# Innate Immunity Part I

A variety of defense mechanisms have evolved to eliminate the different types of pathogen (Fig. 2.2)

* A variety of pathogens
* Categories: extracellular infections and intracellular infections.

1. Recognition of Foreign Molecules by the Innate Immune System

-- mediated by cell surface receptors and by soluble proteins

-- distinguish molecular structures that are not present on host cells (or inside host cells). Many are carbohydrates or lipids that are part of microbial cell walls. Others are nucleic acids with features distinct from mammalian DNA or RNA. As a group these foreign molecular structures = **PAMPs** (pathogen-associated molecular patterns). PAMPs recognized by innate immune receptors are common to many pathogens and stable throughout evolution.

Examples of cell surface receptors (Fig. 2.19):

-- **lectins** that bind carbohydrates: mannose receptor; glucan receptor

-- scavenger receptor: binds negatively charged molecules (i.e. sulfated sugars)

-- **complement receptors** CR3 and CR4 (will describe in next lecture)

-- **Toll-like receptors (TLRs)**

**TLRs**

* a family of signaling receptors, each specific for a different set of microbial products.
* TLRs are transmembrane proteins. (Fig. 2.20)
* Pathogen recognition domain composed of repeated motifs (20-29 aa) that have hydrophobic aa leucines and is termed leucine-rich region (LRR).
* Cytoplasmic domain of TLR is called Toll-interleukin receptor (TIR) domain because it is present in both TLRs and interleukin-1 (IL-1) receptor.
* Some are homodimers (TLR4) and some are heterodimers (TLR1:TLR2).
* **TLR4**. Specific for **LPS** (lipopolysaccharide) and related molecules on Gram-negative bacteria.
* Other members of TLR family sense different microbial constituents, each molecule common to groups of pathogens not found on humans. (Fig. 2.21)
  + TLR3: double-stranded RNA, present in many viral infections.
  + TLR2:TLR6 detect zymosan – derived from yeast cell wall
  + TLR9 detects unmethylated CpG nucleotide motifs abundant in bacteria and viral genomes but not in human DNA.
* The number of TLR is limited (10 in humans), but they recognize features present in a vast number of pathogens.
* Location (Fig. 2.22)
  + TLR 5, 4, TLR1:TLR2, and TLR2:TLR6 – present on the surface of human cells. They detect proteins, carbohydrates, and lipid components.
  + TLR3, TLR7, TLR8, TLR9 – present on the membrane of **endosomes**, they detect nucleic acid of pathogens. Pathogen nucleic acids are released when pathogens are taken up from extracellular environment and degraded.
* Recognition of microbial components by TLR can involved participation of other co-factors called co-receptors. e.g. CD14 and MD2 help TLR4 recognize LPS. (Fig. 2.23)

TLR signaling activates transcription factor **NFB** to cause new gene expression including cytokines (Fig. 2.24)

* Signaling cascade involves MyD88, IRAK4, TRAF6, IB kinase (**IKK**)
* IKK phosphorylates **IB** causing its degradation; this allows NFB to enter nucleus
* NFB turns on cytokine genes and expression of other genes important for immune response
* Most TLR signaling goes activates NFB.
* Exception: TLR3. Instead of NFB this pathway activates the transcription factor **IRF3** (interferon response factor 3) and result in production of antiviral cytokines called **type I interferons**. TLR4 can also use this pathway (the only pathway that can use both). The intermediate steps are different (Fig. 2.26)

1. Phagocytosis

* Macrophages, neutrophils
* Process involves receptor-mediated endocytosis to generate a **phagosome**, then fusion with lysosome to generate a **phagolysosome** where pathogen is destroyed (Fig. 1.17)
* PAMP recognition by TLRs, mannose receptor etc. can trigger phagocytosis (Fig. 2.19)
* Greatly enhanced by **opsonins**, substances that coat and mark pathogens for phagocytosis
* Common opsonins are complement fragments and antibodies that coat pathogen surfaces
* Recognized via complement receptors and receptors for antibody (**Fc receptors**)
* The complement fragment C3b leads to phagocytosis through CR1 (Fig. 2.10)
* Pathogen destruction occurs through multiple mechanisms (Fig. 2.9)
  + Production of oxygen radicals and hydrogen peroxide is caused by a process called “**oxidative burst**” and is mediated by a complex of enzymes called the **NADPH oxidase** (Fig. 2.34)
* Neutrophils even more efficient killers of phagocytosed bacteria (Fig. 2.32), in part due to activation of the NADPH oxidase. Neutrophils not found in healthy tissue; only in infected tissue. Many stored in reserve in the bone marrow.
* Two sequential changes in pH: first pH rises due to consumption of hydrogen ions by the oxidative burst; most bacteria are killed at this stage.
* Then pH decreases to activate proteases and other hydrolytic enzymes to break down components of the dead pathogen. (Fig. 2.33) Then the neutrophil dies. Dead neutrophils = pus. Removed by macrophages.
* Some receptors that mediate phagocytosis also trigger production of cytokines (Fig. 1.17, right)

1. Inflammation

= recruitment and activation of cells and anti-pathogen proteins at site of infection

i. Cytokines, Chemokines and chemotaxis

Overview:

* Upon sensing the presence of pathogens through TLR4 and other receptors, macrophages secrete cytokines. (Fig. 2.27)
* Some cytokines cause blood vessel changes to increase permeability (**IL-1, TNF-**)
* Some cytokines (**CXCL8**) recruit effector cells, mainly neutrophils.
* Infiltrating cells and fluid leakage cause a state of inflammation.
* **Cytokines** are small proteins (~ 25 kDa) that are made by a cell in response to an external stimulus and influence other cells by binding to a specific receptor on their surfaces.

- many of them have the prefix IL for interleukin

* **Chemokines**: mainly involved in directing traffic, i.e. leukocyte migration.
  + Subfamily of cytokines with structurally similarity.
  + Two major subfamilies: CC and CXC. Based on a pair of cysteine residues.
  + Chemokine receptors are GPCR (G protein coupled receptors; Fig. 2.28)
* **Chemotaxis** is the process of cells migrating towards the source of a “chemoattractant”. The cells sense the gradient of chemokine and migrate accordingly.
* Example = CXCL8 (prev. IL-8)
  + Recruits neutrophils from the blood to the infected areas
  + Neutrophils have two receptors CXCR1 and CXCR2 that bind CXCL8
  + Two effects:
    - * Cells’ adhesive properties change 🡪 can leave the blood and enter tissue.
      * Movement is guided toward higher concentration (source) of chemokines. Chemokine present both in solution and attached to the extracellular matrix and endothelial cell surfaces.
      * Complement products (**C3a, C5a**) and bacterial components (fMLP) also can cause neutrophil chemotaxis

*Chemotaxis Movie*

* + IL-1 and TNF-: facilitate entry of neutrophils, NK cells, and other effector cells to the infected area by inducing changes in the endothelial cell walls of the blood vessels.
  + Molecules that contribute to inflammation = inflammatory mediators.
  + TNF-: example of **local** vs. **systemic** function of cytokine. **Septic shock** if systemic production of TNF-. (Fig. 2.29)
  + People who are heterozygous mutant for TLR4 are more susceptible to septic shock because they don’t control local infection before it spreads to the blood.

ii. Fever, acute-phase response

Inflammatory cytokines raise body temp and trigger **acute-phase** response. (Fig. 2.36)

* IL-6, IL-1, and TNF- have systemic effect – fever.
* Temp-control site of hypothalamus and on muscle and fat cells, altering energy mobilization to generate heat.
* Molecules that induce fever are called **pyrogens**. Bacteria are exogenous pyrogens, and certain cytokines are endogenous pyrogens.
* Higher temp inhibits viral and bacterial replication, and adaptive immunity is more effective at higher temp.
* Acute-phase response: Liver secretes acute-phase proteins: Mannose-binding lectin (**MBL**) and C-reactive protein (**CRP**). These help with innate immune recognition and complement activation; will cover in complement section