Effector Mechanisms in Allograft Rejection
Solid Organ and Hematopoietic Stem Cell Transplantation

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Innate and Adaptive Effector Mechanisms

Innate Effectors
- Defensins
- Complement
- Neutrophils
- Eosinophils
- Basophils
- Monocytes, MΦ
- Dendritic cells
- NK Cells

Adaptive Effectors
- T lymphocytes
- Th1 vs. Th2
- CD8+ CTL
- B lymphocytes
- Antibodies
Innate Immunity “Sets the stage” for Rejection

- DAMPs from damaged cells activate TLRs, NODs, NLRs, MBL
- Innate cells secrete pro-inflammatory cytokines
- Leukocyte recruitment
- Dendritic cells migrate to draining lymph nodes
- Lymphocyte activation and differentiation into effector cells
- Activated effector lymphocytes infiltrate allograft

Neutrophils

- Evoke injury in tissues injured by stress, ischemia
- Recruited to allografts early post-tx in response to chemokines, IL-17
- Peri-tubular infiltration seen in AMR
- Secrete chemokines CXCL1-8; degranulation of lysosomal granules → heparan-binding protein, enzymes, reactive oxygen species (ROS)
Innate Cellular Defense Mechanisms

*Defensins* = Lysosomal antimicrobial molecules

- Enzymes – lysozyme, phospholipase
- Nutrient binding proteins – lactoferrin
- Cationic proteins with both hydrophobic and charged regions.
  - Disrupt microbial structure by insertion into phospholipid membranes.
- Defensins evoke chemokines for DC,
- Defensin homologs cross many species.

Monocytes Macrophages

- Recruited to graft by IL-1, IL-6
- Activated by IFN\(_\gamma\), TNF\(\beta\), CD40
- Acquire potent anti-microbial activity, produce ROS
  - Nitric oxide (NO)
  - Superoxide (O\(_2^.-\))
- Produce growth factors – TGF\(\beta\), platelet-derived growth factor
- Recruit lymphocytes via chemokines - RANTES/CCL5
FcR Accessory Cells

- Variety of cells with FcR for different Ig isotypes.
- FcR can be activating (associated with cytoplasmic $\gamma$ or $\zeta$ signaling chain) or inhibitory (ITIM bearing)
  - Mast cells, basophils, eosinophils - Fc$\varepsilon$R
  - Phagocytes, DC - Fc$\gamma$RI, Fc$\gamma$RII-A
  - NK cells - Fc$\gamma$RIII (ADCC)
  - B cells - Fc$\gamma$RII-B1

Antibody-dependent Cell-mediated Cytotoxicity

*Figure 9-34 Immunobiology, 7th ed. © Garland Science 2008*
NK Cells And NKT Cells

- Ability to kill tumor cell lines c/o prior sensitization
- Surveillance for “missing self MHC” (Kärre).
- Evolution of human KIR in parallel with HLA class I.
- Bridge between innate and adaptive responses.

NKT Cells

- Heterogenous subsets of T cells
- Type 1 NKT or iNKT express invariant TCR that recognizes glycolipid Ags presented by CD1d
- Human iNKT can be CD4+, CD8+, CD4-, CD8-
- Found in liver, bone marrow; lower frequencies in thymus, spleen, blood
Potential Roles for NK Cells in Transplantation

- Hematopoietic Stem Cell Transplants
  - Graft vs. leukemia effect
  - Prevention of GVHD
- Solid Organ Transplants
  - Role in acute graft rejection
  - Possible role in tolerance induction

NKG2D Ligands
MIC = MICA/B
MHC class I related proteins:
ULBP
RAE
Inhibitory KIR Ligands

- KIR2DL2/3
- KIR2DL1
- KIR3DL1
- KIR3DL2

NK Cell

- HLA-C^Asn80
- C1: Cw3 group
- HLA-C^Lys80
- C2: Cw4 group
- HLA-Bw4
- HLA-A3,11

Target Cell

NK Licensing

- Not all mature NK cells have iKIR
  - Licensed NK have self-iKIR and are tolerant
  - Unlicensed NK are hyporesponsive
- During development, engagement of iKIR with self-MHC ligand is thought to induce functional capability
- Once licensed, NK cells express at least one iKIR
- Activation post-Tx can license NK to kill allogeneic cells
KIR Expression

- Limited to NK cells, minor subset of CD8+ T cells
- KIR are expressed clonally - individual NK cells have different KIR combinations
  - Exception: 2DL4 expressed on all NK
- Allelic variation may affect expression
  - Eg. 3DL1*002, *01502 associated with high levels of expression
- Gene dose effect

Diversity of KIR Expression

Effect of KIR Incompatibilities in GVH Direction

<table>
<thead>
<tr>
<th>KIR MM in GVH Direction</th>
<th>No</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td># of Transplants</td>
<td>58</td>
<td>34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Donor Alloreactive NK clones</th>
<th>1/58</th>
<th>34/34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant Outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection</td>
<td>15.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Acute GVHD ≥ grade II</td>
<td>13.7%</td>
<td>0%</td>
</tr>
<tr>
<td>Probability of relapse at 5 yrs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>90%</td>
<td>85%</td>
</tr>
<tr>
<td>AML</td>
<td>75%</td>
<td>0%</td>
</tr>
<tr>
<td>Event free survival (5 yr) – AML</td>
<td>5%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Studies showing KIR mm Impact:

Studies with Detrimental or No Impact:
Schaffer, et al. Transpl. 78:1081, 2004

Beneficial Effect of NK alloreactivity likely depends on T cell Depletion, NK licensing, and post-Tx immunosuppression.
Reconstitution of Immune Cells After HSC

Donor Stem Cells ± T cells

Transplant

Stem cell differentiation

Donor T cells mediate GVHD, but also:
- Promote engraftment
- Attack malignant cells (GVL)

T cell depletion prevents GVHD, but abrogates GVL.

NK cells rapidly generated in T depleted BM grafts – may provide GVL.

NK action is directed vs hematopoietic cells and endothelial cells. Lysis of recipient APC may prevent GVHD.

Donor NK Cells

Recipient
Hematopoietic Cells

Targets: AML cells APC T cells
Effect: GVL↑ GVH↓ HvG↓

Inhibitory Signal No Inhibition

Match No Action

MisMatch Killing, Cytokines secreted

AML and other leukemia cells susceptible to lysis express PVR & nectin-2 – recognized by DNAM-1; ALL cells do not & are resistant to lysis.
Ligand Incompatibility Model
Based on HLA matching in GVH direction

![Diagram of ligand incompatibility model](image1)


Receptor- Ligand Model (Missing Ligand)
Based on Donor KIR Genotype and Recipient HLA Phenotype

![Diagram of receptor-ligand model](image2)

Haplotype Model

Considers combinations of A and B haplotypes in HLA matched HCT:
- Best survival with Recipient B+ (BB or AB); Donor B – (AA)*
- Poorest survival with Recipient B- (AA)*; Donor B+ (BB or AB)
Suggests that presence of activating KIR in graft → ↑GVHD & ↓GVL

* 28-30% of donors and recipients have AA genotype


What About NK cells in Solid Organ Transplants?

- Role in rejection
  - Data to date suggest role for NK cells, but they are neither necessary nor sufficient
- Impact likely reflects role of NK cells as “innate inducers” of adaptive immunity
Experiment Evidence for Role Of NK Cells IN Heart Tx Rejection

NK cells provide co-stimulation to T cells; H-2d grafts rejected. With NK cell depletion, cardiac grafts from both strains are accepted.


NK Cells: Allograft Rejection

- NK infiltrate allografts before T cells
- NK secreted IFNγ → ↑ MHC cl I, II expression
- NK cytokines shape T cell response
Evidence for NK Role in Solid Organ Transplants

- Kidney, Pancreas cells express high levels of MICA/B – activating ligands for NKG2 (Kitchens, et al. 2006)
- Better outcomes with renal grafts from donors homzygous for C2 alleles (Kunert, 2007)
- C2 allele on liver grafts → less histological evidence of chronic rejection (Hanvesakul, 2008)
- Retrospective study of 137, HLA-A,-B,-DR compatible renal transplants, KIR-ligand mismatches associated with 25% reduction in 10 year, death censored graft survival (van Bergen, et al., 2011)

Role for NK cells in Tolerance?

- Potential mechanism: destruction of host APC and prevention of direct allo-recognition.
- Host NK engage donor DC in draining lymph nodes.

Modulation of Allo-response by NK Cells


Activation of Adaptive Cellular Response

- Dendritic cells from inflamed graft migrate to regional lymph nodes
- Naïve T cells activated via interaction with DC

- Activated naïve cells differentiate into effector cells
- Activated effector cells up-regulate chemokine receptors and migrate to the allograft in response to chemokine gradients
Impact of Cytokines (Signal 3) on Naïve T Cell Differentiation


Different Th Subsets

- Each has unique transcription factor and cytokine profile
- Factors affecting differentiation:
  - Recipient's immune status
  - Degree of ischemia/reperfusion injury
  - Degree of HLA mismatch


Tfh = follicular helper cell
### Th Subsets in Rejection

<table>
<thead>
<tr>
<th>Factor(s)</th>
<th>Th1</th>
<th>Th2</th>
<th>Th17</th>
<th>Th9</th>
<th>Tfh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducing cytokines:</td>
<td>IL-12</td>
<td>IL-4</td>
<td>IL-6, TGFβ, IL-21, IL-23, IL-1</td>
<td>TGFβ, IL-4</td>
<td>IL-21, IL-6</td>
</tr>
<tr>
<td>Transcript. Factor(s)</td>
<td>T-bet, Stat 1, Stat 4</td>
<td>GATA3, Stat 5, Stat 6</td>
<td>RORgt, RORα, Stat 3</td>
<td>PU.1</td>
<td>Bcl-6, Stat 3</td>
</tr>
<tr>
<td>Cytokines produced:</td>
<td>IFNγ, IL-2, TNF, Lta, Perforin, Granzyme</td>
<td>IL-4, IL-5, IL-10, IL-13</td>
<td>IL-17, IL-21, IL-22, TNF</td>
<td>IL-9, IL-10</td>
<td>IL-21</td>
</tr>
<tr>
<td>Effects:</td>
<td>DTH, Activate: NK, Macrophages, B cell help</td>
<td>Regulation, Pro-inflam. May participate in rejection</td>
<td>Recruitment, mast cells, Induce B cell maturation; AMR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### CD8+ CTL Differentiation

**Interaction with Th1 & APC**

<table>
<thead>
<tr>
<th>Stage</th>
<th>CD62L</th>
<th>CD44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>CD62L&lt;sup&gt;hi&lt;/sup&gt;</td>
<td>CD44&lt;sup&gt;lo&lt;/sup&gt;</td>
</tr>
<tr>
<td>T eff</td>
<td>CD62L&lt;sup&gt;lo&lt;/sup&gt;</td>
<td>CD44&lt;sup&gt;hi&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**2-4 days**

<table>
<thead>
<tr>
<th>CD62L&lt;sup&gt;lo&lt;/sup&gt;</th>
<th>CD44&lt;sup&gt;hi&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restricted to LN/spleen</td>
<td>Circulates widely</td>
</tr>
</tbody>
</table>

CD62L = leukocyte adhesion molecule or L-selectin; binds to CD34 → rolling adhesion.
CD44 – binds to hyaluronin in inflamed tissues.
Clonal Expansion: Effector Cells and Memory Cells

Most effector cells are short-lived

Re-exposure to Ag may be required for survival

Generation of Memory Cells Increases Clonal Frequencies

Clonal Expansion:
Effector Cells and Memory Cells

APC

T eff

T eff

T eff

T eff T

mem

T

mem

T

mem

Most effector cells are short-lived

Re-exposure to Ag may be required for survival
Acute Cellular Rejection
Activated Lymphocyte Infiltration of Tubules and Arterial Endothelium

- Early Adhesion - Selectins & GlyCam, CD34
- Strong Adhesion - LFA-1 & ICAM-1

Chemokines & Receptors

Renal Tubular Epithelium
RANTES (CCL5)
CCR5

Chemokine Receptor Expression: T Effector Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Chemokine Receptors</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>CCR5, CXCR3, CXCR6</td>
<td>Defense vs. intracellular pathogens, autoimmunity</td>
</tr>
<tr>
<td>Th2</td>
<td>CCR3, CCR4, CCR8, CRTh2</td>
<td>Defense vs. parasites, allergy, asthma</td>
</tr>
<tr>
<td>Th17</td>
<td>CCR2, CCR4, CCR6, CCR9, CXCR3, CXCR6</td>
<td>Defense vs. extracellular pathogens, inflammation, autoimmunity</td>
</tr>
<tr>
<td>Th22</td>
<td>CCR4, CCR6, CCR10</td>
<td>Tissue immunity and remodeling</td>
</tr>
<tr>
<td>T reg</td>
<td>CCR2, CCR4, CCR5, CCR6, CCR7, CXCR4</td>
<td>Immunosuppression, tolerance</td>
</tr>
<tr>
<td>Tr1</td>
<td>CCR3, CCR4, CCR5, CCR8, CCR9, CXCR3</td>
<td>Immunosuppression</td>
</tr>
<tr>
<td>Tfh</td>
<td>CXCR5</td>
<td>Promotes B cell immunity</td>
</tr>
</tbody>
</table>

Adapted from: Ding Y, Xu J, Bromberg JS. Trend Immunol. 2012;33:174
CTL and NK Cytotoxicity

• Mechanisms are similar for both cell types.
• Three phases:
  – Binding - TCR:MHC; NK lectin receptor.
  – Secretion of cytotoxic granule components.
  – Destruction of target cell - apoptosis.

CD8 Cytotoxicity - Phases

<table>
<thead>
<tr>
<th>Target Binding</th>
<th>Degranulation</th>
<th>Death Signals</th>
<th>Dissociation</th>
<th>New Target Binding</th>
</tr>
</thead>
</table>
Cytotoxic Mediators

• Perforins - Ca++ dependent, delivery of granule contents
• Granzymes – serine esterases that activate caspases → apoptosis.
• Members of TNF superfamily – cytokines/receptors that mediate inflammation, induce apoptosis.
• B cells require T cell help. Encounter occurs in lymph nodes (or other 2nd lymphoid tissue).
• Functions of Lymph nodes: Trapping and concentration of Ags. Without this:
  Probability of Ag-specific T cell meeting an Ab-specific B cell = $1 \times 10^{-8} - 10^{-12}$
**Cytokines Influencing B Cell Development & Differentiation**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>Co-factor with IL-4, IL-5; growth, differentiation</td>
</tr>
<tr>
<td>IL-4</td>
<td>Proliferation; IgE class switch</td>
</tr>
<tr>
<td>IL-5</td>
<td>Proliferation; IgA class switch</td>
</tr>
<tr>
<td>IL-6</td>
<td>Plasma cell differentiation</td>
</tr>
<tr>
<td>IL-7</td>
<td>Growth factor for B cell precursors</td>
</tr>
</tbody>
</table>

**B Memory Cells**

- Characteristics of memory B cells:
  - IG genes re-arranged
  - Isotype switch
  - Somatic hypermutation
- Poly-clonal activation is thought to be responsible for memory maintenance.
- Subset of long lived plasma cells migrates to bone marrow.
Antibody Mediated Rejection (ABMR)

Banff Criteria for Diagnosis of acute ABMR:
- Detection of HLA donor specific Abs
- Diffuse deposition of C4d in peritubular capillaries
- Histologic evidence of acute tissue injury
- Evidence of graft dysfunction

Note: Banff 2011 conference refined diagnostic criteria with recognition of C4d negative ABMR

Anti-MHC Abs have multiple effects on endothelium aside from MAC
- Platelet activation → release of vWF, P-selectin
- Activation of endothelial cells
  - ↑IL-8, MCP-1
- Activation of other cells via FcR, CR1, CR3, etc.

Murata K, Baldwin WM. Transplant Rev. 2009;23:139

Pathology Induced by anti-MHC class I Ab
A. vWF staining. Mice treated with Ab have ↑vWF and Platelet aggregates (arrows)
B. Increased C4d deposition in antibody treated skin grafts

Tertiary Lymphoid Structures (TLT)

- Lymphoid-like structures found in chronic inflammation sites, including allografts
- Similar to secondary lymphoid organs – follicles, germinal center B cells, follicular DCs, T cell areas, high endothelial venules
- Formation appears to require humoral immunity
- Th17 cells may promote chronic rejection
- However, T regs and IL-10+ B cells are also present

Tertiary Lymphoid Tissue in Chronically Rejected Renal Allografts

**Role of TLT in Chronic Rejection?**

Chronic chemokine production recruits B cells to graft → TLT

1. Aggressive response results in ectopic germinal centers → AlloAb + APC function for T cells
2. Alternatively, B reg cells in TLT may promote tolerance and slow chronic rejection

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**Impact of Lymphocyte Depletion on T Memory Cells**

T cells proliferating under lymphopenic conditions → acquire surface / functional traits of memory cells

- Nominal Ags
- Allo Ags
- Naïve T Cells Potential Precursors
- ATG, Campath

↑ Freq. Alloreactive Memory Cells