The Tale of two Herpes Viruses: CMV and EBV

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Cytomegalovirus

Sharon F. Chen, MD, MS
Cytomegalovirus

CMV is a childhood infection

After primary CMV infection, CMV transitions to latency.
<table>
<thead>
<tr>
<th>Strategy</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>Continuous anti-viral</td>
</tr>
<tr>
<td>Preemptive</td>
<td>Trigger used to start anti-viral</td>
</tr>
<tr>
<td>Hybrid</td>
<td>Mix use dependent on time</td>
</tr>
</tbody>
</table>
## Prevention Strategies - Which one?

<table>
<thead>
<tr>
<th></th>
<th>CMV Syndrome &amp; Disease</th>
<th>Late Onset CMV</th>
<th>Graft Loss, Rejection, Mortality</th>
<th>Leukopenia, Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prophylaxis</strong></td>
<td>-</td>
<td>OR 6.21</td>
<td>-</td>
<td>OR 1.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR 2.07</td>
</tr>
<tr>
<td><strong>Preemptive</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Comparison</strong></td>
<td>No Difference</td>
<td>Prophylaxis higher risk</td>
<td>No Difference</td>
<td>Prophylaxis higher risk</td>
</tr>
</tbody>
</table>

Florescu DF. Clinical Infectious Diseases 2014, 58:785
## Survey of Strategies Used

<table>
<thead>
<tr>
<th>Strategy</th>
<th>% Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>46%</td>
</tr>
<tr>
<td>Preemptive</td>
<td>21%</td>
</tr>
<tr>
<td>Hybrid</td>
<td>33%</td>
</tr>
</tbody>
</table>

LePage AK. Transplantation 2013, 95:1455

Razonable. Am Journal Transplantation 2013, 13:93
CMV is a problem in transplantation because the immune system is not normal.

Should we evaluate the immune response to CMV?
Since a detectable specific T-cell response against CMV is correlated with an appropriate immune control of CMV of the recipient [10], the monitoring of cell-mediated immunity may be useful in establishing the real risk for developing CMV disease after transplantation and, therefore, for individualizing preventive strategies accordingly.
CMV-specific T-cell Monitoring

Rationale:

If left untreated, some patients with asymptomatic CMV “DNAemia” will never progress to CMV disease because their CMV-specific immune response spontaneously controls the virus.
## Spontaneous CMV Clearance

<table>
<thead>
<tr>
<th>CMV Cell-mediated Immunity</th>
<th>Spontaneous CMV Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>24/26 (92%)</td>
</tr>
<tr>
<td>Negative</td>
<td>5/11 (45%)</td>
</tr>
</tbody>
</table>

\( p=0.004 \)

Lisboa LF. Transplantation 2012. 93:195
CMV-specific T-cell Monitoring
Adult Lung Transplant Patients D+/R-
CMV-specific T-cell Monitoring

Clinical Aims:

✓ To determine duration of CMV prophylaxis
✓ To determine risk of developing CMV disease

Challenges:

✓ Standardization of assays to monitor CMV-specific T-cells
✓ Designing intervention trials
The Virus

• In the herpes virus family: HHV4
• Ubiquitous, one of the most common human viral infections
  – 50% infected by 5 years, 90-95% by adulthood
• Spread through body fluids, predominantly saliva
  – Infectivity may last for weeks after primary infection, and can reoccur with reactivation
  – Spread through organ transplantation
• Once primary infection occurs, the virus establishes life long latency in a small population of B cells
Table 1. Clinical spectrum of Epstein-Barr virus (EBV) disease in solid-organ transplant recipients

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical symptoms</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-specific viral infection</td>
<td>Fever, Malaise, Anorexia, Abdominal pain, Diarrhea</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Fever, Malaise, Anorexia, Adenopathy, Tonsillitis/pharyngitis, Hepatosplenomegaly</td>
<td>Proliferation of EBV-positive immunoblasts in the interfollicular region of lymphatic tissue in the absence of effacement and/or destruction of normal tissue architecture</td>
</tr>
<tr>
<td>Post-transplant lymphoproliferative disorders</td>
<td>Fever, Malaise, Anorexia, Diarrhea, Abdominal pain, Hematochezia, Tonsillitis/pharyngitis, Hepatosplenomegaly</td>
<td>Proliferation of EBV-positive immunoblasts and atypical lymphocytes associated with effacement and/or destruction of normal tissue architecture. May be polymorphic or monomorphic in appearance.</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>Rapidly progressive growth of EBV-associated tumors despite reduction or withdrawal of immunosuppression</td>
<td>Indistinguishable from lymphoma in immunocompetent patients</td>
</tr>
</tbody>
</table>
The importance of EBV

• Particularly problematic for pediatric organ recipients
  – Large number experience primary EBV infection post transplant

• The most important long term outcome is Post-transplantation Lymphoproliferative Disease (PTLD)
  – Occurs in 1-15% of liver/renal and up to 6-20% in lung/intestinal/heart
  – Outcomes of PTLD are variable to institutions and are organ-specific
    • Overall ~14%
    • 44% intestinal
    • 31% heart
    • 30% lung
    • 22% liver
    • ~0% renal
  – Prevention is key but early diagnosis is associated with better outcomes, low threshold of suspicion for disease
Risk Factors for PTLD

- Seronegativity at time of transplant-5-7 times increased risk
- Use of T cell suppressive therapy for transplant preparation and post-transplant
- Certain HLA types
- Extremes of age
- Circulating virus at time of transplant
- Viral co-infections
- Persistent low levels of viremia post-transplant
Life Cycle of EBV

1. Modification of B cell development
   - LCL
   - PTLD
   - Re-entry?

2. Escape from Immune surveillance
   - GC B cells
   - Latent Infection: Type 0
     - No Viral protein expression
   - Reactivation?

3. Genomic Instability
   - Hodgkin's lymphoma
   - Burkitt's lymphoma

Aging
Organ transplant
AIDS
PTLD Pathway

- Primary EBV infection → Lytic infection → Latent Infection → Uncontrolled B cell proliferation-immortalization
- Adaptive immunity
- T cell suppression
- Malignancy
- Monoclonal expansion
- Polyclonal expansion
Surveillance

- No established cut-offs signifying PTLD disease or risk but usually detection in more than one sample prompts action
- 100% and 86% for PTLD using a cutoff of 2,000 copies/µg, 100% and 90% for 3,000 copies/µg, and 67% and 94% for 5,000 copies/µg.
- Only decreasing immunosuppression as preemptive therapy has been shown to be effective in reducing EBV disease including PTLD
- Older studies supported IVIg, but newer ones did not

Green, Am J of Transpl: 2006
Plasma vs Whole blood

- Both are good tests and can detect EBV genome by PCR
- Plasma is more specific while whole blood is more sensitive (contains EBV infected lymphocytes)
- Plasma is more stable if sample storage is an issue
### TABLE 3. Common laboratory tests to diagnose and manage EBV-related PTLD

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Specimen types</th>
<th>Analyte</th>
<th>Indications for testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time PCR or similar EBV load assay</td>
<td>Blood, plasma</td>
<td>DNA</td>
<td>Predict current or impending PTLD or assess efficacy of therapy</td>
</tr>
<tr>
<td></td>
<td>Biopsy, aspirate</td>
<td>DNA</td>
<td>Detect and semiquantify EBV</td>
</tr>
<tr>
<td>EBER in situ hybridization</td>
<td>Biopsy, aspirate</td>
<td>RNA</td>
<td>Detect and localize latent EBV</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LMP1, EBNA1</td>
<td>Biopsy, aspirate</td>
<td>Protein</td>
<td>Detect and localize latent EBV</td>
</tr>
<tr>
<td>CD20</td>
<td>Biopsy, aspirate</td>
<td>Protein</td>
<td>Classify PTLD and predict response to anti-CD20 immunotherapy</td>
</tr>
</tbody>
</table>

*a Biopsy or cell aspirate is typically paraffin embedded and sectioned onto glass slides prior to testing.

Gully et al, Clin Microbiol Rev: 2010
Management

• Reduction of immunosuppression first introduced in the 1980s and remains the initial approach
  – 23-86% of organ recipients with non-malignant disease will respond in 2-4 weeks
  – Allows for host immune recovery and persistence for viral control

• Both acyclovir and ganciclovir show in vitro activity against the lytic phase of EBV (replicating), ganciclovir is ~ 10x more potent
  – Neither suppresses EBV-induced B cell proliferation or latent EBV within B cells
  – No prospective studies showing efficacy in treatment of PTLD disease, but routinely used
  – High peripheral viral loads associated with disease or PTLD are present despite antiviral therapy and recent studies show that the majority of these cells are immortalized B cells without lytic EBV activity and therefore not susceptible to antiviral therapy
  – The only role may be by inhibiting the few cells with lytic infectious virus from spreading to new sites
**Management**

- IVIg including CMV-IVIg has been shown in vitro to be effective in controlling EBV infected cells
  - Studies have shown absence of antibodies to one of the EBNA proteins in patients with PTLD
  - Other studies have documented decreasing viral loads with increasing anti-EBNA antibodies (native or transfused)
- Rituximab or anti-CD20 monoclonal antibody is effective and should be used for PTLD that has a strong CD20 phenotype which is not routinely the case
Management

• Chemotherapy, radiation and surgery
  – Radiation and surgery of little value unless only localized disease but typically high viral loads signify systemic disease
  – Chemotherapy is immunosuppression and interferes with host immunity but appear to have a role in malignant forms of the disease
• Trials with cytokines IFN (α or γ) were initially promising with efficacy against the PTLD but increased rates of rejection and this has not been pursued further
The possibility of EBV-specific T cells

- Role for T cells in viral control has been established
- Studies have shown increased CD4+ and CD8+ EBV-specific T cells during treatment with reduced immunosuppression in PTLD patients*
- In addition, rebound viral loads after PTLD treatment were in the presence of strong T cell responses and well controlled
- Successful treatment of SCT recipients with EBV-specific T cells has been reported and in increasing use
- Issue for SOT are complex**
  - Unlike SCT recipients whose PTLD is derived from donor thus T cells from the original source can be obtained and are effective against disease, for SOT recipients the lesions are recipient derived and more likely from previous naïve patients. Therefore developing large quantities of T cells requires in vitro “immunization” of patients T cells before transfer
  - Studies are promising and ongoing

*Wilsdorf et al; Transplantation & Volume 95, Number 1, January 15, 2013
**Green, et al, Ped Trans 1999
### Management

**Table 2. Initial approach to pediatric transplant recipient with non-malignant Epstein-Barr virus–post-transplant lymphoproliferative disorders (EBV–PTLD) at Children’s Hospital of Pittsburgh**

<table>
<thead>
<tr>
<th>Liver</th>
<th>Kidney</th>
<th>Heart</th>
<th>Lung</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune suppression</td>
<td>Stop tacrolimus/CsA(^1), AZA(^2), MMF(^3)</td>
<td>Stop tacrolimus/CsA, AZA, MMF</td>
<td>Stop tacrolimus/CsA, AZA, MMF</td>
<td>Stop tacrolimus/CsA, AZA, MMF</td>
</tr>
<tr>
<td>Steroids at maintenance</td>
<td>Steroids at maintenance</td>
<td>Steroids at maintenance</td>
<td>Steroids at maintenance</td>
<td>Steroids to maintenance</td>
</tr>
<tr>
<td>Antiviral therapy</td>
<td>Ganciclovir IV(^6)</td>
<td>Ganciclovir IV</td>
<td>Ganciclovir IV</td>
<td>Ganciclovir IV</td>
</tr>
<tr>
<td>Clinical F/U, Rejection Surveillance</td>
<td>Monitor LFTs</td>
<td>Monitor renal function (2–3 per week)</td>
<td>Echocardiograms at 1 week then every 1–2 weeks Weekly EBV PCR Daily examination</td>
<td>TBB(^7) 7–10 days into treatment then PRN based on status Weekly EBV PCR Daily examination</td>
</tr>
<tr>
<td></td>
<td>Weekly EBV PCR</td>
<td>Weekly EBV PCR</td>
<td>Surveillance biopsies</td>
<td>Endoscopy with biopsy every 1–2 weeks</td>
</tr>
<tr>
<td></td>
<td>Daily examination</td>
<td>Daily examination</td>
<td>Renal biopsy for suspected ACR</td>
<td>Weekly EBV PCR</td>
</tr>
<tr>
<td></td>
<td>Liver biopsy for suspected ACR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)CsA, Cyclosporin A; \(^2\)AZA, Azathioprine; \(^3\)MMF, Mycophenolate mofetil; \(^4\)CsA level obtained by monoclonal assay; \(^5\)FK, Tacrolium; \(^6\)IV, intravenous; \(^7\)TBB, transbronchial biopsy; \(^8\)CXR, chest radiograph.

- Green, et al, Ped Trans 1999
Viral Load monitoring Post PTLD

- Use of viral load by PCR has been shown to correlate with disease regression and may predict time to rejection
  - Centers using 200 copies/10^5 PBL as threshold for PTLD diagnosis, when levels dropped to this level disease regression was seen and the start of rejection noted
  - The use of post-PTLD viral monitoring has been challenging as rebound levels are seen routinely without recurrent disease
- Repeat disease is seen in <10%