Innate Immunity in Transplantation

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Innate and Adapative Immunity

• **Innate** or “natural” defense mechanisms phylogenetically older; present in some form in all multicellular organisms.

• **Adaptive immunity** evolved only 400 million years ago in higher vertebrates. Mechanisms are inducible (adaptive), specific and can be recalled (memory).

• Both systems are inter-dependent. Induction of adaptive immunity is generally preceded and shaped by innate response.
Innate Immune System: Recognition

- Germline encoded pattern-recognition receptors (PRR) detect invariant motifs
- PRRs detect pathogen associated molecular patterns (PAMPs)
- Other PRRs detect host-derived danger associated molecular patterns (DAMPs)

PRR Categories

1. Trans-membrane PRRs
   - extracellular and endosomal surveillance
2. Intracellular NLRs (NOD-like receptors)
   - Cytosolic surveillance
3. Secreted PRRs that bind to microbial cell surfaces
   - Complement activation, opsonization
Toll-like Receptors (TLR)

- Family of Type I transmembrane receptors
  - Extracellular leucine-rich repeat (LRR) domains
  - Intracellular Toll/IL-1 receptor (TIR) domain
- Mammalian TLR are homologs to Drosophila Toll receptor (discovered in 1996).
  - TIR domain evolutionarily conserved in plants and animals
- Key signal for inflammation/host defense.

IL-1R/Toll-like Receptor Family

- IL-1R1
- IL-18R
- SIGIRR
- Toll

- IG domain
- TIR domain
- Leucine-rich domain
Human TLRs

10 TLRs characterized:

• TLR 1,2,4,5,6,10 expressed at cell surface; recognize bacterial, fungal, parasitic ligands.
• TLR 3,7,8,9 endosomal expression; recognize viral/bacterial DNA and RNA.
• TLR 2 & 4 – danger sensors; recognize damaged cell components.
  – Heat shock proteins (HSP)
  – Heparan sulfate
  – Hyaluronan fragments, fibronectin

TLR: PAMP Specificity
TLR/NLR as Danger Sensors

TLR Expression: Hematopoietic Cells

- Monocytes, macrophages (Mϕ), Neutrophils (PMN) express most TLRs; TLR 2,4 direct migration, pro-inflammatory
- Effector T cells – TLR 2,3,5,9 serve as co-receptors → proliferation, cytokine production
- T regulatory cells – TLR 1,2,4,8
- B cells – high expression of TLR 1,6-10; proliferation, class switching, plasma cell differentiation

Non-hematopoietic TLR Expression

- TLRs expressed on epithelial, endothelial, parenchymal cells of kidney, heart, lung, liver, skin, brain, intestine
- Parenchymal TLRs appear to initiate/modulate local immune responses
- Expression on both hematopoietic and parenchymal cells may be important in transplantation

Examples of Cytosolic PRR

- Retinoic acid inducible gene 1 (RIG-1)
- Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs)
- NALP proteins – NLRS with pyrin domains that form inflammasomes
- Interferon (IFN) stimulatory DNA sensor (ISD)
NOD-like Receptors (NLRs)

- NLR family - 22 human genes
- Characterized by:
  - nucleotide-binding/oligomerization (NACHT) domains
  - leucine-rich repeats (LRR)
  - N-terminal caspase recruitment (CARD) or pyrin (PYD) domains

Schroder K and Tschopp J. Cell 2010;140:821-832

Inflammasomes

- Upon activation, NLRs assemble into inflammasomes
- High molecular weight platforms that activate caspase -1 → secretion of pro-inflammatory cytokines IL-1β and IL-18
- DAMPs,PAMPs, etc. trigger generation of reactive oxygen species (ROS) → inflammasome formation

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Secreted PRR

• Soluble PRR families that recognize PAMPS on microbial surfaces
  – Pentraxins - C-reactive protein
  – Collectins – mannose binding lectin (MBL)
  – Ficolins – similar functions to collectins
• Secreted PRR also bind to apoptotic and necrotic cells
• Trigger phagocytosis and inflammation via Complement activation

C-Type Lectin Receptors

Characteristic feature: Carbohydrate Recognition Domain
CHO Pattern Recognition by MBL

Secreted Innate Receptors
Complement and Lectin Pathways

C1 Complex
Classical Pathway

Mannan Binding Lectin
Lectin Pathway
DAMPS, PAMPS That Activate TLR, NLR in Transplantation

• Endogenous factors – apoptotic cells, HSP, fibronectin, hyaluronan fragments, high extracellular [ATP] (efflux of potassium), reactive oxygen species, heparan sulfate
  – Surgical stress – tissue damage
  – Ischemia reperfusion injury (IRI) – TLR4 and TLR2 (heart, liver, kidney, lung)
• Exogenous – bacterial translocation
  – Skin, small bowel, lung – high levels of commensal bacteria

TLR Signaling:
- cell activation
- initiates secretion of cytokines, chemokines
- augments inflammation
- induction of adaptive immunity

MyD88 – key adapter protein for signal transduction for all TLRs except TLR3 (TRIF)

Signaling → activation of NFkB → IFN
Consequences of TLR/NLR Inflammation

- Chemokine production/secretion – leukocyte recruitment
- Additional pro-inflammatory cytokines
- Complement activation
- Platelet activation/interaction with endothelium
- Augmented by tissue damage - activation of resident mast cells
  - histamine, serotonin, LTB4 (mast cells)
  - heat shock proteins; breakdown products of fibrin, collagen

Cells of Innate Immunity

- Neutrophils, eosinophils
- Monocytes, macrophages, dendritic cells*
- Natural Killer cells (NK)*

* Function as vital links to adaptive immunity
PMN Emigration

- PMN leave blood in response to chemokines
  - IL-8
  - acPGP (acetylated Pro-Gly-Pro derived by proteolysis of collagen)
- Chemokine receptors - CXCR1, CXCR2
- Diapedesis (exit from blood) facilitated by adhesion molecules
  - Selectins
  - Integrins

Neutrophils

- Neutrophils infiltrate allografts with hours post-Tx → chemokines, inflammatory mediators
  - Depletion reduces ischemia-reperfusion injury
  - Depletion/ inhibition of migration synergizes with co-stimulation blockade → increases long term graft survival
  - May potentiate adaptive alloimmune response
**Eosinophils**

- Eosinophils infiltrate allografts during acute rejection (kidney, heart, liver)
  - In mice, eosinophils appear to promote rejection when classical Th1 pathways are blocked
  - Rejection in patients treated with Campath (T cell depleted) is marked by eosinophilic and monocytic infiltration

**Monocytes**

- Unstimulated monocytes function in phagocytosis via mannose & other receptors
- Scavengers for apoptotic cells
- Migrate rapidly to inflamed tissues (may precede PMNs) and differentiate into macrophages and dendritic cells
- Produce IL-1, IL-6, reactive oxygen species (ROS), degradative enzymes
- Now thought that monocytes can distinguish self from non-self – independent of MHC
NK Cells

• Surveillance for “missing self”
• Activated by type I IFNs, IL-12, TNFα
• Early defense vs. viruses
• Key link between innate and adaptive immunity → ‘shaping’ of adaptive response
• NK cells are alloreactive when donor tissue is missing “self” ligands
• Effector functions: Cytolysis, Production of IFNγ, other cytokines

NK Receptors

• Two structural families.
  – Immunoglobulin (Ig) family   KIR
  – C-type lectin    CD94:NKG dimers NKG2D homodimers
• Include both inhibitory and activating receptors.
• C-type lectin receptors are phylogenetically older.

iKIR = Killer cell IG inhibitory receptor
aKIR = Killer cell IG activation receptor
NK Receptors: Structure

C-TYPE LECTIN IMMUNOGLOBULIN GENE FAMILY

Leukocyte Receptor Complex
Human Chr. 19q

Natural Killer Complex
Human Chr. 12

KLR Gene Nomenclature – Human Gene Mapping Workshop
NKC

- Contains 1 CD94 and 5 NKG2 (A/B, C,D,E/H, F) genes.
- Most receptors are heterodimers of CD94 and NKG2 chain.
  - CD94:NKG2A Inhibitory
  - CD94:NKG2C Activating (via DAP10/12)
  - NKG2D:NKG2D Activating (via DAP10)

NKC Ligands

- Activation – CD94:NKG2C; NKG2D
  MICA and MICB – expression ↑ on stressed cells.
- Inhibitory – CD94:NKG2A
  HLA-E + leader sequence peptides from class Ia molecules.
Inhibitory KIR Ligands

KIR Subset: Class I Specificity:

<table>
<thead>
<tr>
<th>KIR Subset</th>
<th>Class I Specificity</th>
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<tbody>
<tr>
<td>KIR2DL2/3</td>
<td>C1: Cw3 related alleles (Ser 77, Asn 80)</td>
</tr>
<tr>
<td>KIR2DL1</td>
<td>C2: Cw4 related alleles (Asn 77, Lys 80)</td>
</tr>
<tr>
<td>KIR3DL1</td>
<td>Bw4 alleles</td>
</tr>
<tr>
<td>KIR3DL2</td>
<td>A3, A11</td>
</tr>
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Cw3 group: Cw1, 3, 7, 8, 2, 13, 14, *1507, 16.
KIR Inhibitory and Activating Ligands


Normal Cell: MHC Class I +
KIR inhibits KAR Activation

Altered, “missing” MHC Class I (-)
No Inhibition → NK Lysis

Regulation of NK Function

DAP12

KIR2DL2/3
KIR2DS1
Activating Ligand

Normal Cell: MHC Class I +
KIR inhibits activation

KIR2DL2/3
KIR2DS1
Activating Ligand only

Altered, “missing” MHC Class I (-)
No Inhibition → NK Lysis
**NK Lysis Depends on Both “Missing Self” and “Induced Self”**

![Diagram showing NK lysis and MHC ligands](image)

- No self MHC
- KIR 3DL
- MICA MICB ULBP

Infection Malignancy Stress

**Contributions of Innate Responses to Alloimmunity**

- Ligands from damaged cells (DAMPS)
  - Hsp70
  - Polysaccharide fragments of heparan sulphate
  - HMGB1/tage
  - Fibrinogen

- Pattern recognition receptors (PIRs)
  - Cell surface – TLRs, CLRs and CD14
  - Intracellular – NOD, NLRs, RLRs, inflammasomes
  - Secreted - MBL, serum amyloid

Innate immune system

Inflammation
- Complement activation
- Leukocyte recruitment
- Clearance and killing
- Adaptive immunity

May potentiate adaptive alloimmunity and prevent tolerance
May break established tolerance

Transition from Innate Defense to Adaptive Alloreactivity

Danger signals
Innate Defense Cells
- PMN, Mϕ, DC
- NK cells
- APC
Pro-inflammatory cytokines
Inflammatory mediators
Chemokines
Lymphocyte Recruitment and Activation – Donor Specific Alloreactivity

Donor peptide
APCs activated via PRRs take up donor HLA antigens
APCs migrate to draining lymph nodes and present donor HLA peptides to CD4+ T cells
Effector T cells migrate to allograft.

• Naïve lymphocytes are recruited to regional lymph nodes by chemokines; up-regulation of adhesion molecules
• Encounter antigen presenting cells (APC) – particularly dendritic cells migrating from the allograft
• Once activated, lymphocytes can “home” or return to sites of inflammation via chemokines/receptors

CCR7, and its ligands, CCL19, CCL21 involved in lymphocyte homing for naïve and regulatory T cells.
Evidence that Innate Responses Contribute to Alloimmunity

- Role in pathogenesis of ischemia/reperfusion injury and GVHD
- Increased expression of TLRs in allografts
- Correlation of TLR alleles with incidence of rejection
- Ability of NK cells to reject bone marrow grafts
- Presence of alloreactive NK cells in rejecting solid organ grafts


Role of TLRs and NLRs in initiation of GVHD

- Pre-Tx conditioning → Inflammation & tissue damage
- Released tissue degradation products and bacterial components activate via TLR and NLRs
- Cytokine, chemokines recruit leukocytes → initiation of GVHD

Increased TLR2 expression on infiltrating leukocytes and damaged tubules during acute rejection (A&B) compared to normal kidney (C&D).


2 loss of function SNPS:
- Asp299Gly
- Thr399Ile

Reduced responsiveness to LPS

Heterozygous recipients had reduced incidence of acute rejection and bronchiolitis obliterans syndrome.

NK cells and “Hybrid Resistance”
Necessary and sufficient for HSCT rejection in absence of T cell immune response

Rejection of Parental strain A bone marrow graft by F1 (AB) NK cells


Innate Immunity and Tolerance

- TLR signaling may disrupt tolerance by inducing differentiation of Th1 effector cells and inhibiting generation of T regs
- Immunoregulatory effects of NK and dendritic cells may favor tolerance
  - Regulation of T cell activation via cytokine production
  - Elimination of donor APCs
  - Inhibition/killing of effector T cells