Role of Innate Immunity in Control of Adaptive Immunity
Innate Immunity

• The burden of pathogen sensing is placed on the innate immune system
  – “Danger hypothesis”

• “Missing Self”
  – Based on the detection of molecular features unique to the host

• Pattern Recognition
  – Based on detection of “suspicious activities” associated with microbial infections and stressed cells
Pattern Recognition Receptors (PRR)

- Germline-encoded receptors

- Recognize pathogen-associated molecular patterns (PAMPs).
  - Patterns that are conserved and required for survival

- Recognize danger-associated molecules (DAMPs).
  - Endogenous factors normally sequestered intracellularly, but released under conditions of cellular stress or injury.

- Include TLR, NLR, RLR, CLR and “Other”
  - Other: Scavenger receptors and C’ receptors
PRR

- **RLR (Retinoic-Acid-Inducible Gene-Like Receptors): Cytoplasmic**
  - RNA-helicase Domain
  - +/- 2 N-terminal CARD
  - RIG-1/MDA-5: ss RNA

- **CLR (C-type lectin Receptors): Membrane**
  - C-type lectin domains
  - Dectin-1: beta-glucan

- **Others: scavenger receptors, C’ receptors**
Consequences of TLR Activation

- Expression of pro-inflammatory cytokines and type I IFNs (IFN-α/β)
- Increased cell migration
- Activation of adaptive immunity
Regulation of TLRs

- TLR activation must be tightly regulated to prevent inflammatory destruction of normal tissue
# TLR Mediated Diseases

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<th>Disease</th>
<th>TLR</th>
<th>Possible mechanism</th>
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<tr>
<td>Bacteria</td>
<td>Sepsis</td>
<td>TLR4</td>
<td>LPS induces inflammatory gene expression and organ failure</td>
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<td>West Nile virus</td>
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<td>Virus double-stranded RNA facilitates infection in the brain</td>
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<td>Plasmodium falciparum</td>
<td>Malaria</td>
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<td>The malaria pigment hemozoin induces inflammatory responses through TLR9</td>
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<td>Candida albicans</td>
<td>Candidiasis</td>
<td>TLR2</td>
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<td><strong>Autoimmunity</strong></td>
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<td>Bordetella pertussis</td>
<td>EAE</td>
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<td>TLR ligands increase innate immunity</td>
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<td>Cardiomyopathy</td>
<td>TLR2,3,4,9</td>
<td>TLR ligands promote dendritic cell function by presenting heart antigens</td>
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<td>ND</td>
<td>Atherosclerosis</td>
<td>TLR4</td>
<td>TLR signals trigger pro-inflammatory responses</td>
<td>23,24</td>
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<td><strong>Chronic inflammation</strong></td>
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<tr>
<td>ND</td>
<td>Asthma</td>
<td>TLR4</td>
<td>LPS induces T(_{H2})-cell responses to inhaled antigens</td>
<td>30,31</td>
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<tr>
<td>Bacteria</td>
<td>COPD</td>
<td>TLR4</td>
<td>LPS exacerbates airway inflammation</td>
<td>34</td>
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</table>

COPD, chronic obstructive pulmonary disease; EAE, experimental autoimmune encephalomyelitis; LPS, lipopolysaccharide; ND, not determined; SLE, systemic lupus erythematosus; T\(_{H2}\), T helper 2.
Regulation of TLRs

- Decoy receptors
  - Soluble competitors
- Intracellular regulators
  - TRIM30α
- TLR downregulation/degradation
  - miRNA

Liew et al., 2005

- Dissociation of adaptor complexes
  - SHIP/NLRX1
- Degradation of signal proteins
  - TRIM30α
- Transcriptional regulation
  - miRNA
  - Inhibition of NF-κB/IRF

Kondo et al., 2012
Inhibition of TLR-mediated NF-kB Activation by TRIM30α
Inhibition of TLR-mediated NF-κB Activation by TRIM30α

Shi et al, 2008
NLR Family

- Detect intracellular pathogens or danger signals
- Present in the cytosol in inactive forms
- Consists of 4 families divided by N-terminal domain structure:
  - NLRA: acidic transactivation domain
    - CIITA
  - NLRB: baculoviral inhibitory repeat domain
    - Naip1-7, aka Birc1a-1g
  - NLRC: caspase-recruitment domain
    - Nod1/2
    - Nlrc3-5
  - NLRP: pyrin domain
    - Inflammasome group: NLRP3
  - Nlrx1: independent member
NLR Protein Family

NLRP1, NLRP3, NLRC4, NAIP, NOD2
Inflammasome function, cell death regulation

NOD2, NLRX1
Mito-signalosome function, Type I interferon, cytokines

NOD1, NOD2
Nodosome, defensins, cytokines, chemokines

CIITA
Transcriptional assembly, promoter activation

CASP-1
Activation

NF-κB, IRF
Regulation

NF-κB, MAPK/JNK
Activation

Regulation of noncanonical NF-κB pathway via NIK

J P Y Ting et al. Science 2010;327:286-290
NOD Proteins

- NOD1/2 expressed in the cytosol
- NOD1/2 recognize peptides derived from degradation of PGN, a bacterial cell wall component
  - NOD1 recognizes iE-DAP (primarily Gram-negative bacteria)
  - NOD2 recognizes MDP (all bacteria)
- Mutations in NOD2 are associated with Crohn’s disease
NOD Signaling

- Ligand binding induces dimerization
- CARD domains of NOD then associate with the RICK (RIP2) through RICK CARD domains
- RICK is activated and mediates polyubiquitylation of IKK leading to activation of NF-κB
- MAPK and JNK pathways and IRF3 are also activated.

Strober et al, 2006
Inflammasome Family

Schroder & Tschopp, Cell 140:821-832, 2010
• **Inflammasome Activation:**
  
  - P2X7-dept pore formation by pannexin-1
  
  - Phagocytosis of particulate agonists and lysosomal rupture
  
  - ROS-dependent activation.

Schroder & Tschopp, Cell 140:821-832, 2010
RLH Family

- Prototype family members: RIG-1 and MDA5
- Primarily detect ssRNA present in the cytoplasm

Akira et al, 2007
Regulation of TLR and RLR Signaling by NLRX1

- NLRX1
  - Member of the NLR family.
  - Blocks activation of TLR4
    - Interferes with TRAF6 activation of NF-κB
  - Mechanism of RLR blocking unclear.
    - May block IPS-1 (MAVS) activation
    - May block NF-κB activation
Regulation of TLR and RLR Signaling by NLRX1
CLR: Dectin 1

- Similar in structure to NKR-like C-lectin family except:
  - No Cys in stalk region, no dimers
  - Contains an hemITAM
- Splice variants exist in humans.
- Involved in fugal recognition-zymosan

Brown, GD, 2006
Dectin 1 Signaling

Dectin1/2 Signal Outcome

• Activation of MAPKs, NFAT and via CARD9, NFkB

• In DCs:
  - TNF, IL-6, IL-23, IL-2 and IL-10
  - Th1 and TH17 responses.
  - Converts Treg to Th17
  - Crosstalk with TLR2/6 needed for TNF, IL-12
Dectin 1: TLR2/6 Crosstalk

Brown, GD, 2006
Control of Adaptive Immune Responses Through PRR

• Hypothesis:
  - Ligand recognition by PRR and subsequent signals induce APC activation and regulate the type of immune response triggered by these cells.
# Effect of PRR Signaling on Adaptive Immune Responses

<table>
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<tr>
<th>PRR Family</th>
<th>PRRs</th>
<th>Ligand</th>
<th>DC or Macrophage Cytokine Response</th>
<th>Adaptive Immune Response</th>
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</thead>
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<tr>
<td>TLRs</td>
<td>TLR2 (heterodimer with TLR1 or 6) Lipopeptides Pam-3-cys (TLR 2/1) MALP (TLR 2/6)</td>
<td>Low IL-12p70 High IL-10 IL-6 (33, 36, 37, 75–81)</td>
<td>Th1 (74) Th2 (33, 36, 37, 77) T regulatory (79–81)</td>
<td></td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA</td>
<td>IL-12p70 IFN-α IL-6 (48–52)</td>
<td>Th1 (48–52)</td>
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<tr>
<td>TLR-4</td>
<td><em>E. coli</em> LPS</td>
<td>High IL-12p70 Intermediate IL-10 IL-6 (33, 36, 37, 76, 78)</td>
<td>Th1 (33, 36, 37, 76)</td>
<td></td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>High IL-12p70 (36, 102) Low IL-12p70 (103, 104)</td>
<td>Th1 (36, 102) Th2 (103, 104)</td>
<td></td>
</tr>
<tr>
<td>TLR7/8</td>
<td>ssRNA Imidazoquinolines</td>
<td>High IL-12p70 IFN-α IL-6 (48–52, 105)</td>
<td>Th1 (48–52, 105)</td>
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<tr>
<td>TLR 9</td>
<td>CpG DNA</td>
<td>High IL-12p70 Low IL-10 IL-6 IFN-α (48–52, 106)</td>
<td>Th1 (48–52, 106)</td>
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<tr>
<td>TLR10</td>
<td>?</td>
<td>?</td>
<td>?</td>
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<tr>
<td>C-type lectins</td>
<td>DC-SIGN</td>
<td>Env of HIV; core protein of HCV; Components of <em>M. tuberculosis</em>; <em>H. pylori</em> Lewis Ag</td>
<td><em>H. pylori</em> Lewis Ag Suppresses IL-12p70 (95) Suppression of TLR signaling in DCs (95, 96)</td>
<td>Th2 (95) T regulatory (96)</td>
</tr>
<tr>
<td>NOD</td>
<td>NOD2</td>
<td>Muramyl dipeptide of peptidoglycan</td>
<td>Induces IL-10 in DCs (97)</td>
<td>Weak T cell response (tolerogenic?)</td>
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<td>Mannose receptor</td>
<td>Mannose receptor</td>
<td>Mannosylated lipoarabinomannans from bacillus Calmette-Guerin and <em>M. tuberculosis</em></td>
<td>Suppression of IL-12 and TLR signaling in DCs (98)</td>
<td>Weak T cell response? (tolerogenic?)</td>
</tr>
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</table>
Role of PRR in Tumor Immunity

- TLR agonists have been used alone or in combination therapy as anti-cancer modalities
  - Coley’s toxin/BCG (crude microbial extracts are effective against bladder cancer. Likely stimulate TLR2/4.
  - TLR7 agonist imiquamod effective in treatment of basal cell carcinoma
  - Many others (CpG) in trial as adjuvant treatment
Role of PRR in Tumor Immunity

• Polymorphisms in TLR 1, 4, 6, 10 are associated with prostate cancer

• Polymorphisms in TLR4 are associated with gastric cancer

• Treatment with TLR agonists have been linked to increased Th1 responses.
  – Increased CD8 primary and memory responses
  – Direct or indirect or both
    • Inhibition of Treg?

• Presence of TLR4 linked to efficacy of chemotherapy?
Radiation/Chemotherapy Efficacy Dependence on TLR4?

Apetoh et al., 2007
HMGB1 Correlates with Immunity

Apetoh et al, 2007
TLR4 mutation associated with metastasis

TLR4 polymorphism Asp299Gly: reduced endotoxin response and reduced ability to bind HMGB1.

Breast cancer patients with TLR4 Asp299Gly polymorphism have increased metastases and reduced long-term survival following chemotherapy.

Apetoh et al, 2007
NALP3 Regulates Anti-Tumor Immunity

Ghiringhelli et al., 2009
NALP3 Controls Response to Chemotherapy

• ATP released from dying cells taken up by DCs via the purinergic receptor P2RX7.

• The presence of ATP in the cytosol induces NALP3 activation.

• Glu496Ala is a loss of function mutation of P2RX7.

Breast cancer patients with P2RX7 Glu496Ala polymorphism have increased metastases and reduced long-term survival following chemotherapy.
TLR Agonists and Cancer

• Many tumor cells express TLRs
• TLRs can contribute to tumor progression, metastasis and survival via induction of NF-κB.
• TLRs can promote Tregs
• TLR stimulation leads to inflammation; inflammation leads to cancer.

Huang et al, 2008
TLR Agonists and Cancer

- Activation of TLR4 can protect HNSCC against chemotherapy and NK cytotoxicity.

Szczepanski et al, 2009