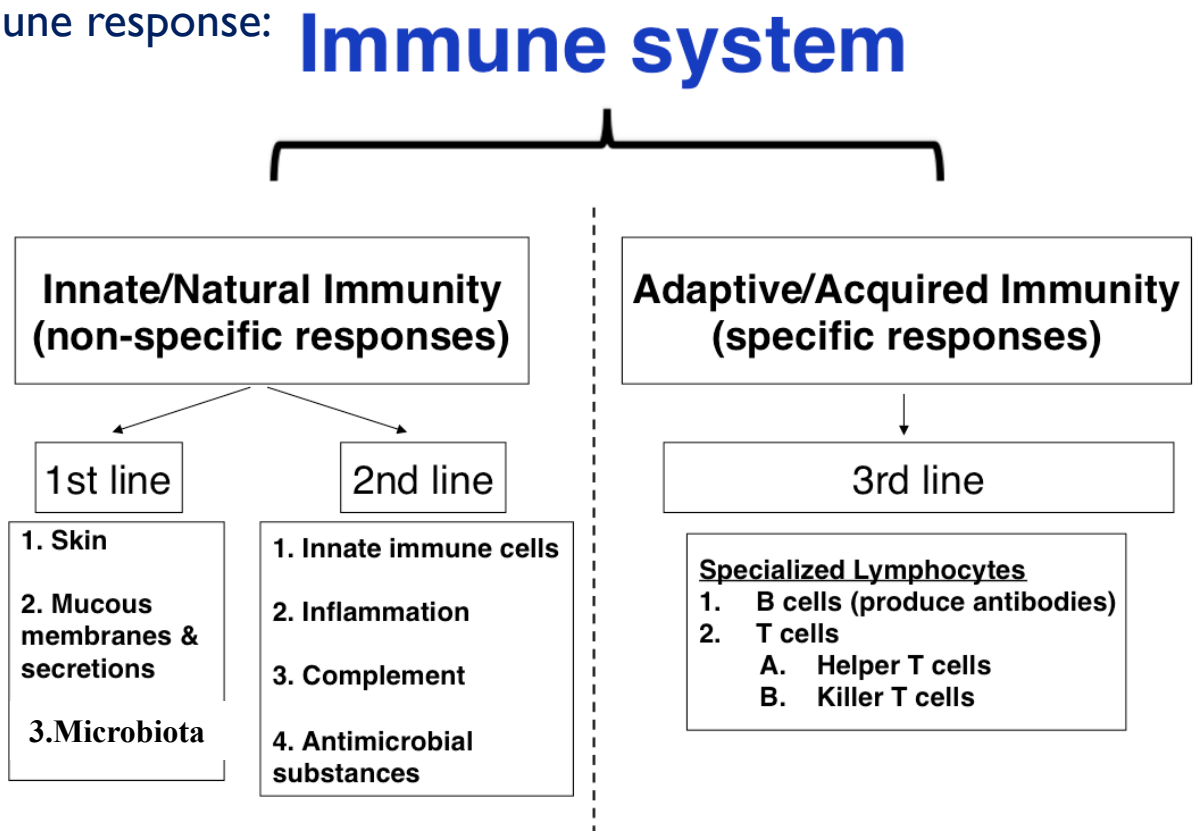
Chapter 9: Pathogen recognition in innate immunity

a.a. 2015-16

Properties of the Host : the immune system

We are constantly being 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:

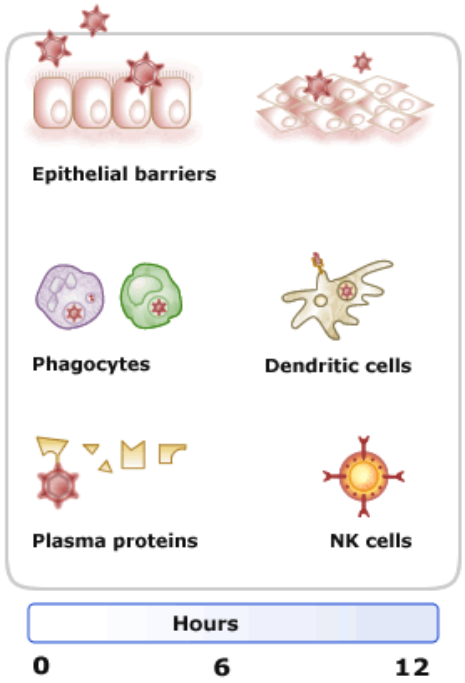


Innate immunity

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.



Innate immune responses **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, it 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

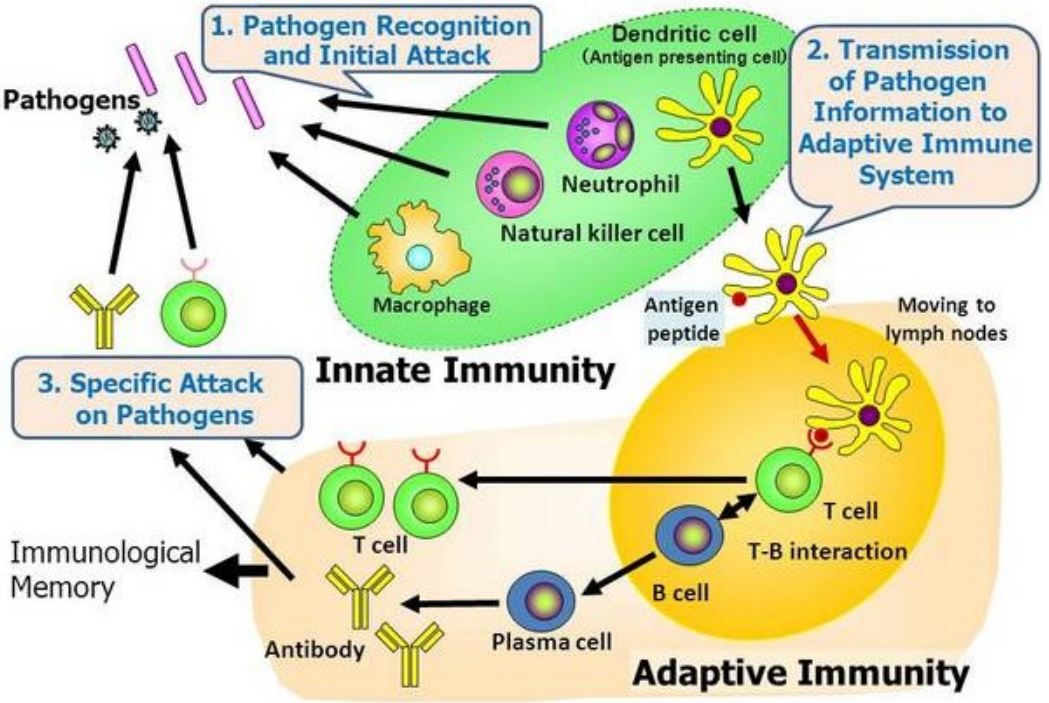
Adaptive mechanisms: not immediately ready to come into play. It must be turned on by host exposure to a pathogen (as during an infection).

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



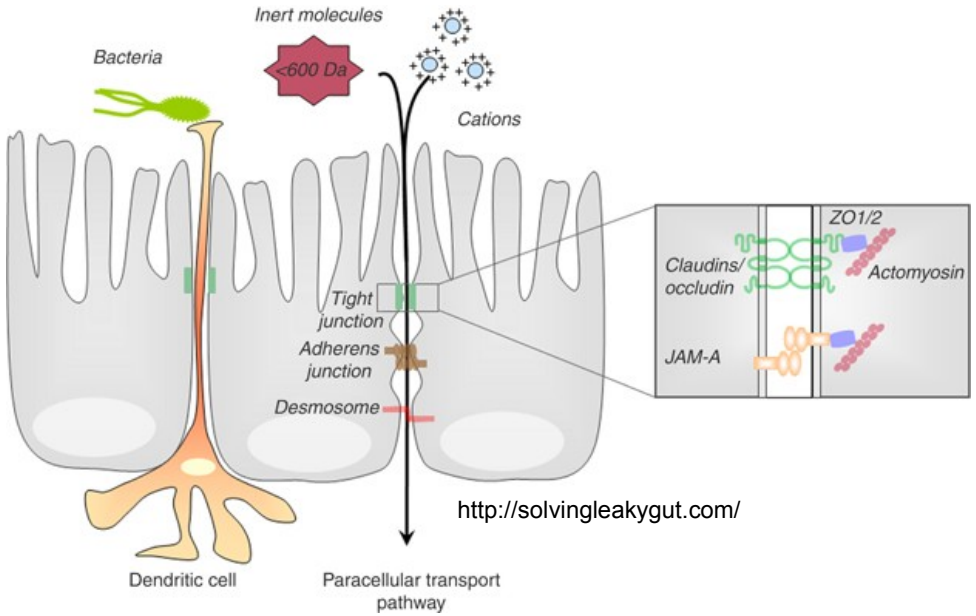
http://akira-pj.lserp.osaka-u.ac.jp/info_en/cafe_third.html

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.

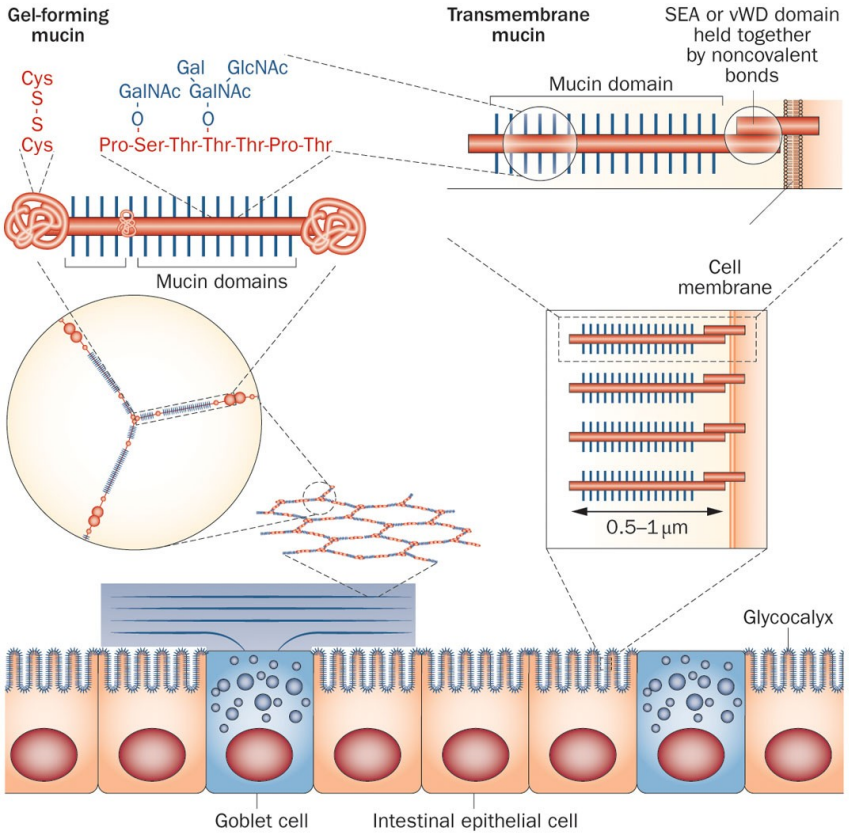
Epithelia surfaces: **tight junctions** between neighboring cells prevent easy entry by potential pathogens.

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.

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.

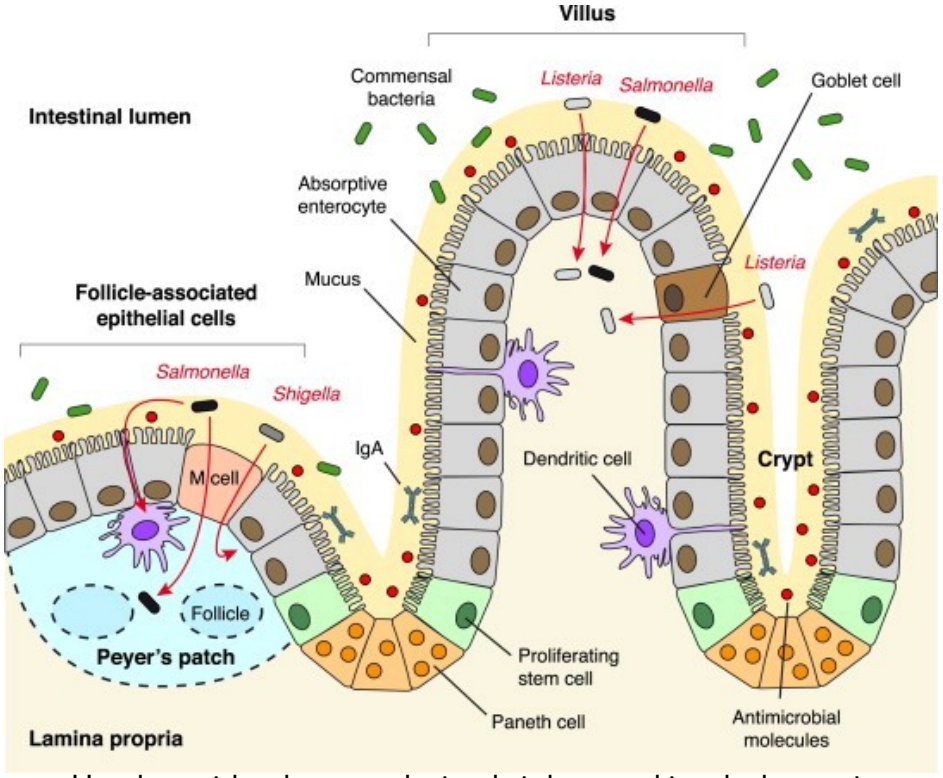
The mucus layer also contains substances such as **lysozyme, lactoferrin, and antimicrobial peptides** that kill pathogens or inhibit their growth.

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.

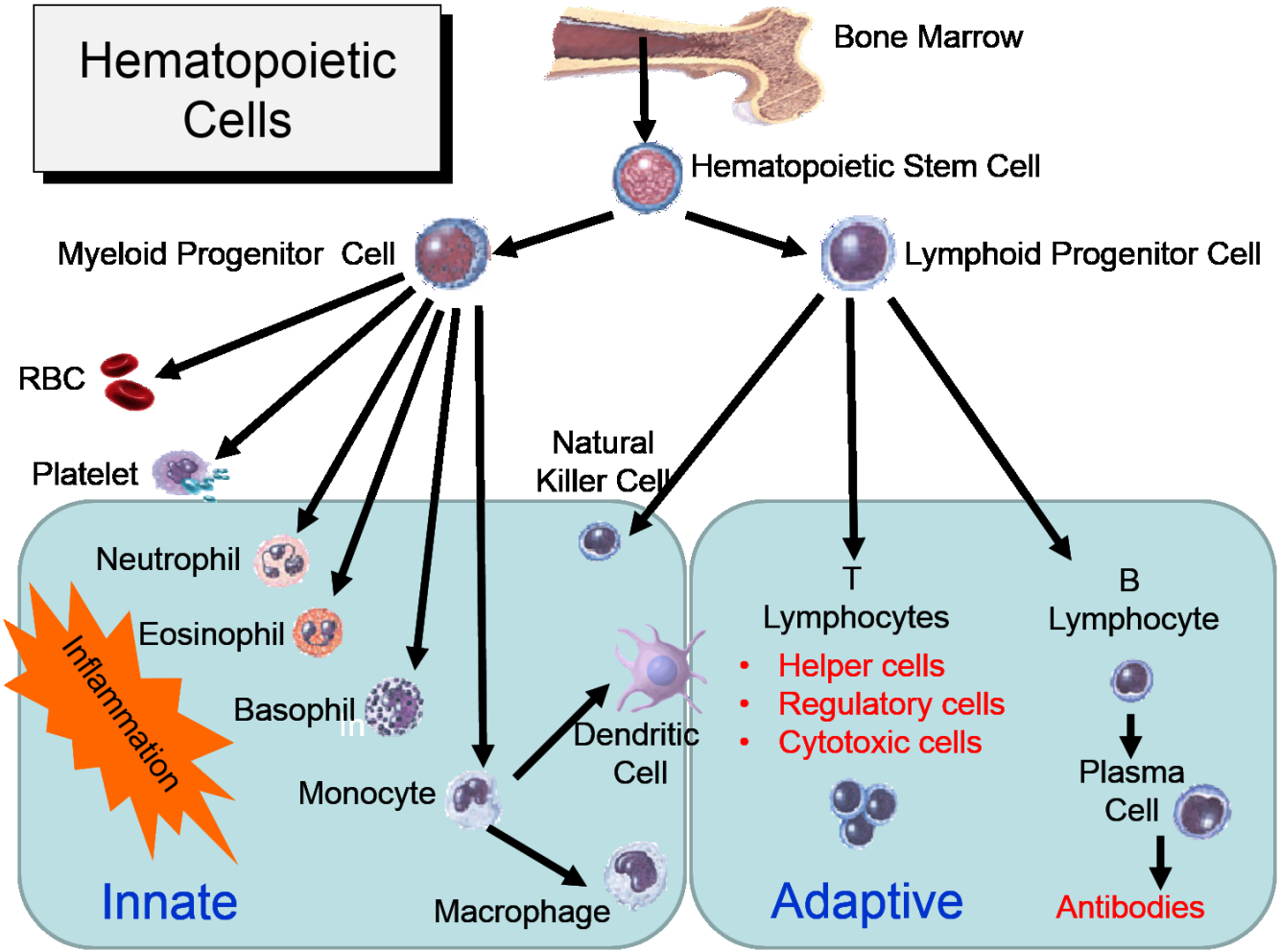
GALT: **gut–associated-lymphoid tissue**: is a specialized lymphoid tissue allowing constant sampling of the luminal microbiota through **M cells**. Of the follicle-associated epithelium. Lymphocytes of GALT make **secretory IgA.**

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, ,

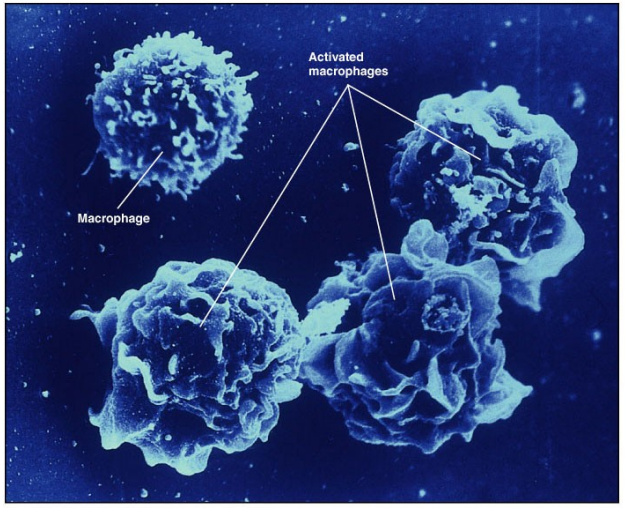
Cells of the Innate and Adaptive Immune Systems



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. Main function: to phagocytize (engulf) pathogens and particles. They also can alert and attract other immune cells Specialized cells: In liver: Kupffer cells, in lungs: alveolar macrophages.



macrophages

Monocytes: reside in the blood, Ingest and kill bacteria, migrate to tissues. Precursors of macrophages.

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 also phagocytic cells, but they have the special ability of initiating an adaptive immune response through a phenomenon called "antigen presentation" and through cytokine production(will be discussed later).

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.

Human alpha defensin 1


Human beta defensin 1

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

Human cathelicidin LL-37

Human Cells Recognize Conserved Features of Pathogens

Innate and adaptive immune systems have to recognize and destroy infective microorganisms, without harming the host. Consequently, the immune systems must be able to distinguish **self from non-self.**

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).

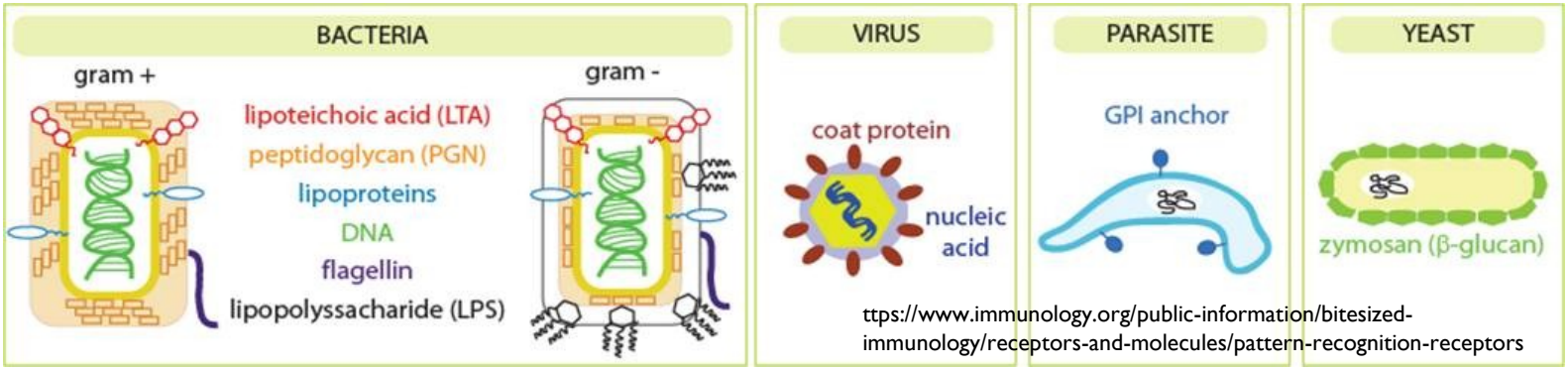
PAMPs are **essential structures** for the survival and therefore are **difficult to alter** for the microorganism. The various classes of pathogen-associated immunostimulants often occur on the pathogen surface in repeating **patterns**

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**: Procaryotic translation initiation differs from eucaryotic 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.



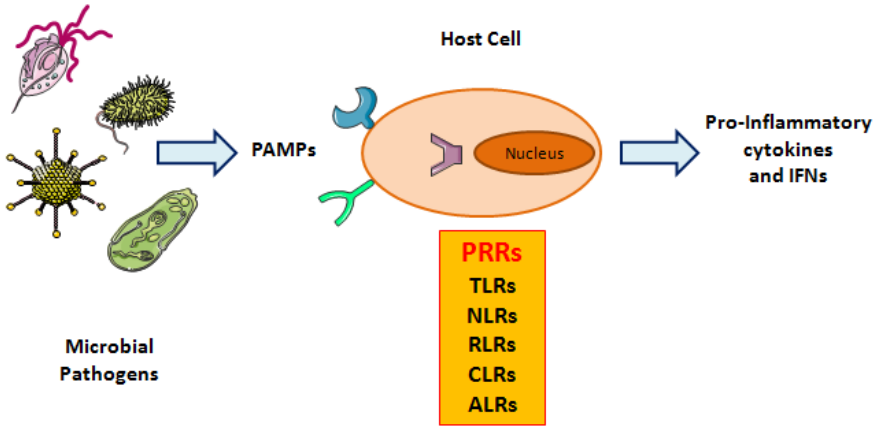
- **Chitin** and other **β-glucans** (zymosan) which constitute cell walls of fungi
- **Short sequences in bacterial and viral DNA**. For example: "**CpG motif**" consists of the **unmethylated** dinucleotide CpG flanked by conserved motif. This sequence is highly less common in vertebrate DNA than in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

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



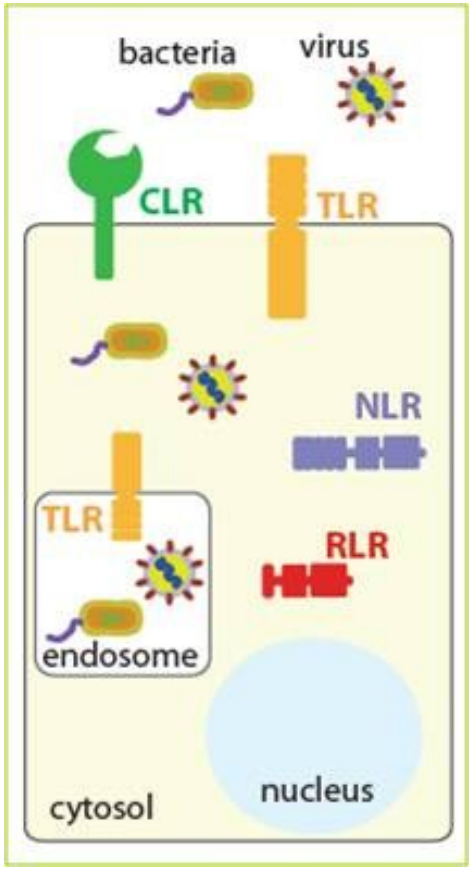
Pattern Recognition Receptors (PRRs)

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

PRRs are **expressed constitutively** 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**)

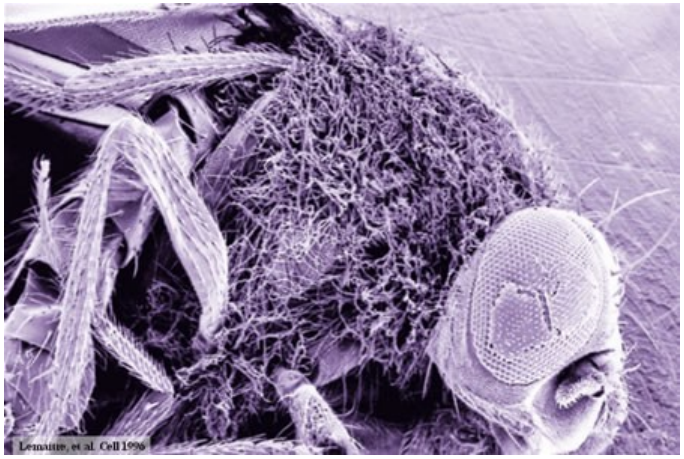
This repertoire of molecules and cells **acts in concert** to form a network of host defense mechanisms. Number of different PRRs are engaged by a given pathogen via various PAMPs, hence securing a rapid and potent inflammatory response and also allowing for some specificity of the response.

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 .

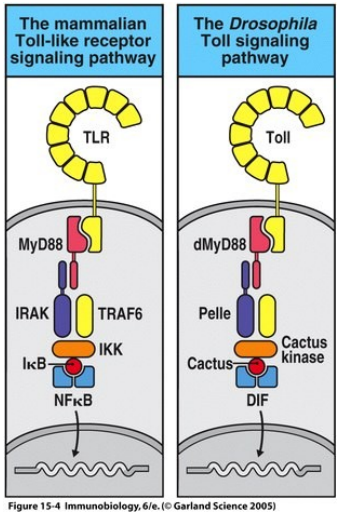
Toll-like Proteins Are an Ancient Family of Pattern Recognition Receptors

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

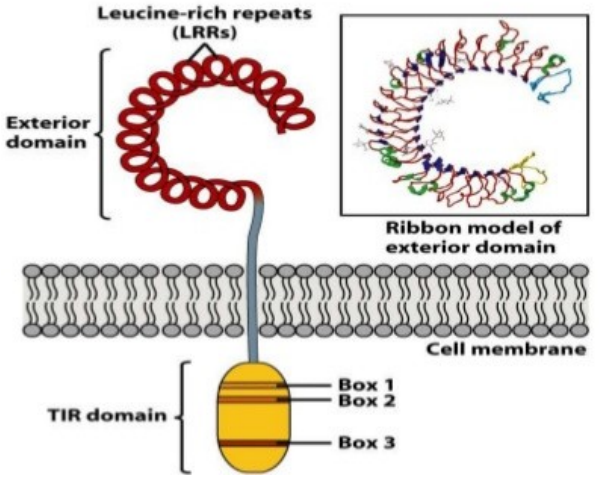


Intracellular signal transduction pathway activated downstream of Toll when a fly is exposed to a pathogenic fungus leads to activates the transcription of various genes, including those encoding the antifungal peptides: defensins.

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.

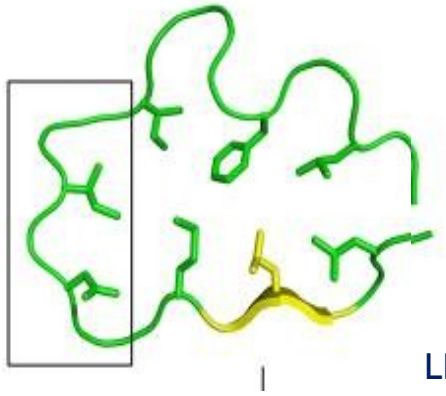


Three domains: 1) the extracellular **Leucine-Rich Repeat (LRR)** domain 2) short cysteine rich patches with short transmembrane portion, and 3) a conserved cytoplasmic signaling domainToll/IL-1R homology (TIR domain).

A

TLR	XLXXLXLXXNXLXXLXXXXFXXLX
RI	XLXXLXLXXNXLXXXXXXXLXXXLXXXX

B

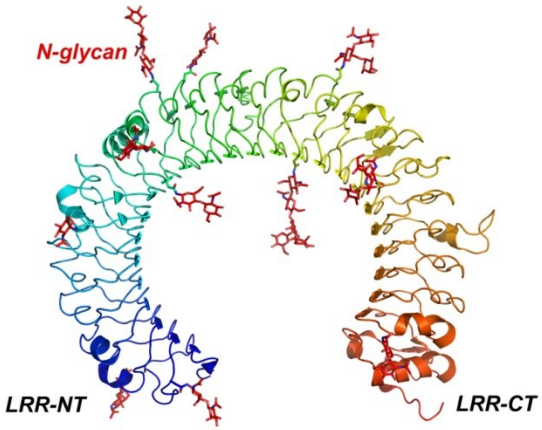


Leucine-Rich Repeat domain is composed of 19–25 tandem repeats of 22–30 residues in length and contains a conserved hydrophobic motif **XLXXLXLXX** spaced at distinctive intervals.

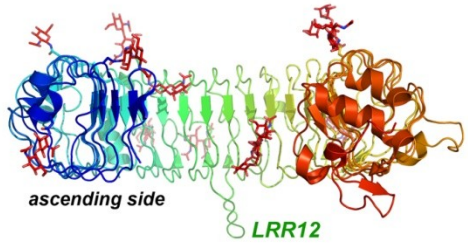
In three dimensions, all LRRs adopt a loop structure, beginning with an extended stretch that contains three residues in the β-strand configuration (yellow).

LRR consensus sequences for TLR3

Structure of the TLR3 extracellular domain

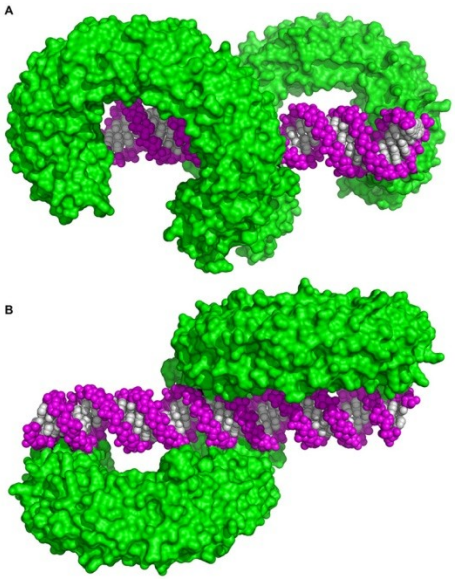


When assembled into a protein, multiple consecutive LRRs form a **solenoid structure**, in which the consensus hydrophobic residues point to the interior to form a stable core and the β strands align to form a hydrogen-bonded parallel β sheet



The structure of a TLR-ECD (hTLR3)

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



Structure of the TLR3/dsRNA complex

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

Upon ligand binding, typically two extracellular domains form an "m"-shaped dimer sandwiching the ligand molecule bringing the transmembrane and cytoplasmic domains in close proximity and triggering a downstream signalling cascade.

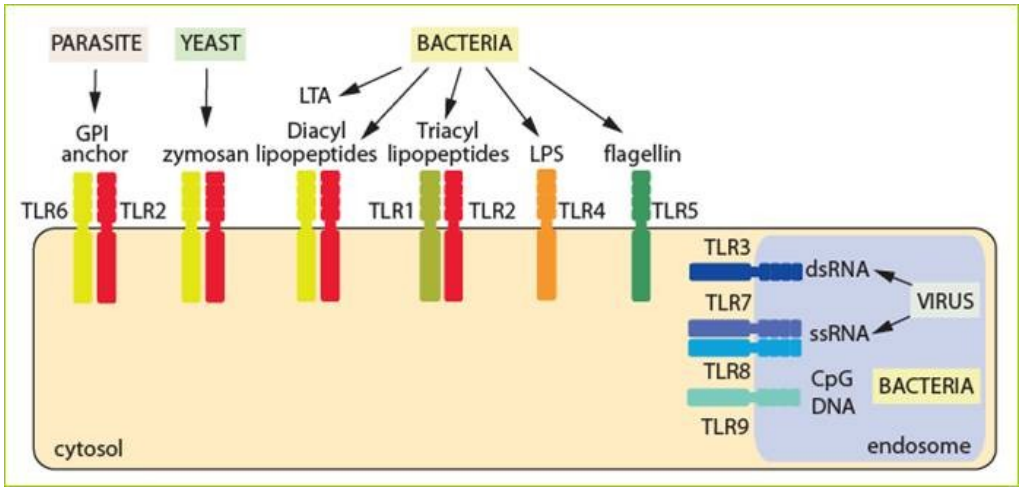
Although the ligand-induced dimerization of these receptors has many common features, the nature of the interactions of the TLR extracellular domains with their ligands varies markedly between TLR paralogs.

Multiple TLRs are expressed in Humans

The human TLR family comprise 10 members (TLR1-TLR10). They are expressed on macrophages, neutrophils, DCs, B cells, specific types of T cells, and even fibroblasts and 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 associated TLR: **TLR1**, **2**, **4, 5** and **6** recognise **microbial surface PAMPs**



TLR2 recognizes its ligands by forming a heterodimer with either TLR1 or TLR6. The resulting complexes recognize distinct ligands: triacyl and diacyl lipopeptides, respectively.

TLR4 recognizes lipopolysaccharide (LPS) on the cell surface. TLR5 recognizes flagellin. TLR-10: orphan receptor, it suppress inflammatory signaling

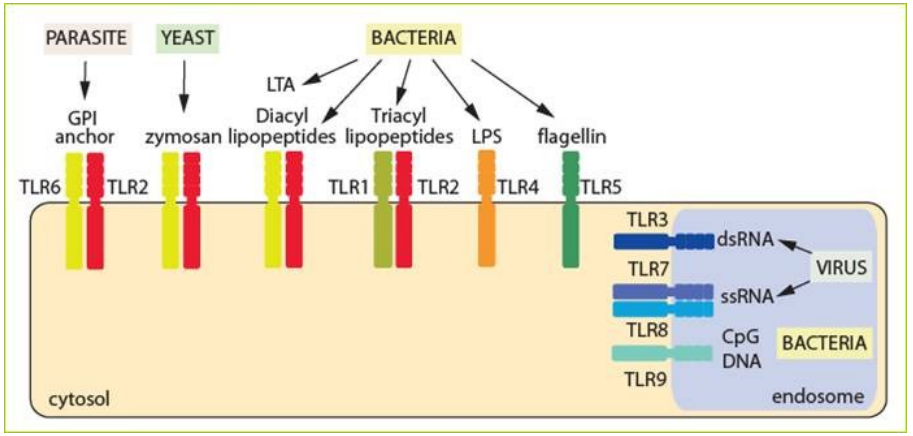
Endosome-associated TLRs

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; It is a dimer

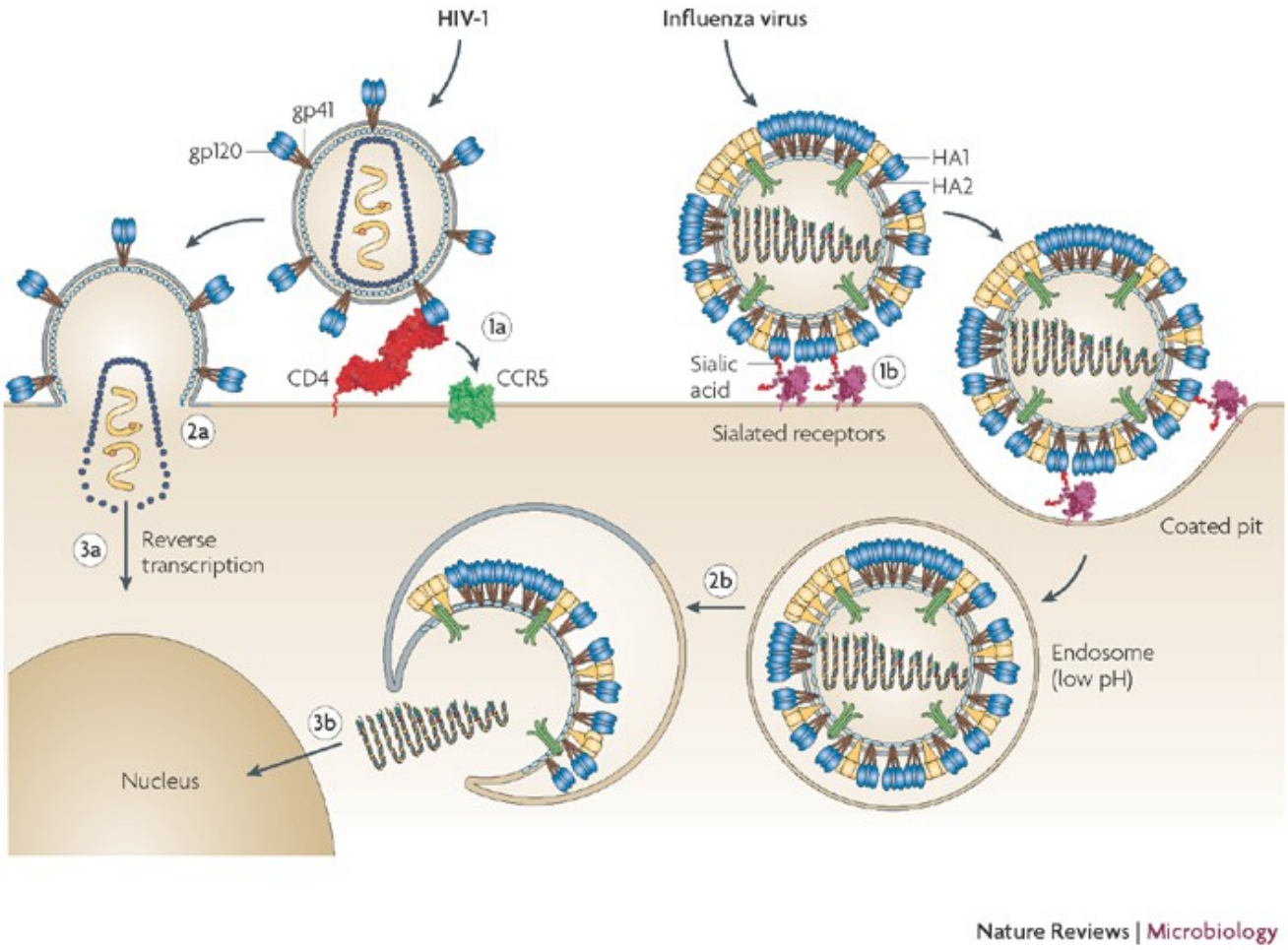
Human **TLR7/8** complex recognize single-stranded (ss) RNAs from RNA viruses.

TLR9 senses unmethylated DNA with CpG motifs derived from bacteria and viruses.



Endosome-associated TLR are essential for virus-induced type I IFN

Eukaryotic viruses entry pathways



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

Signaling transduction pathway of TRLs

TLR-mediated microbial recognition is very important for host defense against pathogens. On the other hand, excess responses to TLR ligands induce lethal septic shock syndrome.

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

The difference in the signaling cascades activated by the individual TLRs can be partly explained by the TIR domain-containing **adaptor molecules. There are multiple adaptor proteins:** TLR signaling is roughly divided into two distinct pathways depending on the usage of the distinct adaptor molecules, **MyD88** and **TRIF.**

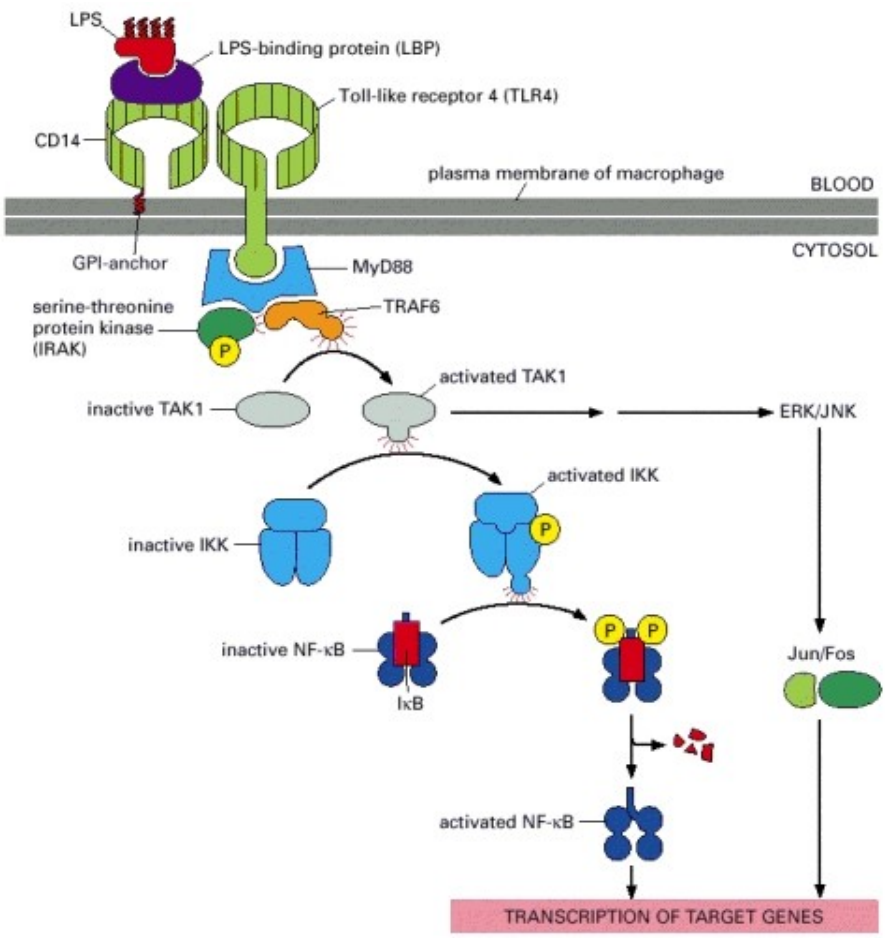
MyD88 and TRIF are responsible for the activation of **distinct signaling pathways**, leading to the production of proinflammatory cytokines (antibacterial response) and type I IFNs (interferons) (antiviral response), respectively.

Activation of TLR4 by bacterial LPS.

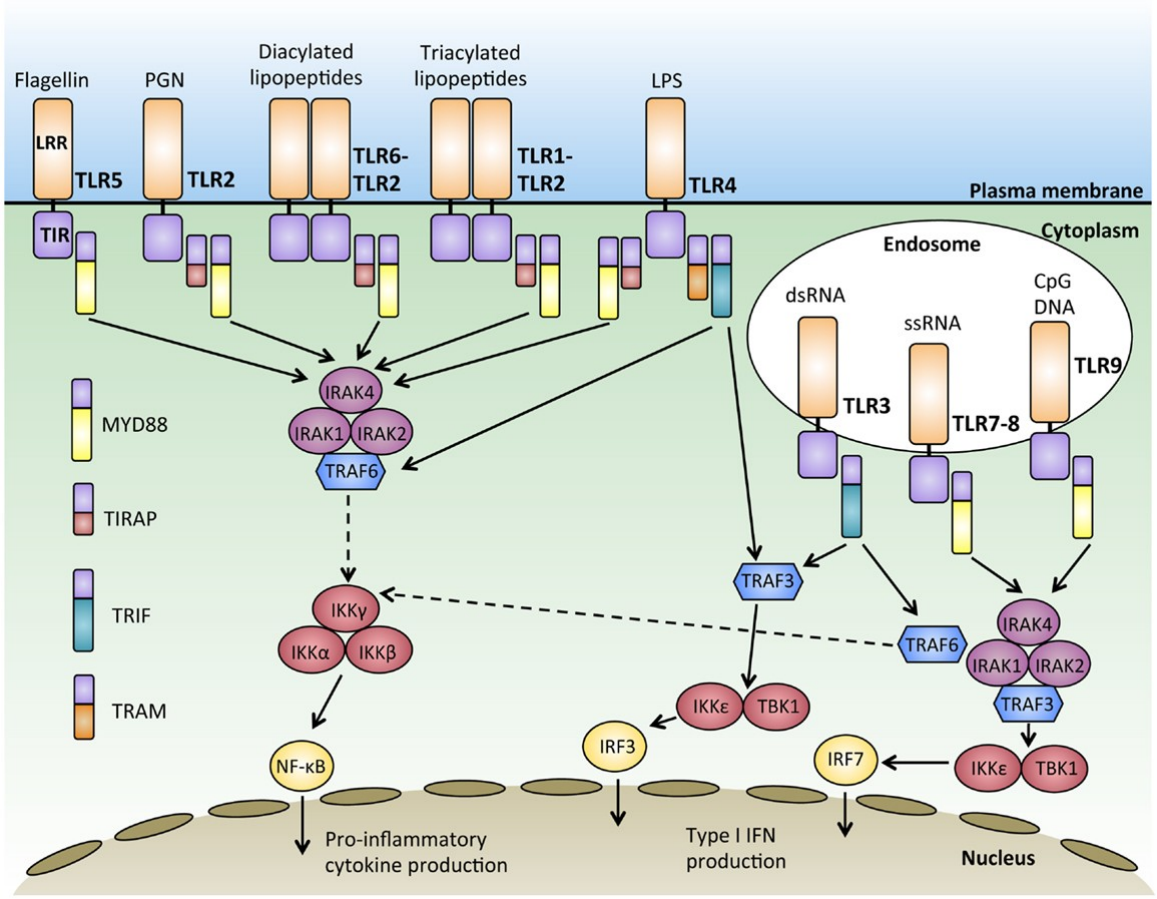
LPS is recognized by the **TLR4** of the phagocytes in conjunction with the cell-surface co-receptor **CD14** and the soluble serum protein **LPS binding protein** (LBP). LBP binds LPS in the blood and then it is bound by the receptor protein CD14 with leucine-rich repeat motifs.

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.



The TLR Signaling Pathways



Front. Immunol., 31 July 2014

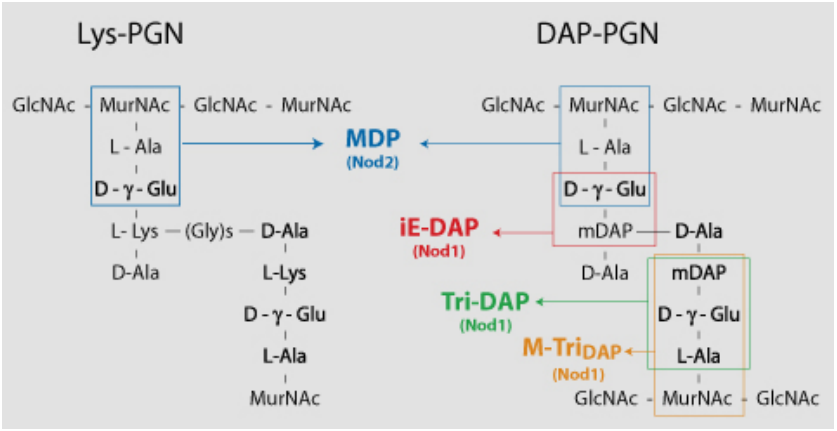
Adaptor molecules MyD88 and TRIF (see the figure) are responsible for the activation of distinct signaling pathways, leading to the production of **proinflammatory cytokines** and type **I IFNs,** respectively.

Stimulation with TLR3, TLR4, TLR7, and TLR9 ligands, but not the TLR2 ligand, induces type I IFN production, in addition to proinflammatory signals.

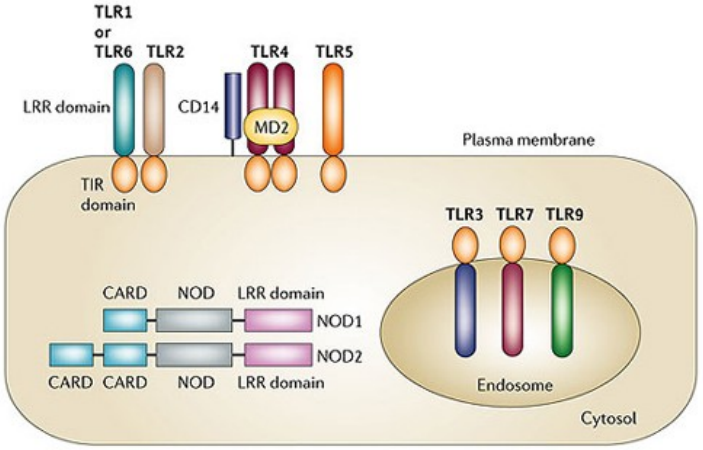
Cytoplasmic Pathogen Recognition System

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

NLRs: have a C-terminal **leucine-rich repeat (LRR) region**, a central nucleotide-binding receptor (NOD) and a N-terminal protein-binding motifs (CARD).



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

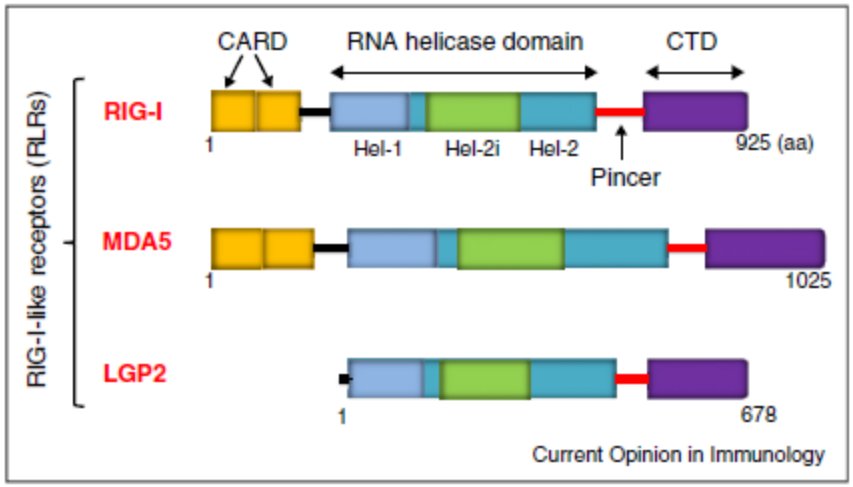


NOD-1 and **NOD-2** through LRR recognize distinct motifs of peptidoglycan (DAP-PGN) and **NOD-2** recognizes muramyl dipeptide (MDP) from bacteria peptidoglycan.
Signal transduction is mediated by CARD domain inducing transcriptional upregulation of proinflammatory cytokine genes.

RLRs and Virus Recognition

The CARD-helicase proteins/ **RIG-I-like** receptor family (RLRs) functions as an **intracellular PRR** serve to recognize viruses that have already entered into the cytoplasm of a cell, through the detection of viral replication by direct interaction with **ssRNA** and **dsRNA.**

RLR receptors (RIG-1) are RNA helicases that contain an N-terminal caspase recruitment domain (CARD) and a central helicase domain with ATPase activity required for RNA-activated signaling.



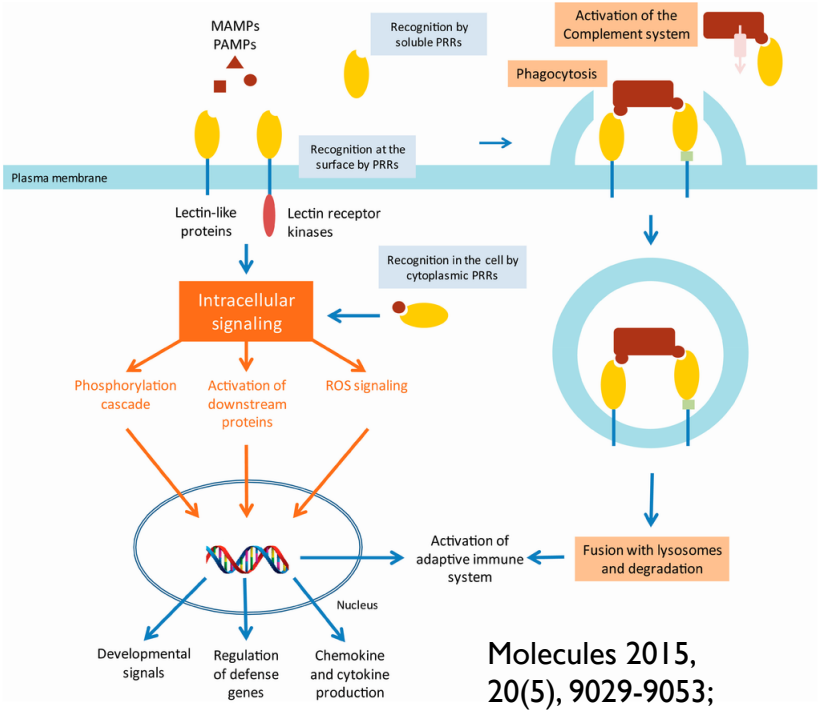
Current Opinion in Immunology 2015, 37:40–45

Binding of dsRNA or 5′-triphosphate RNA to the C-terminal domains of RLRs triggers signaling via CARD-CARD interactions, between the helicase and adaptor proteins, ultimately resulting in an antiviral response mediated by type I IFN production

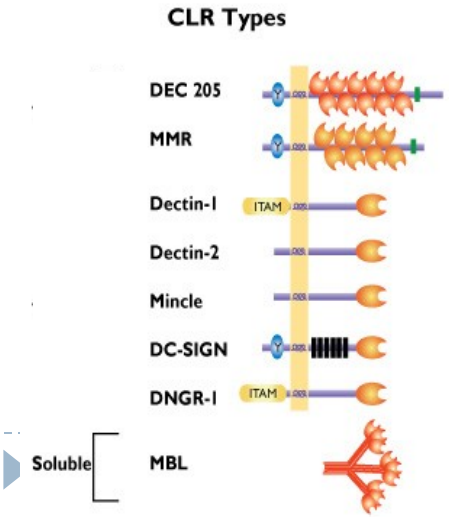
C-type lectin receptors (CLR)

CLRs comprise large family of receptors that bind to carbohydrates in a calcium-dependent manner. The lectin activity of these receptors is mediated by conserved **carbohydrate-recognition domain (CRD).**

CLRs recognize **specific carbohydrate structures** on microorganisms such as viruses, bacteria, and fungi.



Molecules 2015, 20(5), 9029-9053;



CLRs signaling are involved in the **various steps for initiation of innate immune responses** and promote secretion of soluble factors such as cytokines and interferons. CLRs contribute to phagocytosis and antigen presentation.

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

Secreted PRR: The lectin pathway of complement activation

The soluble CLR **Mannose binding lectin** (**MBL**) triggers one of the way to activate complement (**lectin pathway**).

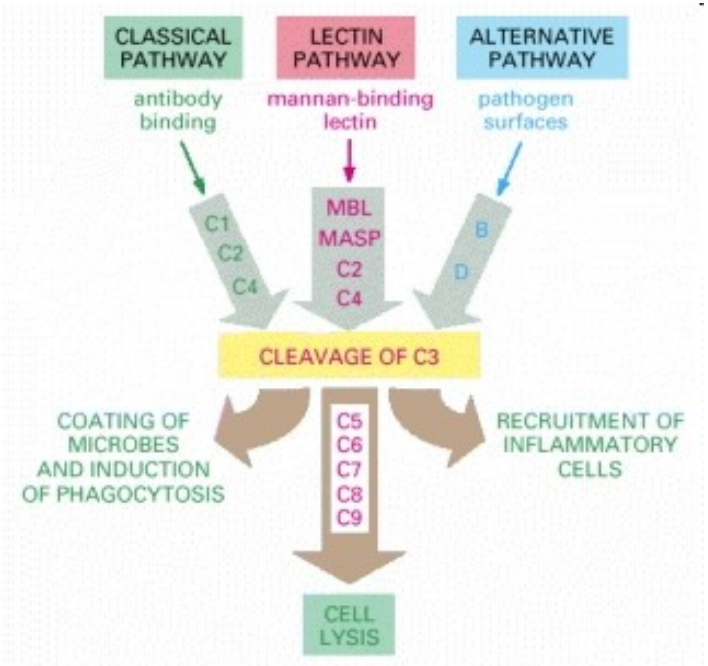
MBL is an acute-phase serum protein that activate complement acting as a PRR.

Three distinct pathways of complement act locally to activate **C3**, which is the pivotal component of complement.

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

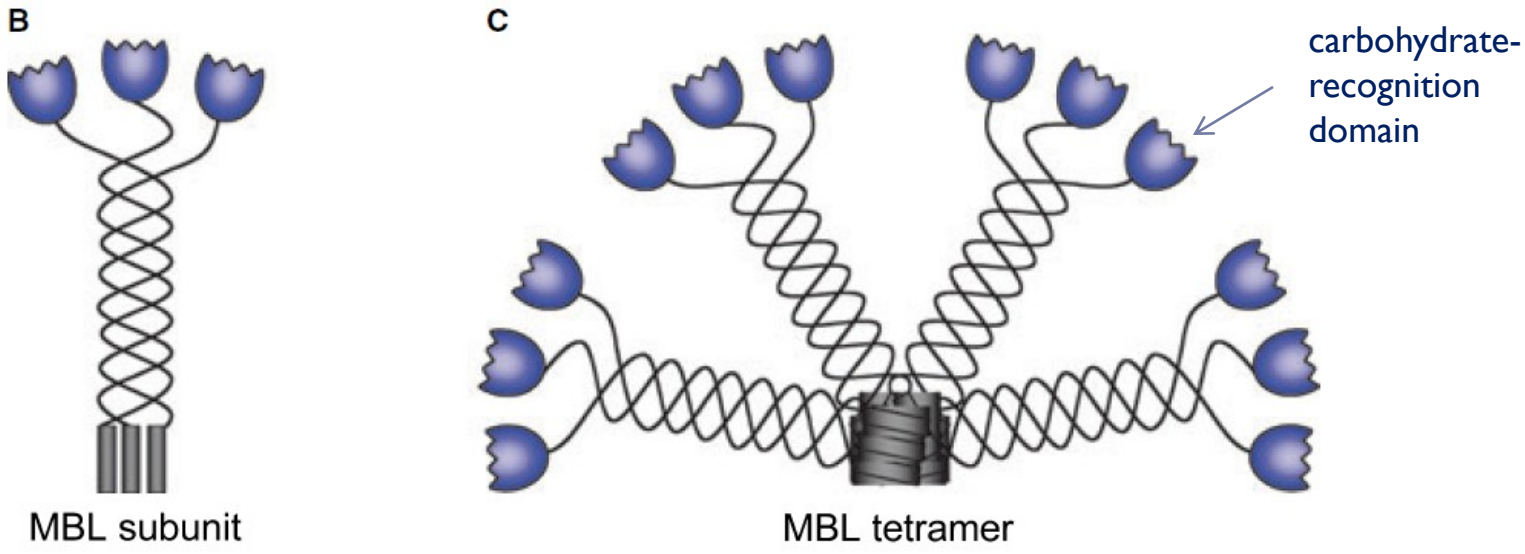
alternative pathway: C3 is spontaneously activated at low levels as C3b by LPS or TA found on the surfaces of bacteria.

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



A large transmembrane channel is formed by a chain of C9 molecules

MBL structure and oligomerization



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Immunological Reviews

MBL: **C-type lectin** forms clusters of 2-9 carbohydrate-binding heads 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.

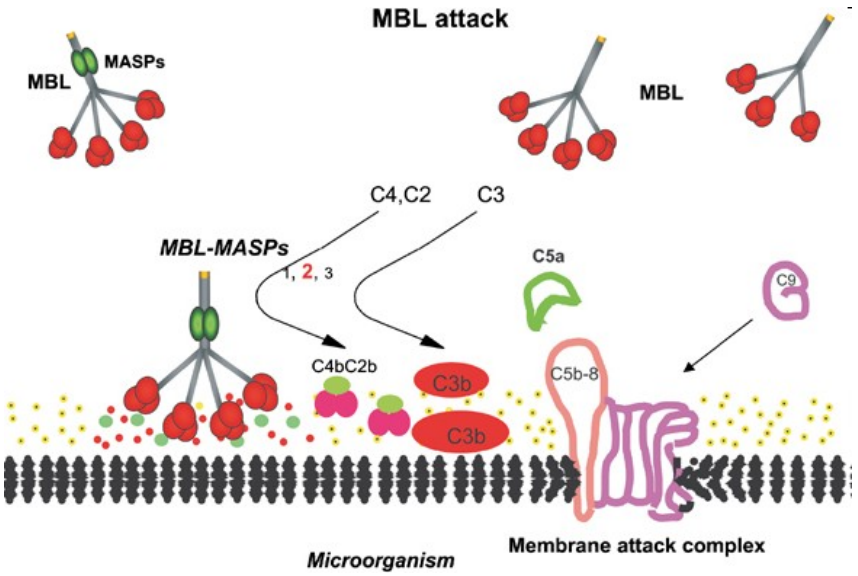
MBL activates lectin-pathway of complement

MBL 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 (**C3 convertase)** cleaves inactive C3 in **C3a** and **C3b.**

C3b binds on the surfaces, acts as **opsonin**, and triggers C5-C9 cascade to form the membrane attack complex, **(MAC)**

C3a (and C5a) is a major proinflammatory molecule (chemoatractant) and acts independently



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.