Immune reconstitution after HSCT

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Outline

The biology of immune development

How to measure immune reconstitution

How immune reconstitution can be improved after BMT
Immune development

Defence against microorganisms

Tolerance to self
Infect strain A mouse with lymphocytic choriomeningitis virus (LCMV)

Infect target cells with LCMV

7 days

LCMV-specific CTLs

Cytotoxicity assay
Coculture CTL and target cells and measure lysis of target cells

CTL | Target cell | Specific lysis
--- | --- | ---
LCMV peptide | Self peptide | CTL recognizes foreign peptide + self MHC

LCMV infected | Yes

Strain A uninfected | No

Strain B LCMV infected | No

Failure to recognize self peptide + self MHC

Failure to recognize foreign peptide + allogeneic MHC
Self MHC restriction in the thymus

- T cells with TCR recognizing self MHC molecules are retained (positive selection)
- Retained T cells with TCR recognizing self peptide associated with self MHC are eliminated (negative selection)
- Self MHC-restricted T cells are released
central deletion in the thymus = mechanism of central tolerance

Thymic stroma

- exogenous antigen expressed in the thymus

- MHC
- TCR

no recognition of self-MHC

Affinity for self-MHC

survival (5%)

death-by-negative selection

TCR affinity/avidity

positive selection

activation-induced apoptosis

Negative Selection
Central thymic deletion is incomplete

**Thymus**
- Autoreactive T cell
  - Negative selection
  - Positive selection
  - "Death by neglect"

**Periphery**
- Autoimmune response
  - Escape
- Tolerance
- Immune response
Regulatory T cells
Haemopoietic reconstitution post-transplant

- Time after therapy:
  - 1
  - 2
  - 3
  - 4
  - 5
  - 10
  - 14
  - 16
  - 18
  - 20
  - 22

- Haemopoietic cells (%):
  - 0.00
  - 25.00
  - 50.00
  - 75.00
  - 100.00
The proportion of CD4+CD25+FoxP3+ cells expand after transplant

Weng et al, PNAS 2007
400cGy + BM
cd

CD25 Ab

T_reg depletion

CD25 Ab
Autologous HSCT is an effective treatment for severe AID

Systemic sclerosis

Farge et al., Ann Rheum Dis 2004
How do we measure immune reconstitution?
Factors Influencing the Rate of Immune Reconstitution

- Age - both of donor and recipient
- Conditioning
- Stem cell source
- T cell depletion
- Infection
- Acute or chronic GvHD
Peripheral T cells

Thymic output

TRECs: TCR rearrangement excision circles
Thymic Output in Normal Individuals

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TREC/100,000 cells

Thymic Output in Normal Individuals
There are known to be 24 TCR Vβ families comprising of a number of subfamilies.

Vβ family bind approx. 100bp 5’ from the V-D junction, giving a PCR product of 320-380bp.

Rearrangement of the TCR gene creates variations in the CDR3 length of the TCRβ of up to 9 amino acids.
Average Range of TCR Repertoire Diversity in Patients Post BMT

- **Children**
- **Adults**
- **GvHD**

**Lower end of normal**
Functional assays to enumerate antigen-specific T cells

- ELISPOT
- Intracellular cytokine staining
- Multimers
ELISPOT assay

Step 1
Coating of plates

Step 2
Incubation of cells

Step 3
Removal of cells and addition of probes for detection of secreted Abs

Step 4
Addition of Streptavidine-conjugated enzyme

Step 5
Addition of substrate for visualization of spots

ICC assay

- Images showing flow cytometry data with IFN-γ and CD8 markers.
Donor Monocyte

Single blood draw

Donor serum

GM-CSF

IL4

1 week

CMV Antigen

Dendritic cell

CD8 T Cell

CD4 T Cell

Pre culture:

Post culture:

Post culture pp65 HLA-tetramer selected:

BV 13

BV 1
Long-term outcome and immune reconstitution after haploidentical stem cell transplant in recipients of allodepleted-T-cells expressing the inducible caspase-9 safety transgene

Conclusions

• Immune reconstitution is a biphasic process (homeostatic expansion and immune development)

• It can be accurately monitored and has predictive value on clinical outcome

• Cellular therapies can be effective but their use is still marred by logistical feasibility and/or selectivity