NK cells and Pattern Recognition Receptors

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Disclosure

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• Baylor College of Medicine/Texas Children’s Hospital

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Gifts
• Nothing to Disclose

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Learning Objectives

Upon completion of this session, participants should be able to:

• Define the role of and key differences among pattern recognition receptors

• Define the major functions of natural killer cells and identify current themes in their biology.
Innate Immunity - Definition

HARDWIRED immune defense against foreign or dangerous material

All function is encoded within the germline DNA

Danger

Foreign
Three paradigms in innate immunity

1. Recognition
2. Amplification
3. Response
Recognition
Distinguishing good from bad

• Pattern recognition
• Danger
  – Foreign (foreign and dangerous)
    • Non-self (yes – think pathogen)
    • But, not all non-self is dangerous (think food)
  – Alarm (think cancer)
  – Damage (think stress)
  – Innate vs adaptive (adaptive cells can use innate systems)
    – Lectins/collectins (MBL)
    – Antimicrobial peptides
• Dedicated Pattern Recognition Receptors (PRRs)
Amplification

Generation of a signal after recognition to enable a response

- **Intracellular**
  - Adaptors, kinases, GEFs
  - Result in $\text{Ca}^{++}$ fluxes, motor functions
  - transcriptional activation.

- **Extracellular**
  - Chemokines
  - Anaphalotoxins C3a, C4a, C5a
Response
Function directed at eliminating or containing danger

- **Inflammation**
  - Effects on local physiology (vascular permeability, blood flow, endothelial activation)

- **Innate effector mechanisms**
  - Soluble proteins (antimicrobials, complement, apoptosis inducing)
  - Phagocytosis - reactive metabolites
  - Cytotoxicity

- **Initiation of adaptive immunity**
  - Cytokines (polarize T cells, increase adhesion)
  - Chemokines - Recruit adaptive immune cells
  - Costimulation - to adaptive immune cells
  - Antigen processing
Pattern Recognition

• A central theme in innate immunity
  – Inherent means to call immunity into action

• Pattern Recognition Receptors (PRRs)
  – Germline DNA-encoded means for immediate recognition of danger
  – Arguably appreciated decades
  – Defined as such after discovery of Toll-like receptor (TLR) system

• PAMP – pathogen-associated molecular pattern

• DAMP – Danger-associated molecular pattern
Pattern Recognition Receptors

- Five broad structurally defined families
  - Leucine rich repeat (LRR) containing
    - Toll-like receptors (TLRs), NOD-like receptors (NLRs)
  - RNA-sensing RIG-I-like receptors (RLRs)
    - Retinoic acid inducible gene I (RIG-I)
  - DExD/Hbox Helicases (DDX)
  - Pyrin and HIN domain-containing (PYHIN)
  - C-type lectin receptors (CLRs)
    - Dectin-1

- Sub-cellular location specific
  - Cell surface (TLRs, CLRs)
  - Endosomal (TLRs)
  - Cytoplasmic (RLRs, NLRs)
Toll-like receptors (TLR)

The beginning of PRR and the most established LRR-containing PRRs

B. Lemaitre - Nature Immunol 4:521
Toll-like receptors (TLRs) - Recognition

Leucine Rich Repeats (LRR)
19-25 tandem copies of LRR
XLXXLXLXX

Toll/IL-1 receptor (TIR) Domain
Box1, Box2, Box3

10 human TLRs (1-10), TLR11-13 are mice only!
### TLRs and their exemplary ligands

Recognize both **PAMPs** and **DAMPs**

<table>
<thead>
<tr>
<th>TLR</th>
<th>Location</th>
<th>Ligand</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1/2</td>
<td>surface</td>
<td>Lipoarabinomannan, Triacyl lipopetides</td>
<td>Mycobacteria, Bacteria</td>
</tr>
<tr>
<td>TLR2±6</td>
<td>surface</td>
<td>Zymosan, Peptidoglycan, HSP70</td>
<td>Fungi, Bacteria, Host</td>
</tr>
<tr>
<td>TLR3</td>
<td>endosomal</td>
<td>ds RNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR4</td>
<td>surface</td>
<td>lipopolysaccharide, RSV fusion protein, HSP70</td>
<td>Gr- bacteria, RSV, Host</td>
</tr>
<tr>
<td>TLR5</td>
<td>surface</td>
<td>Flagellin</td>
<td>Flagelated bacteria</td>
</tr>
<tr>
<td>TLR6/2</td>
<td>surface</td>
<td>Diacyl lipopeptides</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td>TLR7/8</td>
<td>endosomal</td>
<td>ss GU RNA, Short dsRNA, Imidazoquinolones</td>
<td>Viruses, Synthetic</td>
</tr>
<tr>
<td>TLR9</td>
<td>endosomal</td>
<td>Unmethylated CpG motifs</td>
<td>Bacteria, DNA viruses</td>
</tr>
<tr>
<td>TLR10</td>
<td>Unknown</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>
TLR signal generation to NF-κB after ligation

Cell membrane

TLR 5/10/11

TLR 2

TLR 1/6

TLR 4

CD14

MD2

MyD88

MyD88

MD2

Endosome

Cytoplasm

MyD88

MyD88

Endosome

NF-κB binding motif

Nucleus

New gene transcription

TLR 7/8/9

IRAK1

IRAK2

IRAK4

TRAF6

TRAF3

TRIF

RIP1

TIRAP

MyD88

MyD88

TAB1

TAB2

IκB

= Ubiquitin

= phosphorylation

IKK-α

IKK-β

NEMO

IκB

NF-κB

TAK1
TLR induction of other transcription factors

**Cell membrane**
- TLR 7/8/9
- Endosome
- MyD88
- IRAK1/4
- TRAF6
- IRF7

**Cytoplasm**
- IRAK4
- TRAF6
- IRF5
- MAPK
- AP-1

**Nucleus**
- New gene transcription

**TLR 5/10/11**
- TLR 2
- TLR 1/6
- CD14
- MD2
- TIRAP
- TRIF
- TRAM
- TRAF3
- TBK1
- IRF3
- AP-1

**Endosome**
- TRIF
- TRAM
- TRAF3
Important targets of TLR-induced transcription factors
Amplification - Response

- **NF-κB** - ggg ACT TTC C (ggg RNN YYC C, R=purine Y=pyrimidine)
  Pro inflammatory cytokines - TNF, IL-1, IL-6, IL-12,
  Adhesion molecules, Antimicrobial peptides,
  Chemokines, iNOS, Other transcription factors (IRFs)
  Apoptosis regulators, Complement components (some)
  Antigen processing machinery, Immunoglobulin genes

- **Interferon regulatory factor (IRF)3** - IFNβ, chemokines
- **IRF7** - IFNα, IFNβ, chemokines
- **IRF5** - Pro inflammatory cytokines
- **AP-1** - Pro inflammatory cytokines
Question #1

Which TLR can directly sense danger in the human extracellular environment?

A) TLR1 and TLR3
B) TLR3 and TLR7
C) TLR8 and TLR9
D) TLR7 and TLR11
E) TLR11 and TLR13
### Primary Immunodeficiencies that affect TLR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease</th>
<th>Susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\kappa B\alpha$</td>
<td>ED with immunodeficiency</td>
<td>Pyogenic bacteria and mycobacteria</td>
</tr>
<tr>
<td>NEMO</td>
<td>Immunodeficiency with or without ectodermal dysplasia</td>
<td>Pyogenic bacteria and mycobacteria (NTM)</td>
</tr>
<tr>
<td>MyD88</td>
<td>MyD88 deficiency</td>
<td>Pyogenics and NTM</td>
</tr>
<tr>
<td>IRAK4</td>
<td>IRAK4 deficiency</td>
<td>Pyogenic bacteria</td>
</tr>
<tr>
<td>Unc93B</td>
<td>HSV encephalitis</td>
<td>Herpes Simplex Virus</td>
</tr>
<tr>
<td>TRAF3, TRIF, TBK1</td>
<td>HSV encephalitis</td>
<td>Herpes Simplex Virus</td>
</tr>
<tr>
<td>TLR3</td>
<td>HSE, VZV cerebritis</td>
<td>HSV and VZV</td>
</tr>
<tr>
<td>TLR4</td>
<td>TLR4 deficiency</td>
<td>N. Meningitidis sepsis</td>
</tr>
</tbody>
</table>
NOD-like receptors NLRs

- Nucleotide-binding oligomerization domain (NOD)
- Cytoplasmic sensing
- Nucleotide binding domain and a LRR
  - Can include a CARD or Pyrin domain
- Over 20 different NLRs
  - Ligation leads to inflammasome induction
    - cell death (pyroptosis)
    - proinflammatory cytokine (IL-1B, IL-18)
    - Procaspase-1 secretion
Major NLR PRR types

• NOD family
  – NOD2 binds peptidoglycan Muramyldipeptide
    • Mutated in Crohn’s disease (<30%)

• NALP family (AKA NLRP)
  – Nacht domain leucine-rich repeat and PYD containing proteins (NALPs)
  – NALP3 (cryopyrin, NLRP3)
    • Recognizes Alum (vaccine adjuvant)
    • Mutated in autoinflammatory diseases
    • Muckle-wells, CINCA, Familial cold autoinflammatory syndrome
  – NALP1 (NLRP1)
    • Recognizes bacterial muramyldipeptide
PYHIN - PRRs

- **PYrin and HIN domain-containing**
- **AIM (absent in melanoma) family**
  - non-NLR but functional overlap
  - AIM2 – First identified cytosolic DNA sensor
- **IFI16 (Interferon-γ inducible protein 16)**
  - Nuclear localization
  - Senses viral DNA
  - Signals through STING (Stimulator of InTerferon Genes protein) to produce type-I interferon
NLR inflammasome

Uric Acid

dsDNA

Cell membrane

Endosome

Destabilized endosome

NLRP3

AIM2

ASC

Pro-Caspase1

Pro-IL-1β

IL-1β

IL-1β

Cytoplasm

Nucleus
RLR PRRs

• RNA-sensing RIG-I-like receptors
• Cytoplasmic sensors
• Viral RNA sensors (dsRNA, cytosolic DNA)
• RIG1 (retinoic acid inducible gene I)
  – Discovered for TLR-independent sensing of viral RNA\(^1\)
  – RNA helicase
  – TLR-independent induction of IFN by dsRNA
  – Recognizes in vitro transcribed dsRNA, influenza, paramyxovirus\(^2\)
• MDA5 (Melanoma-differentiation associated gene 5)
  – RNA helicase that complexes with RIG1
  – Recognizes poly I:C, picornavirus\(^2\)
  – Both unwind dsRNA to enable signaling through assembled complex via CARD domain\(^3\)

DDX PRRs

• DDX family (DExD/H box helicases)
  – DDX3, DDX9, DDC36, DDX41, DDX60
• Cytoplasmic sensor
• Sense cytosolic DNA and cyclic dinucleotides (CDNs)
• Activate STING (Stimulator of InTerferon Genes protein)
• STING activation leads to type-I interferon production – STING can also bind CDNs

1 Zhang, et. al. Nat. Immunol 2011 12:959
RLR and DDX cytoplasmic sensors

Virus

Cell membrane

dsRNA

DDX

STING

RIG1

MDA5

IPS1

TBK1

IRF3

IRF7

IKK-ε

NEMO

IKK-α

IKK-β

FADD

TRAF6

RIP1

Nucleus

Cytoplasm

Type-I Interferon

CDN

dsRNA
Question #2
Which of the following is true regarding the NLR and PYHIN PRR-induced inflammasome

A) It cleaves Caspase1 to Caspase2
B) After ligand recognition the receptors move into the endolysosome
C) It leads to the active form of IL-1β
D) It uses Syk to access NLRP3
C-type lectin receptor (CLR) PRRs

- Cell surface sensors
- Dectin-1/2
  - recognizes fungal cell wall $\beta$-glucan (mold allergen uptake)
  - Induces – Syk/CARD9
  - Pathway promotes TH-17 response
- DC sign
  - Recognizes sugars containing mannose and fucose
  - Binds facilitates cell entry of allergens (Arah1/Der p1/2)
- Mannose Receptor
  - Sugars terminating mannose, fucose, or $N$-acetylglucosamine
  - Broad pathogen recognition - Includes candida
  - Facilitates allergen cell entry – Der p1/2, Ara h1
CLR fungal sensing

Dectin1 → Fungal glycans → Src

Dectin2 → Fungal glycans → FcγRI → Syk

NLRP3 → Syk → CARD9, BCL10, MALT1

NF-κB binding motif

IL-1β
Chronic Mucocutaneous Candidiasis

• Breakdown in CLR anti-candidal pattern recognition (or downstream signaling and functions)

• Chronic non-invasive Candidal infections
  – Mucosal and/or dermal candidiasis

• Dectin-1 mutation (truncation)
  • Fails to bind β-glucan
  • Strictly mucocutaneous

• CARD9 mutation (no expression)
  • Some invasive infection – also with dermatophytes
Question #3
Which of the following represents a valid extracellular pattern recognition receptor – ligand pair?

A) Dectin1 – dsRNA
B) TLR4 – Lipoarabinomannan
C) DDX41 - cyclic dinucleotide
D) TLR6 – Zymosan
E) Dectin2 – Uric Acid
Natural Killer (NK) Cells

NK cells are lymphocytes capable of being specifically activated or inhibited after the ligation of germline-encoded receptors.

Cytotoxicity
- Contact dependent danger recognition
- Antibody dependent

Cytokine Production
- inflammation
- promoting immunity
- immunoregulatory subsets

Costimulation
- contact dependent stimulation
NK cell functional subsets

Human NK cell development

Requires IL-15

NK cell inhibition

Activation receptor ligand

Class I MHC
NK cell inhibition
NK cell inhibition

Activation (lysis) receptor

Inhibitory KIR
NK cell inhibition
NK cell inhibition
NK cell inhibition
NK cell inhibition
NK cell inhibition
NK cell inhibition
NK cell inhibition

Activation receptor ligand

Class I MHC

Activation (lysis) receptor

Inhibitory KIR
NK cell activation - “missing self”
NK cell activation - “missing self”

Activation receptor ligand
NK cell activation - “missing self”
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NK cell activation - “missing self”
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NK cell activation - “missing self”
NK cell cytotoxicity – 1.5hr timelapse
Measuring cytotoxicity

% lysis = 100 \times \frac{(\text{Experimental release} - \text{Spontaneous release})}{(\text{Total release} - \text{Spontaneous release})}
### Selected NK cell activation receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>CD</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIII</td>
<td>CD16</td>
<td>IgG Fc</td>
</tr>
<tr>
<td>DNAM-1</td>
<td>CD226</td>
<td>Nectin-2/PVR (CD112, CD155)</td>
</tr>
<tr>
<td>2B4</td>
<td>CD244</td>
<td>CD48</td>
</tr>
<tr>
<td>NKG2D</td>
<td>CD314</td>
<td>MICA/B, ULBP</td>
</tr>
<tr>
<td>NKG2C/CD94</td>
<td>CD159c/CD94</td>
<td>HLA E</td>
</tr>
<tr>
<td>NKp46</td>
<td>CD335</td>
<td>Hemagglutinin</td>
</tr>
<tr>
<td>NKp44</td>
<td>CD336</td>
<td>Hemagglutinin</td>
</tr>
<tr>
<td>NKp30</td>
<td>CD337</td>
<td>BAT3, B7-H6</td>
</tr>
<tr>
<td>Tactile</td>
<td>CD96</td>
<td>CD155</td>
</tr>
</tbody>
</table>
KIR: Restraining NK cell function
KIR: Restraining NK cell function

Activation receptor ligand

Activation (lysis) receptor

Class I MHC

Inhibitory KIR
KIR: Restraining NK cell function
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KIR: Restraining NK cell function
The KIR Family

KIR2D

KIR3D

Transmembrane domain
ITIM
Question #4

NK cell cytotoxicity can be accessed after which of the following events:
A) Long tail KIR are ligated in excess of NKp46
B) Lytic granules are exhausted
C) NKp46, NKG2D and K2DS1 are all ligated simultaneously
D) KIR2DL1, KIR2DL4 are ligated simultaneously
Current (but old enough) concepts in human NK cell biology

- Linkages between KIR “haplotype” and disease
- Regulatory NK cells
  - Costimulation
  - High potency cytokine producing subsets
- ILC (innate lymphoid cell) family (ILC1)
  - Original members of a diverse group
- Licensing
  - Need to see self MHC to be enabled
  - Relevance to HSCT – human data
- “Memory”
  - “adaptive” like features
  - Contact hypersensitivity, viral infections
  - Human data growing (CMV, EBV, Hanta, ChikV, HIV)
# KIR associations with human disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>KIR allele</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C</td>
<td>KIR2DL3</td>
<td>KIR2DL3 and HLA-C1 homosygosity with rapid viral clearance</td>
</tr>
<tr>
<td>AIDS</td>
<td>KIR3DS1</td>
<td>Presence with delayed progression, absence with rapid progression</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>KIR2DS</td>
<td>Presence without cognate ligand associated with disease</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>KIR2DS1, KIR2DL5</td>
<td>Presence associated with disease</td>
</tr>
<tr>
<td>Diabetes (type 1)</td>
<td>KIR2DS2</td>
<td>Presence along with cognate ligand associated with disease</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>KIR2DS2</td>
<td>Presence of KIR2DS2 without KIR2DL2 associated with disease</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>KIRDS</td>
<td>Absence of multiple KIRDS and HLA Cw2/4/5/6 associated with disease</td>
</tr>
<tr>
<td>Recurrent miscarriage</td>
<td>KIRDL</td>
<td>Absence of multiple KIRDL associated with recurrent spontaneous abortion</td>
</tr>
</tbody>
</table>
NK cells in hematopoietic stem cell transplantation for malignancy

Donor

NK cell
- KIR2DL1
- KIR2DL3
- KIR3DL1

Recipient

Leukemia Cell
- HLA-Cw4
- HLA-Cw1
- HLA-Bw4

HLA identical

No lysis

HLA mismatch

Lysis
NK cells as costimulators

• Expression of central costimulatory ligands
  • CD252 (OX40)
  • CD154 (CD40L)
  – In vitro evidence for costimulation
  – Costimulatory “triumvirates”
  • Strong in vivo animal evidence and human histology
Footpad injected NK (green) and DC (red) in popliteal lymph node

Regulatory NK cell subsets

- NK1- IFN-γ, TNF secreting
  - Induced by IL-12
  - Tbet positive

- NK2 - IL-5/IL-13
  - Induced by IL-4
  - Found in allergic rhinitis

- NKreg – Regulatory and IL10/TGFβ producing
  - IL-2-induced

- NK22 - IL-22 secreting (LTi-like)
  - under distinct transcriptional regulation (RORγt)
  - Found in mucosal tissues
  - Stimulate IL-10 production locally

- NK17 – IL-17 family secreting
  - RORγt+, CCR4+, IFN-γ+

Deniz, et. al. JACI 2013 132:527
NK cell “licensing”

• KIR and MHC are inherited as separate loci (chr19 and chr6)
• Enabling for cytotoxicity requires that an NK cell have seen “self” at some point and receive an effective inhibitory signal via KIR-MHC-I interaction.
• NK cells that have not had an inhibitory KIR signal exist, but are “not licensed to kill” – i.e., anergic
• Can be overcome by extreme inflammation – thus most germane to surveilance
Question #5

The engagement of ITIM containing KIR on NK cells is a **NOT** requirement for which of the following processes?

a) Inhibition of NK cell activation
b) Success in bone marrow transplantation for hematologic cancer
c) Licensing of NK cells
d) Induction of SHP-1 phosphatase activity
NK cell memory

- NK cells can fulfill the postulates of memory
  - Antigen-specific expansion
  - Contraction
  - Antigen-specific recall
- Human markers of NK cell memory
  - CD57
  - NKG2C (CMV)
  - CXCR6 (liver memory NK cells)
- Demonstrated in:
  - Viruses - CMV, HIV, HBV, Hantavirus, Chikungunya virus
  - Contact hypersensitivity – mostly mouse, some Ni in human
- Can be mimicked with cytokine activation
- Molecular determinants unknown
Evidence for Human NK cell “Memory”


NKT cells

- T cells that express NK cell markers (i.e., not NK cells)
  - Range from <1 to 15% of PBL
- iNKT cells (i=invariant) express limited TCR repertoire
  - Vα24/Jα18 and Vβ11
  - 0.01-0.1% of PBL
  - CD4+ or CD4- (most CD8-)
  - Stimulated by αGalCer and iC3b
    - Pollen lipids (olea europaea)*
    - Milk Sphingomyelin in food allergy**
  - Produce IFN-γ/TNF or IL-4/IL-13
  - Recognize glycolipid antigen in context of CD1d
  - Play an essential role in mouse asthma models
  - Can be common among T cells in BAL from asthma patients (NEJM 2006 354:1117)

*JACI – 2013 121:1393, **JACI – 2011 128:102
Question #6
Which of the following accurately describes memory NK cells?

A) They recombine V/D/J segments at the NK cell receptor locus to sustain memory
B) They utilize a single and uniform receptor to promote recall responses to diverse viral pathogens
C) After human viral infections they can proceed through expansion, contraction, and recall
D) They require IL-15 and IL-18
Conclusions

- Innate immunity is a HARDWIRED defense that functions through recognition of danger
- There are 5 general types of pattern recognition receptors to hardwire innate recognition of danger
  - TLR, RLR, CLR, DDX, PYHIN
- NK cells are “innate” lymphocytes that bridge to adaptive immunity
  - Mediate cytotoxicity – balance for activation
  - Restrained by healthy environment
  - Multiple roles in inflammation and costimulation
  - Can have memory characteristics