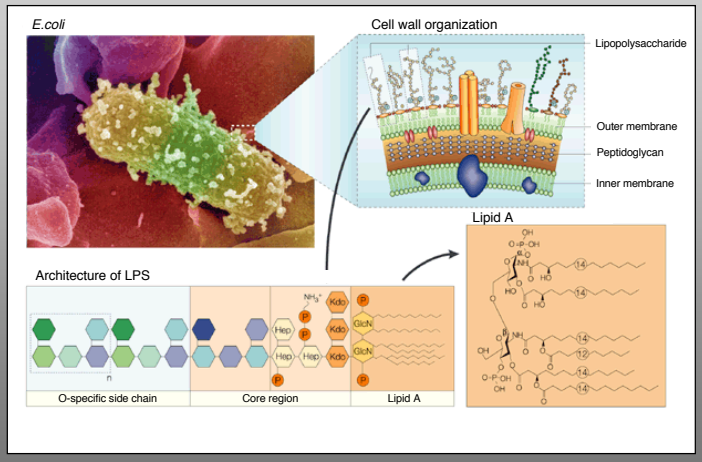
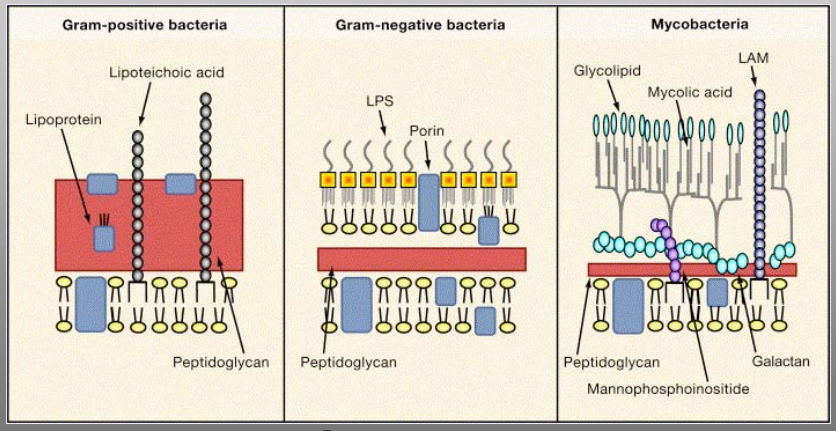
The Innate Immune Response is Conserved Throughout Evolution and is Triggered by Pattern Recognition

Lipopolysaccharide = Lipid + Polysaccharide



From: Beutler and Rietschel, *Nature Reviews Immunology* **3**; 169-176 (2003)

Diversity of Pathogen-associated Molecular Patterns (PAMPs)

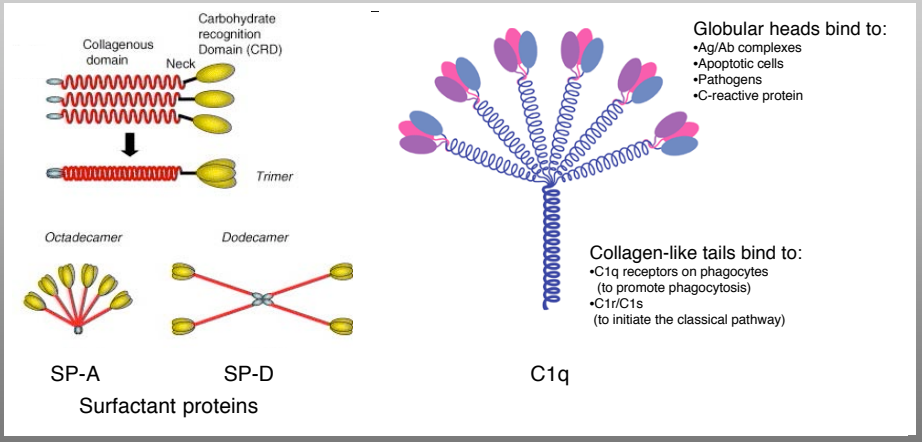


From: Akira et al., *Cell* 124:783, 2006

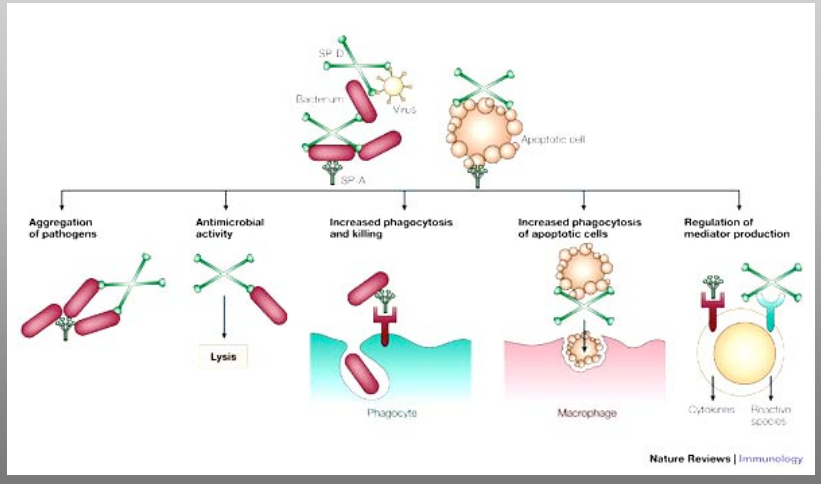
Innate Immune Receptors for PAMPs

- Toll-like receptors (TLRs)
- Complement
- Collectins (e.g., Surfactant Protein-A)
- Scavenger receptors
- Pentraxins (e.g., CRP)
- Lectins (e.g., Dectin-1)
- CD14
- NOD-like receptors (NLRs)
- RIG-1-like receptors

Collectins and Innate Immune Recognition

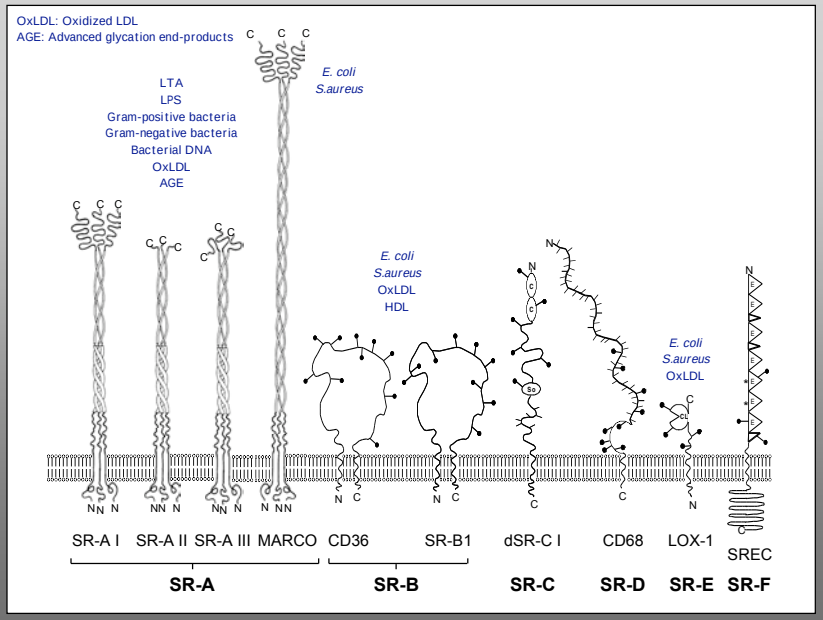


Some Functions of Collectins

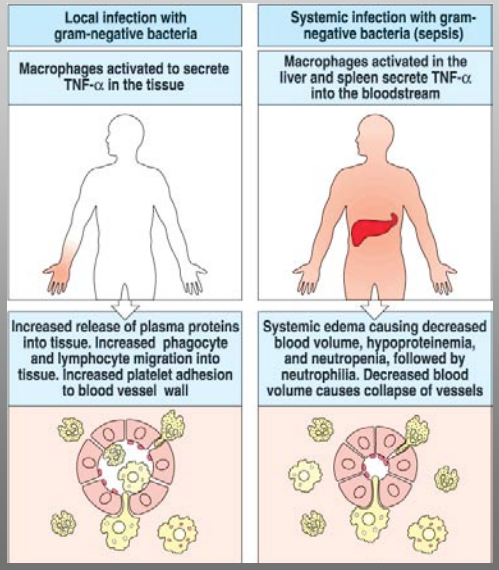


From: Wright, *Nature Rev. Immunol.* **5**:58, 2005

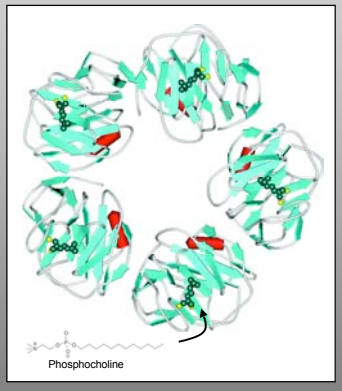
The Scavenger Receptor Superfamily Recognizes PAMPs

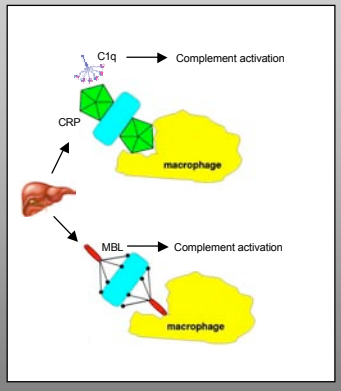


Innate Immune Receptors Also Trigger a Systemic Response to Infection



Innate Immune Functions of C Reactive Protein (CRP), an Acute Phase Protein Synthesized by Hepatocytes

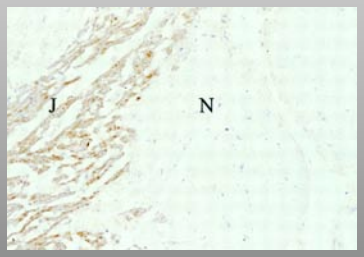




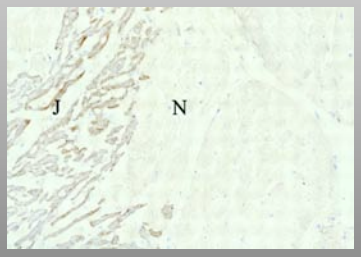
CRP acts as a bridge between phosphocholine on bacterial targets and C1q

Adapted from: Black et al., *J. Biol. Chem.* 47:48487, 2004

Co-localization of CRP and Activated Complement in Infarcted Human Myocardium



CRP

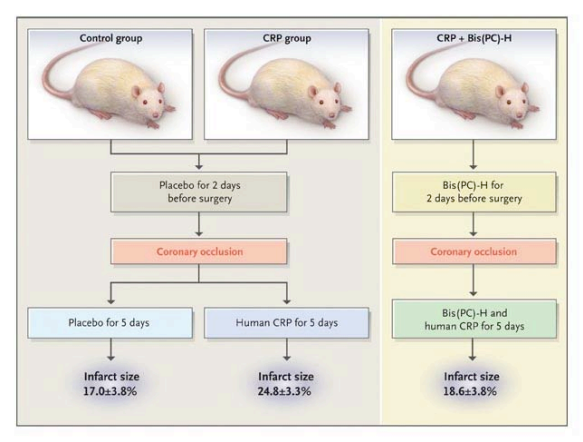


C4d

J = jeopardized; N = normal

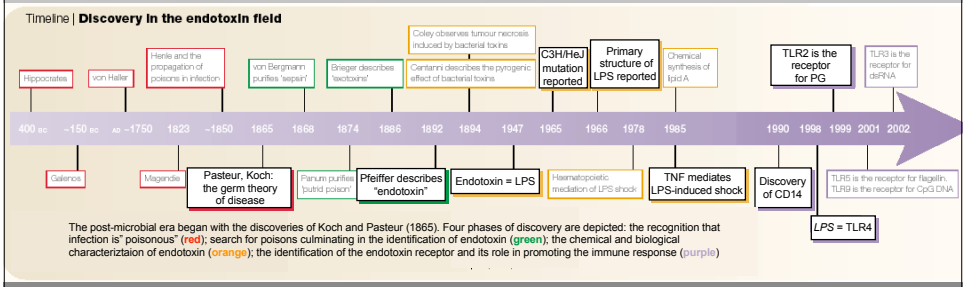
From: Nijmeijer et al., *Am. J. Pathol.* 163:269, 2003

Treatment of Experimental Myocardial Infarction with a CRP-binding Analog of Phosphocholine Limits Infarct Size



From: Kitsis and Jialal, *New Engl. J. Med.* 355:513, 2006

History of Endotoxin Research



Modified from: Beutler and Rietschel, *Nature Reviews Immunology* **3**; 169-176 (2003)

letters to nature

A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity

Ruslan Medzhitov*, Paula Preston-Hurlburt & Charles A. Janeway Jr*

*Section of Immunobiology, Yale University School of Medicine, and * Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011, USA*

Induction of the adaptive immune response depends on the expression of co-stimulatory molecules and cytokines by antigen-presenting cells. The mechanisms that control the initial induction of these signals upon infection are poorly understood. It has been proposed that their expression is controlled by the non-clonal, or innate, component of immunity that preceded in evolution the development of an adaptive immune system in vertebrates[1]. We report here the cloning and characterization of a human homologue of the *Drosophila* toll protein (Toll) which has been shown to induce the innate immune response in adult *Drosophila*[2–4]. Like *Drosophila* Toll, human Toll is a type 1 transmembrane protein with an extracellular domain consisting of a leucine-rich repeat (LRR) domain, and a cytoplasmic domain homologous to the cytoplasmic domain of the human interleukin (IL)-1 receptor. Both *Drosophila* Toll and the IL-1 receptor are known to signal through the NF-κB pathway[5–7]. We show that a constitutively active mutant of human Toll transfected into human cell lines can induce the activation of NF-κB and the expression of NF-κB-controlled genes for the inflammatory cytokines IL-1, IL-6 and IL-8, as well as the expression of the co-stimulatory molecule B7.1, which is required for the activation of naive T cells.

The Toll protein controls dorsal–ventral patterning in *Drosophila* embryos and activates the transcription factor Dorsal upon binding to its ligand Spätzle[8]. In adult *Drosophila*, the Toll/Dorsal signalling pathway participates in an anti-fungal immune response[2]. Signalling through Toll parallels the signalling pathway induced by the IL-1 receptor (IL-1R) in mammalian cells: IL-1R signals through the NF-κB pathway, and Dorsal and its inhibitor Cactus are homologous to NF-κB and I-κB proteins, respectively[9,10]. Moreover, the cytoplasmic domain of *Drosophila* Toll is homologous to the cytoplasmic domain of IL-1R (ref. 9). Remarkably, the tobacco-virus-resistance gene that encodes N-protein is also similar to Toll in that it contains both a Toll signalling domain and an LRR domain[11]. It thus appears that the immune-response system mediated by Toll represents an ancient host defence mechanism[4] (Fig. 1). To investigate the possibility that this pathway has been retained in the immune system of vertebrates, we used sequence and pattern searches[12] of the expressed-sequence tag (EST) database at the National Center for Biotechnology Information (NCBI). A search with a sequence profile of the Toll/IL-1R signalling domain identified a matching sequence in the EST database derived from human fetal liver/spleen library (Genebank accession number H48602, corresponding to clone 202057 from the IMAGE consortium[13]). Sequencing of this clone showed that it corresponds to the 3′ untranslated region (UTR) and the C terminus of the coding region of the messenger RNA. A fragment of the clone amplified by using the polymerase chain reaction (PCR) was used to screen a human spleen complementary DNA library by hybridization, and the 5′ end of the cDNA was cloned using the 5′-RACE technique as described[14,15]. The full-length 4,874-base-pair (bp) cDNA clone contained an open reading frame of 2,523 bp which encoded an 841-amino-acid protein chain (Fig. 2a), as well as a LINE-1 reverse transcriptase pseudogene in the 3′-UTR. Alignment of the sequences of the human and *Drosophila* Toll proteins shows that there is homology over the entire length of the protein chains (Fig. 2b). Notably, the similarity between the cytoplasmic domains is higher than between the human proteins Toll and IL-1R (not shown). The extracellular domain of human Toll (hToll) contains 21 tandemly repeated leucine-rich motifs separated by a non-LRR region, similar to *Drosophila* Toll (dToll). At the N-terminal end of the LRR domain there is a 31-amino-acid long N-flanking region that is also present in several other LRR-containing proteins, for example RP105, Decorin and Biglycan[16]. The C-terminal end of the LRR domain is flanked by a cysteine-rich domain which is also present in dToll and some other transmembrane proteins[16,17].

To examine the expression pattern of hToll, a 720-bp cDNA fragment was used to probe northern blots containing poly(A)$^+$ RNA from several organs. Most organs expressed two mRNA species: one of ~5 kilobases (kb) was predominant in most tissues except peripheral blood leukocytes (PBL), and corresponded to the length of the cDNA that we cloned. The lower band was ~4 kb long and this band was predominant in the PBL. The 4-kb band was not detectable in kidney, and liver did not contain any mRNA at all (Fig. 3). We also tested different mouse and human cell lines for expression of hToll mRNA by using PCR with reverse transcription (RT-PCR). We found mRNA for hToll in monocytes, macrophages, dendritic cells, γδ T cells, Th1 and Th2 α/β T cells, a small intestinal epithelial cell line, and a B-cell line (data not shown). The hToll gene is expressed most strongly in spleen and PBL (Fig. 3); its expression in other tissues may be due to the presence of macrophages and dendritic cells, in which it could act as an early-warning system for infection. Alternatively, hToll may be widely expressed because hToll signals through the conserved NF-κB pathway (see below) and NF-κB is a ubiquitous transcription factor.

To characterize hToll functions and see whether it can induce transcription of immune response genes like dToll, we generated a dominant-positive mutant of hToll because the natural ligand of hToll is unknown. To produce a constitutively active mutant of hToll, we made use of genetic information from dToll: analysis of ventralizing mutants in *Drosophila* embryos had identified the function of the ectodomain C-flanking cysteine-rich region in dToll[18] as controlling the activity of dToll in signal transduction. In three dominant

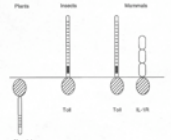
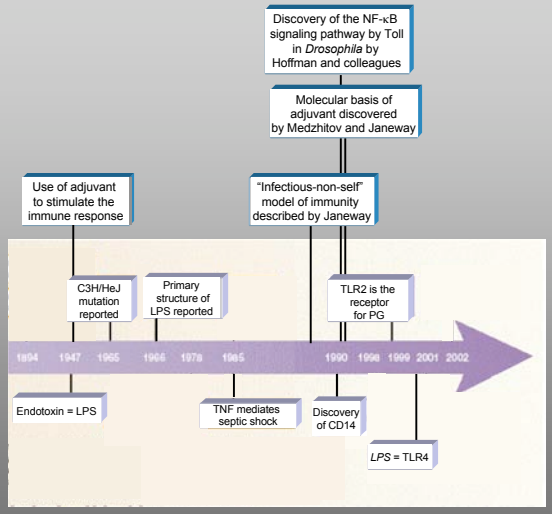


Figure 1 Ancient immune defence systems of plants, insects and vertebrates. A homologous immune response system based on the Toll signalling domain is used in plants, insects and vertebrates. In mammals, Toll induces signals required for the activation of both an innate and an adaptive immune response (see text). The figure is modified from ref. 6. Diagonal hatching represents the Toll signalling domain; striped rods, leucine-rich repeat domains; black rectangles, C-terminal cysteine-rich domains; white ellipsoids, immunoglobulin domains.

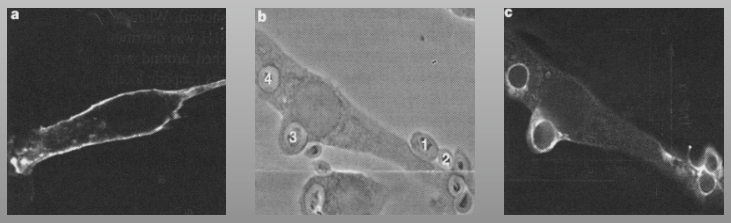
A Re-interpretation of the Endotoxin Research Timeline



Modified from: Beutler and Rietschel, *Nature Reviews Immunology* **3**; 169-176 (2003)

Primitive Specificity in Target Recognition by the Innate Immune System

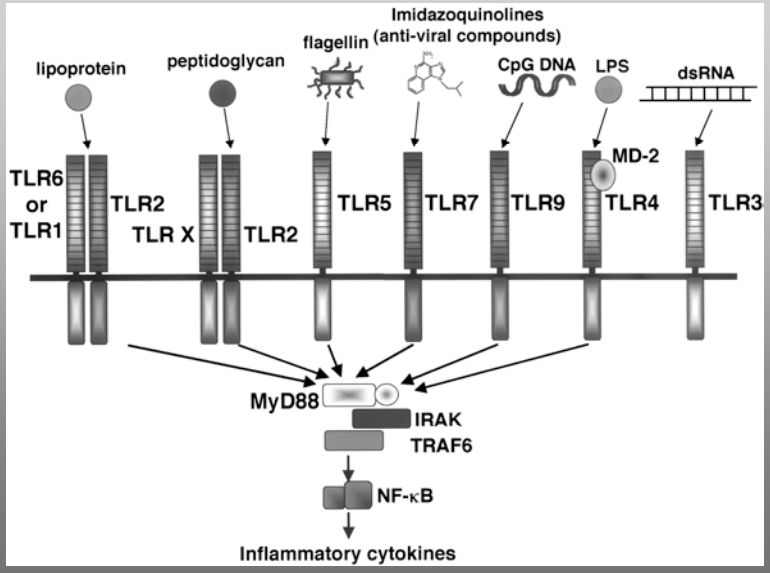
Recruitment of TLR2 to Yeast Phagosomes



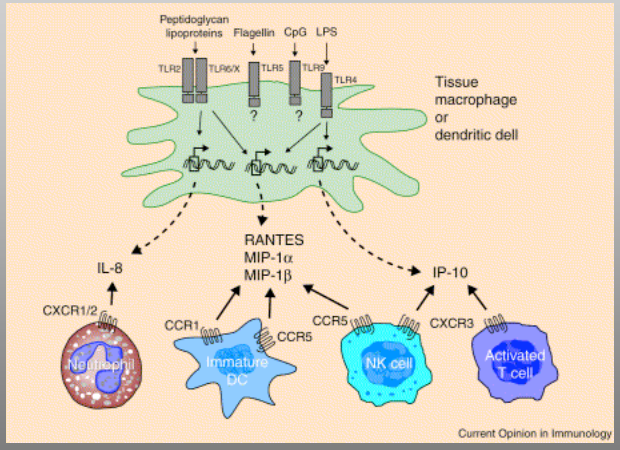
TLR2 Phase Contrast TLR2

From: Underhill et al., *Nature* 401:811, 1999

Ligand Specificity of TLRs

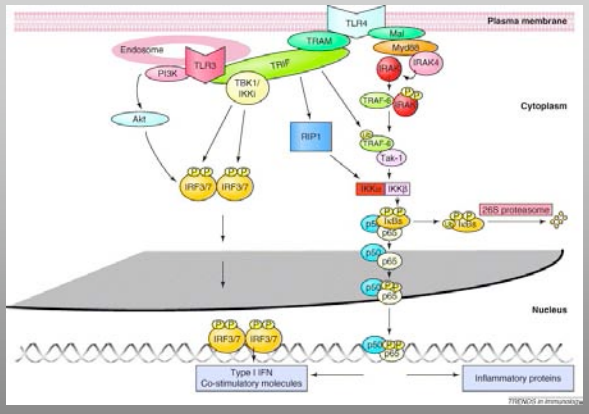


Specificity of TLR Transcriptional Programs



From: Luster, *Curr. Opin. Immunol.* 14:129, 2002

TLR Signaling: Two Major Pathways

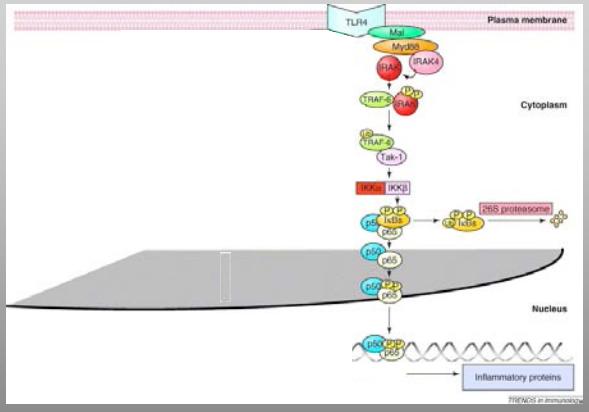


Cartoon of major signal transduction pathways following engagement of TLRs. TLR4 is the major sensor of LPS. TLR3 recognizes dsRNA and is important in the anti-viral response. The IRF pathway leading to production of Type I IFNs (i.e., IFN-α/β) is particularly prominent in a minor subset of dendritic cells (called "plasmacytoid DCs") that are the major source of these IFNs in response to viral infections.

Do not memorize this cascade but rather appreciate that it consists of two parallel pathways, one that activates NF-κB, leading to production of pro-inflammatory proteins, and one that activates the IRF pathway, leading to production of Type I IFNs.

From: Moynagh, *Trends Immunol.* 26:469, 2005

TLR Signaling: Two Major Pathways

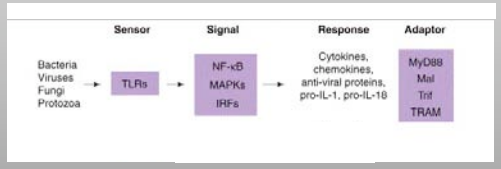


Cartoon of major signal transduction pathways following engagement of TLRs. **TLR4** is the major sensor of LPS. **TLR3** recognizes dsRNA and is important in the anti-viral response. The IRF pathway leading to production of Type I IFNs (i.e., IFN-α/β) is particularly prominent in a minor subset of dendritic cells (called "plasmacytoid DCs") that are the major source of these IFNs in response to viral infections.

Do not memorize this cascade but rather appreciate that it consists of two parallel pathways, one that activates NF-κB, leading to production of most pro-inflammatory proteins, and one that activates the IRF pathway, leading to production of Type I IFNs.

From: Moynagh, *Trends Immunol.* 26:469, 2005

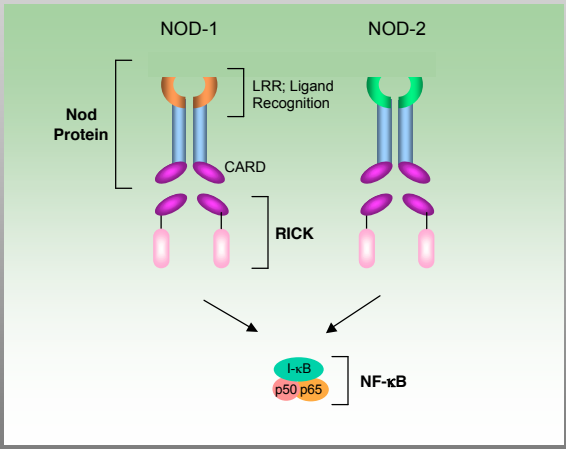
TLRs Sense Microbial Pathogens and Trigger Expression of Pro-inflammatory Cytokines and Chemokines



Adapted from: Creagh and O'Neill, *Trends Immunol.* 27:352, 2006

Newly Recognized Components of the Innate Immune System

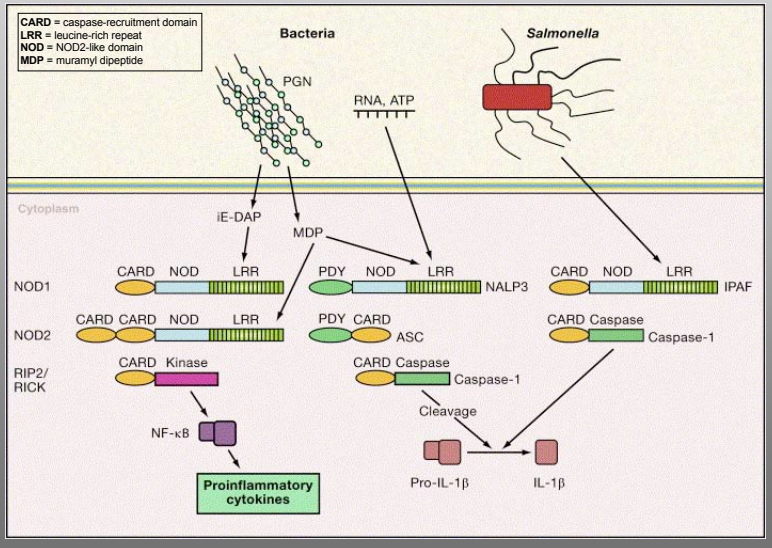
NOD Proteins: Intracellular Peptidoglycan Sensors



Polymorphisms in *Nod-2* are associated with up to 30-40% of cases of Crohn's disease (an inflammatory bowel disease)

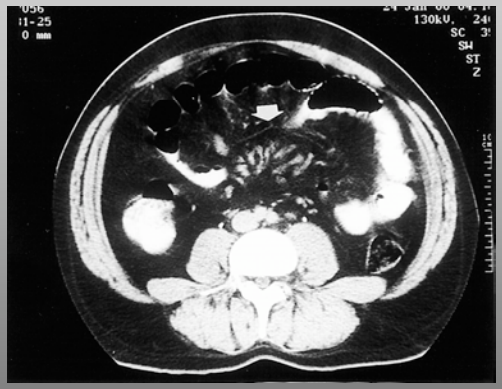
CARD, caspase-recruitment domain; **LRR**, leucine-rich repeat; **RICK**, a CARD-containing protein kinase

Cytosolic Bacterial Recognition Systems and "the Inflammasome"



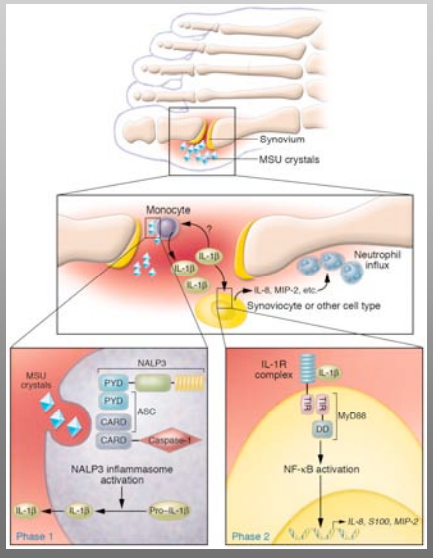
From: Akira et al., *Cell* 124:783, 2006

Mutations in Pyrin, Another CARD-containing Innate Immune-like Protein, is Responsible for Familial Mediterranean Fever



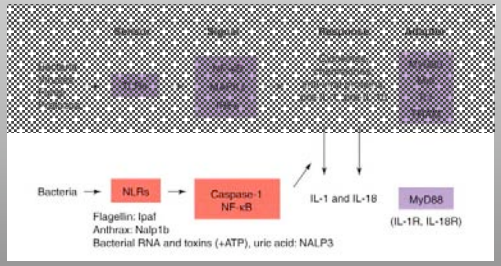
Contrast-enhanced abdominal CT from a 31 year-old patient with Familial Mediterranean Fever suffering an acute attack of abdominal pain, nausea, vomiting, and arthritis. Note mesenteric vessel with thickened mesenteric fold (*white arrow*). Histopathology demonstrated neutrophilic infiltrate and associated vasculitis. Treatment with an IL-1 receptor antagonist (Anakinra) resulted in prompt cessation of symptoms.

Pathogenesis of Gout Uncovered in 2006: Monosodium urate crystals activate the inflammasome



From: Martinon and Glimcher *J. Clin. Invest.* 116:2073, 2006

Nod-like Receptors (NLRs) Sense Microbial Products, Activate the "Inflammasome," and Trigger Maturation of IL-1

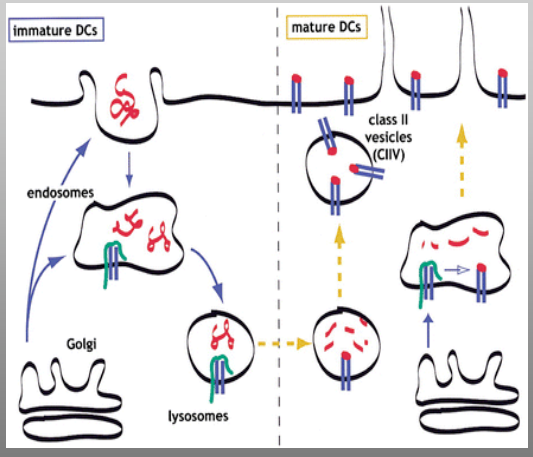


Adapted from: Creagh and O'Neill, *Trends Immunol.* 27:352, 2006

The Dendritic Cell and Development of The Primary Immune Response:

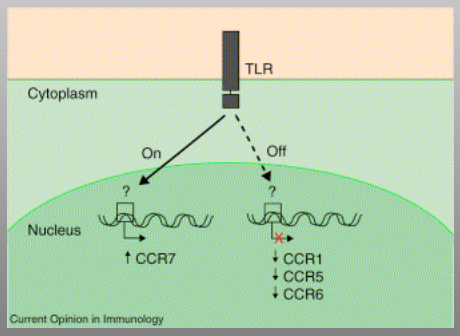
Wisdom Through Maturity

Dendritic Cell Maturation



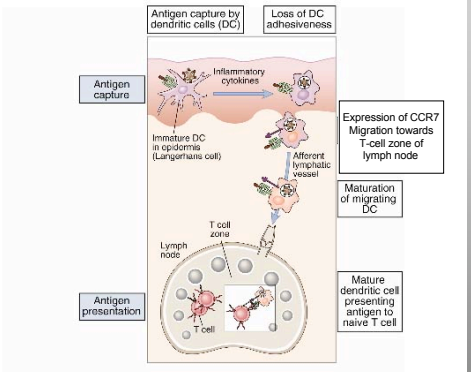
From: Mellman & Steinman, *Cell* 106:255, 2001

The Innate Immune Response Orchestrates DC Trafficking to Secondary Lymphoid Organs



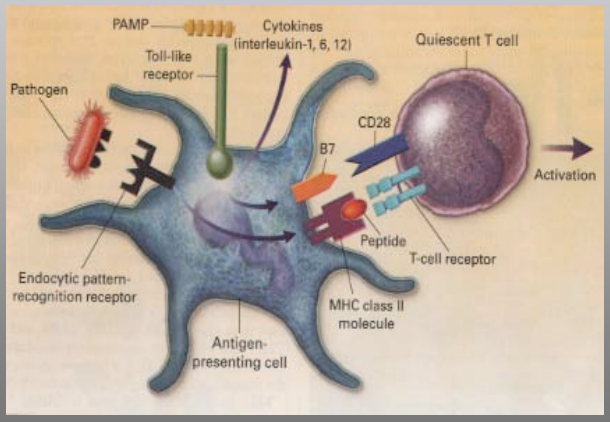
From: Luster, *Curr. Opin. Immunol.* 14:129, 2002

Functional Differences Between Immature and Mature DCs

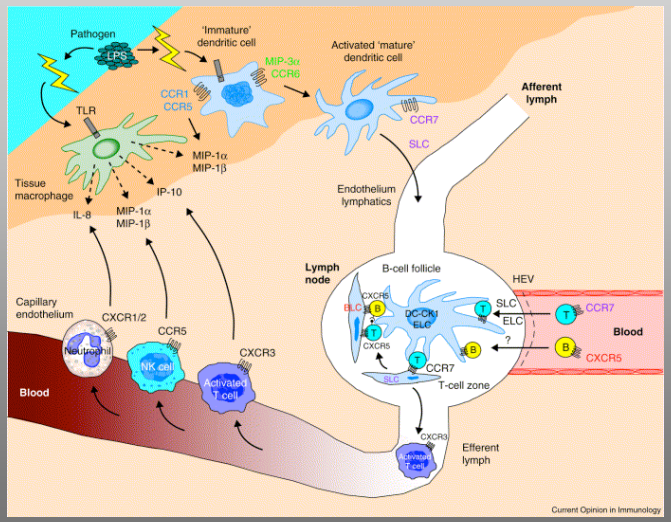


	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life on surface	~10 hr	>100 hr
Number of surface molecules	~10^6	~7×10^6

The (Primary) Acquired Immune Response is Initiated by Innate Immune Recognition

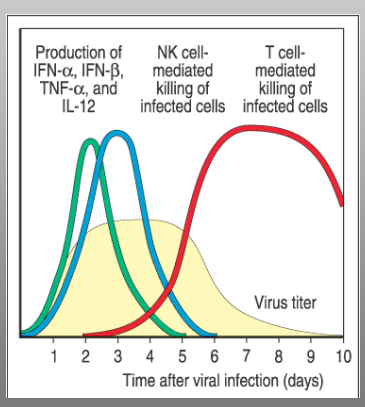


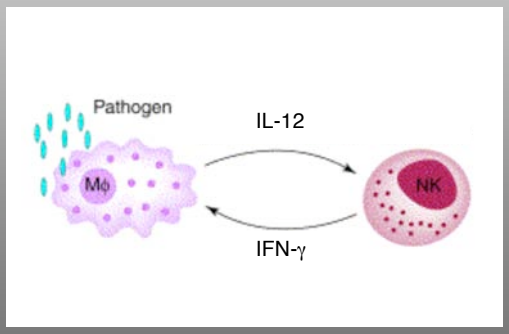
Chemokines Direct Trafficking of Immune Cells



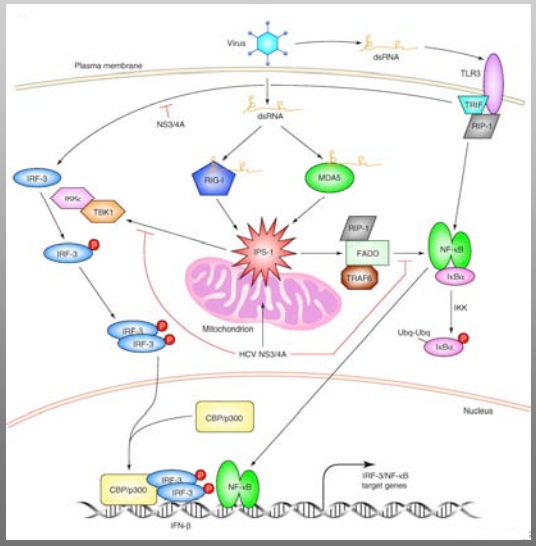
From: Luster, *Curr. Opin. Immunol.* 14:129, 2002

The Early Antiviral Response: Cytokines of the Innate Immune System





Innate Immune Receptors for dsRNA Cooperate to Initiate the Immune Response to RNA Viruses

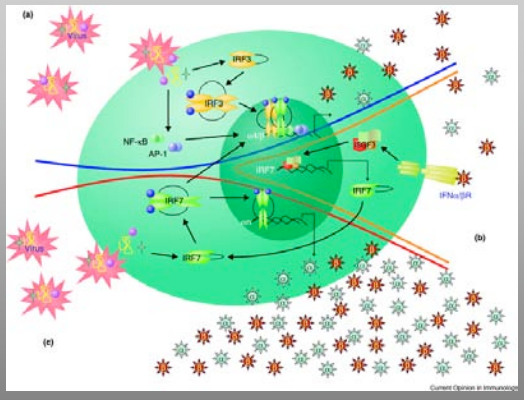


Double-stranded RNA products of virus infection bind to RIG-I or MDA5, which in turn bind to IPS-1 via CARD domain interactions. This complex then signals the activation of IKK-ε and TBK1 or other kinases to phosphorylate IRF-3, possibly through direct recruitment of signaling effectors, leading to IRF-3 dimerization, nuclear translocation and assembly onto the IFN-β enhancer. IPS-1 might also signal the activation of the IKK complex via direct binding of IKK components or through recruitment of RIP-1, FADD and/or TRAF6, causing the phosphorylation of IκB, the inhibitor of NF-κB. Phosphorylated IκB is then ubiquitinated and targeted to the proteosome for degradation, releasing the active NF-κB complex to translocate to the nucleus. During virus infection, dsRNA products can signal through TLR3 to activate IRF-3 and NF-κB by the actions of the TRIF adaptor protein and RIP-1, respectively.

N.B.: Do not memorize this cartoon, but appreciate how cytosolic dsRNA receptors (RIG-1, MDA5) and plasma membrane-associated dsRNA receptors (TLR3) cooperate to activate IRF- and NF-κB-dependent gene expression.

From: Johnson and Gale, *Trends Immunol.* 27:1, 2006

The Antiviral Response: a Cascade of Transcriptional Events

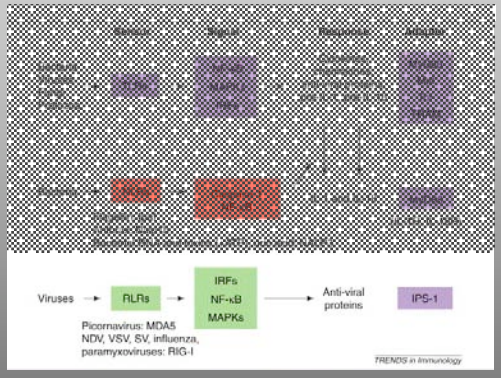


Some targets of IRFs

Gene	Function
p21	Cell cycle arrest
IL-15	NK cell maturation
FasL	Cell death
IL-12	Th1 immune response

Multiphasic induction of murine type I IFN genes can be divided into three phases. (a) The immediate early phase. Virus infection stimulates a phosphorylation cascade, leading to the activation of at least three families of transcription factors, including NF-κB, AP-1 and IRF3. Activation of the IFN-α promoter requires all three transcription factors. (b) IRF7 induction phase. Secretion of early IFN produces an autocrine response through stimulation of the JAK-STAT pathway. Among the pathway's target genes is IRF7, itself. (c) Delayed early (amplification) phase. Many members of the IFN-α gene family possess promoter binding sites for activated IRF7 and become transcriptionally active.

RIG-1-like Receptors (RLRs) Sense Viral Products, Activate the IRF Pathway, and Trigger and Trigger Production of Antiviral Proteins



Adapted from: Creagh and O'Neill, *Trends Immunol.* 27:352, 2006

Summary

1. Innate immunity is conserved throughout evolution and is triggered by recognition of "pathogen-associated molecular patterns" (e.g., LPS) by "pattern recognition receptors."

2. Collectins (e.g., SP-A, C1q, MBP) recognize carbohydrates on pathogen surfaces and perform multiple anti-microbial functions (e.g., opsonization). Collectins are essential for innate immunity, but also help clear apoptotic debris.

3. Members of the Scavenger Receptor superfamily recognize bacteria as well as glucose-modified proteins and oxidized lipoproteins. They are implicated in the response to infection as well as atherosclerosis and other degenerative diseases.

4. TLR4 is the major LPS receptor in mammalian cells. TLR4 triggers activation of NF-κB (leading to production of TNF-α, for example). Other TLRs recognize additional microbial products. NOD-like receptors (NLRs) are intracellular sensors of bacterial products that activate the "inflammasome," triggering caspase-dependent maturation of IL-1.

5. Dendritic cells undergo a maturation program: immature DCs, which traffic to the periphery, capture antigen, and mature DCs, which traffic to the lymph node, present antigen. Innate immune stimuli trigger DC maturation, which upregulates co-stimulatory molecules and facilitates antigen presentation. Thus, the innate immune response ushers in the acquired immune response.

6. NK cells, a component of innate immunity, especially to viruses, represent an early source of IFN-γ and serve to stimulate macrophages and DCs in inflammatory sites. Additional components of the antiviral response include intracellular dsRNA sensors (RIG-like proteins) that activate the IRF pathway to signal antiviral gene expression.