Priming of Type-1 EBV-specific CD8+ T cell from EBV− pediatric HTx recipients for cellular immunotherapy for PTLD

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PTLD

- Life-threatening complication after SOTx
- Triggered by EBV infection in chronically IS recipients
- Highest incidence in pediatric SOTx patients (EBV−)
- Current treatments not always effective (25% refractory cases)
Goal of our study

- Adoptive immunotherapy with EBV-specific CTL for refractory PTLD in pediatric SOTx patients

- Establish protocols for efficient priming of naïve T cells into EBV-specific Type-1 CTLs
Adoptive immunotherapy with CTLs

**In BMTx successful**

- PTLD are exclusively of donor origin mandating ex vivo generation of donor-derived EBV specific CTLs from healthy EBV positive subjects.

**In SOTx more challenging**

- PTLD are exclusively of recipient origin (IS) mandating ex vivo generation of patient-derived EBV specific CTLs. EBV negative Tx patients very challenging

Hypothesis

**Effective anti-EBV Type-1 immunity**

- **LCL**
- **PBMC (EBV⁻)**
- **DC**

**Type-1 polarizing cytokines**

Brombacher F, Trends in Immunology, 2003
Our previous data:

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Effectiveness</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LCL-based protocols</strong></td>
<td>- not effective at priming Type-1 EBV-specific T cell responses in EBV− adults or children</td>
<td>Popescu I. et al., <em>Amer J. of Transpl.</em> 2003, 3 (11) : 1369 -1377</td>
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<td></td>
<td>- effective only in the presence of IL-12p70</td>
<td>Metes D. et al., <em>Transplantation</em> 2000, 70(10): 1507-1515</td>
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<tr>
<td><strong>DC-based protocols</strong></td>
<td>- effective at priming Type-1 EBV-specific T cell responses in EBV− healthy or SOTx adults</td>
<td>Popescu I. et al., <em>Amer J. of Transpl.</em> 2003, 3 (11) : 1369 -1377</td>
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<td>- effective for EBV+ pediatric SOTx patients</td>
<td>Popescu I. et al., <em>Amer J. of Transpl.</em> 2007, 7(5):1215-1223</td>
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<td></td>
<td>- what about EBV− pediatric SOTx patients?</td>
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</table>
Patient Demographics

<table>
<thead>
<tr>
<th>PEDIATRIC HTx PATIENTS*</th>
<th>PATIENTS</th>
<th>SEX</th>
<th>AGE</th>
<th>EBV STATUS Pre-Tx</th>
<th>EBV STATUS Post-Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=4</td>
<td>1F / 3M</td>
<td>10.2 ± 6.2</td>
<td>Serology (VCA IgG)</td>
<td>PCR (EBV DNA)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*All patients are HLA-A2 and are on Tacrolimus-based IS, with variable use of anti-proliferative agents and/or steroids.
Methods

Ex vivo co-culture

PBMC

IL-4
GM-CSF

EBV sup

LCL

iDC

IL-1β
IL-6
TNFα

IFNγ

DC1

EBV Pep

2h

DC1+

Peptides

Ly

IL-12

Yield/phenotype

CD8+ T cells (EBV Pep)
IFN-γ ELISPOT
T-bet/IL-12Rβ2

10 days/21 days

Ex vivo co-culture

Slide 8
EBV-specific CTL yield after *in vitro* stimulation

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**EBV-specific CTL yield**

- **LCL**
- **LCL+IL-12**
- **DC/pept.**
- **DC/pept.+IL-12**

**Yield expansion (x)**

Time of EBV stimulation (days)

![Graph](https://example.com/graph.png)

- **DC/pept.**
- **DC/pept.+IL-12**
- **LCL**
- **LCL+IL-12**

**p < 0.02**

**Slide 9**
Phenotype of *in vitro* generated CTLs

![Graph showing the phenotype of in vitro generated CTLs with different conditions.](image-url)
LCL can prime naïve CD8+ T cells into Type-1 EBV-specific CD8+ T cells only in the presence of exogenous IL-12p70

Slide 11
Cytokines that regulate Type-1 responses

IL-12Rβ1

Naïve T cells

IL-12p70

IL-27R

Naïve T cells

T-bet

Th1 Cell

T-bet

β2

β1

β2

β1

IFN-γ

IL-27

IL-27R
Kinetics of T-bet induction in CD3+ T cells by DC vs LCL protocols

Days

T-bet (%)
Kinetics of IL-12Rβ2 expression on CD3+ T cells by DC vs LCL

![Graph showing IL-12Rβ2 expression over days](image-url)

- T(DCpept)
- T(DCpept)+IL12
- T(LCL)
- T(LCL)+IL12

Days vs IL-12Rβ2 (%)
Modulation of IL-12Rβ2 expression on CD8^+ CTLs by DC vs LCL

**DC/pep**

- Block IL-12
- Block IL-27
- Block IFN-γ

**LCL**

- Block IL-12
- Block IL-27
- Block IFN-γ

*IL-12Rβ2 (%)*

- Media
- +IL-12p70

*\(p < 0.05\)
**\(p < 0.02\)
***\(p < 0.002\)
Proposed model for naïve precursor priming into Type-1 EBV-specific CD8+T cells

DC1

IL-12p70

MHC I

CD86

CD40

IL-12p70

IL-27

T-bet

CD8

IL-12Rα

IFN-γ

LCL

IL-12p70

MHC I

CD86

CD40

IL-12Rβ2

IFN-γ

T-bet

IL-12Rα

IL-12p70

NK

IL-27
The role of Type-1 polarizing cytokines on IFN-γ production by \textit{in vitro} primed EBV-specific CD8+ T cells using DC vs LCL protocols

![Graphs showing IFN-γ production](image-url)
Conclusions

• DC1 can not trigger up-regulation of IL-12Rβ2 expression on naive CD8+ T cells, nor Type-1 CD8+ T cell priming (IFN-γ production)

• Addition of exogenous IL-12p70 is not effective at up-regulating IL-12Rβ2 expression, nor at Type-1 CD8+ T cell priming (IFN-γ production)

• The inducible expression of IL-12Rβ2 and subsequent IFN-γ release by naïve CD8+ T cells using in vitro DC protocols is possible, and dependent on exogenous IL-27 and IFN-γ

• These results (lack of Type-1 priming by DC) are observed only when samples from pediatric EBV- Tx patients are used, suggestive of an age-dependent deficiency in Type-1 polarizing cytokines by DC and T cells
Conclusions cont’

• LCL (and not DC) via endogenous IL-27 may trigger up-regulation of IL-12Rβ2 expression on naive CD8+ T cells, rendering them responsive to IL-12p70

• Further addition of exogenous IL-12p70 is critical for effective Type-1 CD8+ T cell priming (IFN-γ production), since LCL are not an important source of IL-12p70

• LCL (unlike DC) support NK cell expansion, and exogenous IL-12p70 further activates NK cells to secrete IFN-γ

• The inducible expression of IL-12Rβ2 and subsequent IFN-γ production by naïve CD8+ T cells when using LCL protocols is dependent on IL-27 and IFN-γ (indirectly stimulated by exogenous IL-12p70)

• LCL + IL-12p70 protocol is currently used to prime EBV-specific CTLs for adoptive immunotherapy for refractory PTLD in pediatric HTx patients.
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