</tr>
<tr>
<td>PTLD mortality</td>
<td>6/11 (55%)</td>
</tr>
</tbody>
</table>

Comoli, et.al. Amer.J.Transplant. 2007

Pediatric Haplo -HSCT

<table>
<thead>
<tr>
<th></th>
<th>Preemptive TX</th>
<th>Prompt TX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viremia</td>
<td>12/27 (44%)</td>
<td>3/65 (35%)</td>
</tr>
<tr>
<td>PTLD</td>
<td>4/12 (33%)</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>PTLD mortality</td>
<td>0/4 (0%)</td>
<td>3/3 (100%)</td>
</tr>
</tbody>
</table>

4 patients developed PTLD resistant to Rituximab and were salvaged with CTL
Preemptive therapy: unresolved issues

- Untreated PTLD has a very high MR: >90%
- Rituximab is highly effective if used early: 70-100% RR
  
  But...many patients get treated unnecessarily to prevent PTLD in a single patient
  
  EBV infected B cells may become CD20\(^-\) and thus resistant to rituximab
  
- EBV-CTLs are highly effective
  
  But...are also time consuming, expensive, & require a GMP facility
  
  Cannot be derived for all patients
Bottom Line?

1. **Monitor** at risk patients by weekly Q-PCR

2. **Pre-emptive treatment** when viremia reaches threshold
   - Rituximab (+/- ganciclovir, IVIG)

3. **Discontinue** immunosuppression (if possible)

4. **Consider DLI** (Donor Lymphocyte Infusion)
   - or EBV specific **CTL**, if available

5. **Chemotherapy** for evidence of clonal malignancy
Risk Factors for developing PTLD: Summary

- T cell depletion of the stem cell product
  - Haploidentical HSCT
  - HLA mismatched donors
- T cell immunosuppression
  - ATG, OKT3, α-CD3
- NMA / RIC regimens
- Grade III-IV GVHD
- EBV serologic status of donor and recipient
  - seropositive donor and naïve recipient
- Primary immunodeficiency disorders
- Age: children at higher risk than adults
References