Translating EBV Immunology from Bench to Bedside:
Somnium Animadverto
A Dream Realized
Epstein-Barr Virus

- Human herpesvirus
- 90% population infected
- Associated with many other cancers
- Latent infection...persists for life
Epstein-Barr Virus

Glandular Fever (Infectious Mono)

- Posttransplant Lymphoma
- Nasopharyngeal Carcinoma
- Hodgkin’s Lymphoma
- Gastric Carcinoma
- Nasal T cell Lymphoma
- Burkitt’s Lymphoma
- Breast Cancer???
Immune Control of Epstein-Barr virus

EBV or B cell

B cell Blast (EBNA1-6, LMP1&2)

Proliferation

CD8+ T cell response controls EBV infection

CD8+ T cell = CTL
Viral Gene Expression of EBV-associated malignancies

Latency I
Burkitt’s Lymphoma
EBNA1 EBERs

Latency II
Hodgkin’s lymphoma
Nasopharyngeal carcinoma
EBNA1 EBERs LMP1 LMP2

Latency III
Post-transplant lymphomas
EBNA1-6 EBERs LMP1 LMP2
EBV-Associated Cancers: QIMR Immunotherapy Program

Post-transplant lymphomas
Hodgkin's Lymphoma
Nasopharyngeal Carcinoma
Post-transplant lymphoproliferative disease (PTLD)

Organ Transplant

EBV-infected cells proliferate

Polyclonal lymphomas

Intensive Immunosuppression

Loss of Virus-specific CD8+ T cells
Genesis of PTLD in Transplant Patients

**Stem Cell Tx**
- EBV transferred in the graft
- Includes EBV+ B Cells
- Tx recipient EBV- specific CTL immunity
- PTLD emerges of donor origin

**Solid Organ Tx**
- EBV transferred from the graft
- Solid organ
- EBV+ cells in graft
- EBV donated with graft is released
- Infects recipient B cells
- EBV CTL immunity
- PTLD emerges of recipient origin
1. Lower Immunosuppression
2. Chemo/radiotherapy
3. Treatment with anti CD20

Adoptive transfer EBV-specific CTL: a Treatment Option?
Adoptive immunotherapy for BMT/Stem Cell Tx Patients

~100% success rate (>50 patients)

Cliona Rooney, Helen Heslop et. al
Problems of Generating EBV-specific CTLs from Solid organ Tx Recipients

1. Patients under immunosuppression
   Will CTL expand?

2. Allospecific T cells
   Potential to cause graft rejection
Expansion of EBV-specific CTLs in the laboratory

EBV-infected B cells

Mix and Culture (3 weeks)
(responder : stimulator ratio 40:1)

Add rIL-2

After 4-6 weeks

40-50 fold expansion of killer T cells

Inject these cells back into the patient

Patients white blood cells
Liver CT scan Pre & Post CTL immunotherapy

Preinfusion          Postinfusion
2 weeks             20 weeks

Khanna et. al. PNAS
Heart Tx

Female, 53
1996

3 years later

EBV+ve

EBV+ve PTLD

Nodular, large B cell
s.c. lymphoma on
back & buttock

>16 lesions of
recipient origin

EBV+ve Lymphoma
Re-emerged

Drug Resistant PTLD
Scan of PTLD before and after transfer

Preinfusion

Postinfusion
Limitations of CTL-based immunotherapy

T cell expansion takes 6-8 weeks – Too long some Patients

Heterogeneity in PTLD and their susceptibility to T cell therapy
# PTLD Ig Phenotype and EBV gene expression

<table>
<thead>
<tr>
<th>Case</th>
<th>Ig phenotype</th>
<th>EBV Ag Expression</th>
<th>Ig genotype</th>
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<tr>
<td>A</td>
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<td>+</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>B</td>
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<td>+</td>
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<td>C</td>
<td>n.d.</td>
<td>+</td>
<td>Naïve B cell</td>
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<td>Memory B cell</td>
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<td>H</td>
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<td>Post-GC, non-Ag-selected</td>
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<td>K</td>
<td>l</td>
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<td>k</td>
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</table>

PTLD Ig Phenotype and EBV gene expression

**Polyclonal**

**Naïve B cell**

**Memory B cell**

**Post-GC, non-Ag-selected**

**Post-GC, sterile**
EBV-specific T cells from allogeneic HLA matched healthy donors?

Safe - Yes
GvHD - No
Therapeutic - Yes
Prophylactic - Yes

Haque and Crawford, 2003 Lancet
Gandhi et al. 2007, AJT In press
EBV-specific Allogenic T cells for PTLD immunotherapy

Haque et al., Blood (2007) In press
EBV-specific Allogenic T cells for PTLD immunotherapy

Total number of PTLD patients treated: 28

Number of responders (%)

- 5wk
- 6mo
- <16
- 16-49
- ≥50
- <2
- >2
- 1
- >1

Haque et. al., Blood (2007) In press
CT Scan of PTLD before and after allogeneic EBV CTL Immunotherapy
New Strategies in PTLD immunotherapy

Australasian Allogeneic EBV T cell bank

PBMC → In vitro expanded EBV-specific CTLs → T cell culture → Adoptive immunotherapy

EBV-infected B cells

CHAT Therapy
Chemotherapy → anti-CD20 → T cell therapy

Viral Gene Expression of EBV-associated malignancies

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LMP1
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EBNA1
EBERs

Latency III
Post-transplant lymphomas
EBNA1-6
EBERs
LMP1
LMP2
Hodgkin’s Lymphoma

Stages

I 1 lymph node (LN) region
II >1 LN region; same side of diaphragm
III involvement on both sides of diaphragm
IV extra-nodal site (s)

A absence of “B” symptoms
B weight loss and fevers

40-50% HL are EBV-positive
(100% in HIV-infected individual)
Study Design:

90 patients enrolled (32 Female and 58 Male)
Median age 32yrs (range 6-76)

36 newly-diagnosed (ND)
8 relapsed (RL)
46 long-term survivors (LTS).

97% were EBV-seropositive.

LMP1 and EBER staining revealed that 21/59 (35%) patients had EBV-positive HL

Loss of LMP1/2-specific T cell Function in HL Patients

Loss of LMP1/2-specific T cell Function in HL Patients

Pre-Rx n=87(28)
6m n=45(14)
12m n=41(14)
remission n=136(37)
healthy n=41(20)

* * *

SFC/10^6 PBMC

LMP1 & 2 (p<0.0001) EBNA3/4/6 (p=0.3575) Lytic (p=0.083)
HL Patients show normal Frequency of LMP-specific T cell precursors

% Tetramer +ve CD8+ T cells

Ratio of γ-IFN producing and tetramer +ve cells

p=0.047
Potential Mediator: LAG-3

LAG-3 T cell

APC MHC class II

Blocks maturation of APC

Blocks T cell response

Potential Mediator: LAG-3
LAG-3 expression on T cells is co-incident with loss of EBV-specific T cell function
Galectins and Tumour Immune Regulation

[Tumoral lysis]

Primary tumour

Eosinophils

IL5

Th2 CD4+

B cell

IL4

IFNγ, perforins, granzymes

T cell

Gal-2

Gal-3

Gal-9

Gal-1

Apoptosis

Tumour-immune escape

Th1 CD8+

Th1 CD4+

Dendritic cell

IL12

B7

CD40

MHC

Tumour antigens
Galectin-1 and Hodgkin lymphoma

Number of infiltrating CD8+ T-cells within HRS rich areas

P < 0.002

Blood (2007) In press
Galectin-1 inhibits EBV-specific T cell function

Galectin-1 inhibits EBV-specific T cell function.
Mechanism for HL immunoevasion

Galectin-1  
IL-10  
IL-13  
Active STAT3

HRS Cell

CD4 T cells  
LAG-3-, CD25- CTLA4-

Loss of T cell function

Active STAT3

CD4 T cells  
LAG-3+, CD25+ CTLA4+

Blocks DC maturation
Hodgkin’s “Axis of Evil”

pSTAT3 $\rightarrow$ Galectin-1 $\rightarrow$ LAG-3

Loss of
T cell function
Designing therapeutic vaccine for EBV+ HL and NPC

E1-LMPpoly™
Our approach

Identify CD8+ T cells epitopes within LMP1 and LMP2
E1-LMPpoly™

The polyepitope is incorporated into the delivery vector

Adenochimera™
Preclinical testing of E1-LMPpoly™

Check for
(a) LMP1 & 2-specific T cell responses
(b) EBNA1-specific T cell responses
(c) Protection from tumour challenge
LMP/EBNA1-specific T cell response in E1-LMPpoly™ immunized mice

AdE1-LMPpoly

IFN-γ SFC/10^6 cells

EBNA1

Control

CD8-PerCP

CD4-FITC

5.94

1.76

7.54

0.01
Stimulation with E1-LMPpoly™ restores T cell function in HL/NPC patients

Stimulation with E1-LMPpoly™ restores T cell function in HL/NPC patients

Adoptive transfer of E1-LMPpoly T cells reduces viral load

EBV-infected B cells

Tumour growth for 14 days

Adoptive immunotherapy

Monitor DNA load

AdE1-LMPpoly

Control (PBS)
Phase I testing of E1-LMPpoly™-stimulated T cells in HL/NPC patients (June 2007)

A collaborative study between ACVD and HKU
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“Success consists of going from failure to failure without the loss of enthusiasm”
Winston Churchill