Immunology for the Rheumatologist

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Learning Objectives

We spend much of our time as rheumatologists dealing with the immune system gone awry. We rarely study the normal function of the immune system. Why is it important to have an understanding of immunology? Abnormal immune responses are the cause of many of our inflammatory diseases with serious morbidity and mortality. Antibodies are in widespread use to treat immunologic diseases. Understanding immunology helps us to better understand the diseases that we treat and their current therapies. It also prepares us for advances in understanding the immune mechanisms of inflammatory and autoimmune diseases and therapeutic options for these diseases in the future.

• 1) Review the innate immune system
• 2) Discuss acute gout as an example of a disease driven by aberrant innate immune function
• 3) Review the adaptive immune system
• 4) Discuss the details of T-cell function
• 5) Discuss the immunopathogenesis of rheumatoid arthritis
• 6) Review the treatment of rheumatoid arthritis from an immunologic perspective
Disclosures

- Speakers’ Bureau: Abbvie, Amgen, BMS, Celgene, Crescendo, Genentech, Janssen, Lilly, Merck, Novartis, Pfizer, Quest, Sanofi-Regeneron
Role of the immune system

- **Defense against infection**
- Surveillance against tumors
- Recognizes and reacts against foreign proteins and tissues

- *How do perturbations in the normal immune system result in disease?*
- *How can we modulate the immune response to help our patients?*
Components of the Immune System

• Physical Barriers-part of innate immune system
  – Skin
  – Epithelial membranes

• Innate Immune System
  – “Non-specific” response
  – Involves both immune & non-immune cells
  – Immediate response
  – Response = inflammation
    cytokines/chemokines & co-stimulatory molecules

• Adaptive Immune System
  – Specific recognition
  – Immune cells only (T-, B-cells)
  – Delayed response
  – Response = clonal expansion & effector cytokine secretion
  – Memory
Mechanisms of inflammation
Compare and contrast gout and rheumatoid arthritis
Two arms of the immune system

Innate (acute) Immunity:
• First response—12+ hours
• Gout is an example of this response

Adaptive (acquired) immunity
• Takes time to develop
• RA is an example of this response
Cells of the Immune System (Leukocytes)

Innate response

- Neutrophil (Phagocytosis, Degranulation)
- NK cell (ADCC)
- Basophil (Histamine Release)
- Eosinophil (IL-5 producers)
- Macrophage (APC)

Adaptive response

- Dendritic cell (APC)
- Tc lymphocyte (CD8+)
- B lymphocyte
- Th lymphocyte (CD4+)

Adapted from Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003 p25
Two Arms of the Immune System: Innate and Adaptive Immunity

Innate immunity:
- Epithelial barriers
- Phagocytes
- Complement
- NK cells

Adaptive immunity:
- B lymphocytes
- Antibodies
- T lymphocytes
- Effector T cells

Prevent infections
Eliminate microbes
Antibodies block infections and eliminate microbes
T lymphocytes eradicate intracellular microbes
Innate Immunity: General features

- Initial response to microbes
- Recognizes structures shared by classes of microbes
- Receptors encoded in germline, limited diversity
- Consists of epithelial barriers, phagocytes (neutrophils, monocytes and macrophages), NK cells, dendritic cells
- Complement system
- Cytokines + chemokines such as TNFα, IL-1, IL-6, IL-10, IFNγ
- *All defenses without MEMORY*
Danger Is All Around Us

- Physical Damage
  - Tissue injury
  - Cell death

- Chemical Insults
  - Environmental toxins
  - Self-inflicted toxins

- **Infection**
  - Bacteria
  - Viruses
  - Parasites
  - Fungi
Sensing Danger/Danger Signals
a.k.a. “pathogen-associated molecular *patterns*”
“danger-associated molecular *patterns*”

- Unique microbial structures
  - Bacterial cell wall components (LPS, PGN)
  - Microbial proteins (flagellin, zymosan, toxins)

- Nucleic acids
  - Double stranded RNA
  - CpG DNA
  - Viral and Microbial RNA

- Necrotic cell ATP

- Uric acid

- Hyaluronan fragments

- Cytochrome c
**Pattern Recognition Molecules (PRMs)**

- *Toll-like Receptors (TLRs)*
- NOD-like Receptors (NLRs)
- RIG-I-like Receptors (RLRs)

- Pentraxins
- *Complement cascade*
- Collectins
- Ficollins

- C-type lectins
- Scavenger receptors
- Inflammation
- Opsonization
- Phagocytosis
Recognition of pathogens by Toll-like receptors

- TLRs mediate innate immune response
- Found on macrophages, neutrophils, and dendritic cells
- Recognize distinct pathogen-associated molecular patterns conserved in microbes, eg, lipopolysacharides, lipoproteins, viral ds-RNA
Macrophage Function

The macrophage expresses several receptors specific for bacterial constituents:
- TLR
- LPS receptor (CD14)
- mannose receptors
- CR3
- glucan receptor
- scavenger receptor

Bacteria bind to macrophage receptors

Macrophage engulfs and digests bound bacteria:
- phagosome
- lysosome
- phagolysosome

Figure 2.19 The Immune System, 3rd ed. (© Garland Science 2009)
Macrophage Function

On sensing microbial products, macrophages secrete a variety of pro-inflammatory cytokines

- **IL-6**
  - activates vascular endothelium and increases vascular permeability, which leads to increased entry of complement and cells to tissues and increased fluid drainage to lymph nodes

- **TNF-α**
  - activates vascular endothelium
  - activates lymphocytes
  - local tissue destruction
  - increases access of effector cells

- **IL-1β**
  - activates vascular endothelium
  - activates lymphocytes
  - local tissue destruction
  - increases access of effector cells

- **CXCL8**
  - chemotactic factor recruits neutrophils and basophils to site of infection

- **IL-12**
  - activates NK cells

**Local effects**

**Systemic effects**

- Fever induces acute-phase protein production by hepatocytes
- Fever mobilization of metabolites
- Fever production of IL-6

*Figure 2.27 The Immune System, 3rd ed. (© Garland Science 2009)*
COMPLEMENT-3 distinct ways to activate all lead to C3b

- **Initiation of complement activation**
  - Alternative pathway: Microbe
  - Classical pathway: Antibody
  - Lectin pathway: Mannose-binding lectin

- **Early steps**
  - C3b is deposited on microbe

- **Late steps**
  - C3b: Inflammation
  - C3b: opsonization and phagocytosis
  - C5a: Inflammation
  - Complement proteins form membrane attack complex
  - Lysis of microbe

Effector functions
Both Classical and Alternative Complement Pathways Coat Microbe With C3b

**Classical Pathway**
- Antigen–antibody complex
- C1, C2, C4
- 3 subcomponent proteins

**Alternative Pathway**
- Cell wall polysaccharides
- Factor B + Factor D
- C3b circulating in serum
- Factor P

**Inflammation:**
- Increase of blood vessel permeability, chemotactic attraction of phagocytes

**Opsonization:**
- Immune adherence

**Cytolysis:**
- Loss of cellular contents through transmembrane channel formed by membrane attack complex C5–C9

© BENJAMIN/CUMMINGS
Classical Complement Pathway is Triggered by Antibodies Binding to Foreign Cells

1. Once antibodies recognize and attach to the antigen, complement protein C1 binds to two adjacent antibodies.

2. C1 acts as an enzyme that splits the C2 and C4 proteins into fragments. Fragments C2b and C4b combine to form another enzyme, which splits C3 into two fragments. The active fragment is called C3b.
Classical and Alternative Complement Pathways Cause *Inflammation, Opsonization, and Cytolysis*
The Membrane Attack Complex

C5

C5a

C5b

C6, C5b, C7, C8

70-180 Å
Functions of NK Cells

(A) NK cell

Virus-infected cell

Killing of infected cells

(B) NK cell

Macrophage with phagocytosed microbes

IFN-γ

IL-12

Killing of phagocytosed microbes

<ADCC>
Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Antibody binds antigens on the surface of target cell

Fc receptors on NK cell recognize bound antibody

Cross-linking of Fc receptors signals the NK cell to kill the target cell

Target cell dies by apoptosis

Figure 9.43 The Immune System, 3ed. (© Garland Science 2009)
Mechanisms of Acute Gouty Inflammation:
Disorder of Innate Immunity

• Acute onset, self limited

• Urate is the inflammatory stimulus, resolves when urate is is removed

• Predominant neutrophil response. No lymphocytic reaction

• No autoantibody formation
How Does a Crystal Incite Inflammation?
Components of the Innate Immune System that Respond to DAMPS**

**Toll-like receptors**
Lipoteichoic acid, endotoxin, flagellin, viral RNA, viral/bacterial DNA, **MSU/CPPD crystals**

**Nod-like receptors**
Bacterial products (*S. aureus*, Listeria, anthrax lethal toxin, flagellins, etc.), stress, K+ efflux inducing agents, **MSU/CPPD crystals**

**DAMPS = Danger-Associate Molecular Patterns**
Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)

Toll-like receptors

14 members of TLR family

How TLR senses the presence of DAMPs is not clear

MyD88

1 Toll/IL-1 receptor (TIR) domain
2 Death domain
3 Leucine-rich domain

Toll/IL-1 receptor (TIR) domain
Death domain
Leucine-rich domain
**Innate Immunity Sensors – Pattern Recognition Molecules (PRMs)**

22 members of the NLR family in humans- Ligands include:

NALP1: anthrax lethal toxin
NALP3: *S. aureus*, *Listeria*, uric acid crystals, “stress”
NOD2: muramyl dipeptide
IPAF and NAIP5: *Legionella* flagellin

**NOD-like receptors (NLR)**

Cytoplasmic equivalent of TLR

Nucleotide binding (NACHT) domain
Pyrin domain
Leucine-rich repeat
Caspase recruitment domain
Apoptosis inhibition domain
MSU Crystals

CD14

TLR2/4

MyD88

NFκB Mediated Cell Activation

Pro-IL1β Gene Transcription

Pro-caspace 1

NALP3

ASC

Caspase 1

Monocyte

Synovial Fluid

Endothelium/Leukocyte

IL-1β

IL-1R

Endothelial Activation

Leukocyte Migration

Edwards NL. Crystal-Induced Joint Disease, ACP Medicine Textbook, 2012
Adaptive Immunity

- Delayed response to a specific antigen demonstrating the features of SPECIFICITY and MEMORY

- Consists of lymphocytes and their products

- Utilizes specific receptors (T & B) generated by somatic mutation during development - i.e., system learns from what it sees

- Must be re-invented every generation!!
Time course of innate and adaptive immune responses

Mouse model of a viral infection
Three Strategies to Combat Microbes

• Secreted antibodies bind to extracellular microbes, block their ability to infect host cells, and promote their ingestion and subsequent destruction by phagocytes

• Phagocytes ingest and kill microbes—helper T cells enhance the killing by phagocytes

• Cytotoxic T cells destroy cells infected by microbes that are inaccessible to antibodies
Classes of Lymphocytes - Recognize Different Types of Antigens

- **Recognize soluble or cell surface Ags**
- **Recognize Ags on surface of APC’s**
- **Recognize Ags on infected cells**
- **Recognize changes on surface of infected cells**
**T helper cells**

- Help B cells to produce antibodies
- Help phagocytes to destroy ingested microbes

T helper cells have many different functions based on the profile of cytokines they produce: e.g. Th1 cells activate MØ function

*More to come*

**T cytotoxic cells**—induce cell death in target cells via cytotoxic granule release
T Cell Immunity (cell-mediated)

- T lymphocytes mature in the Thymus
- They express a specific receptor that binds antigen, called the T Cell Receptor (TCR)
- There are 2 main types:
  - CD8+ Cytotoxic T cells (Tc)
  - CD4+ Helper T cells (Th)

Adapted from Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003
CD4 Subsets: Generation and Function

**Naive CD4⁺ T cell**

- IFN-γ, IL-12
- IL-4
- TGFβ, IL-6
- IL-6, IL-21

**Th1**
- STAT1
- STAT4
- T-bet
- IFN-γ, TNF-α
- Defense against intracellular parasites

**Th2**
- STAT6
- GATA3
- IL-4, IL-5, IL-6, IL-13
- Allergy, Asthma, Controls parasites and extracellular pathogens

**Th17**
- STAT3
- RORγt
- IL-17A, IL-17F, IL-21, IL-22
- Defense against pathogens, Autoimmunity, Transplantation rejection and Cancer

**Treg**
- STAT3
- FOXP3
- TGFβ, IL-10
- Immune homeostasis, Maintains tolerance

**Tfh**
- STAT3
- CXCR5
- Bcl6
- Help germinal centre B cells to make antibodies, Affinity maturation and antibody class switching
<table>
<thead>
<tr>
<th>Types of effector T cell</th>
<th>CD8 cytotoxic T cells</th>
<th>CD4 Th1 cells</th>
<th>CD4 Th2 cells</th>
<th>CD4 Th17 cells</th>
<th>CD4 regulatory T cells (various types)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (CTL)</td>
<td>KILL virus-infected cells</td>
<td>Activate infected macrophages</td>
<td>Provide help to B cells for antibody production</td>
<td>Enhance neutrophil response</td>
<td>Suppress T-cell responses</td>
</tr>
<tr>
<td>Function in adaptive immune response</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>VIRUSES (e.g. influenza, rabies, vaccinia)</td>
<td>MICROBES that persist in macrophage vesicles (e.g. mycobacteria, Listeria, Leishmania, Pneumocystis)</td>
<td>HELMINTH parasites</td>
<td>EXTRACELLULAR bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some intracellular bacteria</td>
<td>Extracellular bacteria (e.g. Salmonella enterica)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Figure 8-1 Immunobiology, 7ed. (© Garland Science 2008)
T follicular helper cells migrate to follicles
T follicular helper cells

Trigger formation and maintenance of germinal centers
Stimulate plasma cell development
Stimulate development of memory B cells
B cells and Humoral Immunity

• Major limb of adaptive immunity

• Immunoglobulin is structurally homologous to T cell receptor and also produced via somatic recombination

• Provides surveillance against blood born pathogens (bacteria, virus, parasites etc)

• Directly linked to innate immunity through complement activation
B-Cell Immunology: Lineage\textsuperscript{1,2}

- B cells develop in the bone marrow and migrate to the peripheral lymphoid organs, where they can be activated by antigens\textsuperscript{2}
- Activated B cells proliferate and differentiate into long-lived memory cells and antibody-secreting plasma cells\textsuperscript{2}

**B-Cell Activation**

**Recognition phase**
- Resting IgM⁺, IgD⁺ mature B cell
- Activated B cell
- Helper T cells, other stimulus
- Antigen

**Activation phase**
- Clonal expansion
- IgM-expressing B cell
- IgG-expressing B cell
- High-affinity IgG-expressing B cell
- Memory B cell
- Affinity maturation
- Antibody secretion
- Isotype switching

**Remark:**
- High-affinity IgG-expressing B cell
- IgG-expressing B cell
- High-affinity IgG
Roles of Mature B Cells

Role of B cells in immune-mediated inflammation in the RA synovium

- Cytokine production
- Autoantibody production and self-perpetuation
- Antigen presentation

**Antigen presentation**
- B cell
- Cartilage
- Bone

**Autoantibody production**
- Antigen-presenting B cells
- Plasma cells
- RF immune complexes
- Complement activation
- IL-1
- TNF-α

**Antigen presentation**
- RF
- Immune-complexed antigen
- Autoreactive B cell
- Antigen presentation

**Cytokine production**
- B cell
- Dendritic cell
- Lymphotoxin
- Cytokines
- IL-6
- IFN-γ
- TNF-α

**Formation of new lymphoid structures**
- Amplification of inflammation and damage

**Role of B cells in immune-mediated inflammation in the RA synovium**
**Targets of Rituximab**

1,2

**Expression of CD20 During B-Cell Maturation**

(CD=cluster of differentiation)

- Rituximab binds specifically to the CD20 antigen located on pre-B and mature B lymphocytes.
- CD20 is not found on hematopoietic stem cells, pro-B cells, normal plasma cells, or other normal tissues.
- Long-lived plasma cells do not express CD20 and are not directly targeted by Rituximab.

Antibody Function

**Neutralization**
- Specific antibody prevents bacterial toxins from interacting with the cell.

**Opsonization**
- Specific antibody enhances the phagocytosis of bacteria by macrophages.

**Ingestion and destruction by phagocyte**
- Bacterial toxins
  - Cell with receptors for toxin
- Bacteria in extracellular space
  - Macrophage
- Complement

Figure 1-29  The Immune System, 2/e (© Garland Science 2005)
T Cell Function
MHC Based Antigen Presenting Cell-Lymphocyte Interactions

MHC II interacts with CD4+ lymphocyte

MHC I interacts with CD8+ lymphocyte
MHC Restriction

![Diagram of MHC restriction](image_url)

Figure 3-30 The Immune System, 2/e © Garland Science 2005
T cell activation

TCR ligation alone is insufficient to activate T cells

Safety control

Second signal is essential

CO-STIMULATORY

Failure to co-stimulate results in ignorance, anergy or apoptosis
Co-stimulation

T Helper Cell Differentiation Driven by the Cytokine Milieu

- Th1
- Th2
- Th17
- Treg

IL-12 → Th1
IL-4 → Th2
TGFβ, IL-6 → Th17
TGFβ → Treg

APC

Curtsinger et al., 1999 Journal of Immunology, 162:3256–3262
Abatacept: A Human Immunoglobulin Receptor Fusion Protein

CTLA4

Abatacept (CTLA4Ig)

IgG1

External domain

External

Cell membrane

Internal

IgG1 = immunoglobulin G1.

Heavy-chain constant region
Mechanism of Action of Abatacept

Without Abatacept
- DC
- T cell
- CD80/86
- CD28
- Activated T cell

With Abatacept
- DC
- T cell
- Abatacept
Specificity of Immune Response
Cytokines in RA

• Drive inflammation
• Drive joint damage
• Drive systemic manifestations
Targeted Therapies

• Treatments for rheumatoid arthritis (RA) prior to 1998 were discovered fortuitously.

• Now, due to a greatly improved understanding of the immunopathogenesis of the disease, we have developed targeted therapies.

• These include tumor necrosis factor α (TNFα), Interleukin-6, and Intracellular Signaling.
Tumor Necrosis Factor $\alpha$ (TNF$\alpha$)

- Expressed as a transmembrane protein
  - Cleaved by TACE on cell surface
- Active protein is trimeric
  - 157 amino acids / monomer
  - Unglycosylated
  - One intrachain disulfide per monomer for stability
- Binds p55 (ubiquitous) and p75 receptors (hematopoietic cells)
  - Receptors present on virtually all cells (200 – 10,000!!)

Synthesis and Function of TNFα

Activated macrophage

pathogens

Interferon γ

Transmembrane TNFα

Soluble TNFα

Receptors crosslinked by TNFα

Target cell

Induction of mediators

TNFα receptor

TNF receptors phosphorylate each other—induces signaling

Key Actions of TNFα in RA

- **Macrophages**
  - proinflammatory cytokines
  - chemokines
  - Increased inflammation

- **Endothelium**
  - adhesion molecules
  - vascular endothelial growth factor
  - Increased cell infiltration
  - Increased angiogenesis

- **Synoviocytes/Chondrocytes**
  - acute phase response
  - metalloproteinase synthesis
  - Increased CRP in serum
  - Articular cartilage degradation

INTERLEUKIN 6 IS AN IMPORTANT CYTOKINE IN RA
Functions of IL-6
IL-6 affects a broad range of cells and tissues. It can do so because of its unique signaling mechanism. Classical (cis-) signaling.
Trans-signaling
Probably more important in chronic inflammatory diseases
Intracellular Signaling
7 different cytokine receptors

- IL-17 cytokine receptors
- Types I and II cytokine receptors
- TNF receptors
- Chemokine G protein coupled receptors
- Ig receptors
- TGF-B receptors
Cytokines Signal Through Different Pathways
Signaling by Different Cytokines Requires Unique JAK Pairings

JAK-STAT Signaling
Insights into the Initiation and Progression of RA
Etiopathogenesis of RA

- Genetic predisposition
  - **HLA-DRB1**
  - **PTPN22**

- Environment
  - Prior infections
  - Hormones
  - **Smoking**

- Autoimmunity
  - CD4+ helper and TH17 T cells
  - B cells, plasma cells, and autoantibodies
  - Proinflammatory cytokines: TNF-α, IL-1, IL-6, IL-17

References:

Genetic Predisposition to RA

• Strongest genetic factor associated with an increased risk of developing RA:
  - Polymorphisms at the *HLA-DRB1* locus (chr. 6p21.3) encoding HLA Class II β-chain molecules

• Proteins (MHC molecules) involved in antigen presentation to T cells

• MHC molecules containing the shared epitope are able to accommodate citrullinated peptides in the binding site

Shared Epitope -- part of the binding site of an MHC class II molecule
Immunologic Tolerance

• Definition:
  - specific unresponsiveness to an antigen that is induced by exposure of lymphocytes to that antigen

• Significance:
  - All individuals are tolerant of their own antigens (self-tolerance); breakdown of self-tolerance results in autoimmunity
HOW IS TOLERANCE BREACHED??

IE-WHY DOES THE RA PATIENT RECOGNIZE SELF AS FOREIGN??
PADI Citrullination is the Target Resulting in Breaching Tolerance in RA

• Citrullination occurs at sites of inflammation¹

• PADI post-translationally modifies proteins¹-³

• Peptidyl arginine amino acid residues are modified to citrulline residues¹-³

• This process occurs in multiple proteins³

RA in many pts characterized by autoantibodies that target citrulline-containing proteins

CITRULLINATION

A

\[ \text{L-arginine} \xrightarrow{\text{PAD, Ca}^{2+}} \text{L-citrulline} \]

L-arginine (+ charged)

L-citrulline (neutral)

B

Substrate binding partner

1) Protein unfolding
2) Proteolytic degradation
3) Loss intramolecular interactions

Substrate

\[ \xrightarrow{\text{PAD, Ca}^{2+}} \text{Citrullination} \]
Shared Epitope

- Part of the MHC class II molecule
- Able to bind citrullinated self-antigens
Etiopathogenesis of Rheumatoid Arthritis


FLS = fibroblast-like synoviocyte; MMP = matrix metalloproteinase; MΦ = macrophage.

Genetics

Environment

↓

Adaptive Immunity

Innate Immunity

Rheumatoid factor
Autoantibodies

Activates

Inflammation
Joint damage

Cytokines
MMPs

RA-associated Self-antigens

Dendritic cells

Plasma cells

B cells

T cells

T

Macrophages

MΦ

B

PC

Adaptive Immunity

Innate Immunity
There are multiple cell types and cytokines involved in RA. Understanding immunology allows a better understanding of the disease and its treatment.

Key cytokines in chronic inflammatory arthritis:

- TNF-α
- IL-1
- IFN-γ
- IL-6
- RANK-ligand
- IL-17