How do primitive metazoans survive without an acquired immune system?

How important is innate immunity for higher metazoans?

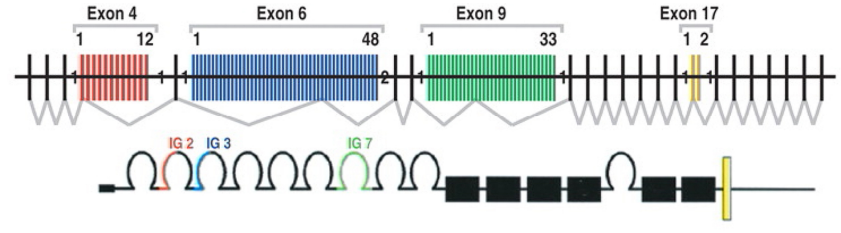
Mechanisms of Generating Immunological Diversity in Primitive Metazoans

Taxonomic group	Genetic variability (polymorphism+polylocism)	Somatic variability			
		Alternative splicing	Mutation, conversion	Rearrangement	Combinatorial association of peptides
Nematodes	Lectins				
Mollusks	Fibrinogen-related proteins (immunoglobulin SF), lectins (s)	Fibrinogen-related proteins (immunoglobulin SF)	Fibrinogen-related proteins (immunoglobulin SF)		
Arthropods	Thioester-containing proteins, peptidoglycan binding proteins (s), Gram-negative binding protein, lectins (s), Toll-like receptor (leucine-rich repeat)	Dscam (immunoglobulin SF) (s), peptidoglycan binding proteins (s)			
Echinoderms	Scavenger receptor cysteine rich (s), 185/333	Scavenger receptor cysteine rich (s), 185/333			
Prochordates	Variable region-containing chitin binding protein (immunoglobulin SF), lectins (s), Toll-like receptors (leucine-rich repeat)				
Agnathans	Agnathan paired receptors resembling Ag receptors (immunoglobulin SF), variable lymphocyte receptor (leucine-rich repeat)		Variable lymphocyte receptor?	Variable lymphocyte receptor	
Gnathostomes	Many immunoglobulin SF multigene families (s), Toll-like receptor (leucine-rich repeat), lectins (s), peptidoglycan binding proteins (s), complement, major histocompatibility complex class I and II	Immunoglobulin SF families (s)	Immunoglobulin (s)	Immunoglobulin (s), T cell receptor	Immunoglobulin (s), T cell receptor, Toll-like receptors (leucine-rich repeat), major histocompatibility complex class I and II

Red, membrane-associated; **Black**, soluble

From: Du Pasquier *Science* 309:1826, 2005

Dscam, a Secreted Opsonic Protein with Extensive Alternative Splicing in *Drosophila*

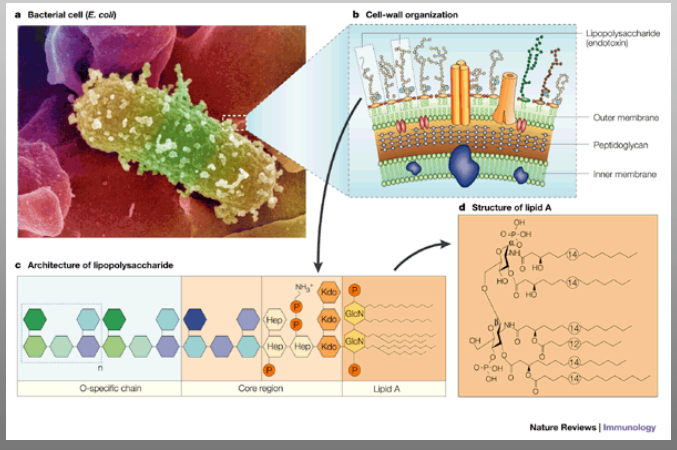


From: Watson et al., *Science* 309:1874, 2005

The Innate Immune Response to Bacterial and Fungal Infections

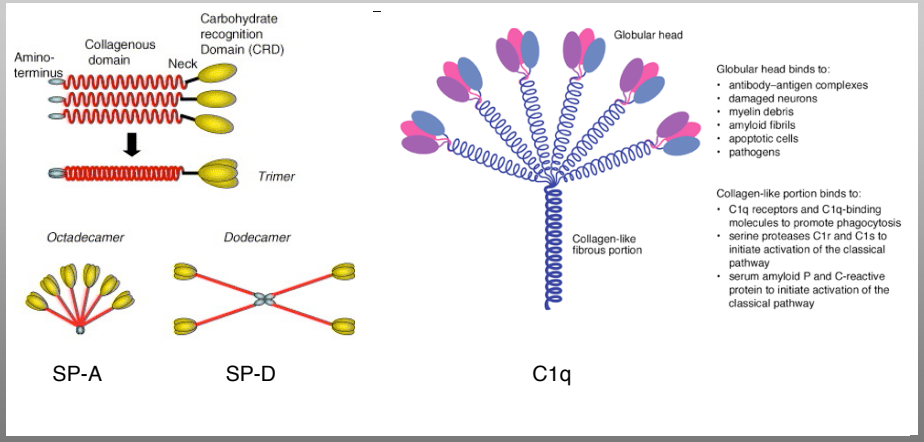
The Innate Immune Response is Conserved Throughout Evolution and is Triggered by Pattern Recognition

Lipopolysaccharide is Composed of Lipid and Polysaccharide

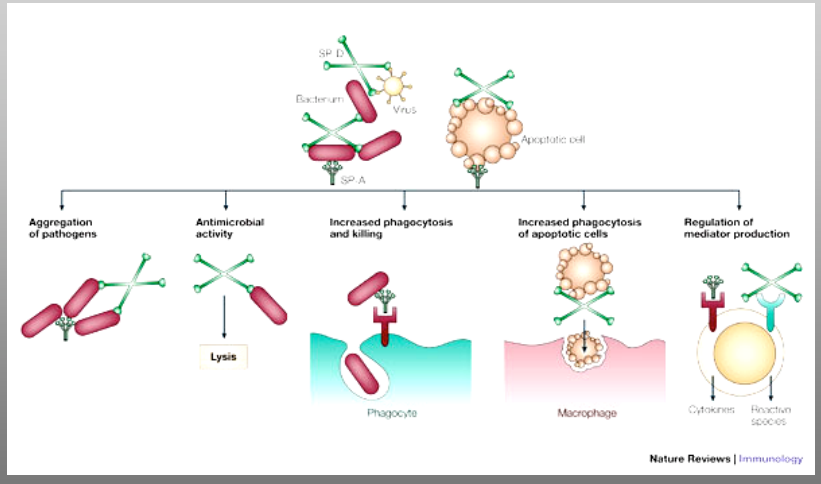


From: Beutler and Rietschel, *Nature Reviews Immunology* **3;** 169-176 (2003)

Collectins and Innate Immune Recognition

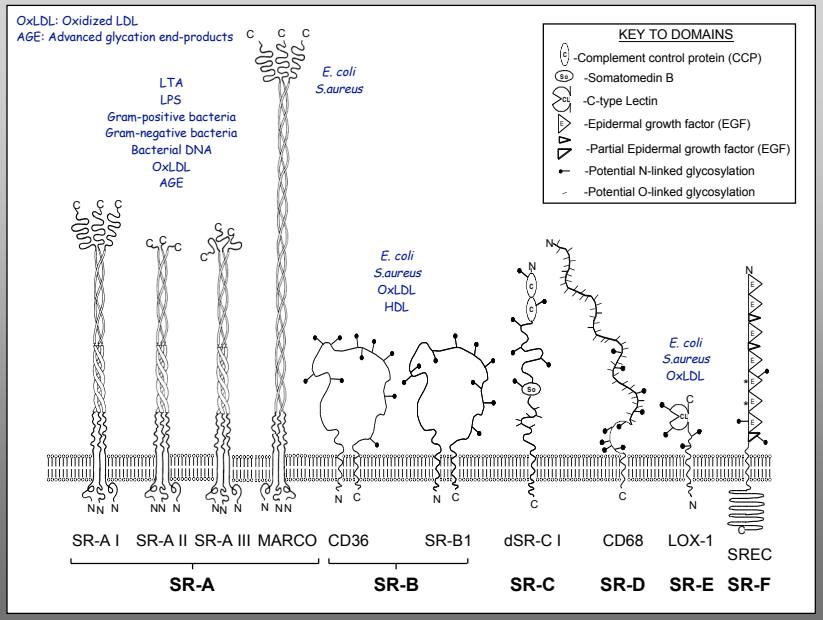


Some Functions of Collectins

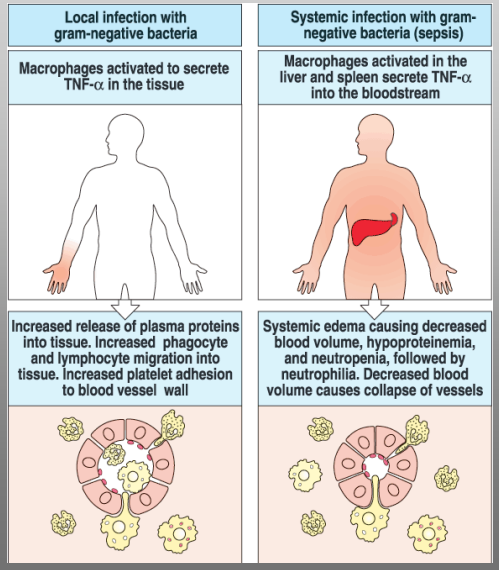


From: Wright, *Nature Rev. Immunol.* **5**:58, 2005

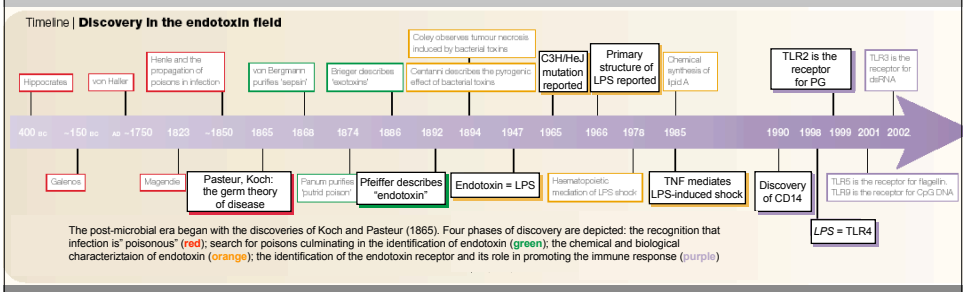
The Scavenger Receptor Superfamily



Receptors Important in The Systemic Response to Infection



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 I 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[10]. It thus appears that the immune-response system mediated by Toll represents an ancient host defence mechanism[9] (Fig. 1). To investigate the possibility that this pathway has been retained in the immune system of vertebrates, we used sequence and pattern searches[11] 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[12]). 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[13,14]. 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 51-amino-acid long N-flanking region that is also present in several other LRR-containing proteins, for example RP105, Decorin and Biglycan[15]. 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, γ/δ 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

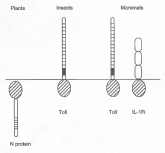


Figure 1 Ancient immune defence systems of plants, insects and vertebrates. A homologous immune response system based on the Toll signalling domain is used in plants, insects and vertebrates. In mammals, Toll induces signals required for the activation of both an innate and an adaptive immune response (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

Discovery of the NF-κB signaling pathway by Toll in *Drosophila* by Hoffman and colleagues

Molecular basis of adjuvant discovered by Medzhitov and Janeway

Use of adjuvant to stimulate the immune response

"Infectious-non-self" model of immunity described by Janeway

Timeline | **Discovery in the endotoxin field**

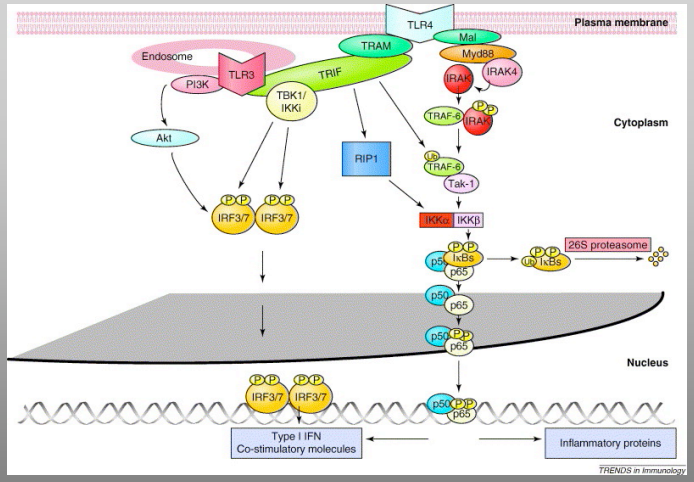
Hippocrates
Galenos
von Haller
Magendie
Henle and the propagation of poisons in infection
Pasteur, Koch: the germ theory of disease
von Bergmann purifies 'sepsin'
Panum purifies 'putrid poison'
Brieger describes 'leucotoxins'
Pfeiffer describes "endotoxin"
Coley observes tumour necrosis induced by bacterial toxins
Centanni describes the pyrogenic effect of bacterial toxins
Endotoxin = LPS
C3H/HeJ mutation reported
Haematopoietic mediation of LPS shock
Primary structure of LPS reported
Chemical synthesis of lipid A
TNF mediates LPS-induced shock
TLR2 is the receptor for PG
Discovery of CD14
LPS = TLR4
TLR3 is the receptor for dsRNA
TLR5 is the receptor for flagellin. TLR9 is the receptor for CpG DNA

400 BC | ~150 BC | AD ~1750 | 1823 | ~1850 | 1865 | 1868 | 1874 | 1886 | 1892 | 1894 | 1947 | 1965 | 1966 | 1978 | 1985 | 1990 | 1998 | 1999 | 2001 | 2002

The post-microbial era began with the discoveris of Koch and Pasteur (1865). Four phases of discovery are depicted: the recognition that infection is" poisonous" (**red**); search for poisons culminating in the identification of endotoxin (**green**); the chemical and biological characteriztaion of endotoxin (**orange**); the identification of the endotoxin receptor and its role in promoting the immune response (**purple**)

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

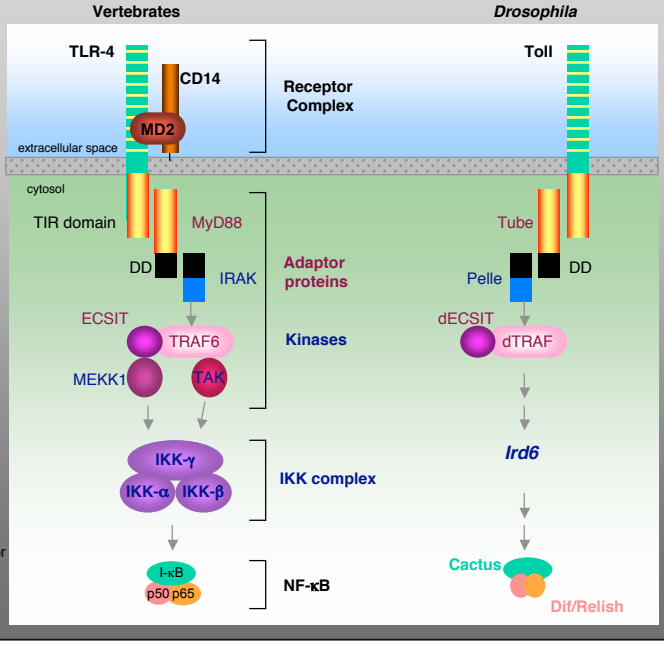
TLR Signaling: Not So Simple



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.

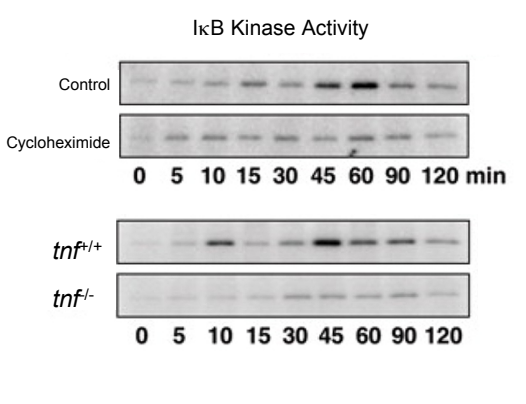
From: Moynagh, *Trends Immunol.* 26:469, 2005

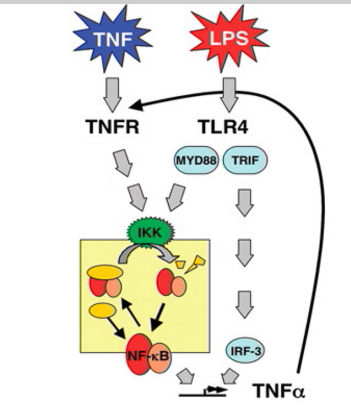
Selected Pro-inflammatory TLR Signaling Components



TIR = Toll/IL-1 receptor
DD = Death domain
IKK = I-κB kinase

Positive Feedback Loops in Innate Immunity



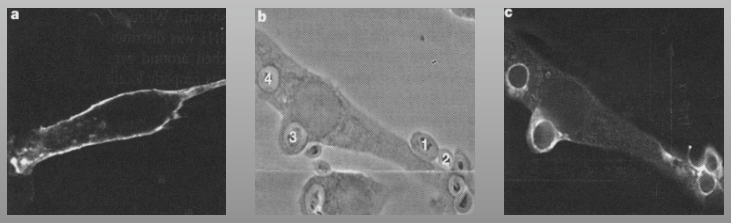


The existence of multiple, overlapping positive feedback loops helps explain why targeting any one pro-inflammatory mediator is typically ineffective in the treatment of severe, systemic inflammation (e.g., during Gram-negative septicemia).

From Werner et al., *Science* 309:1826, 2005

Primitive Specificity in Target Recognition by the Innate Immune System

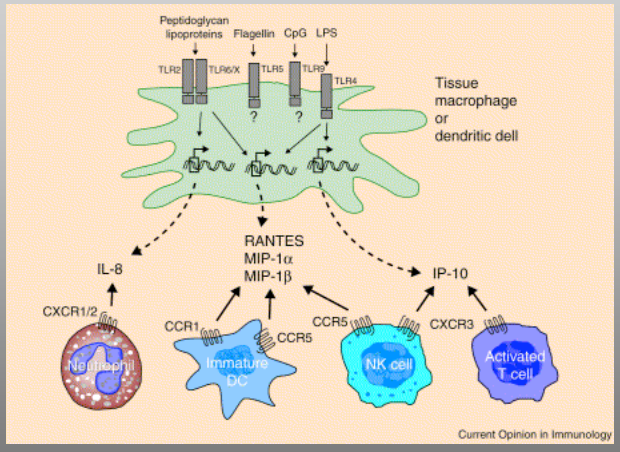
Recruitment of TLR2 to Yeast Phagosomes



TLR2 Phase Contrast TLR2

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

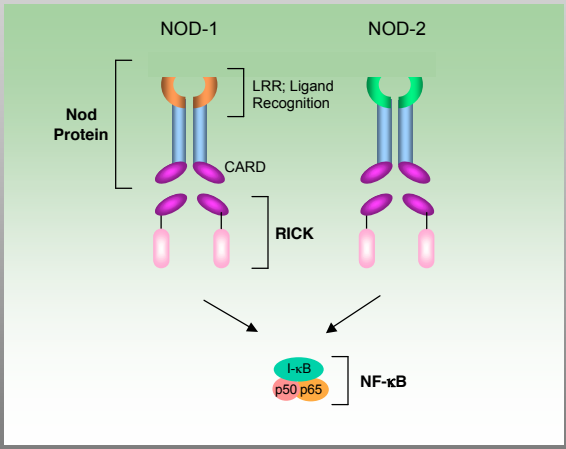
Specificity of TLR Transcriptional Programs



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

Newer Innate Immune Proteins

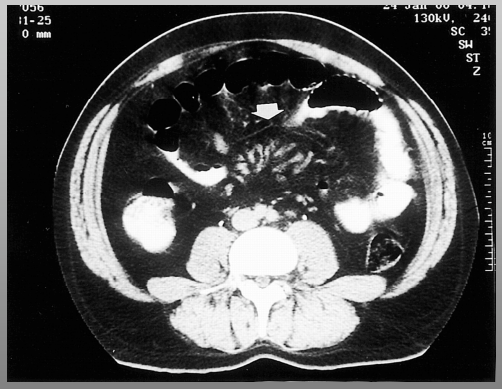
NOD Proteins: Intracellular Peptidoglycan Sensors



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

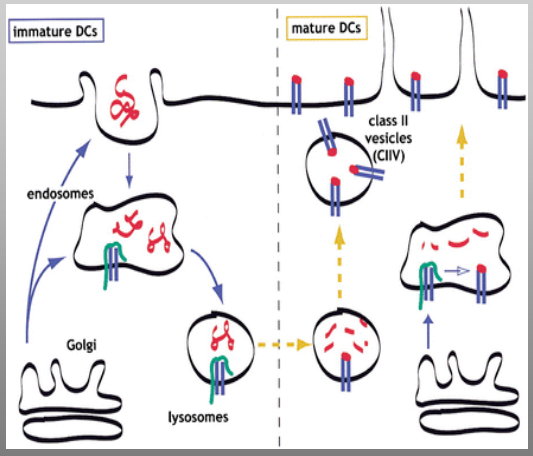
Mutations in Pyrin, Another CARD-containing Innate Immune-like Protein, is Responsible for Familial Mediterranean Fever



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.

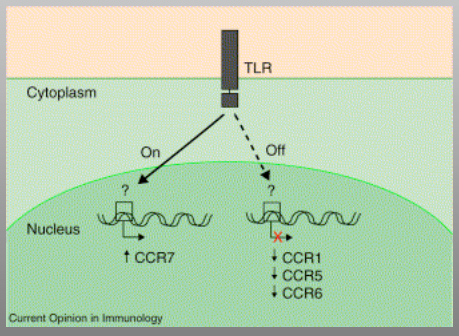
The Dendritic Cell and Development of The Primary Immune Response

Dendritic Cell Maturation



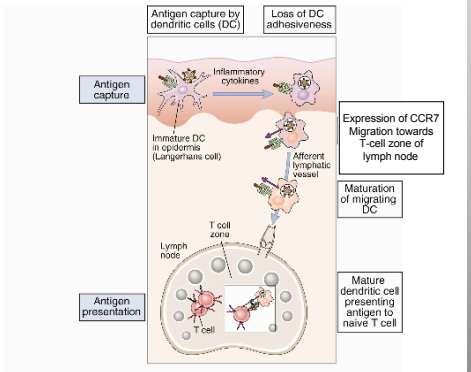
From: Mellman & Steinman, *Cell* 106:255, 2001

The Innate Immune Response Orchestrates DC Trafficking to Secondary Lymphoid Organs



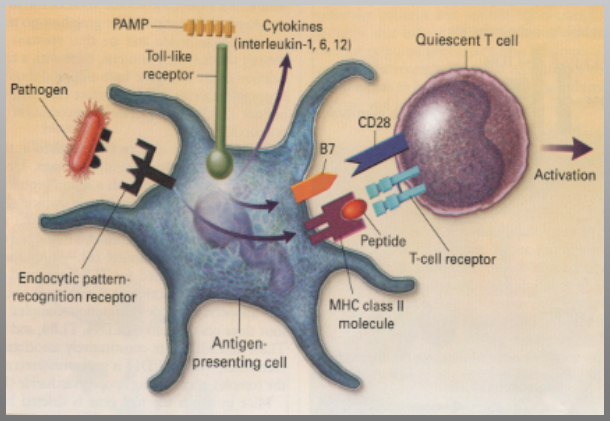
From: Luster, *Curr. Opin. Immunol.* 14:129, 2002

Functional Differences Between Immature and Mature DCs

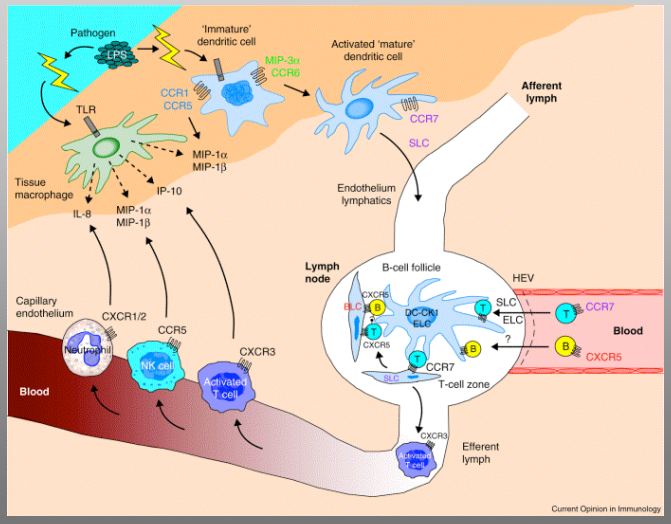


	Immature dendritic cell	Mature dendritic cell
Principal function	Antigen capture	Antigen presentation to T cells
Expression of Fc receptors, mannose receptors	++	–
Expression of molecules involved in T cell activation: B7, ICAM-1, IL-12	– or low	++
Class II MHC molecules		
Half-life on surface	~10 hr	>100 hr
Number of surface molecules	$\sim 10^5$	$\sim 7 \times 10^6$

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

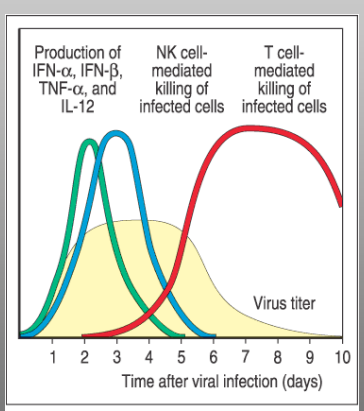
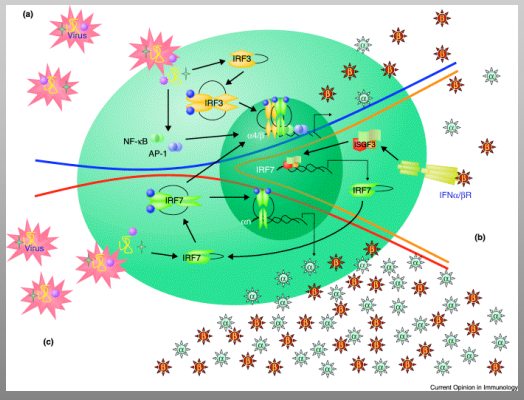


Fig 2.41 © 2001 Garland Science

The Antiviral Response: a Cascade of Transcriptional Events

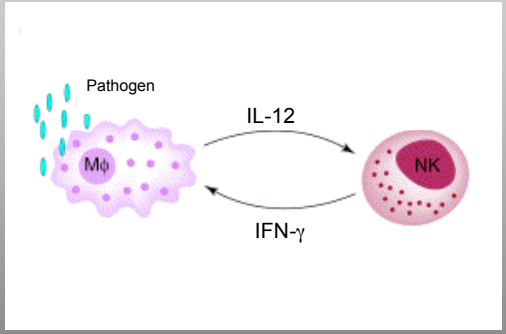


Some targets of IRFs

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.

NK Cells are an Important Early Source of IFN-γ



Modified from: Cooper et al., *Trends Immunol.* 22:633, 2001

Summary

1. Innate immunity is conserved throughout evolution and is triggered by recognition of repetitive 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 (AGEs) 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. Via engagement of a series of adaptor proteins and kinases, it triggers activation of NF-κB (leading to production of TNF-α, for example) and the IRF pathway (and production of IFN-α/β).

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.

6. NK cells, a component of innate immunity, especially to viruses, represent an early source of IFN-γ which serves to stimulate macrophages in inflammatory sites.