Innate Immunity Part II
Inflammation, continued

iii. Neutrophil homing and diapedesis
Homing of neutrophils to inflamed tissues involves altered interaction with vascular endothelium (the cells lining the blood vessels).
- Interaction between complementary pairs of adhesion molecules, one on leukocyte surface and the other on vascular endothelial cells or other tissue cells.
- 4 structural classes of proteins. (Fig. 2.30)
  - **Selectins**: lectins with specificity for oligosaccharides on ligands such as vascular addressins.
  - Carbohydrates on molecules such as vascular addressins
  - **Integrins**: α and β chains. Binds protein ligands, many of which are Ig superfamily members. LFA-1
  - **Immunoglobulin (Ig) superfamily members**: extracellular domain has ~100 aa in length. ICAM-1
- Extravasation: process of neutrophil migrating out of blood capillaries and into tissues (Fig. 2.31). 4 steps:
  - **Rolling adhesion**: Interaction between neutrophil and vessel wall that slows down neutrophils. Selectins on vascular endothelium bind to the carbohydrate side chains of sialyl-Lewis^x\ (s-Le^x) on neutrophils.
  - **Tight binding**: Integrin LFA-1 on neutrophils and adhesion molecule on endothelium (ICAM-1, member of Ig superfamily). In absence of inflammation and chemokines, LFA-1 bind weakly but CXCL8 changes conformation of LFA-1 and allows tight binding to ICAM-1.
  - **Diapedesis**: Crossing of blood vessel wall. Squeezing through between endothelial cells. Reaches the basement membrane. Secretes proteases that break down the basement membrane.
  - **Migration**: Movement toward infected area. Gradient of CXCL8 and other chemoattractants.

D. Complement System and Defensins
Complement
- Soluble proteins that are constitutively made by the liver and are present in the blood, lymph, and extracellular fluid.
- Most important step in complement activation is the cleavage of C3 to generate C3a and C3b (Fig. 2.3). This is called **complement fixation**.
  - C3a diffuses away to recruit phagocytes
  - C3b becomes covalently attached to pathogen surface, marking for phagocytosis (opsonization)
- There are three pathways of complement activation (Fig. 2.5): alternative, lectin, and classical pathways.
  - They differ in mechanism of pathogen recognition
  - They all converge at the level of C3 cleavage
  - Each pathway uses a different protein complex to cleave C3; these are called: C3 convertases
The 3 pathways are activated sequentially during an immune response.

Three functions of complement activation: recruitment of inflammatory cells (i.e. phagocytes), opsonization, perforation (poking holes) of pathogen membrane (Fig. 2.5)

Basic concepts and definitions:
- Many complement components are proteolytic enzymes (proteases); they circulate as zymogens (inactive form).
- Some complement components have internal thioester bonds that are considered “high-energy” as they are subject to nucleophilic attack by water (hydrolysis) or by molecules on the surface of pathogens – the latter event leads to covalent attachment of the complement fragment (Fig. 2.4) and activation of the protease.

Recognition mechanisms of the three pathways
1. Alternative pathway
   - C3 molecules are constantly being cleaved in plasma and lymph at a low rate by the soluble C3 convertase.
   - usually C3b is released as soluble molecule due to attack by water (Fig. 2.4, top)
   - if this occurs near a pathogen surface, C3b becomes attached to surface (Fig. 2.4, bottom)
   - C3b recruits Factor B which is then cleaved by Factor D to generate two fragments, Ba and Bb (Fig. 2.8)
   - Bb binds to C3b to form C3bBb, the alternative C3 convertase (Fig. 2.7)
   - C3bBb cleaves many more molecules of C3 to deposit more C3b, amplifying the pathway (Fig. 2.8).

2. Lectin pathway
   - Mannose-binding lectin (MBL) (Fig. 2.37)
     - Binds to mannose-containing carbohydrates of bacteria, fungi, protozoa, and viruses.
     - Structure is like a bunch of flowers. Each stalk is a triple helix made from three identical polypeptides (like collagens).
     - Each MBL has 5-6 “flowers”. Each flower has 3 pathogen binding sites. Each MBL has 15-18 pathogen attachment sites.
     - Some human cells have mannose, but their geometry does not allow binding of MBL.
     - MBL is a member of collectin family (properties of collagen and lectin).

   - MBL binding to pathogen activates protease MASP-2 (Fig. 2.37, 2.40) to cleave C4
   - Leads to covalent binding of C4b on pathogen and formation of C4bC2a, the classical C3 convertase (same in lectin pathway as classical pathway) (Fig. 2.41)
   - MBL produced by liver cells (hepatocytes) during acute-phase reaction (Fig. 2.38). This is why lectin pathway becomes important later than alternative pathway.
3. Classical pathway
- can be initiated by antibody (Ig)-coating of pathogens (later lecture) or by C-reactive protein (CRP) produced during acute-phase response (Fig. 2.38)
- CRP or antibodies bind to C1q, a collectin with similar structure to MBL (Fig. 2.42)
- CRP binds to phosphocholine component of LPS on pathogen surface and recruits C1q (Fig. 2.43)
- C1q cleaves C4 to deposit C4b and form the classical C3 convertase C4bC2a (Fig. 2.43)

Opsonization
- Complement coats the surface of bacteria and extracellular virus and makes them more easily phagocytosed. Without this coating many bacteria are resistant to phagocytosis (especially those with thick polysaccharide capsules).
- Most important: C3 (patients lacking other components are often mildly affected – those without C3 has severe infections).

C5 and the terminal components of the complement cascade
- Cleavage of C5 generates C5b and C5a
- the alternative 3 convertase binds some of the C3b that it cleaves
- this molecule (C3b)2Bb is the alternative C5 convertase (Fig. 2.12)
- C5b can initiate formation of the membrane attack complex (Fig. 2.13) that can poke holes in the cell wall and plasma membrane of certain bacteria
- Other components are C6, C7, C8 and C9, also known as the terminal components of complement (Fig. 2.11)
- Dramatic pictures (Fig. 2.13) but most pathogens handled with no problems by humans with deficiency in C6-C9. Exception: Neisseria
- C5a, like C3a, recruits inflammatory cells. These also increase vascular permeability and microbicidal activity of macrophages (Fig. 2.15). C5a and C3a are known as anaphylotoxins because can cause a toxic loss of blood pressure when over-produced.
- Many different types of cells have receptors for C3a and C5a

Regulatory proteins
- Complementary control proteins regulate complement reactions to prevent destruction of host cells and depletion of C3 from body fluids
- Two classes: (Fig. 2.9)
  - Plasma proteins that interact with C3b attached to human and microbial cell surface
    - Factor H and factor I: factor H binds to C3b and facilitates C3b cleavage by factor I to produce iC3b, which cannot become C3 convertase – reduces number of C3 convertase on microbial surface.
  - Membrane proteins on human cells that prevent complement fixation
    - DAF and MCP disrupt C3 convertase
    - CD59 blocks membrane attack complex (Fig. 2.14)
Defensins
- A major family of antimicrobial peptides (Fig. 2.18)
- Two classes
  • α-defensins
  • β-defensins
- Amphipathic – surface has both hydrophobic and hydrophilic regions. This allows penetration of microbial membrane.
- α-defensins
  • Expressed mainly by neutrophils and by Paneth cells (specialized epithelial cells of the small intestine situated at the base of the crypts between intestinal villi). (Fig. 2.17)
- β-defensins
  • Expressed by a broad range of epithelial cells (especially those of the skin, respiratory tract, and urogenital tract).

E. Innate immunity to viruses: Type I interferons and NK cells
- Innate immune response controls intracellular pathogens e.g. viruses through secretion of type I interferon (IFN-α and IFN-β) that interfere with viral replication.
  - (Fig. 2.44) Viral infection triggers the phosphorylation, dimerization, and nuclear translocation of the transcription factor IRF3 that works in concert with NFκB and AP-1 to transcribe the IFN-β gene. Sensing mechanism includes TLR3 etc.
  - Secreted IFN-β works in a paracrine manner to help uninfected cells become resistant to infection.
  - Additionally, IFN-β works in an autocrine manner to mobilize IRF7 to the nucleus where it transcribes IFN-α.

Functions of type I IFNs: (Fig. 2.45)
- Make healthy cells resistant to infection
- Make virus-infected cells more vulnerable to attack by killer lymphocytes
- Type I IFNs together with interleukin-12 (IL-12) activate NK cells
- Almost all human cells can be infected with a virus, and almost all are equipped to make interferons and their receptors.
**NK cells** provide an early defense against intracellular infections. (Fig. 2.47)
- Larger than B and T, well-developed cytoplasm containing toxic granules.
- People who lack NK cells have persistent viral infection, particularly herpes viruses.
- Provide protection against intracellular pathogens e.g. viruses through production of cytokines as well as lytic activity.
- Type I IFN’s simulate NK cells and enhance lytic activity while IL-12 favors production of cytokines.
- **IFN-γ (type II IFN)** is a principal cytokine released by NK cells. NK cells are responsible for early secretion of IFN-γ that serves to activate macrophages to produce additional inflammatory cytokines & help activate T cells during adaptive immune response.

**NK cell receptors** (Fig. 2.48)
- NK cells do not have surface receptors that arise from gene rearrangement.
- Most NK cell receptors fall within two categories.
  - Immunoglobulin-like receptors
  - Lectin-like receptors (many bind proteins though)
- Balance between activating receptors and inhibitory receptors on NK surface. (Fig. 2.48, 2.49)
- When an NK cell interacts with a healthy cell, the combined signals it receives from its inhibitory & activating receptors block attack.
- When a NK cell encounters a virus-infected cell, the balance of activating and inhibitory signals is altered to favor NK cell attack.
- Example
  - **NKG2D**: activating lectin-like NK receptor
  - Binds to **MIC-A** and **MIC-B** which are produced in response to stress such as infection