**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**:  and  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-Lewisx (s-Lex) 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.
1. Complement System and Defensins

**Complement**

* Soluble proteins that are made constantly 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). Nomenclature: usually larger fragment is “b” and smaller is “a”

- 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 C3 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. 2.11, 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.

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
	+ -defensins
	+ -defensins
* amphipathic – surface has both hydrophobic and hydrophilic regions. This allows penetration of microbial membrane.
* **-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)
* -defensins
	+ Expressed by a broad range of epithelial cells (especially those of the skin, respiratory tract, and urogenital tract).
1. 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-** and **IFN-)** 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 NFB and AP-1 to transcribe the IFN- gene. Sensing mechanism includes TLR3 etc.

- Secreted IFN- works in a **paracrine** manner to help uninfected cells become resistant to infection.

- Additionally, IFN- works in an **autocrine** manner to mobilize IRF7 to the nucleus where it transcribes IFN-.

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- (type II IFN)** is a principal cytokine released by NK cells. NK cells are responsible for early secretion of IFN- 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