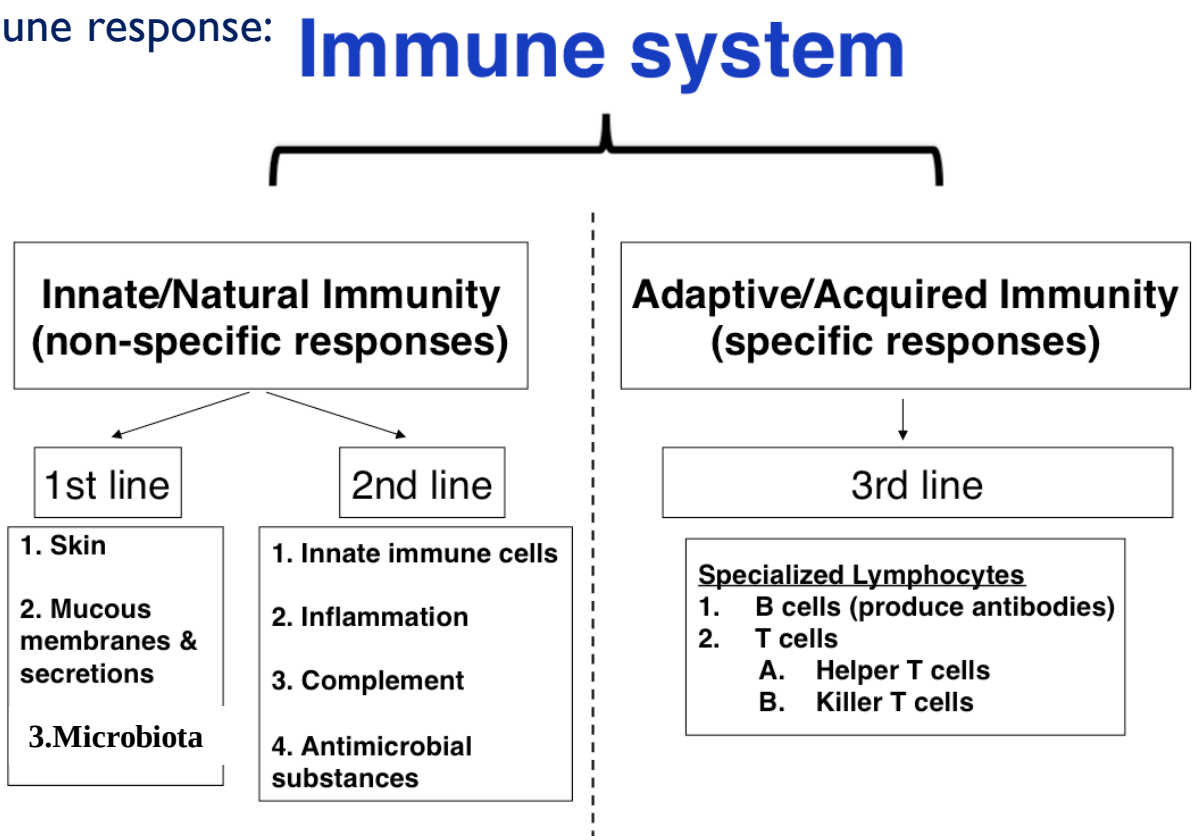
Chapter 6: Pathogen recognition in innate immunity

a.A 2020-21

Properties of the Host: the immune system

We are constantly exposed to millions of potential pathogens daily, through contact, ingestion, and inhalation, but our immune system enables us to resist infections.

Infections triggers the immune response:

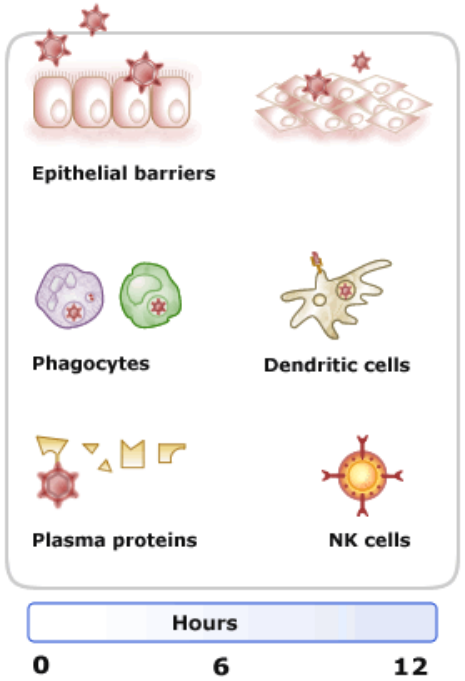


Innate immunity

•Innate immunity responses are the first defense against invading pathogens and spring into action immediately after an infection begins.

•During the first critical hours and days of exposure to a new pathogen, we rely on our **innate immune system** to protect us from infection.

•Innate immune responses are "**Inherent to the host**". They do not depend on the host's prior exposure to the pathogen and are **not specific to a particular pathogen**.



•They responses rely on the body's ability to recognize **conserved features of pathogens** that are not present in the uninfected host. Basic mechanisms that regulate them are conserved.

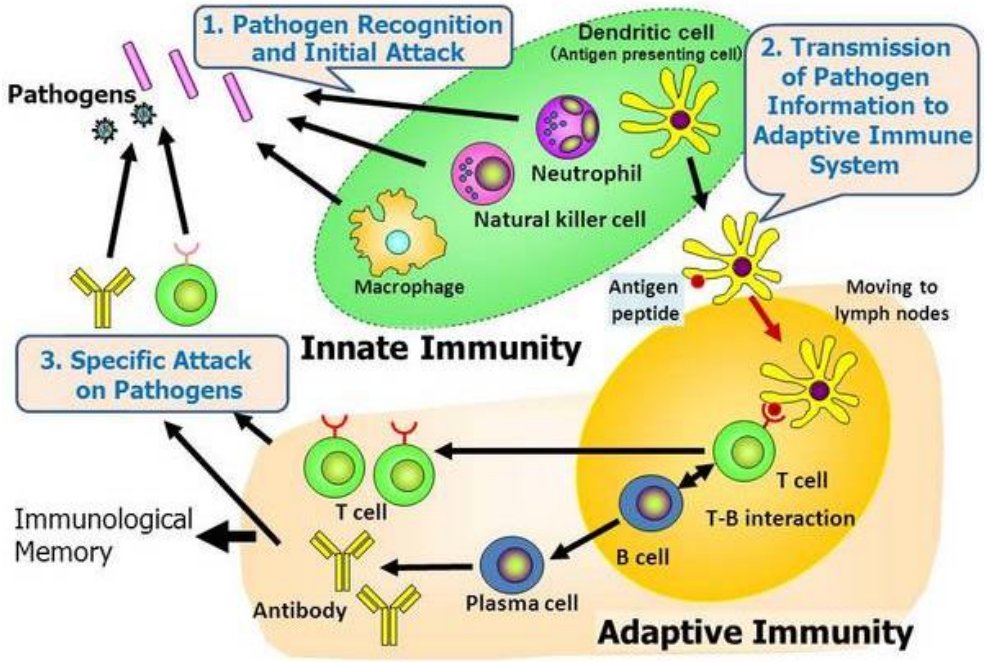
•When activated, innate immunity triggers inducible mechanisms: complement cascade, phagocytosis, and produces inflammatory response.

•In vertebrates induction of innate immune response is required to initiate specific adaptive immune responses.

Adaptive immunity

Adaptive mechanisms: not immediately ready to come into play. It must be turned on by host exposure to a pathogen (as during an infection).

Responses take at least some days to activate.
Highly specific: **directed against the invading pathogen** (immunological response based on antibody)
Diversity is extensive and resulting in a wide range of antigen receptors.
Memory is present: subsequent exposures to the same agent induce amplified responses



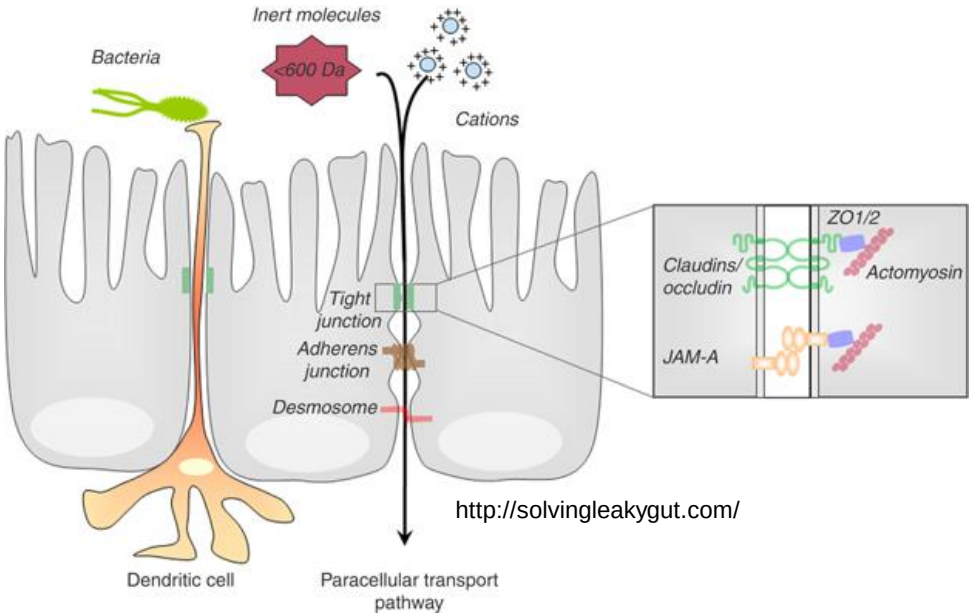
http://akira-pj.lserp.osaka-u.ac.jp/info_en/cafe_third.html

Epithelial Surfaces Help Prevent Infection

The skin and other epithelial surfaces, including those lining the lung and gut provide a **physical barrier** between the inside of the body and the outside world. No microbs can penetrate intact human skin, events have to breach the normal integrity of the skin.

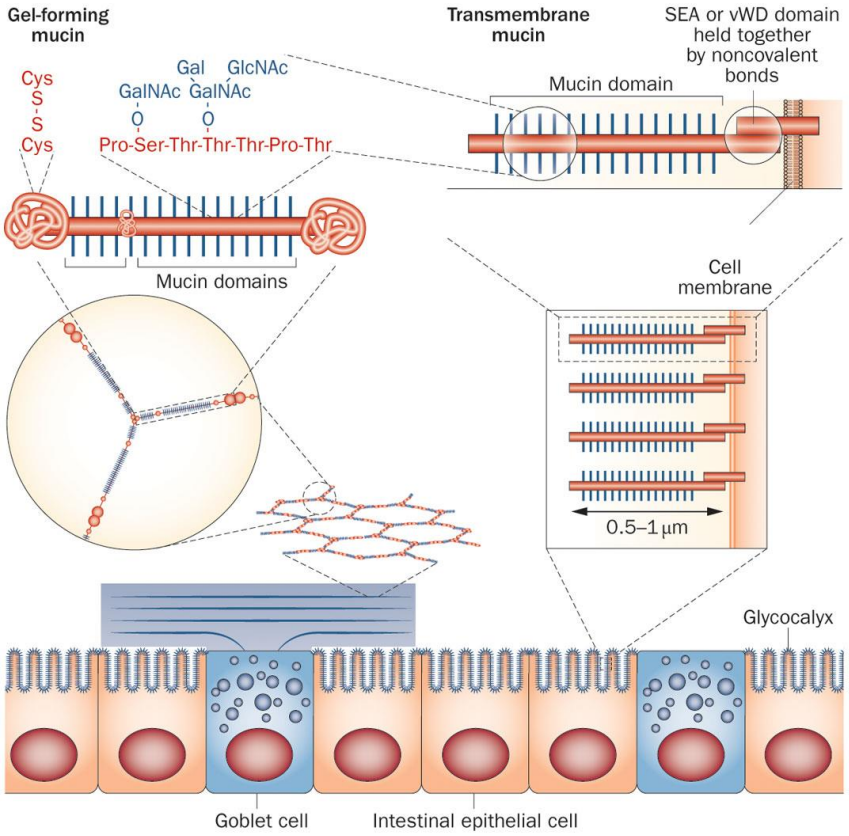
Epithelia surfaces: **tight junctions** between neighboring cells prevent easy entry by potential pathogens.

The interior epithelial surfaces are also covered with a **mucus layer** that protects these surfaces against microbial insults. It also facilitates their **clearance by beating cilia** on the epithelial cells.



Gel-forming mucin and transmembrane mucins attached to the apical membrane of cells

All the parts of mucosal surfaces are protected by mucin.



Nat. Rev. Gastroenterol. Hepatol. doi:10.1038/nrgastro.2013.

Mucins are a family of high MW glycoproteins. Mucin polymers are packed in the granules of the **goblet cells** found among the epithelia.

Two types: **gel-forming and transmembrane mucins.** N-termini and C-termini In gel-forming mucins are held together with numerous disulphide bonds.

Mucin's key characteristic is to form **gels** for protection and lubrification.

Secretion expands to form flat ring-like structures that stack under each other.

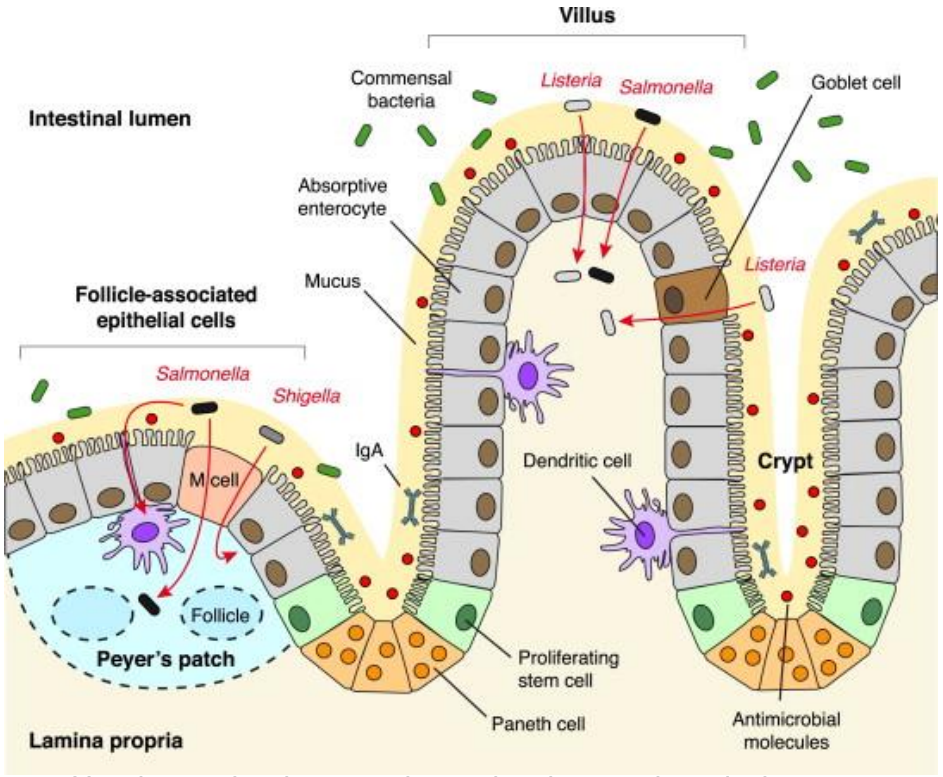
The mucus layer also contains substances such as **lysozyme, lactoferrin, and antimicrobial peptides** that kill pathogens or inhibit their growth.

The epithelium of the small intestine

Intestinal epithelial cells maintain a physical barrier against normal microbiota, The epithelium of the small intestine is composed of absorptive enterocytes, goblet cells, **M cells**, as well as proliferating stem cells and **Paneth cells** located in intestinal crypts. **Peyer's patches** are a component of GALT found in the lining of the small intestines

GALT: **gut–associated-lymphoid tissue**: is a specialized lymphoid tissue allowing constant sampling of the luminal microbiota through **M cells**. Of the follicle-associated epithelium. Lymphocytes of GALT make **secretory IgA.**

Translocated bacteria (by transcytosis) are thus exposed to macrophages, dendritic cells (DCs), and B. lymphocytes, they are usually captured, killed, processed, and presented to the immune system.



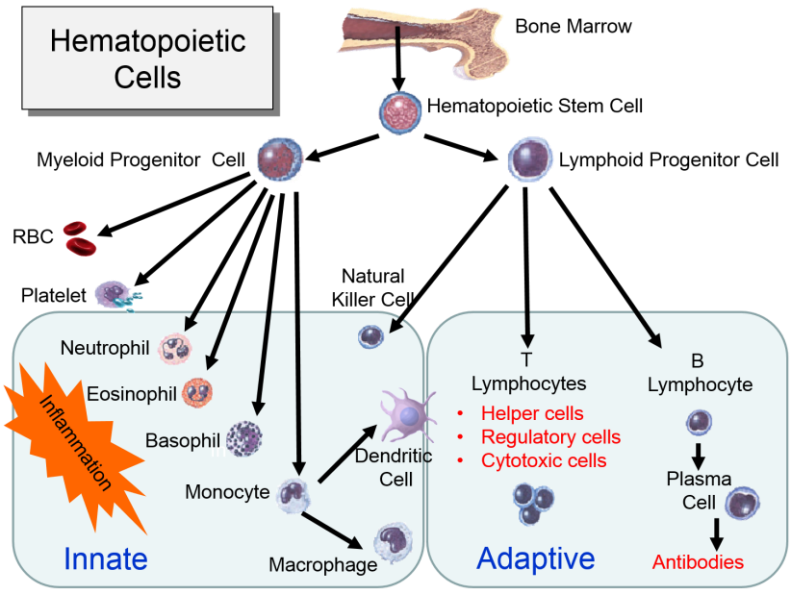
How bacterial pathogens colonize their hosts and invade deeper tissues
David Ribeta, b, c, , , Pascale Cossarta, b, c, ,

Cells of the innate immune system

Skin and mucosal surfaces are high effective in preventing pathogenic bacteria from entering in the tissue and blood but from time to time, bacteria succeed in breaching these surfaces. Bacteria that get this far encounter a formidable defense force: the phagocytic cells and NK cells

Macrophages reside in tissues throughout the body. These **long-lived cells** patrol the tissues of the body. The phagocytize (engulf) pathogens and particles, and also can alert and attract other immune cells. In liver: Kupffer cells, in lungs: alveolar macrophages. **Monocytes:** reside in the blood, Ingest and kill bacteria, migrate to tissues. Precursors of macrophages and dendritic cells.

Neutrophils, are differentiated, short-lived cells, which are abundant in blood but rapidly recruited to sites of infections. Neutrophils can phagocytize particles, release a respiratory burst and produce inflammatory cytokines.



Dendritic Cells are phagocytic cells that have the special ability of initiating an adaptive immune response through a phenomenon called "antigen presentation" and through cytokine production.

Non-cellular Systems of the Innate Immune Response

Besides cells, there are also defenses in your body that are ready to react to pathogens as soon as they are encountered. These systems rely on small proteins that are found within the body fluids.

Complement system: The liver synthesizes the proteins of the complement system and they work in concert to aid in phagocytosis, bacteria lysing and immune cell attraction. It is a self-assembling machine that starts to assemble as soon as the first proteins are bound and in place.

Acute Phase Proteins: produced by the liver during inflammation when pro-inflammatory cytokines are produced. Many are designed to coat pathogens and have chemotactic properties. Some inhibit microbial growth by sequestering iron from the environment.

Anti-microbial Peptides: they function as natural antibiotics and our produced by cells that protect the external surfaces and internal surfaces such as the skin and the gastrointestinal system.

Human alpha defensin 1


Human beta defensin 1

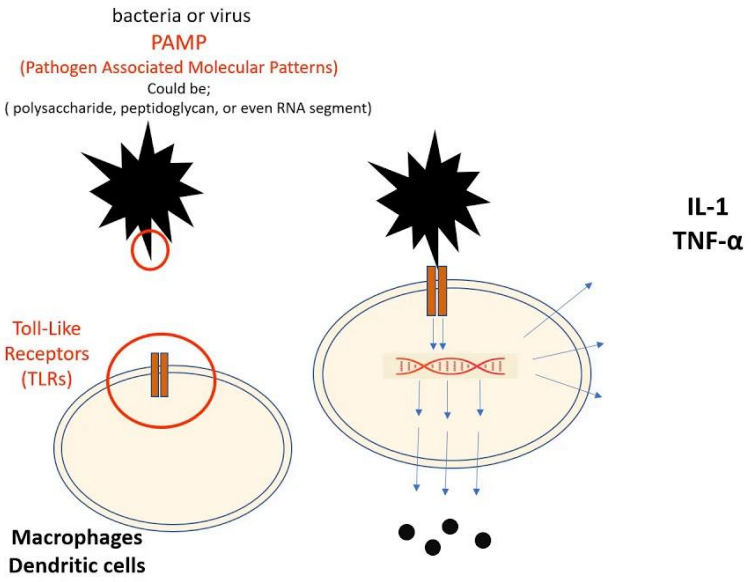
Human cathelicidin LL-37

Human Cells Recognize Conserved Features of Pathogens

The innate immune system relies on the recognition of particular types of molecules or parts of them that are **common to many pathogens** but are absent in the host. Overall these molecules are called **pathogen-associated molecular pattern** (**PAMP**). The term Microbe-Associated Molecular pattern, MAMP, has also been proposed.

PAMPs are **essential structures** for the microorganism survival and therefore they are **difficult to alter**. The various classes of pathogen-associated immunostimulants often occur on the pathogen surface in repeating **patterns.**

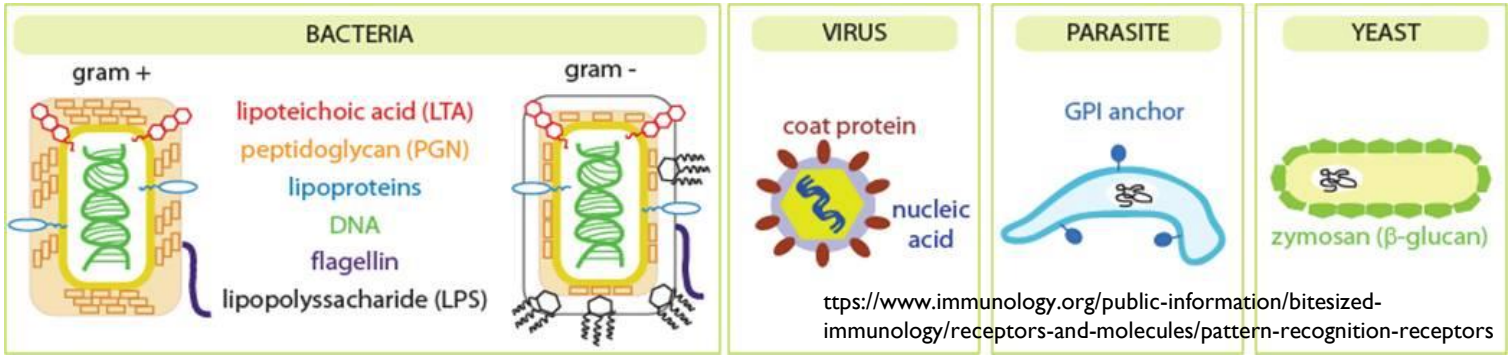
Various types of **PAMPs are** able to stimulate two types of innate immune responses: **inflammatory responses** (discussed below) and **phagocytosis** by cells such as neutrophils and macrophages.



Pathogen-associated molecular pattern

Microbial-associated immunostimulants are of various types:

- **Formylmethionine-containing peptides**: Prokaryotic translation initiation differs from eukaryotic translation initiation in that formylated methionine. Any peptide containing formylmethionine at the N-terminus must be of bacterial origin.
- **The peptidoglycan cell wall** as well as **lipopolysaccharide** (LPS) on Gram-negative bacteria and **teichoic acids** on Gram-positive bacteria. **Flagellin** is also recognized.



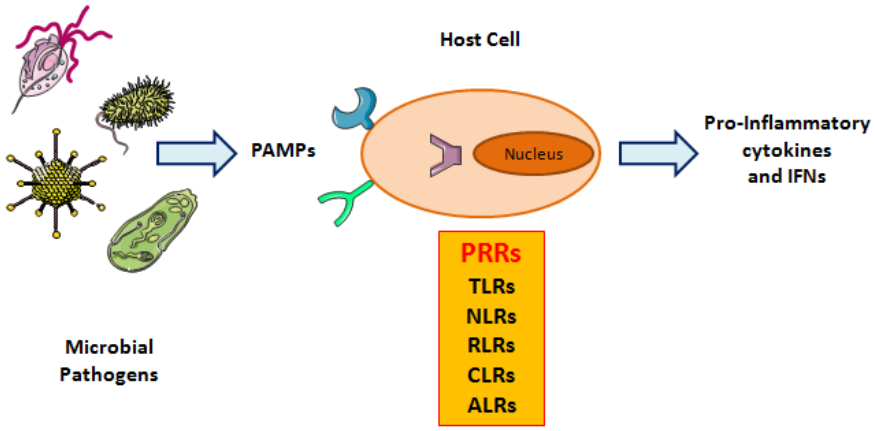
ttps://www.immunology.org/public-information/bitesized-immunology/receptors-and-molecules/pattern-recognition-receptors

- **Chitin** and other **β-glucans** (zymosan) which constitute cell walls of fungi
- **Short sequences in bacterial and viral DNA**. For example: "**CpG motif**" consists of the **unmethylated** dinucleotide CpG flanked by conserved motif. This sequence is highly less common in vertebrate DNA than in bacterial DNA.
- **Ss** and **Ds RNA** of viral origin.

PRRs: Pattern Recognition Receptors

The innate immune system recognizes various classes of PAMPs/MAMPs via a limited number of well-conserved ancient receptors: **pattern-recognition receptors** (**PRRs**).

PRRs are germline encoded, independent of immunologic memory and typically not clonally distributed.



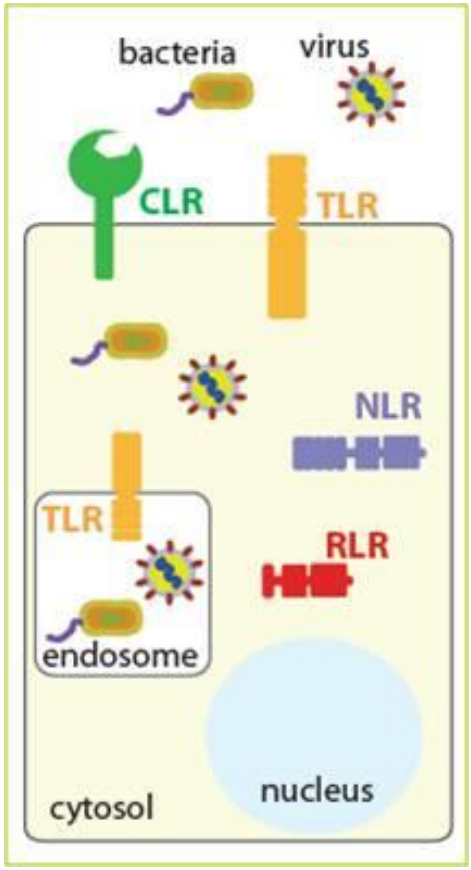
Pattern Recognition Receptors (PRRs)

http://www.inbionet.eu/outreach-news/the-innate-immune-system/n

PRRs are **constitutively expressed** in the host. They are mainly expressed by antigen presenting cells such as dendritic cells and macrophages, but they are also found in other immune (neutrophils, monocytes, limphocytes) and non-immune cells.
The complex interactions between components allows a certain degree of pathogen-specific tailoring of the innate immune responses.

Different class of PRRs act in concert

PRRs are **strategically localized** in the cell. There are present at the cell surface to recognize extracellular pathogens such as bacteria or fungi, in the endosomes where they sense intracellular invaders such as viruses and finally in the cytoplasm.



The PRR are divided in four families:
Toll-like receptors (**TLR**)
Nucleotide oligomerisation receptors (**NLR**)
RIG-I like receptors (**RLR**)
C-type lectin receptors (**CLR**)

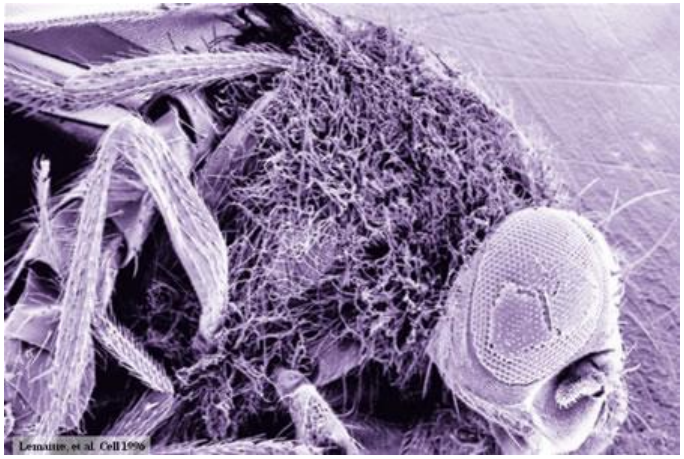
This repertoire of molecules and cells **acts in concert** to form a network of host defense mechanisms. Number of different PRRs are engaged by a given pathogen via various PAMPs, hence securing a rapid and potent inflammatory response and also allowing for shaping and differentiate the response.

The recognition of PAMPs by PRRs leads to production of pro-inflammatory cytokines, IFNs, activation of phagocytes which promotes activation of antigen-presenting cells that elicits adaptive immunity .

Toll-like Proteins Are an Ancient Family of Pattern Recognition Receptors

Many of the mammalian cell-surface pattern recognition receptors responsible for triggering host cell gene expression in response to pathogens are members of the **Toll-like receptor** (**TLR**) family.

Toll receptor has been originally discovered in *Drosophila*. It is involved in the adult fly's resistance to fungal infections.



Toll was discovered in 1996 when in drosophila a developmental gene toll, was knocked out. When this gene was knocked out it was apparent that the animals or the fruit flies succumbed to massive fungal infection

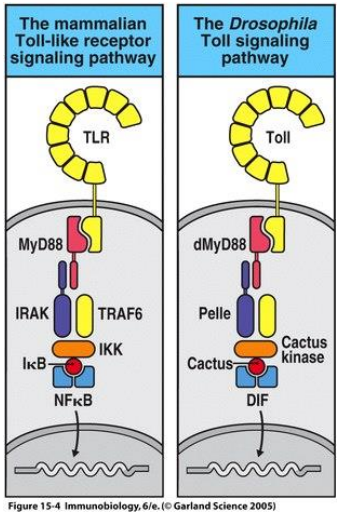


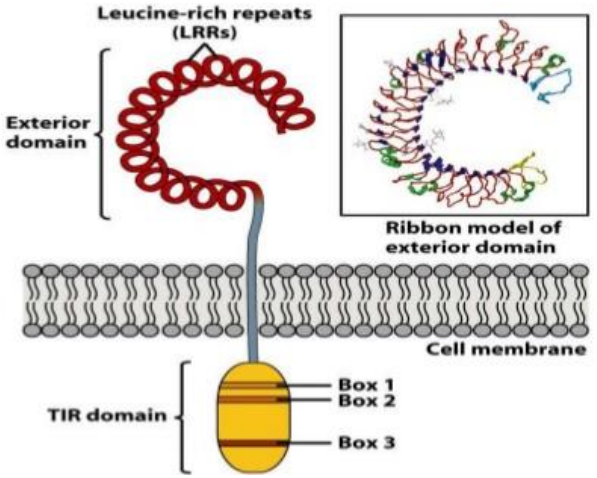
Figure 15-4 Immunobiology, 6/e. (© Garland Science 2005)

Intracellular signal transduction pathway activated downstream of Toll when a fly is exposed to a pathogenic fungus leads to activates the transcription of various genes, including those encoding the antifungal peptides: defensins.

TLRs are evolutionarily conserved from the worm *Caenorhabditis elegans* to mammals. The human TLR family comprise 10 members, 12 in mouse.

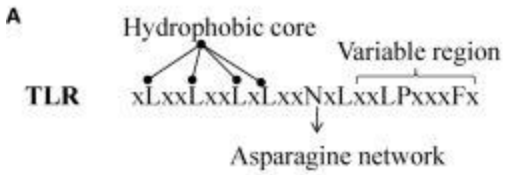
The structure of a toll-like receptor

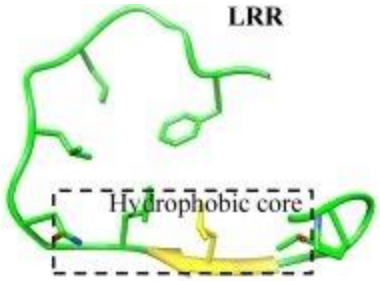
Toll-like receptors (**TLRs**) are type I integral membrane receptors, non-catalytic receptors that recognize structurally conserved molecules derived from microbes.



Three domains: 1) the extracellular **Leucine-Rich Repeat (LRR)** domain 2) short cysteine rich patches with short transmembrane portion, and 3) a conserved cytoplasmic signaling domainToll/IL-1R homology (TIR domain).

Leucine-Rich Repeat domain is composed of 19–25 tandem repeats of 22–30 residues in length and contains a conserved hydrophobic motif **XLXXLXLXX** spaced at distinctive intervals.



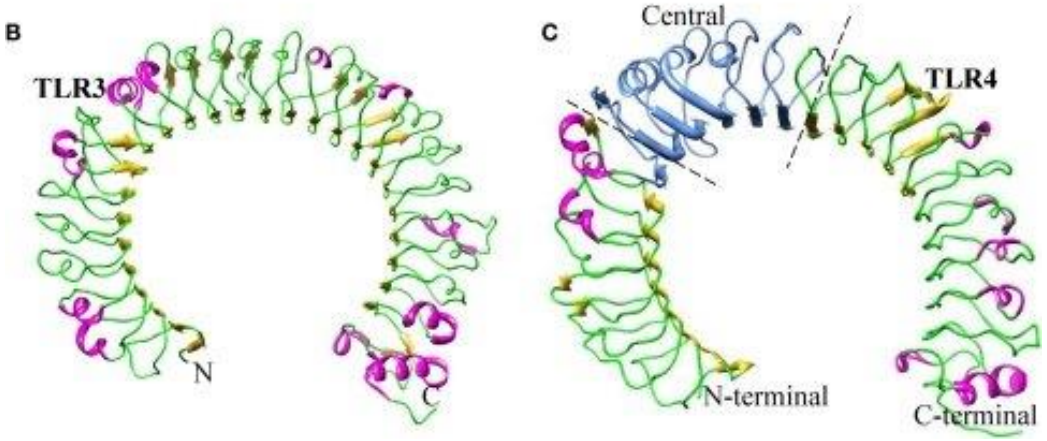


All LRRs adopt **a loop structure** (in figure TLR4), beginning with an extended stretch that contains three residues in the β-strand configuration (yellow), followed by an alpha-helical motif.

B. Manavalan et al. 2011Frontiers in Physiology 2:41
DOI: 10.3389/fphys.2011.00041

The green regions form the surfaces involved in ligand binding.

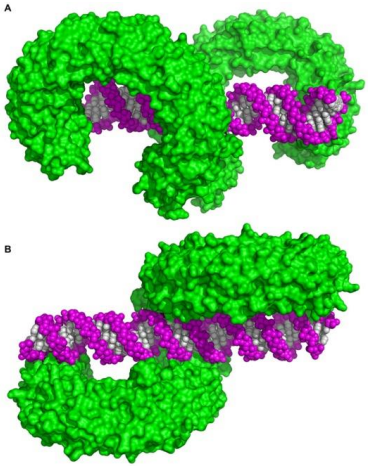
Structure of the TLR extracellular domain



B. Manavalan et al. 2011Frontiers in Physiology 2:41
DOI: 10.3389/fphys.2011.00041

When assembled into a protein, multiple consecutive LRRs form a **solenoid structure**, in which the consensus hydrophobic residues point to the interior to form a stable core and the β strands align to form a hydrogen-bonded parallel β sheet

Structure of the TLR3/dsRNA complex



Structure. 2011 Apr 13; 19(4): 447–459.

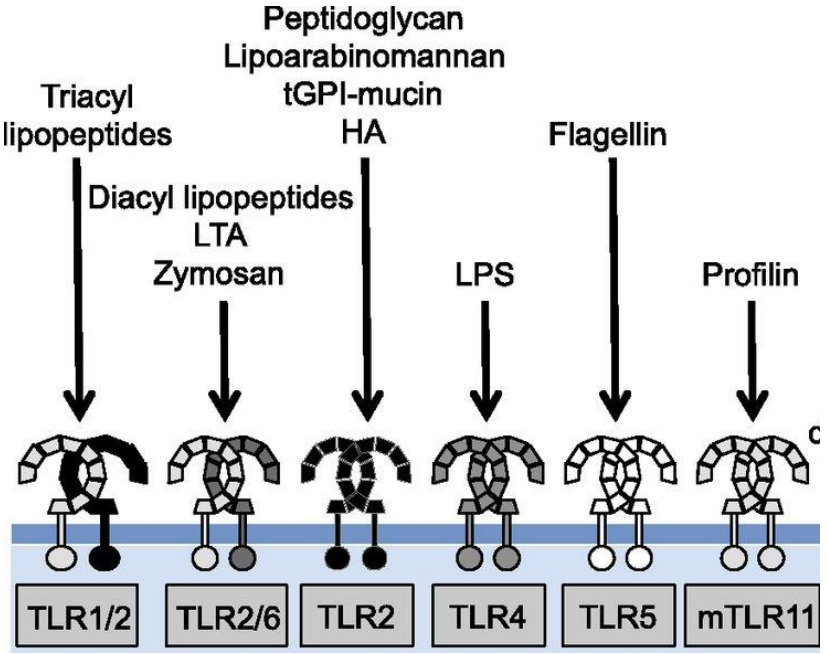
In TLR signaling interaction of an agonist with the LRR either induces the formation of a receptor dimer or changes the conformation of a pre-existing dimer in such a way that it brings two intracellular TIR domains of the TLRs to interact physically.
This simple rearrangement serves as a nucleating act for the recruitment of downstream signaling adapter proteins.

Multiple TLRs are expressed in Humans

The human TLR family comprise 10 members (TLR1-TLR10). They are expressed in different amount and distribution on macrophages, neutrophils, DCs, B cells, specific types of T cells, and intestinal epithelial cells.

Expression of TLRs is not static but rather is modulated rapidly in response to pathogens, a variety of cytokines, and environmental stresses.

Plasma membrane-associate TLRs: **TLR1**, **2**, **4, 5** and **6** that recognize **microbial surface PAMPs.** They functions as homodimer or heterodimers.



TLR2 recognizes its ligands by forming a heterodimer with either TLR1 or TLR6. The resulting complexes recognize distinct ligands: TLR1/2 triacyl lipopeptides and lipoproteins, TLR2/6 diacylipoproteins and zimosan

TLR4 recognizes lipopolysaccharide (LPS) on the cell surface. TLR5 recognizes flagellin. mTLR-11(m=mouse) recognizes toxoplasma profilin.

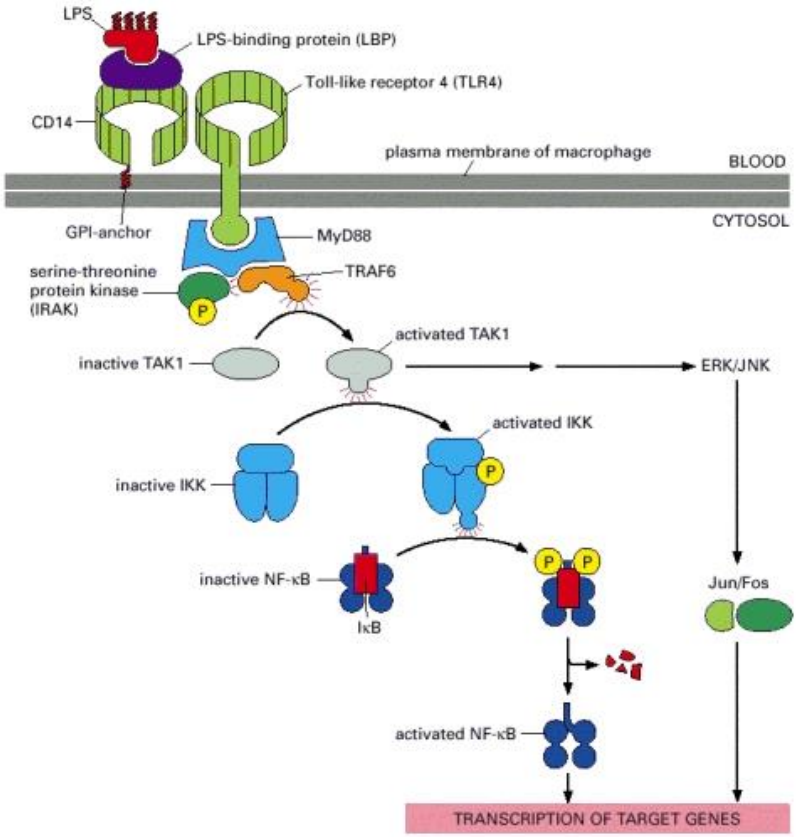
Activation of TLR4 by bacterial LPS

Missense mutation in mouse TLR-4 determines alterations in the response to LPS causing increased risk of sepsi.

LPS is recognized by the **TLR4** of phagocytes in conjunction with the cell-surface co-receptor **CD14** and the soluble serum protein **LPS binding protein** (LBP). LBP binds LPS in the blood and then it is bound by the receptor protein CD14 with leucine-rich repeat motifs.

TLR-4 binds to the CD14:LBP:LPS leading to recruitment of the adaptor proteins **MyD88** to the cytoplasmic domain of TLR4. The complex initiates a signaling cascade of phosphorylation events leading to activation of the kinase **IκK.** IκK phosphorylates **IκB**, an inhibitor bound to the transcription factor **NF-κB**.

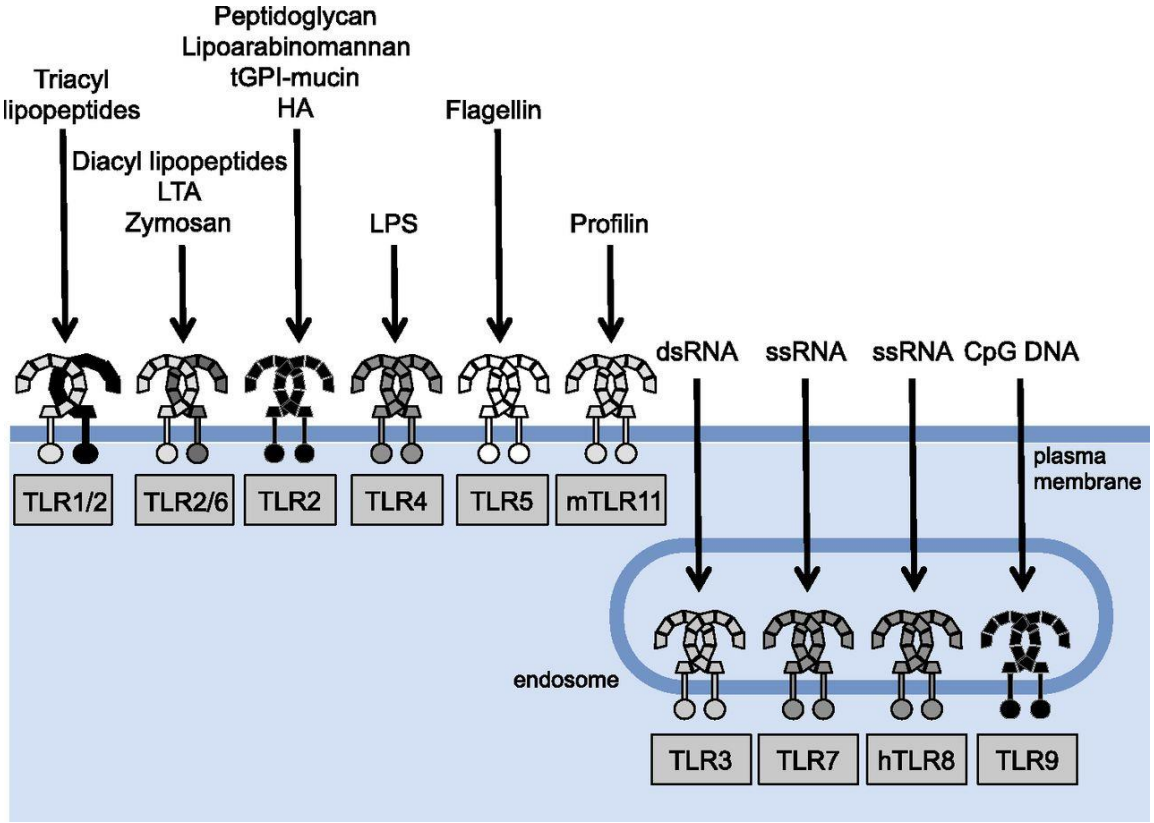
P-IκB is degraded, releasing NF-κB, which migrates to the nucleus where it activates the transcription of **proinflammatory genes.**



Endosome-associated TLRs

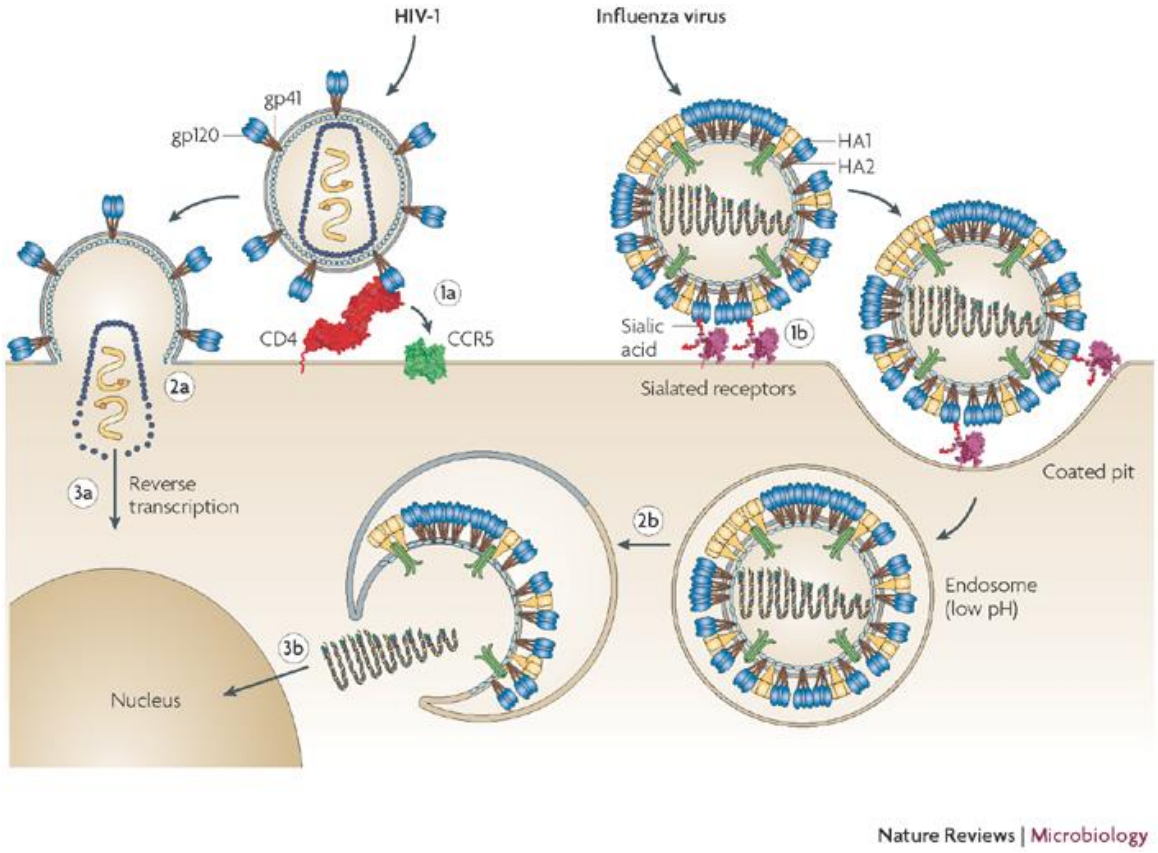
A set of TLRs, comprising TLR3, TLR7, TLR8, and TLR9, recognize nucleic acids derived from viruses and bacteria, as well as endogenous nucleic acids in pathogenic contexts.

Endosome-associated TLR:
TLR3 detects viral double-stranded (ds) RNA in the endosome;
Human heterodimeric **TLR7/8** complex recognize single-stranded (ss) RNAs from RNA viruses.
TLR9 senses unmethylated DNA with CpG motifs derived from bacteria and viruses.



Eukaryotic viruses entry pathways

Viruses are obligate intracellular parasites and so need to get inside cells in order to replicate, make new copies of themselves and spread infection. Virus entry can be a highly co-ordinated and complex process:

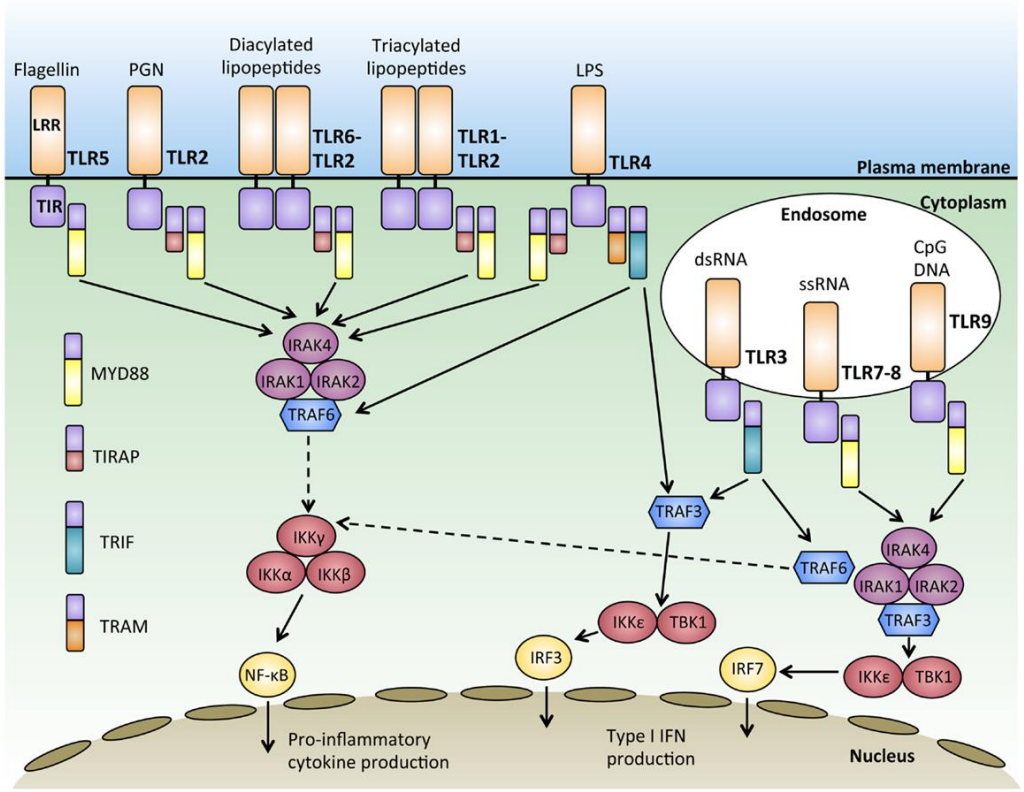


Some viruses can enter directly at the cell surface (e.g. HIV-1). Other viruses exploit receptor- mediated endocytosis (e.g. influenza virus) to bring them inside the cell, where viruses can eventually break free and begin replication.

Signaling transduction pathway of TRLs

Recognition of PAMPs by TLRs leads to **transcriptional upregulation** of distinct genes, depending on the TLRs and cell types involved.

TLR signaling is roughly divided into two main distinct pathways depending on the usage of the distinct adaptor molecules. Adaptor molecules **MyD88** and **TRIF** (see the figure) are responsible for the activation of distinct signaling pathways, culminating in the activation of transcription factors such as nuclear factor-κB (NFκB) and interferon-regulatory factors (IRFs), which regulate the production of **pro-inflammatory cytokines** and **type I interferon** (IFNs) (interferon α and β) respectively.



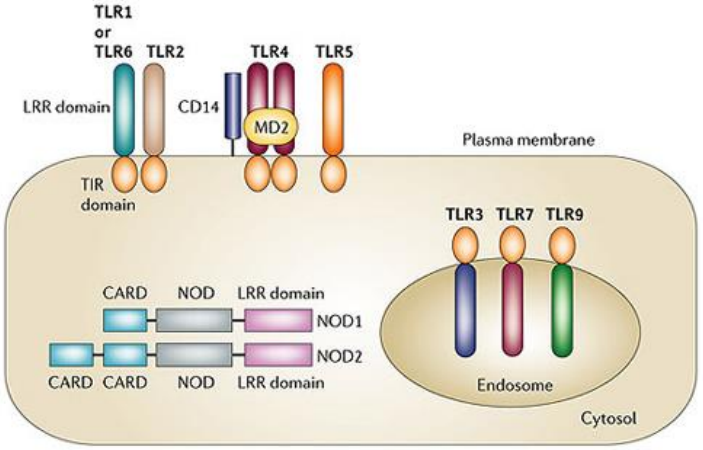
Stimulation with TLR3, TLR4, TLR7, and TLR9 ligands, induces type I IFN production, in addition to proinflammatory cytokines .

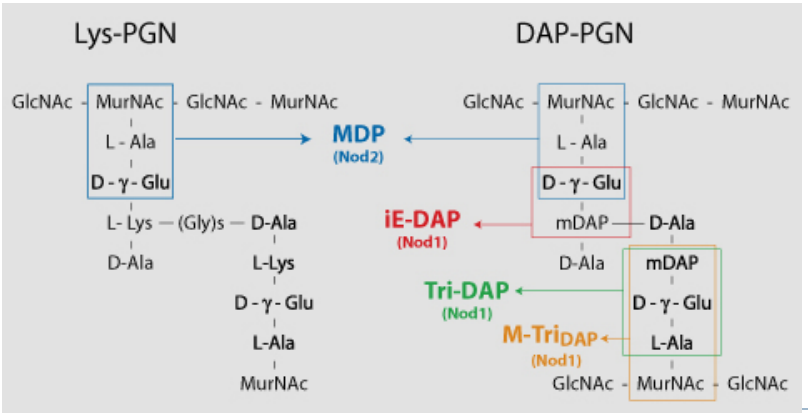
Front. Immunol., 31 July 2014

Cytoplasmic Pathogen Recognition System

Pathogens that have invaded the cytosol are detected by a large family of cytoplasmic PRRs, which activate a number of signaling pathways.
NLR (**NOD-like receptors)** and **RLR** (**RIG-I-like** receptor family) are sensors for cytoplasmic pathogens.

NLRs: cytoplasmic proteins having a variety of functions in regulation of inflammatory and apoptotic responses. NLRs: C-terminal **leucine-rich repeat (LRR) region**, a central nucleotide-binding receptor (NOD) and a N-terminal protein-binding motifs (CARD). Signal transduction is mediated by CARD domain inducing transcriptional upregulation of proinflammatory cytokine genes.





NOD-1 and **NOD-2** through LRR recognize distinct peptidoglycan motifs (DAP-PGN) and **NOD-2** recognizes muramyl dipeptide (MDP) from bacteria peptidoglycan.

γ-D-glutamyl-meso-diaminopimelic acid (DAP)

RLRs and Virus Recognition

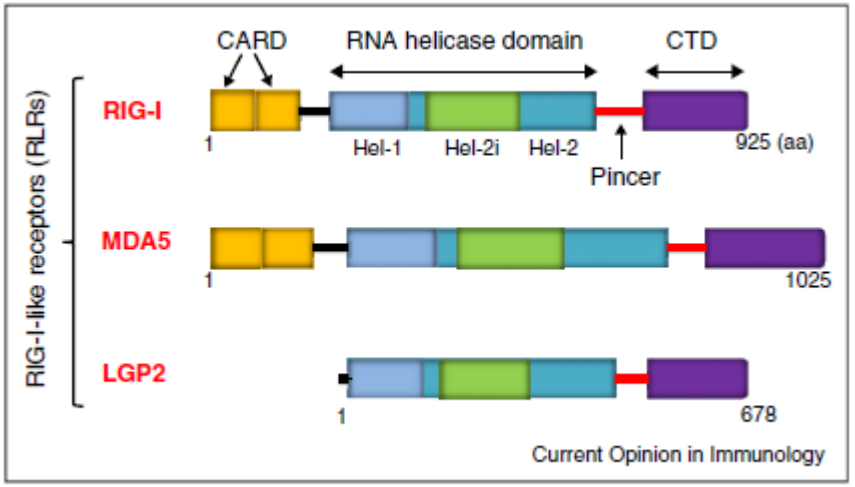
RLRs: The CARD-helicase proteins/ **RIG-I-like** receptor family functions as an **intracellular PRR** and are sentinels for intracellular **viral RNA** that is a product of viral infections. RLR directly interact with **ssRNA** and **dsRNA.**

RLR receptors (RIG-1) are modular proteins that contain:

- a central helicase domain with ATPase activity
- A C-terminal domains (CTD) that bind to dsRNA or 5′-triphosphate RNA .

These two domains work together to detect immunostimulatory RNAs.

n N-terminal caspase recruitment domain (CARD) which mediate downstream signal transduction.



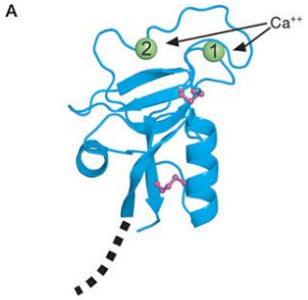
Current Opinion in Immunology 2015, 37:40–45

Activation of C-terminal domains of RLRs triggers signaling via CARD-CARD interactions, ultimately resulting in an antiviral response mediated by type I IFN production.

C-type lectin receptors (CLR)

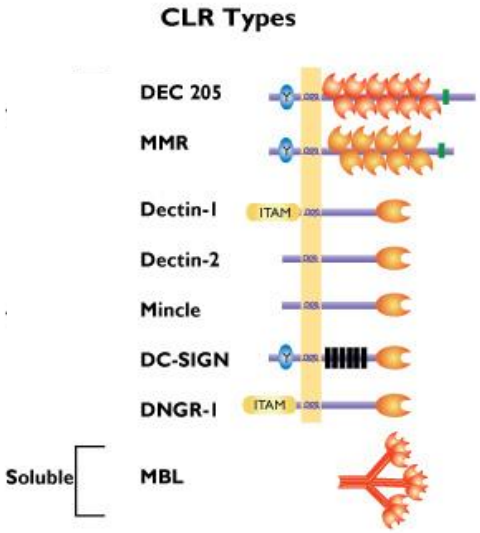
CLRs comprise large family of receptors that recognize specific **carbohydrate structures** (lectin activity) on microorganisms such as viruses, bacteria, and fungi.
The lectin activity of these receptors is mediated by conserved **carbohydrate-recognition domain (CRD).**

CLRs include transmembrane proteins containing several CRDs (Macrophage mannose receptor MMR, and DEC-205) or single CRD-like domains such as Dectin-1 (specific receptor for β-glucans, Dectin-2 (the functional receptor for α-mannans).



carbohydrate-recognition domain (CRD).

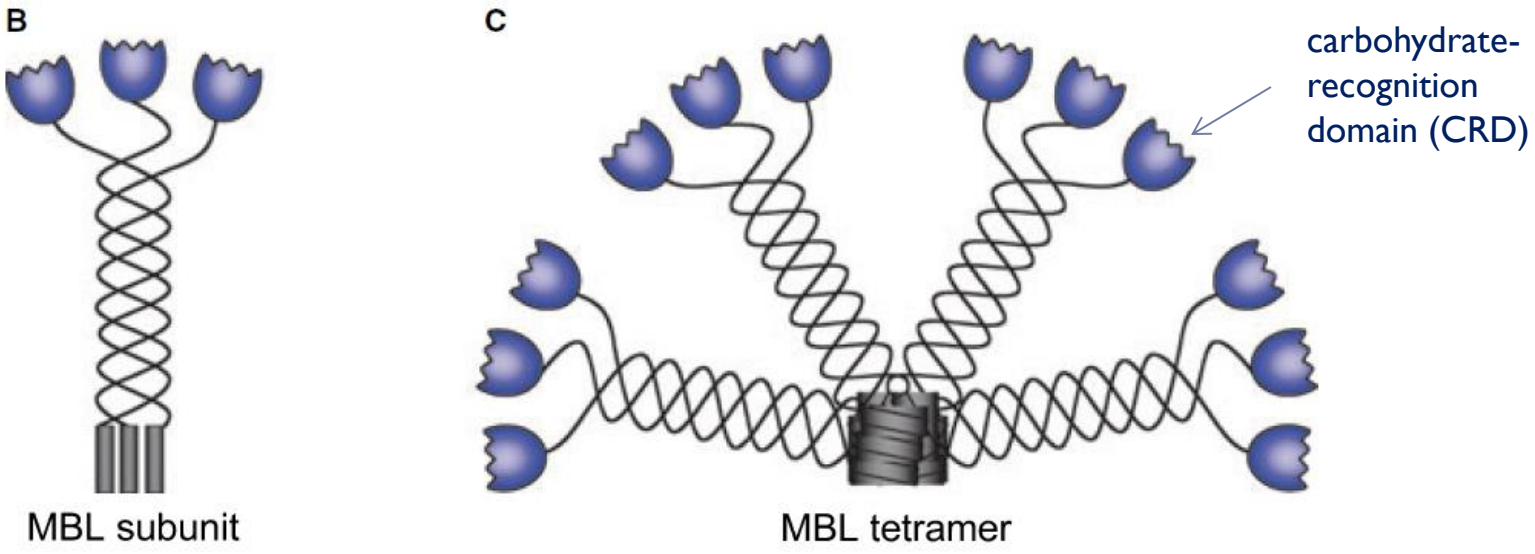
Detection of P/MAMPs by membrane bound CLRs initiate both intracellular signaling cascade and the phagocytosis of the pathogen.



http://www.invivogen.com/review-clr

Mannose-binding lectin (MBL) is a C-type lectin (collectins) with functions of soluble receptors for bacteria carboydrates. **MBL** is an acute-phase serum protein that triggers one of the way to activate complement. Soluble lectin CLR can recognize PAMPs and subsequently bind to receptors that will trigger phagocytosis and activate the complement system (**lectin pathway**).

MBL structure and oligomerization



modified from 2009 John Wiley & Sons A/S
Immunological Reviews

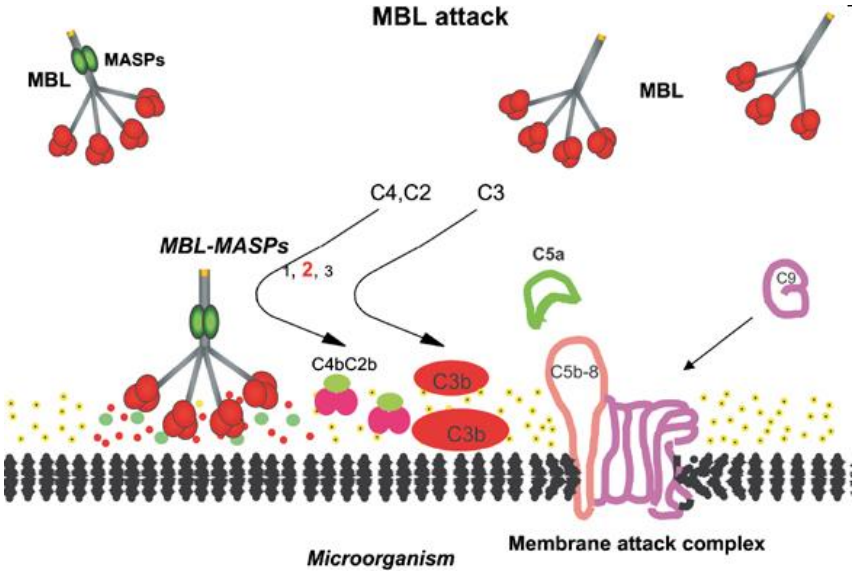
MBL: as other collectins contain a collagen-like domain and usually assemble in large oligomeric complexes. MBL clusters of 2-9 carbohydrate-binding heads (CRD) around a central collagen-like stalk. This assembly binds specifically to mannose and fucose residues in bacterial cell walls that have the **correct spacing and orientation** to match up perfectly with the number of carbohydrate-binding sites, providing a exemplary model of PRR.

MBL activates lectin-pathway of complement

MBL when is activated by the binding of mannose-containing glycans, complexed with the serine protease **MASPs** binds to sugar arrays on a microorganism.

Associated MASP is responsible for the release of C2a and C4b from the inactive complement factors C2 and C4.

The active complex C2aC4b form the **C3 convertase** that in turn cleaves C3 in **C3a** (chemoattractant) and **C3b** (opsonin and C5 convertase).



P Garred, F Larsen, J Seyfarth, R Fujita and H O Madsen. Genes and Immunity 2010

MBL-mediated complement attack complex. a complement attack through MASP. MASPs denote MBL-associated serine proteases.

Cellular responses to recognition of pathogens

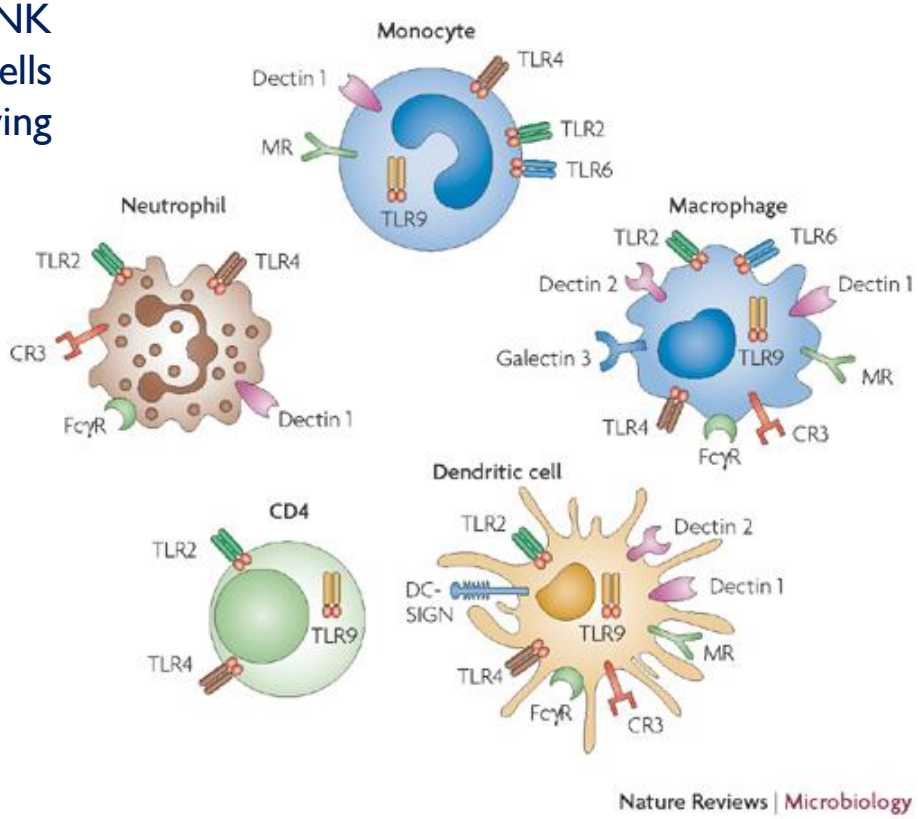
Neutrophils, macrophages, dentritic cells and NK cells, (and partially CD4 T lymphocytes) are the cells that "sense" the presence of pathogens having different types and sets of PRRs:

Phagocytic receptors: Fc receptor for antibodies (FcγR); C3b receptor (CR3); MR= mannose receptor (MR), dectins (CLR).

Activation receptors: TLRs, f-Met Receptor. They mainly activate production of soluble mediators.

Adhesion receptors: integrins and selectins.

Others: MHC class I and II



PRRs have 3 functions: detect unusual molecular patterns, sense the extent of tissue damage and determine the **class** of immune response. Specialized cells of central immunity such as dendritic cells and T and B cells are principle players in integrating these TLR signals into a specific immune response.

Proinflammatory signaling pathways

NF-κB, and **IRF3/7** play pivotal roles due to their capacity to stimulate the production of proinflammatory mediators, including cytokines and IFNs respectively.
NF-κB proinflammatory signaling pathways induced by PRRs upregulate these innate immune responses:

- proinflammatory cytokines: IL-1, IL-6 and TNF-α, chemokines including IL-8
- upregulation of adhesion molecules (selectins)
- Upregulation of immunoreceptors: cytokine and chemokine receptors, TLRs.

Overall these mediators have these effects on other immune cells:

Activation and recruitment of leukocytes to sites of infection (inflammation),
Enhanced phagocytosis of microbes,
Activation of complement- or NK cell-mediated cellular lysis
Enhanced antigen presentation

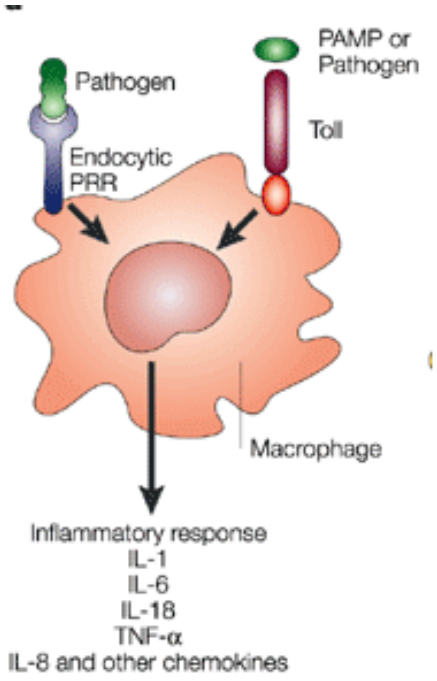
Tight regulation is essential to ensure the strong, albeit transient, nature of these responses, and this is achieved via amplification early during infection, as well as restriction and downregulation when needed at later stages.

Proinflammatory signaling pathways leads to release proinflammatory cytokines

Proinflammatory signaling pathways leads to release **proinflammatory cytokines** and other mediators that set up a state of inflammation in the tissue:

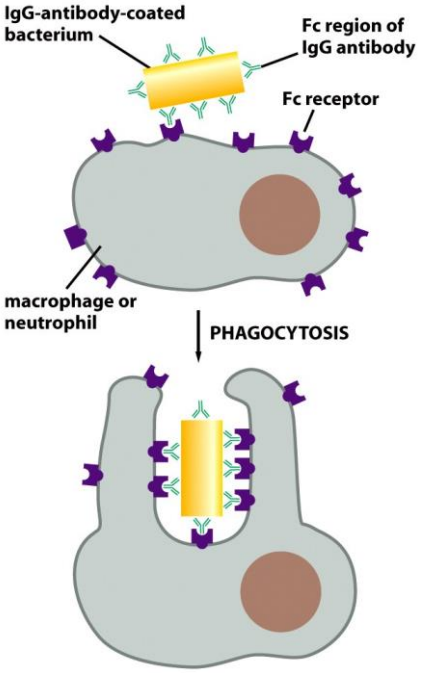
Cytokines: small proteins (~25 kDa) released by various cells in the body, usually in response to an activating stimulus.
They mainly act in an **autocrine** and **paracrine** manner affecting the behavior of adjacent cells by binding to specific receptors.
Main cytokines secreted in response to PRRs stimulation: interleukin-1 **IL-1**, interleukin-6 **IL-6** and tumor necrosis factor (**TNF-α)** and many others (IFNγ, interleukins IL-4, IL-10, IL-12, IL-18 and TGFβ.)

Chemokines: cytokines that have **attractant properties**, inducing cells with the appropriate receptors to migrate towards their source: interleukin-8 (**IL-8**).
Other factors (**vasoactive peptides**) increase vascular permeability.



Receptor-activated phagocytosis

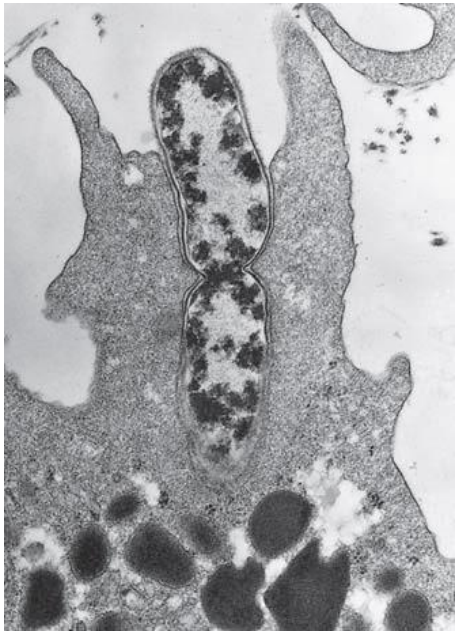
Phagocytosis is a remarkably complex and versatile process: it contributes to innate immunity through the ingestion and elimination of pathogens.



Antibody-activated phagocytosis

Phagocytes have several PRRs that bind specifically to certain PAMPs inducing the phagocytosis (mannose receptor, dectins).

Opsonins (Fc receptor, C3b receptor) can be deposited onto foreign surfaces and serve as adaptors that bind and activate potent phagocytic receptors



Membrane protrusions surround the bacteria and absorb the bacteria into the phagosome, which is formed by the fusion of cell membranes. Actin cytoskeleton governs particle engulfment. A variety of signaling cascades can be activated during this process.

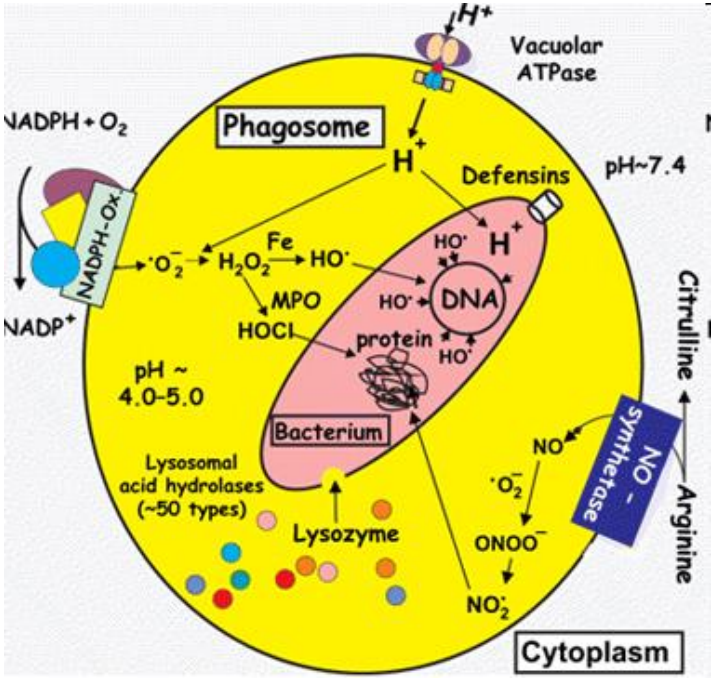
The macrophages and neutrophils produce many toxic compounds

Once the pathogen has been phagocytized, macrophages and neutrophils have an impressive armory of weapons to kill it. The phagolysosomes contain:

non oxidative killing effectors: lysozyme and acid hydrolases to degrade bacterial cell walls and proteins, antimicrobial peptides.

oxidative killing mechanisms: **NADPH oxidase complex** that catalyzes the production of highly toxic oxygen-derived compounds: superoxide anion ($\bullet O_2^-$), hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO•.) *NO* synthase for the production of nitric oxide (NO).

competitors: Fe^{2+} binding proteins.



Toxic oxygen- and nitric-derived compounds are generated in a process known as the "*respiratory burst*"

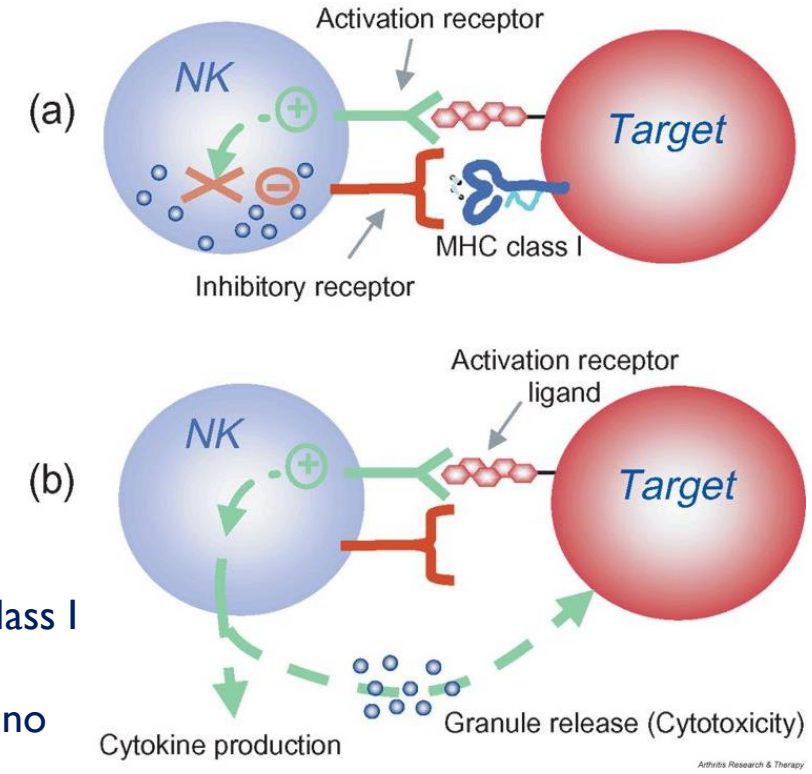
Chronic Granulomatis Disease (CGD), a primary immunodeficiency that affects phagocytes, defects in NAPDH oxidase

Functions of Natural killer cells

Natural killer (NK) cells are large, granular, bone marrow-derived lymphocytes that do not express T or B cell receptors.

Rapid response to infected or transformed cells either by killing the abnormal cells and by releasing chemokines and cytokines.
NK functions must be carefully regulated to prevent damage to normal tissues

NK cell responses result from the integration of signals from both cytokine receptors and germline-encoded NK cell inhibitory and activation receptors.



(a) Inhibitory NK cell receptors recognize self MHC class I and restrain NK cell activation. **(b)** In the absence or downregulation of MHC class I, stimulatory signals are no longer suppressed, resulting in NK cell responses

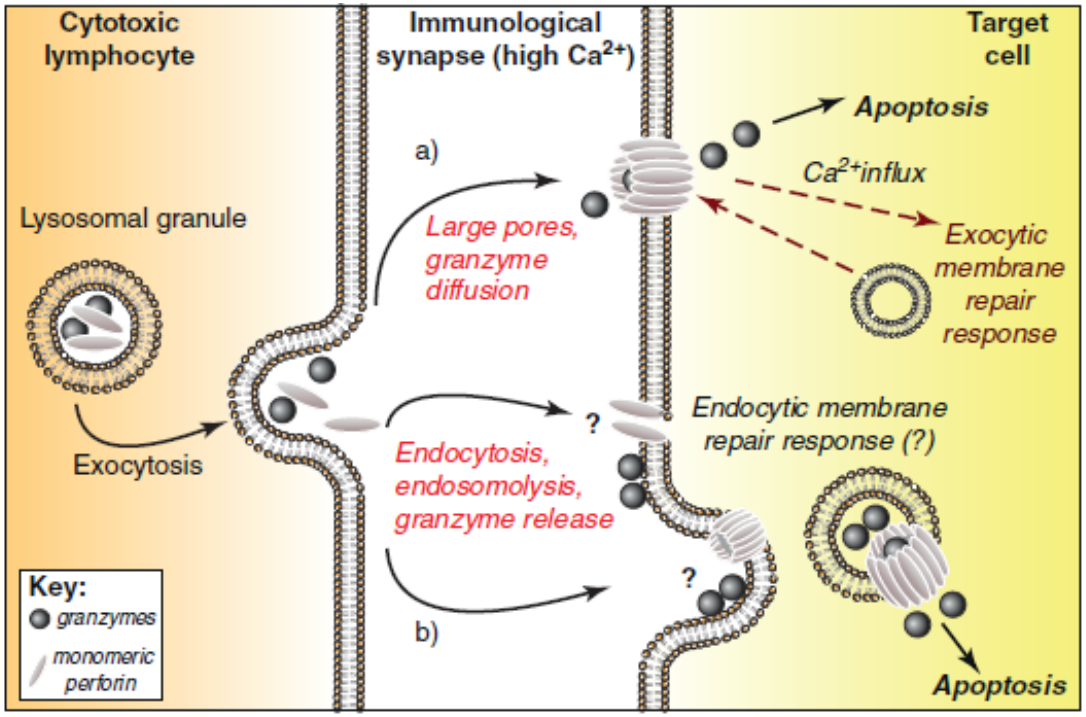
Cytotoxic mechanism of NK cells

NK cells and CTLs (cytotoxic lymphocytes) use a common mechanism of cytotoxicity, involving the regulated exocytosis of toxic effector molecules

Cytotoxic lymphocytes use the highly toxic pore- forming protein **perforin** to eliminate dangerous cells, while remaining refractory to lysis.

Following exocytosis, perforin delivers the proapoptotic protease, **granzyme B**, into the target cell.

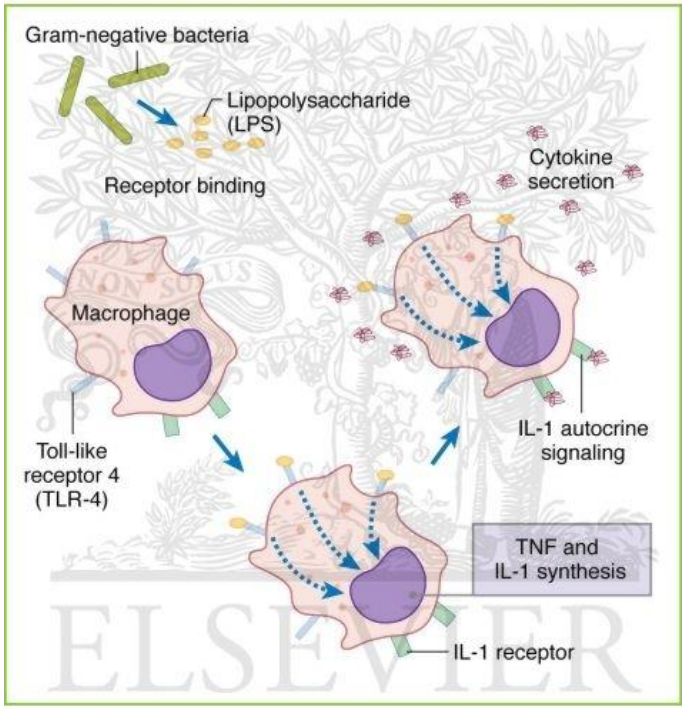
a) and b) Putative mechanisms of synergy between perforin and granzymes



The dark side of the innate defenses: septic shock

I**nflammatory responses,** which are so effective at controlling local infections, can have disastrous consequences when they occur in a disseminated infection in the bloodstream, a condition called ***sepsis*.**

Bacterial LPS has the ability to induce a dramatic systemic reaction known as septic shock. This syndrome is the result of **overwhelming secretion of cytokines**, particularly of **TNF-α** and **IL-1**, often as a result of an uncontrolled systemic bacterial infection. The systemic release of proinflammatory signaling molecules into the blood causes dilatation of blood vessels, loss of plasma volume, and widespread blood clotting, which is an often fatal condition known as ***septic shock*.**



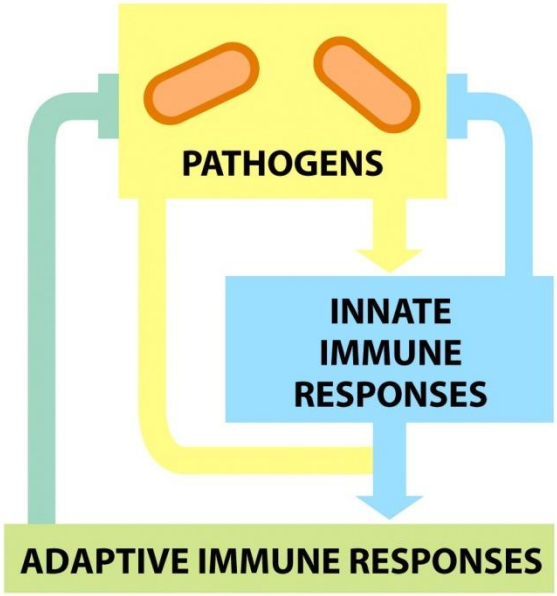
Although mutant mice lacking **TLR-4** function are resistant to septic shock they are highly sensitive to LPS-bearing pathogens such as *Salmonella typhimurium*.

Bridging Innate and Adaptive Immune Defenses

Whereas the innate immune responses are general defense reactions, the adaptive responses are **highly specific** to the particular pathogen that induced them, and they provide long-lasting protection.

The adaptive immune system is composed of many millions of **lymphocyte** clones, with the cells in each clone sharing a unique **cell-surface receptor (TCR)** that enables them to bind a particular antigen.

In vertebrates, pathogens, together with the innate immune responses activate, stimulate adaptive immune responses, which then work together with innate immune responses to help fight the infection.



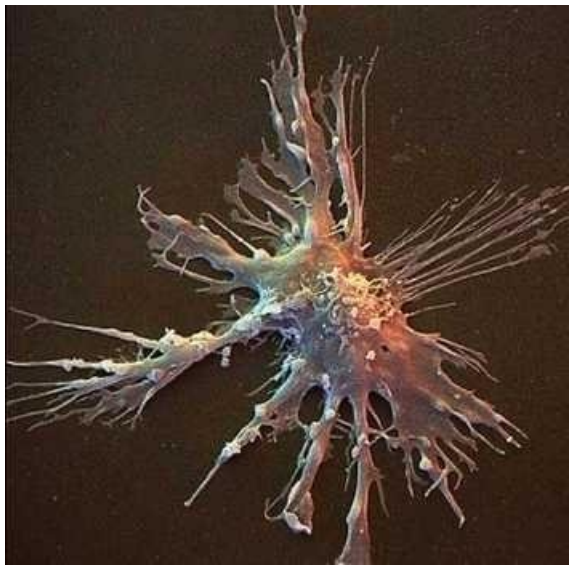
The cells of the vertebrate innate immune system that respond to PAMPs and activate adaptive immune responses most efficiently are **dendritic cells.**

Dendritic cells a bridge between innate and acquired immunity

Dendritic cells (**DCs**) are the most important **antigen-presenting cells**.

Immature DCs: are specialized phagocytic cells resident in most tissues, long-lived, turning over at a slow rate.

From bone marrow iDCs migrate to their peripheral stations, their role is to survey the local environment for pathogens. They express **high levels of TLRs** and other PRRs.

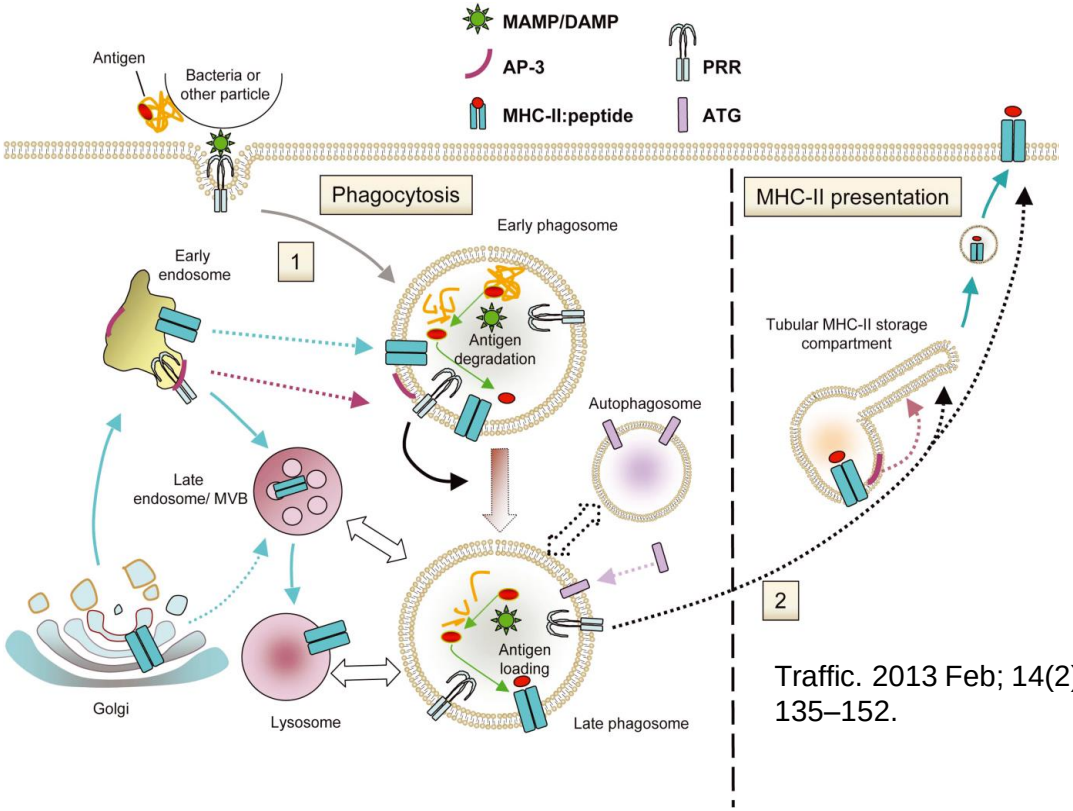


http://www.realscience.us/2011/10/03/nobel-prize-in-medicine-goes-to-immunologists/

iDCs become **mature** when they recognize PAMPs released from pathogens. During this process, DCs upregulate **antigen presenting molecules** such as **MHC class II** and **costimulatory molecules** (including CD80, CD86, and CD40), and secrete cytokines that influence both innate and adaptive immune responses, making these cells essential gatekeepers that determine whether and how the immune system responds to the presence of infectious agents.

Model of phagosomal antigen processing and presentation by MHC-II.

CDs engulf pathogens processing their antigens on the cell surface in association with MHC class II



Traffic. 2013 Feb; 14(2): 135–152.

MHC-II: molecules composed of 2 integral membrane chains, α and β. The peptide-binding pocket is comprised of the membrane distal domains of both chains.

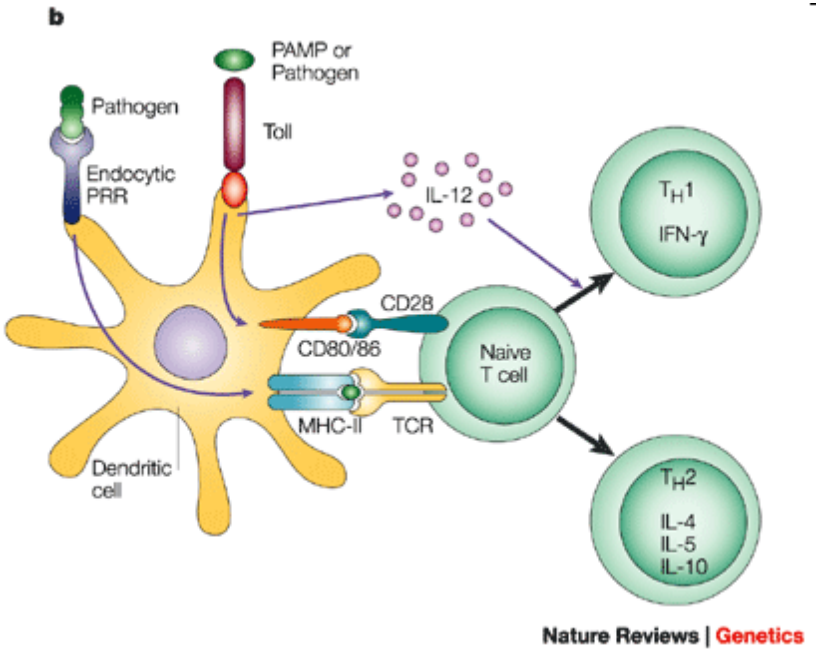
Phagocytized antigen captured by APCs is degraded in phagosomes.

The phagosomes matures by the acquisition of content (including MHC-II) from early and late endosomes and lysosomes.

Antigen is loaded onto MHC-II molecules predominantly in late phagosomes.

From late phagosomes, peptide-loaded MHC-II molecules are delivered to the cell surface by mechanisms that are still poorly understood where they are available to stimulate antigen-specific T cells with cognate receptors.

DCs present antigenic peptides in the context of relevant MHC molecules



DCs are endowed with the ability to stimulate naïve $CD4^+$ T lymphocytes into **T helper** (**Th**).

Each lymphocyte carries cell-surface receptors (**TCR**) of a single specificity, so that the total repertoire of receptors can recognize virtually any antigen

Stimulation require two signals:

1) an antigen-specific signal provided through the T cell receptor (**TCR**) which interacts with **antigen-MHC molecules** on the membrane of APC.

2) the co-stimulatory signal, an antigen-nonspecific signal and provided by the interaction between co-stimulatory molecules expressed on the membrane of APC and the T cell.

T cells interact with APC by releasing cytokines. APC also release cytokines.

Slides aggiuntive di ripasso utili (non in programma d'esame) per comprendere gli argomenti descritti in questo capitolo

Scheme of the complement activation systems

The complement system consists of about 20 interacting soluble proteins that are made mainly by the liver and circulate in the blood and extracellular fluid.

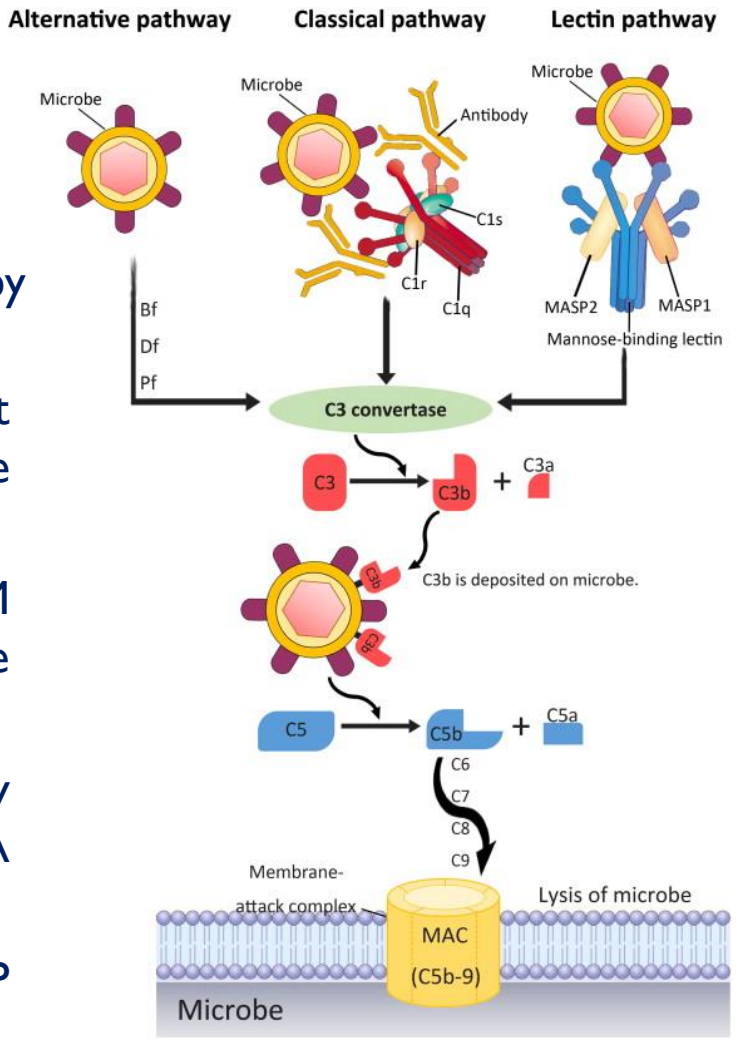
The principal stages in complement activation by the classical, lectin, and alternative pathways

Three distinct pathways of complement act locally to activate **C3** (C3a+C3b), which is the pivotal component of complement:

classical pathway: activated by IgG or IgM antibody molecules of the adaptive immune system bound to the surface of a microbe.

alternative pathway: C3 is spontaneously activated at low levels as C3b by LPS or TA found on the surfaces of bacteria.

Lectin pathway: activated by MBP/MASP complex (later)



S. Zhang and P. Cui. Develop.& Comp.. Immunol. 46, 1, 2014, Pages 3-10

The complement system

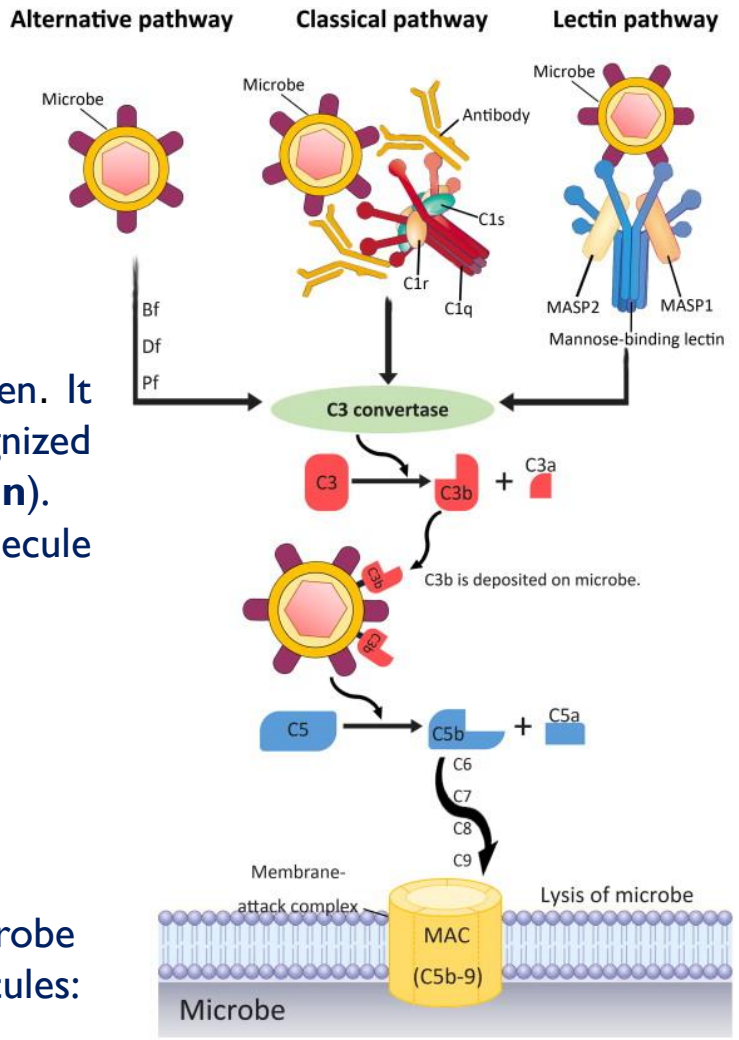
Three distinct pathways of complement act locally to activate **C3** (C3a+C3b), by the activity of the protease C3 convertase.

The early components and C3 are all proenzymes, which are activated sequentially by proteolytic cleavage (proteolytic cascade).

C3b, binds covalently to the surface of the pathogen. It acts as a protease (**C5 convertase**) but it is recognized by specific receptors (C3bR) on phagocytes (**opsonin**). **C3a** is a major proinflammatory molecule (chemoatractant) and acts independently.

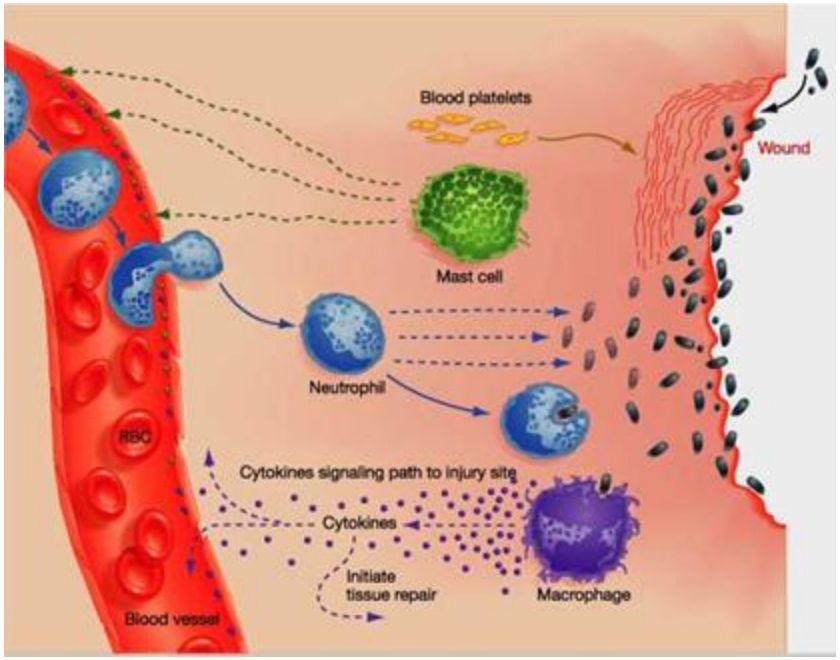
C5 convertase causes the cleavage of the **C5** to produce **C5a** (chemoatractant) and **C5b** which remains bound to C3 on the cell surface and assembles other late factors (C6, C7, C8)

A large transmembrane channel on microbe membrane is formed by the addition of C9 molecules: membrane attack complex, **(MAC)**



Inflammatory responses and recruitment of leukocytes to the site of infection

Inflammatory responses are characterized by: **increased permeability of the blood vessels** leading to increased local blood flow and the leakage of fluid. (pain, redness, heat, and swelling at the site of an infection). **Mast Cells:** secrete factors that mediates vasodilation and delivery of blood factor. **Platelets** from blood release blood-clotting proteins at wound site.

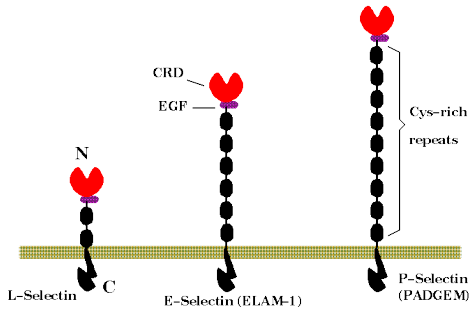


Released cytokines (IL-1, TNFα) and chemotactic factors (chemokines, C3a C5a). cause the endothelial cells of blood vessels near the site of infection to express cellular adhesion molecules**. Increased adhesive properties** of the endothelium cause circulating leukocytes to stick to the endothelial cells of the blood vessel wall and migrate between them to the site of infection, to which they are attracted by chemokines.

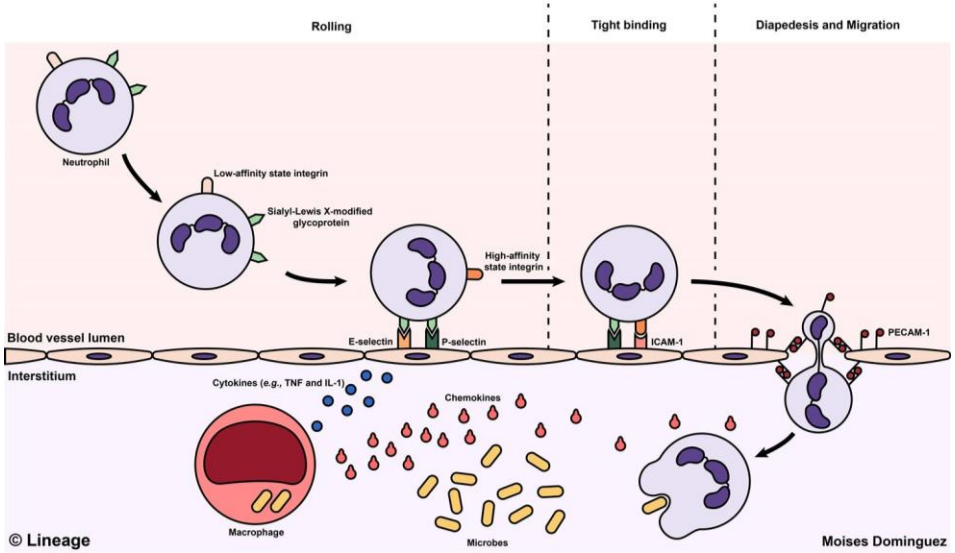
Neutrophils, which are recruited into the infected tissue in large numbers in the early phase are the principal cells that engulf and destroy the invading micro-organisms.

Leukocytes extravasation

Leukocyte rolling occurs due to transient interactions between **E-selectins** present on endothelial cells and **selectin ligands** (sialyl-Lewis^x) expressed on leukocytes.
Tight binding: some high affinity state **integrins** on the surface of leukocytes bind to members of the Ig superfamily (ICAM-1) on the surface of endothelial cells. This arrests the motion of the rolling cells.



The selectins are a family of mammalian adhesion proteins, with three members.

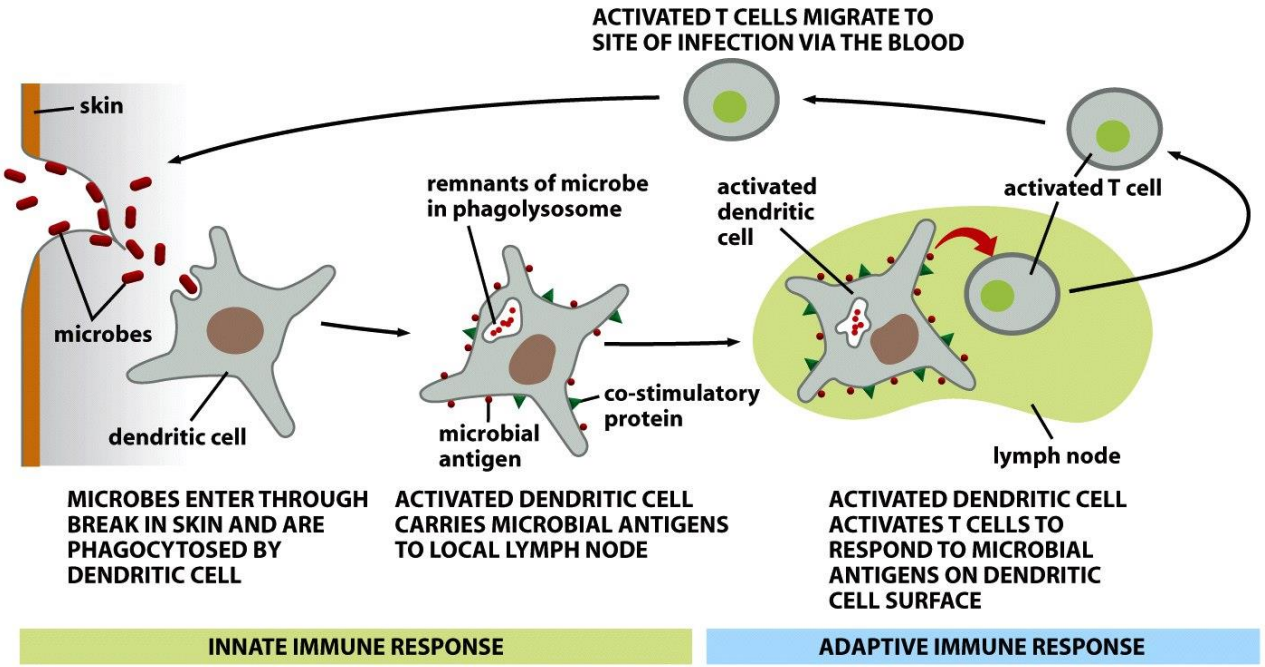


https://step1.medbullets.com/pathology/106005/leukocyte-extravasation

Leukocytes enter the tissues by secreting proteases to breach the endothelial basement membrane (diapedesis). The influx of neutrophils is followed a short time later by monocytes that rapidly differentiate into macrophages.

The adhesion defects result in poor leukocyte chemotaxis: Leukocyte adhesion deficiency (LAD)

How the innate immune system can help activate the adaptive immune system.



Following antigen uptake by DCs, these cells become activated, express receptors for specific chemokines and are attracted to migrate to regional lymph nodes to present antigenic peptides in the context of relevant MHC molecules.

The microbial PAMPs activate the **DCs** so that they, in turn, can directly activate the **T cells** in peripheral lymphoid organs to respond to the microbial antigens displayed on the dendritic cell surface.