Immunology for the Rheumatologist

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We spend much of our time as rheumatologists dealing with the immune response gone awry.

We rarely study the normal function of the immune system.

Recommended Reading and Reference:

Basic Immunology
Functions and Disorders of the Immune System 3rd Edition

Abul K. Abbas + Andrew H. Lichtman
Saunders Elsevier

What's the reason to have an understanding of immunology?

- Abnormal immune responses are the cause of many inflammatory diseases with serious morbidity and mortality
- Antibodies are in widespread use to treat immunologic diseases
- Understanding immunology helps us better understand the diseases we treat and their current therapies and…….
- It prepares us for advances in understanding immune mechanisms of inflammatory and autoimmune diseases and therapeutic options for these diseases in the future

Rheumatic Disease

- Characterized by the inflammatory damage of tissue
- Clinical features of specific diseases reflect:
  - Stimuli that initiate and propagate the inflammatory response
  - Particular tissues that are targeted
  - Predominant inflammatory effector mechanisms
- Understanding of these mechanisms is the key to rational therapy

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?***

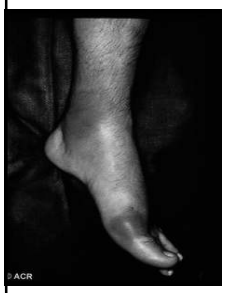
Components of the Immune System

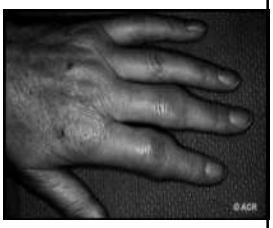
- Physical Barriers-part of innate immune system
  - Skin, epithelia
  - Commensal bacteria
- Innate Immune System
  - "Non-specific" response
  - Involves both immune & non-immune cells
  - Immediate response
  - Response = inflammation
    cytokines/chemokines & co-stimulatory molecules
- Adaptive Immune System
  - Specific recognition
  - Immune cells only (T-, B-cells)
  - Delayed response
  - Response = clonal expansion & effector cytokine secretion
  - Memory

Clinical features that provide clues to the nature of the immune response

- Acute vs chronic
  - Gout vs RA
- Identification of exogenous stimuli
  - Uric acid, foreign antigens (drugs, microbes)
- Target tissues affected
- Genetics
  - MHC, complement proteins, signaling molecules
- Cellular nature of the response
  - Neutrophils, Lymphocytes

Mechanisms of inflammation
Comparing and contrasting gout and rheumatoid arthritis



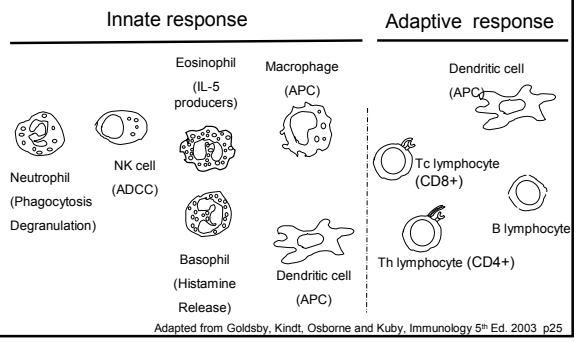


Two arms of the immune system

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

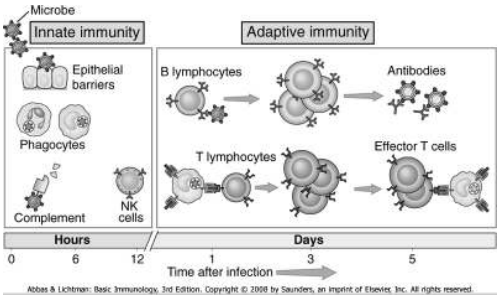
Adaptive (acquired) immunity
- Takes time to develop
- RA is an example of this response

Cells of the Immune System (Leukocytes)



Adapted from Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003 p25

Two arms of the Immune System: Innate and Adaptive Immunity



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***
  - Microorganisms (bacteria, yeast, fungus, etc.)
  - Viruses
  - Parasites

Sensing Danger/Danger Signals
a.k.a. "pathogen-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

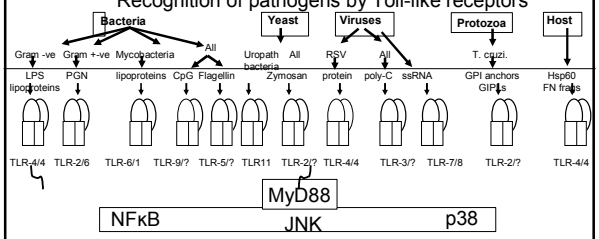
Pattern Recognition Molecules (PRMs)

- ***Toll-like Receptors (TLRs)***
- NOD-like Receptors (NLRs) } inflammation, Apoptosis
- RIG-I-like Receptors (RLRs)

- Pentraxins } opsonization, ***Complement cascade***
- Collectins
- Ficollins

- C-type lectins } phagocytosis
- Scavenger receptors

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
- Stimulate NF-κB associated cytokines, eg, TNFα, IL-1β, IL-8 via activation of stress kinases
- Shares signaling apparatus with IL-1R adapter protein MyD88
- ***Hydroxychloroquine works via Toll-like receptors***

Macrophage Function

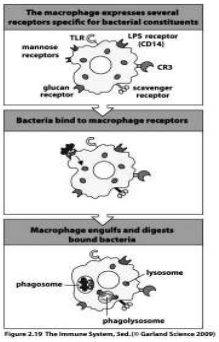


Figure 2.19 The Immune System, 3ed.(© Garland Science 2009)

Macrophage Function

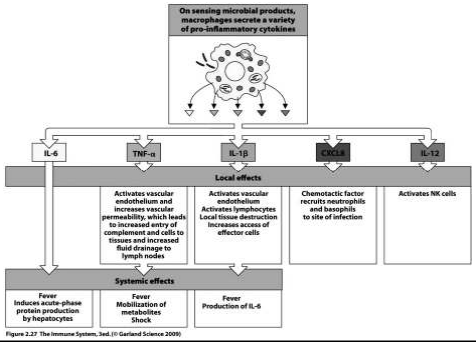
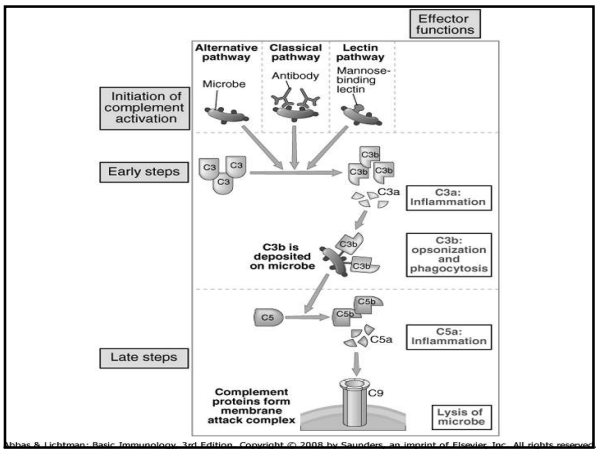
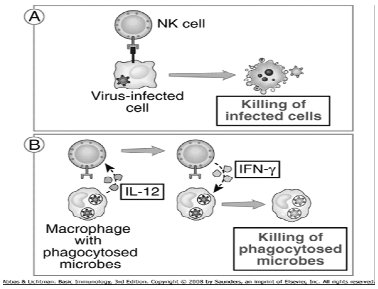


Figure 2.27 The Immune System, 3ed.(© Garland Science 2009)



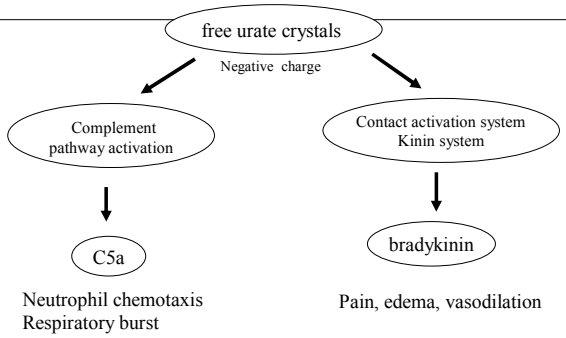
Functions of NK Cells



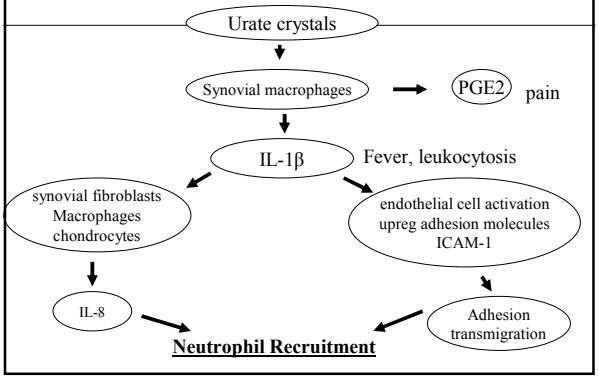
Mechanisms of Acute Gouty Inflammation: Disorder of Innate Immunity

- Acute onset, self limited
- Urate is the inflammatory stimulus, resolves when urate is removed
- Predominant neutrophil response. No lymphocytic reaction
- No autoantibody formation

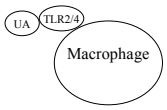
Urate interactions with humoral mediators



Urate interactions with synovial cells



Urate stimulates synovial macrophages through toll-like receptors



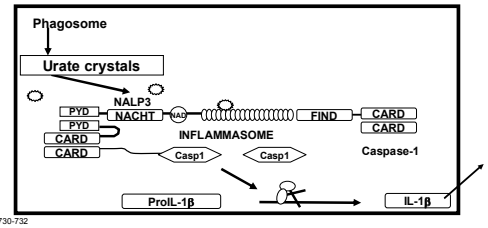
IL-1β, IL-8

TLR2/4 on Mφ

- Urate crystal stimulation is specifically mediated by toll receptors demonstrated by knock out mice and blocking studies
- Urate crystals and not antibodies or proteins coating urate crystals mediate the response

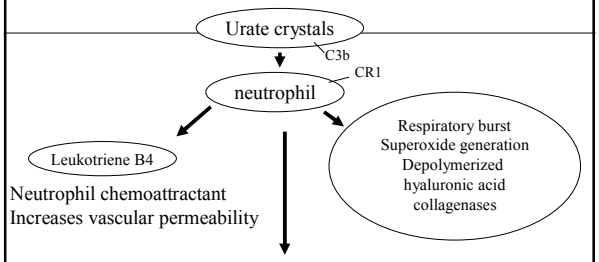
IL-1β and the Inflammasome in Gout

- Inflammasome: Complex of intracellular proteins in neutrophils and macrophages involved in activation of the innate immune system
- Uric acid crystals, bacterial toxins (with ATP) and bacterial RNA activate the inflammasome
- Activated inflammasome leads to production of interleukins, particularly IL-1β which is excreted from the cell.



NEJM 2006 355:730-732

Neutrophil Interactions and Effector Functions

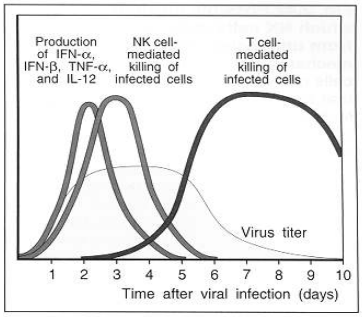


Phagocytosis of Urate crystals
CR1/C3b

Release of lysosomal enzymes
Cell death

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-ie system learns from what it sees
- Must be re-invented every generation!!

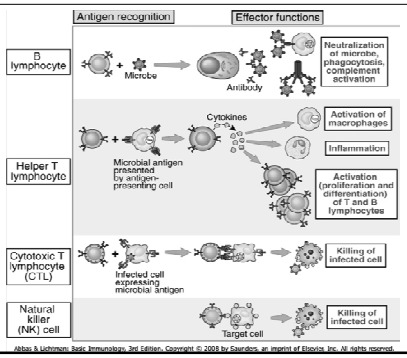


Time Course of innate and adaptive immune response

Three Strategies to Combat Microbes

- Secreted antibodies bind to extracellular microbes, block their ability to infect host cells, 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



T Cell Function

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

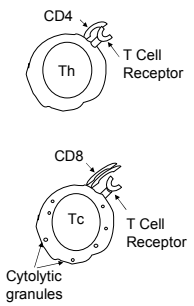
More to come

T cytotoxic cells- induce cell death in target cells via cytotoxic granule release

Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003 Chapter 2, 13

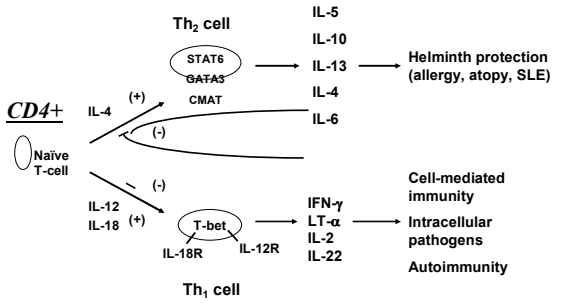
T Cell Immunity (Cell-mediated)

- 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

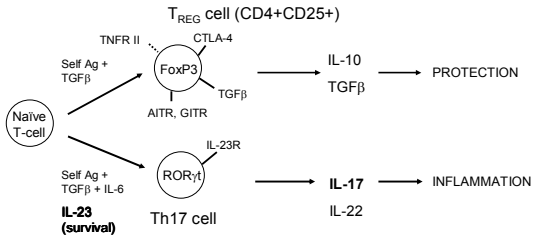
Th1/Th2 paradigm



Schulze-Koops H, *et al. EULAR, 2006,* Amsterdam, #SP0130. Zhu J, *et al. Cell Res* 2006;16:3

T cell subsets: Th17 and T_{REG} cells

- Th17 cells are abundant in gut, maintain mucosal homeostasis
- Th17 upregulated in inflammatory diseases: MS, RA, SLE, IBD
- IL-23 important for the maintenance of the Th17 phenotype



Betelli E, *et al. Nature* 2006;441:235-8; Ivanov II, *et al. Cell* 2006;126(6):1121-33; Tesmer L, *et al.* ACR, Washington DC, 2006; #SUN0297

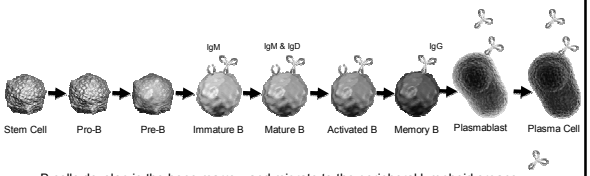
	CD8 cytotoxic T cells	CD4 T_H1 cells	CD4 T_H2 cells	CD4 T_H17 cells	CD4 regulatory T cells (various types)
Types of effector T cell					
Main functions in adaptive immune response	Kill virus-infected cells	Activate infected macrophages Provide help to B cells for antibody production	Provide help to B cells for antibody production, especially switching to IgE	Enhance neutrophil response	Suppress T-cell responses
Pathogens targeted	Viruses (e.g. influenza, rabies, vaccinia) Some intracellular bacteria	Microbes that persist in macrophage vesicles (e.g. mycobacteria, *Listeria*, *Leishmania donovani*, *Pneumocystis carinii*) Extracellular bacteria	Helminth parasites	Extracellular bacteria (e.g. *Salmonella enterica*)	

Figure B-1 Immunobiology, 7ed. (© Garland Science 2008)

B cells and Humoral Immunity

- Major limb of adaptive immunity
- Immunoglobulin is structurally homolgous 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

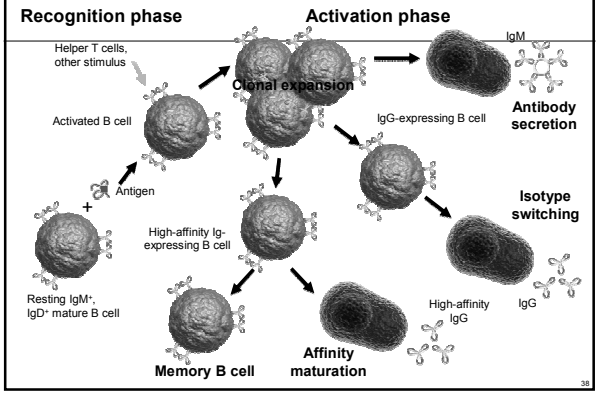
B-Cell Immunology: Lineage[1,2]



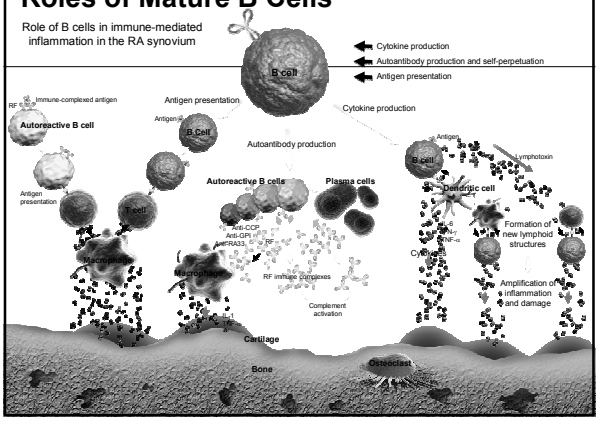
- B cells develop in the bone marrow and migrate to the peripheral lymphoid organs, where they can be activated by antigens[2]
- Activated B cells proliferate and differentiate into long-lived memory cells and antibody-secreting plasma cells[2]

1. Roitt et al, eds. *Immunology*. 6th ed. 2001.
2. Murphy K et al. eds. *Janeway's Immunobiology*. 7th ed. New York, NY: Garland Science, Taylor & Francis Group, LLC; 2008:323-377.

B-Cell Activation

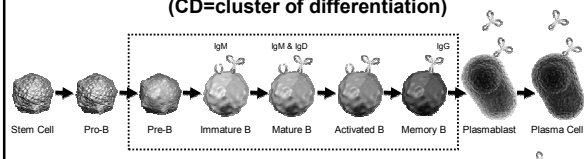


Roles of Mature B Cells



Targets of Rituxan[1,2]

Expression of CD20 During B-Cell Maturation[1] (CD=cluster of differentiation)



- Rituxan 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 Rituxan

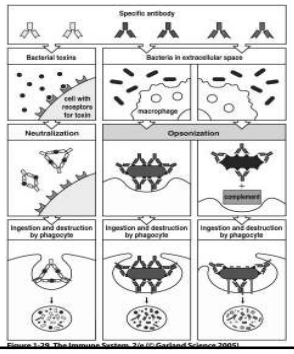
1. Roitt et al, eds. *Immunology*. 6th ed. 2001.
2. Murphy K et al. eds. *Janeway's Immunobiology*. 7th ed. New York, NY: Garland Science, Taylor & Francis Group, LLC; 2008:323-377.
3. Rituxan® (rituximab) Full Prescribing Information. Genentech USA, Inc., and Biogen Idec Inc. October 2009.

Plasma Cells and Memory B Cells

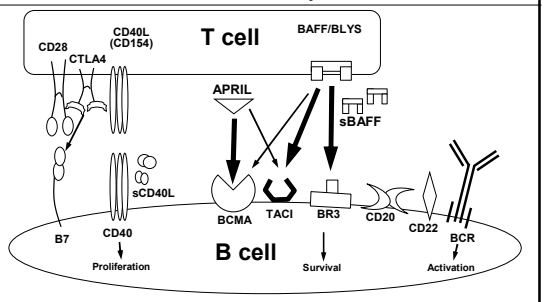
- Plasma cells[1]
  - –Short-lived – remain in lymphoid organ
  - –Long-lived – migrate to bone marrow and can persist for years (or even decades)
- Memory B cells[1,2]
  - –Long-lived
  - –Circulate through immune system compartments
  - –Are rapidly recruited by re-exposure to specific antigen

1. Weinstein E, et al. *Rheum Dis Clin North Am*. 2004;30:159-174.
2. Murphy K, et al. eds. *Janeway's Immunobiology*. 7th ed. New York, NY: Garland Science, Taylor & Francis Group, LLC; 2008.

Antibody Function



Targeting of B Cells Through Diverse Receptors

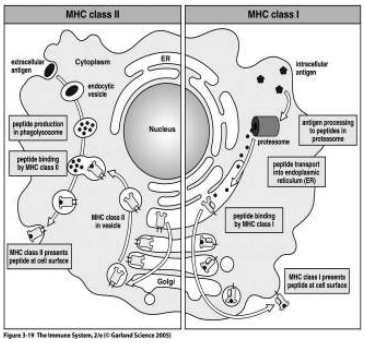


1. Miller JP et al. *J Immunol*. 2006;176:6405-6410.
2. Roitt I et al, eds. *Immunology*. 6th ed. London, England: Mosby International Ltd; 2001.
3. Takemura S et al. *J Immunol*. 2001;167:4710-4718.
4. Rodriguez-Pinto D. *Cell Immunol*. 2005;238:67-75.

T Cell Function

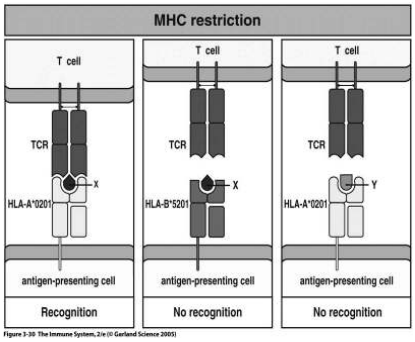
MHC Based Antigen Presenting Cell-Lymphocyte Interactions

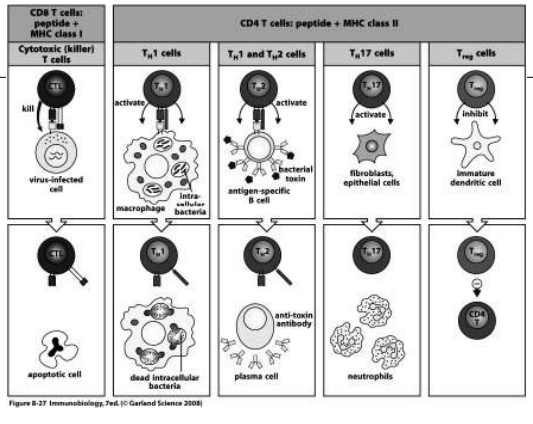
MHC II interacts with CD4+ lymphocyte



MHC I interacts with CD8+ lymphocyte

MHC Restriction





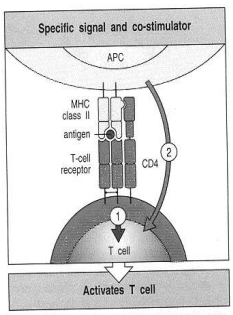
T cell activation

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



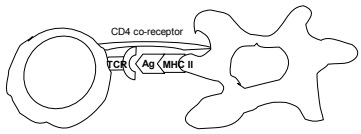
T Helper Cell Activation

- 3 signal hypothesis
  - Antigen binding to TCR (with MHC)
  - T cell co-stimulation (e.g. B7/CD28 binding)
  - Cytokine signal to drive differentiation

Curtsinger *et al.*, 1999 Journal of Immunology, 162:3256–3262
reviewed by Gutcher and Becher, 2007 Journal of Clinical Investigation 117(5):1119-1127

T-Cell Activation - Signal 1

- APC presents processed antigen fragments to the TCR in complex with APC expressed MHC class II protein
- CD4 acts as a co-receptor for TCR activated cell signaling

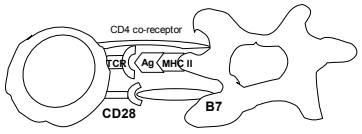


Th lymphocyte (CD4+) Dendritic cell (APC)

Goldsby, Kindt, Osborne and Kuby, Immunology 5th Ed. 2003 Chapter 10
Gutcher and Becher, 2007 Journal of Clinical Investigation 117(5):1119-1127

T-Cell Activation - Signal 2

- APC cell surface ligand B7 binds to the T cell CD28 receptor
- CLTA-4 can bind in the place of CD28 to inhibit T cell activation



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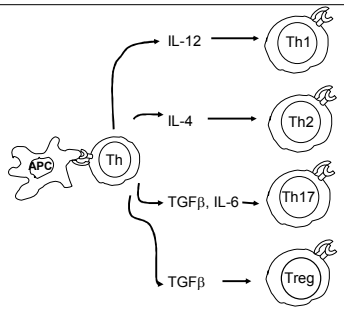
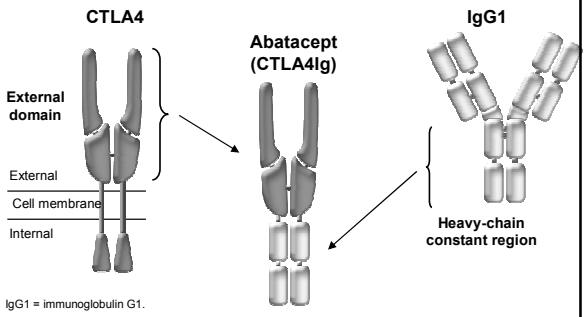
T Helper Cell Differentiation Signal 3-Delivered by APC



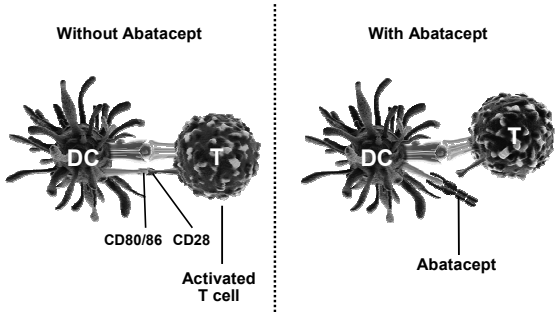
Curtsinger *et al.*, 1999 Journal of Immunology, 162:3256–3262
Gutcher and Becher, 2007 Journal of Clinical Investigation 117(5):1119-1127

Abatacept: A Human Immunoglobulin Receptor Fusion Protein

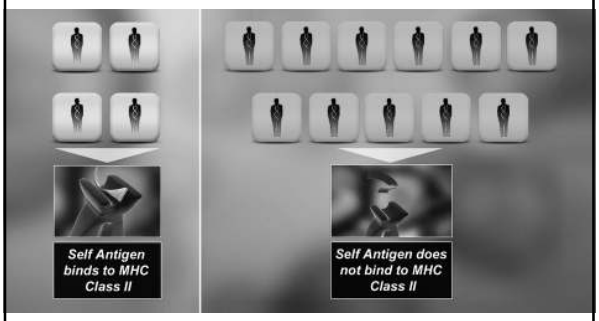


IgG1 = immunoglobulin G1.

Mechanism of Action of Abatacept



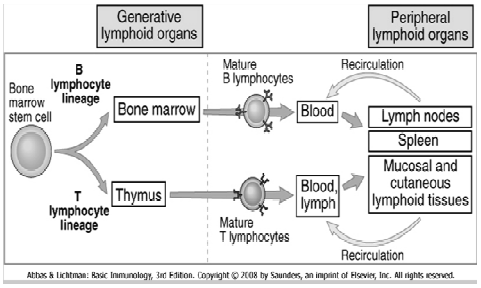
Shared Epitope-part of the MHC class II molecule
Shared Epitope-able to bind citrullinated self Ags
Patients with shared epitope==yellow



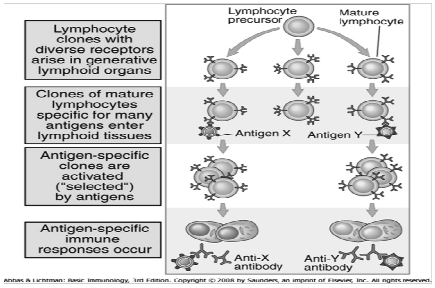
Properties of Adaptive Immune Response

Feature	Functional significance
Specificity	Ensures that distinct antigens elicit specific responses
Diversity	Enables immune system to respond to a large variety of antigens
Memory	Leads to enhanced responses to repeated exposures to the same antigens
Clonal expansion	Increases number of antigen-specific lymphocytes to keep pace with microbes
Specialization	Generates responses that are optimal for defense against different types of microbes
Contraction and homeostasis	Allows immune system to respond to newly encountered antigens
Nonreactivity to self	Prevents injury to the host during responses to foreign antigens

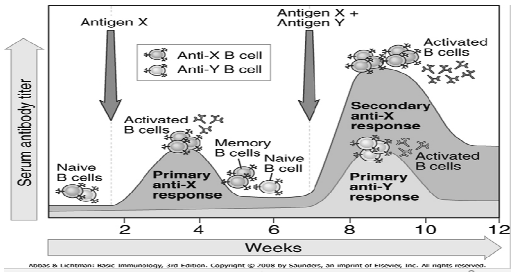
Lymphocyte Maturation



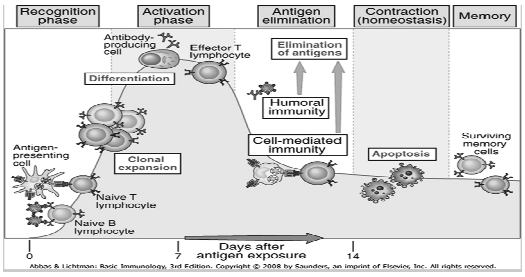
Clonal Selection



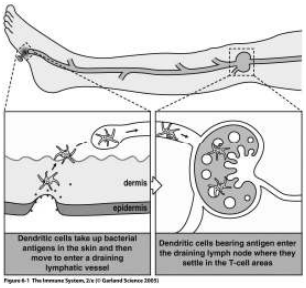
Specificity of Immune Response



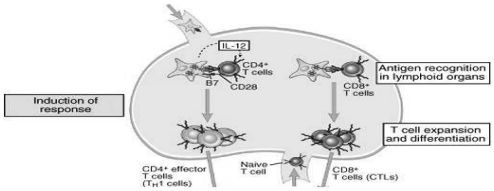
Phases of Immune Response



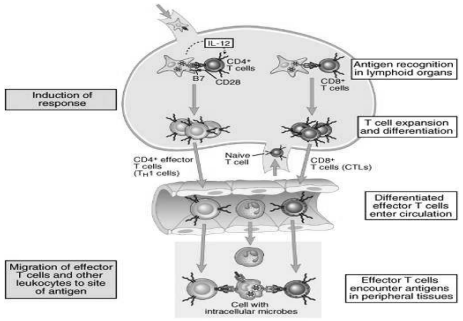
Dendritic cell (APC) action



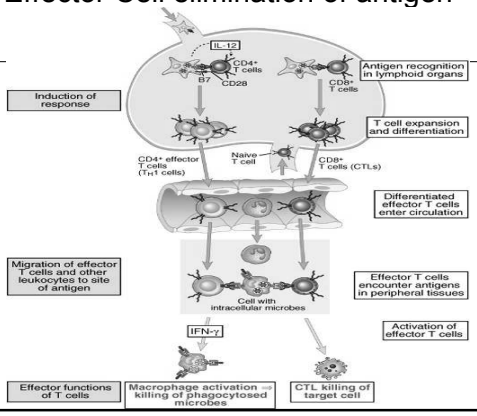
Induction of Immune Response



Migration of effector cells and leucocytes

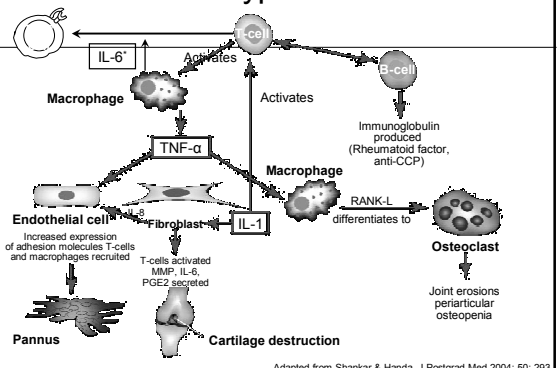


Effector Cell elimination of antigen



Adaptive immunity in rheumatoid arthritis

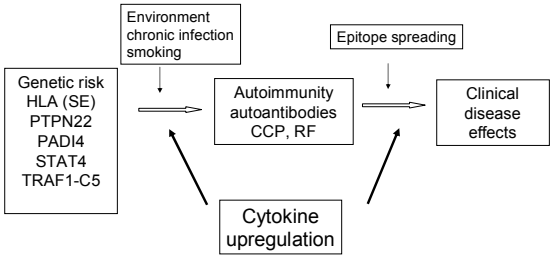
T-cell mediated hypothesis of RA



Adapted from Shankar & Handa, J Postgrad Med 2004; 50: 293

*Bettelli E et al., *Nature*. 2006;**441**:235–238, *Veldhoen *et al.*, 2006 Immunity 24:179-189

Pathogenesis of RA: Overview



Pathophysiology of RA

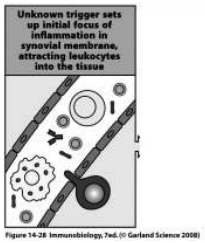


Figure 14-28 Immunobiology, 7ed. (© Garland Science 2008)

Pathophysiology of RA

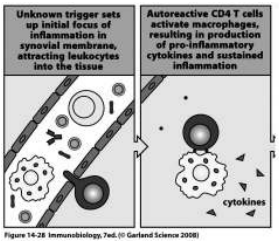


Figure 14-28 Immunobiology, 7ed. (© Garland Science 2008)

Pathophysiology of RA

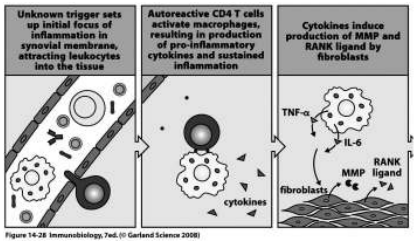


Figure 14-28 Immunobiology, 7ed. (© Garland Science 2008)

Pathophysiology of RA

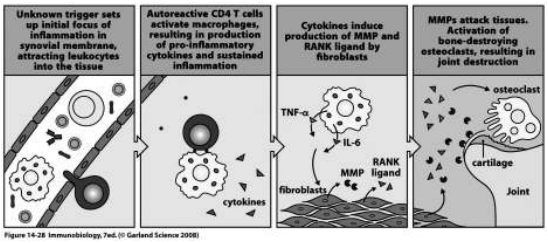
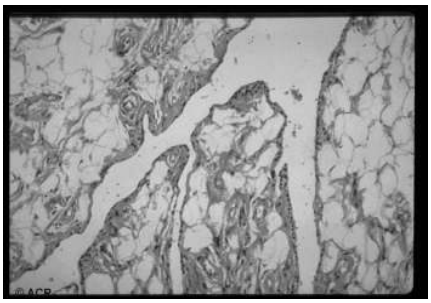
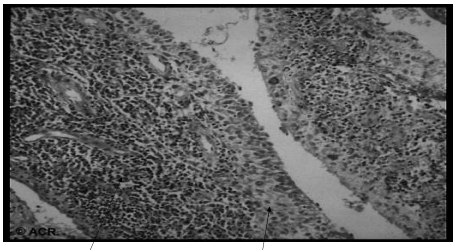


Figure 14-28 Immunobiology, 7ed. (© Garland Science 2008)

Normal Synovium



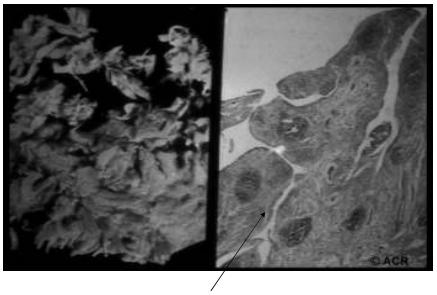
Rheumatoid Synovitis



Lymphocyte recruitment into subsynovial space

Hypertrophy of synovial lining cells

Rheumatoid Synovitis



Prominent nodal lymphoid architecture

RA synovitis: Evidence for an antigen driven adaptive immune process

- Persistence of autoantibodies
- Affinity maturation of autoantibodies
- Association with MHC class II molecules HLA DRβ1 alleles, shared epitope located in the antigen binding portion of the molecule
- Upregulation of Th17 cells
- Lymphoid architecture of RA synovium
- Limited T cell receptor repertoire early in disease, then epitope spreading

QUESTIONS ?