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Primer

Toll-like receptors

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Beginning with the physical barrier presented by the epithelium, infectious agents such as viruses and bacteria encounter an array of cellular and molecular countermeasures that evolved within the host to resist them. Host immune responses are of two types, termed innate and adaptive. Immediate defensive responses, which include inflammation, phagocytosis of pathogens, and recruitment of a variety of immune cells, are employed against all classes of microbe, irrespective of prior exposure, and are collectively termed the innate immune response. Innate immunity is evolutionarily ancient, and selected mechanisms are known to be conserved from plants to humans. In contrast, the adaptive immune response is mobilized over a more protracted timescale, is influenced by prior exposure, and, by virtue of antigen-specific receptors generated through somatic DNA recombination within lymphoid clones, is highly specific at the molecular level, often to the point of specificity for a particular microbial species. Adaptive immunity evolved relatively recently and exists only in vertebrates. In mammals, the immune response encompasses the innate and adaptive responses, and, although cross-talk occurs between them, each can be carried out independently by distinct cellular and molecular mechanisms.

Both innate and adaptive immune responses depend on distinguishing self from non-self. For the innate response, a family of cellular receptors called Toll-like receptors (TLRs), which recognize molecules unique to microbes, constitutes the primary strategy for self versus non-self discrimination. The study of TLRs as critical innate immune activators began with classical genetic studies that led to the identification of Toll-like receptor 4 (TLR4) as the receptor for lipopolysaccharide (LPS), a structural component of the outer membrane of Gram-negative bacteria. Recognition of a total of 10 human and 12 mouse TLRs and their distinct microbial ligands followed soon after. The family of TLRs is now known to represent the major microbe-sensing system in mammals, detecting molecules derived from viruses, fungi, bacteria, and protozoa. Intracellular NOD-like receptors, receptors of the retinoic acid-inducible gene I (RIG-I)-like helicase family, C-type lectin receptors, and a subset of the eIF2α kinases also act as microbe sensors. However, some of these sensing systems display TLR dependence, and none is able to fully compensate for a lack of TLR signaling, a condition that results in severe immune deficiency. Here, we review basic concepts of TLR biology, including the ligands, structural properties, cellular localization, and signaling pathways of these receptors. We discuss physiological responses to TLR activation, collectively termed the inflammatory response, and the connection between TLRs and autoimmune and autoinflammatory disease.

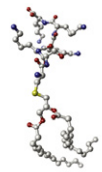
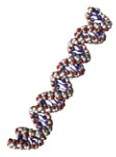
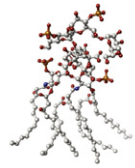
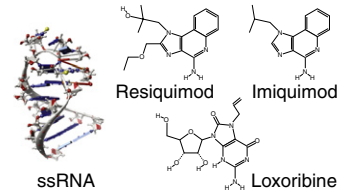
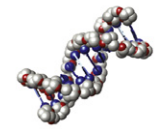
TLRs sense molecular signatures of microbes

TLRs are single-pass type I transmembrane-spanning proteins characterized by multiple extracellular leucine-rich repeats (LRRs) and a single intracellular Toll/interleukin-1 (IL-1) receptor (TIR) domain that is homologous to the intracellular domain of IL-1 receptor family members. In mice and humans combined there are 13 paralogous TLRs; 10 in humans and 12 in mice. Each recognizes and is activated by a small collection of microbe-derived molecules, as determined through studies of targeted mutant mice lacking individual TLRs. Lipopeptides and other components of Gram-positive bacterial cells activate TLR2 in conjunction with either TLR1 or TLR6; LPS is detected by TLR4 (discovered by forward genetic studies as mentioned above); flagellin is detected by TLR5; poly I:C, a double-stranded RNA (dsRNA) analog, is detected by TLR3; unmethylated DNA and CpG-oligodeoxynucleotides (CpG-DNA) are detected by TLR9; and single-stranded RNA and its synthetic analogs resiquimod, imiquimod, and loxoribine activate TLR7 (Figure 1A). The ligands for TLR8, TLR10 (only present in humans), and TLR11–13 (only present in mice) remain unknown.

Seven TLRs have recognized ligands, and collectively these receptors detect all known infectious agents via their clusters of extracellular LRRs. The LRR module has been conserved in plants, insects, and mammals as a recognition and binding element for microbial molecules. In some cases, TLR ligands must initially or exclusively interact with transmembrane or membrane-associated accessory proteins or co-receptors that affect the conformation of TLRs and thereby permit downstream signaling. These accessory proteins include MD-2 (which is necessary for TLR4 to bind LPS) and CD14 (which allows TLR4 to distinguish between smooth (containing long *O*-polysaccharide chains) and rough (lacking *O*-polysaccharide chains) LPS chemotypes). CD14 and CD36 both serve to augment, but are not absolutely required for, TLR2 signaling. Granulin and high mobility group (HMG) B proteins have been proposed to deliver CpG-DNA to TLR9 through an ability to bind simultaneously to both CpG-DNA and TLR9.

The three-dimensional crystal structures of the ectodomains of TLR3, TLR4, TLR2–TLR6, and TLR2–TLR1 revealed a curved solenoid shape characteristic of LRR-containing proteins. TLRs form homodimers or heterodimers induced by the simultaneous binding of ligands to residues in the LRRs of distinct receptor chains. TLRs utilize diverse strategies for ligand recognition (Figure 1B). For example, TLR3 interacts with dsRNA using lateral surface-exposed sites on the concave side of each ectodomain. In contrast, TLR2–TLR1 and TLR2–TLR6 heterodimers interact with lipopeptides via a hydrophobic pocket formed by residues on the convex side of each ectodomain near the center of the dimer. In the case of TLR4, the interface between TLR4 and its required co-receptor MD-2 must first form, followed by LPS-induced dimerization of two TLR4–MD-2 dimers. LPS simultaneously contacts a hydrophobic pocket within MD-2 (from one dimer) and the convex

A

Receptor	TLR2/1 or 2/6	TLR3	TLR4	TLR5	TLR7	TLR9
Ligands	Lipopeptides	Poly I:C, dsRNA	LPS	Flagellin	ssRNA, resiquimod, imiquimod, loxoribine	Unmethylated DNA, CpG-DNA
Source	Gram-positive bacteria, fungi	Viruses	Gram-negative bacteria	Bacterial flagellum	Viruses	Bacteria
Examples	 Pam_2CSK_4	 dsRNA	 LPS	 Flagellin	 Resiquimod Imiquimod ssRNA Loxoribine	 CpG-DNA

B

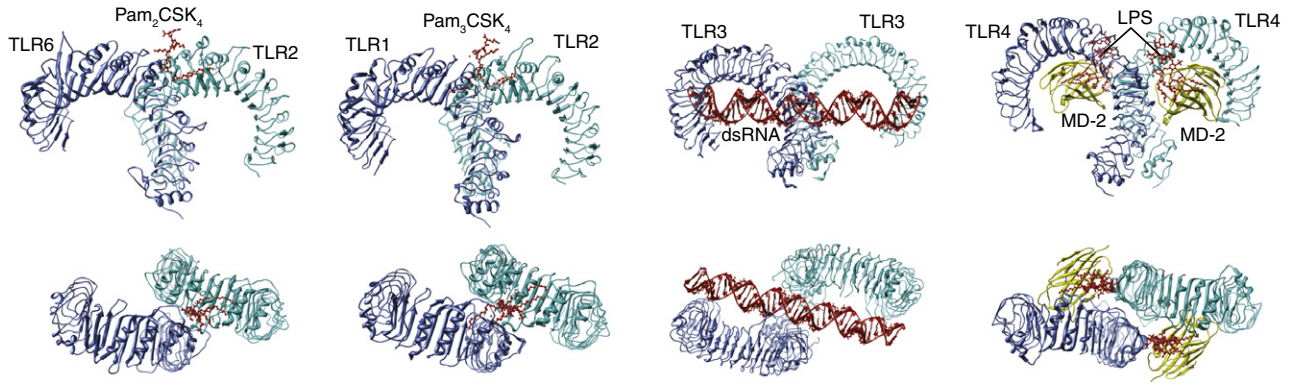


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Figure 1. TLR ligands and interactions with receptors.
(A) Three-dimensional structures of the lipopeptide Pam_2CSK_4 (from 3A79), double-stranded RNA (from 3CIY), LPS (from 3FXI), flagellin (3K8V), a tRNA as a model of single-stranded RNA (2L9E), and unmethylated CpG-DNA (from 3QMB). Gray, blue, red, orange, and yellow spheres represent carbon, nitrogen, oxygen, phosphorus, and sulfur atoms, respectively. The chemical structures of resiquimod, imiquimod, and loxoribine are also shown. Possible microbial sources of ligands are indicated. (B) Structures of TLR2–TLR6–Pam_2CSK_4 lipopeptide (3A79), TLR2–TLR1–Pam_3CSK_4 lipopeptide (2Z7X), TLR3-dsRNA (3CIY), and TLR4–MD-2–LPS (3FXI). Side view (upper panels) and top view (lower panels) are shown. Protein Databank ID numbers are indicated in parentheses. (All figures were generated with UCSF Chimera.)

surface of TLR4 (from the other dimer). Despite the variation in modes of ligand recognition, all known TLR dimer structures display the same arrangement, with the two carboxy-terminal tails closely juxtaposed and the amino termini at opposite ends of the dimer. This conformation may be required to bring the intracellular TIR domains into close proximity to initiate signaling.

The response elicited by TLR activation: inflammation

TLRs act principally to initiate an innate immune response, and inflammation is the central hallmark of this response. TLRs have also been described as 'necessary' or 'required' for an adaptive immune response. This conclusion may initially have been prompted by the long-known fact that molecules ultimately recognized as TLR ligands, including LPS, poly I:C, and CpG-DNA, do indeed enhance the adaptive immune response, i.e. have adjuvant properties. However, there remains no evidence of adaptive immune failure in the absence of TLR signaling. To the contrary, robust adaptive immune responses, including antibody production and activation of cytotoxic T lymphocytes, occur after infection in animals that lack TLR signaling. Classical adjuvants, including Freund's complete adjuvant, elicit strong antibody responses in animals that lack TLR signaling. Moreover, some adjuvants that contain TLR ligands are able to initiate adaptive immune responses in the absence of TLR signaling as well, suggesting that they have multiple mechanisms of action. Adaptive immune responses are elicited by a range of mechanisms, and not merely by TLR signaling.

Systemic inflammation induced by TLRs results primarily from the activation of macrophages and neutrophils, cell types with specialized functions in innate immunity (Figure 2). TLR ligands cause macrophages to produce inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1 and IL-6. Cytokines act on immune cells by binding to specific cell-surface receptors, which elicit complex cellular responses. Inflammatory cytokines produced by activated macrophages near the site of infection promote inflammation by increasing the permeability of the vascular endothelium to plasma, increasing the propensity of neutrophils to bind to the microvascular endothelial surface and move out of the vascular system by diapedesis, and causing the release of antimicrobial peptides and small molecules such as leukotrienes

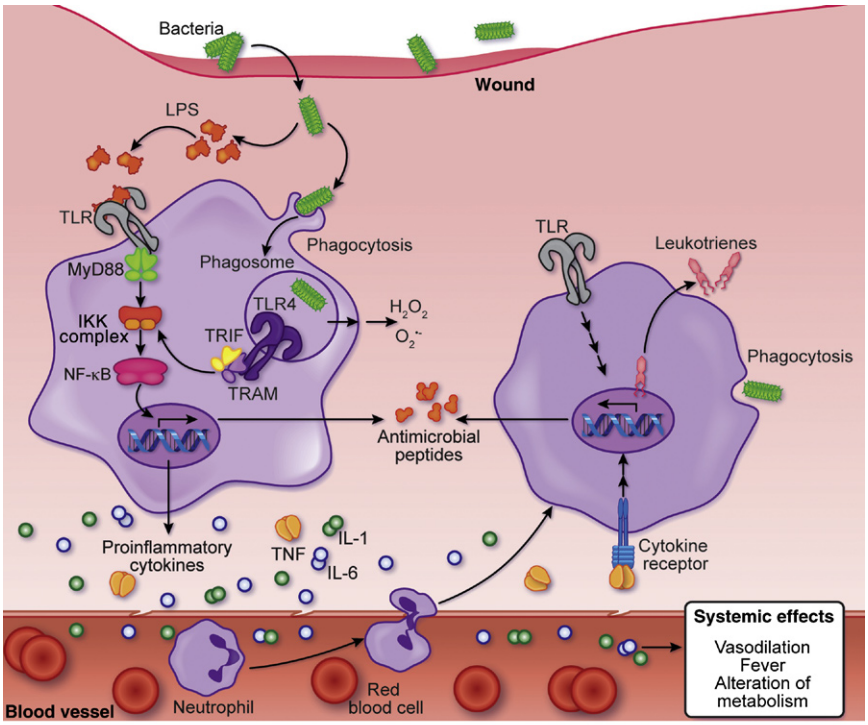

Figure 2. TLR signaling elicits inflammation.
Pathogens, such as bacteria, may enter the host through a breached epithelial barrier, leading to activation of macrophage TLRs by pathogen-derived molecules, including LPS. TLR signaling leads to the production of inflammatory cytokines, which act near the site of infection to recruit neutrophils and induce neutrophil production of antimicrobial molecules, including peptides, reactive oxygen species (e.g. H_2O_2 and superoxide anion), and leukotrienes. Cytokines also travel throughout the body and induce systemic effects. Both macrophages and neutrophils act to limit infection by phagocytosis of pathogens.

(lipid mediators of inflammation) and reactive oxygen species from neutrophils (Figure 2). Operating at sites distant from the infection, cytokines also mediate systemic effects, such as fever, and may cause generalized vasodilation and reduce the contractile strength of the heart. Cytokines may also alter host metabolism, usually opposing the effects of insulin and encouraging a catabolic state.

In addition to producing cytokines (and thereby influencing neutrophils, endothelial cells, and other cells of the host), macrophages directly engulf microbes and generate reactive oxygen species and antimicrobial peptides. They are commonly the principal host cells for pathogens that are not easily eradicated, including various fungi, mycobacteria, and protozoa.

TLRs are expressed by both macrophages and neutrophils. They are also expressed by dendritic cells, specialized phagocytes that primarily serve to stimulate adaptive immune responses by activating T lymphocytes through cell-surface presentation of antigens. As discussed below, a subset of dendritic cells, the plasmacytoid dendritic cells (pDCs), require a special set of proteins to properly establish the TLR signaling system that detects nucleic acids. It may be inferred that different cell types transport and utilize TLRs in somewhat different ways. Certain TLRs are expressed in B cells, which can be activated by TLR ligands to promote mitogenesis. Many 'non-immune' cell types, including epithelial cells, neurons, astrocytes, and fibroblasts, also express TLRs and respond to their activation. In some cases, as in the gut epithelium, the response is proliferative. In other cases, it resembles the classical inflammatory response. The expression and function of TLRs in diverse cells implies that the innate immune response results from the concerted actions of multiple cell types rather than a few specialized immune cell types.

TLR signaling

Signal transduction from TLRs ultimately induces the expression of numerous genes required for the inflammatory response, including inflammatory cytokines, chemokines (a large family of cytokine molecules that engage G-protein-coupled receptors), antimicrobial molecules (e.g. hydrolytic enzymes, peptides, proteases), and major histocompatibility (MHC) and costimulatory molecules important for adaptive immune activation. TLR signaling depends critically on a total of four adaptor proteins— MyD88, TIRAP (also called MAL), TICAM1 (also called TRIF), and TICAM2 (also called TRAM) — that directly bind to activated TLRs and recruit downstream signaling components. Ligand binding to TLRs is believed to alter the association or conformation of TLR ectodomains, changes that are propagated to the intracellular TIR domain of the TLR. Through unknown mechanisms, the TIR domains of adaptors then associate with receptor TIR domains. Some structural data have recently revealed a helical 'myddosome' as the active adaptor conformation.

With the exception of TLR3, which signals exclusively via TICAM1, all TLRs utilize a MyD88-dependent pathway resulting in the production of TNF-α, IL-1, IL-6, and other cytokines dependent on NF-κB, and also trigger mitogen-activated protein (MAP) kinase cascades that lead to activation of AP-1 and cyclic AMP (cAMP) response element-binding protein (CREB). MyD88 can, when stimulated by some of the TLRs, also activate the transcription factor interferon regulatory factor 7 (IRF7), leading to the induction of type I interferons (IFNs), which are especially potent anti-viral cytokines. The TICAM1-dependent pathway utilized by TLR3 is able to activate NF-κB, and additionally activates IRF3 to induce type I IFNs. TLR4 can also activate the TICAM1-dependent pathway from within late endosomes following phagocytosis (Figure 2). A basic delineation of the pathways is depicted in Figure 3. The details of TLR signaling have been intensively investigated, and much is known about the cell-type- and stimulus-dependent nuances of the pathways. Stepping back from these particulars, a key point to appreciate is the

importance of phospho- and ubiquitin-dependent interactions for signaling from all TLRs; the activities of kinases and ubiquitinases dominate the TLR signaling cascades and may allow for enormous signal amplification, in that a few activated TLR complexes are able to cause dramatic transcriptional changes within a cell.

Post-transcriptional changes also occur within cells in response to TLR signaling. Well known is the enhanced stability and translational efficiency of TNF-α mRNA, which is poorly transcribed in resting cells because of a blockade imposed by an AU-rich element in the 3' untranslated region. The RNA-binding protein tristetraprolin appears to control TNF-α mRNA stability, whereas T-cell intracellular antigen-1 (TIA-1) controls translational efficiency. Other cytokine mRNAs bear a similar AU-rich element and may be subject to similar control. Still other molecules, such as the zinc-finger-containing ribonuclease Zc3h12a, respond to TLR signaling and govern the expression of IL-6 by causing mRNA decay.

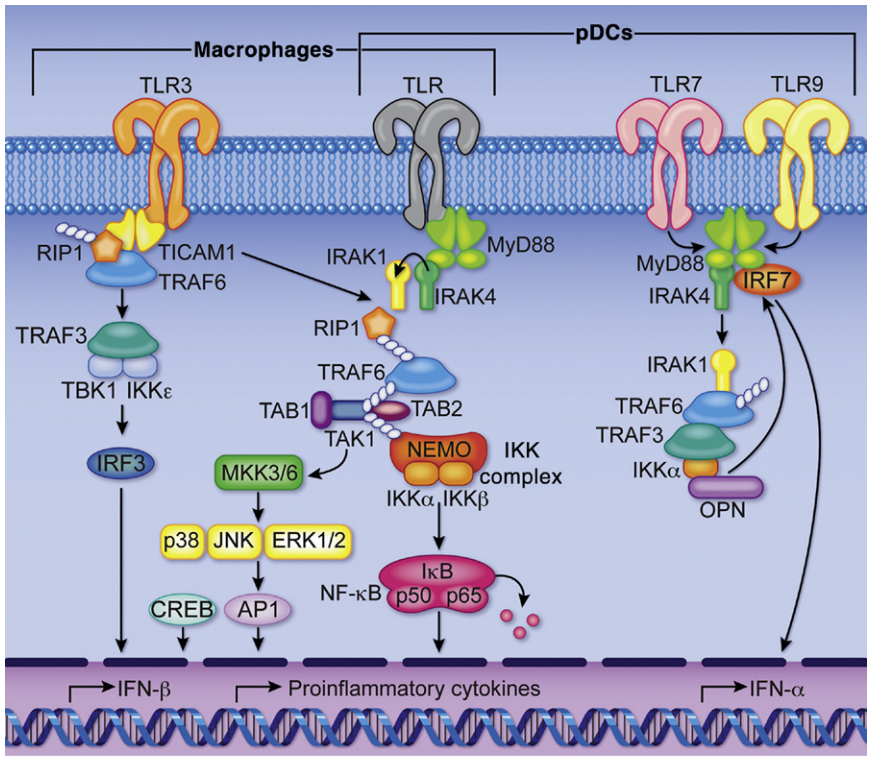

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Figure 3. TLR signaling.
The MyD88-dependent pathway leading to proinflammatory cytokine production (center) is activated in macrophages by all TLRs except TLR3. Upon TLR activation, MyD88 recruits IRAK4 through death-domain interactions. IRAK4 phosphorylates IRAK1 and IRAK2 that, in turn, activate the E3 ubiquitin ligase TRAF6, which together with UBC13 and UEV1A (not depicted) polyubiquitinates NEMO and itself. TRAF6 also activates TAK1. The TAK1-associated TAB2 protein serves as a receptor for the polyubiquitin chains on TRAF6 and NEMO and holds these proteins together. TAK1 phosphorylates IKKβ, activating the IKK complex to phosphorylate IκB leading to IκB degradation and release of NF-κB. TAK1 also activates MKK3 and MKK6, resulting in activation of CREB and AP1. NF-κB, CREB, and AP-1 transcription factors induce transcription of proinflammatory cytokines, such as TNF-α and IL-1. Signaling from TLR3 in macrophages (left) induces IFN-β through a complex of TICAM1, TRAF6, and RIP1; the complex associates with TRAF3 to activate TBK1 and IKKε, which, in turn, phosphorylate IRF3. Upon TLR3 activation, TRAF6 and RIP1 can also activate NF-κB and MAPKs to induce proinflammatory cytokines. pDCs utilize a distinct pathway to elicit large amounts of type I IFN, primarily IFN-α (right). Activation of TLR7 or TLR9 in pDCs recruits MyD88 and IRAK4, which then interact with TRAF6, TRAF3, IRAK1, IKKα, osteopontin (OPN), and IRF7. IRAK-1 and IKKα phosphorylate and activate IRF7, leading to transcription of type I IFN. pDCs also induce proinflammatory cytokine production downstream of TLR7 and TLR9 by signaling through the MyD88-dependent pathway resulting in NF-κB activation (center).

Compartmentalization of TLRs that sense nucleic acids

An important feature of the TLR signaling system is that nucleic-acid-sensing TLRs (TLR3, TLR7, TLR8, and TLR9) are localized intracellularly, whereas the other TLRs (TLR1, TLR2, TLR4, TLR5, and TLR6) are expressed predominantly on the cell surface. TLRs 3, 7, 8, and 9 detect ligands and signal from the endosomal compartment, where they are trafficked from the endoplasmic reticulum (ER) via the secretory pathway. The general chaperones Gp96 and PRAT4A are necessary for trafficking many of the TLRs, including TLR7 and TLR9 to endolysosomes, and TLR1, TLR2, and TLR4 to the plasma membrane. A twelve-pass transmembrane-spanning protein called UNC93B1 directly binds and is required for endolysosomal localization of TLR3, TLR7, and TLR9.

Because of their responsiveness to nucleic acids, the intracellular TLRs are particularly important for detecting viruses, some of which cannot be sensed by any other mechanism. However, intracellular TLRs are equally sensitive to host DNA, and trigger innate immune responses if exposed to it. Since host DNA is usually excluded from endolysosomes, the compartmentalization of TLR3, TLR7, TLR8 and TLR9 is thought to prevent innate immune activation by host DNA that could lead to the development of autoimmune disease. The strong drive to avoid this outcome is underscored by additional restrictions on signaling from these TLRs. For example, their activation and signaling also require endosomal acidification, and typically occur during a particular stage of endosome maturation. Endosomal acidification may contribute to the processing, such as cleavage, of the TLRs for efficient signaling. Protease-mediated cleavage of TLR7 and TLR9 occurs in endolysosomes but not in the ER, and in the case of TLR9 increases its binding to CpG DNA and permits recruitment of MyD88 and initiation of signal transduction. Cleavage of TLR3 has not been reported.

TLR signaling in pDCs

pDCs express TLR7 and TLR9 to the exclusion of all other TLRs, and upon TLR7 or TLR9 activation are capable of producing type I IFN in quantities

at least three orders of magnitude greater than that produced by any other cell type. These properties make pDCs uniquely adapted for sensing and responding to viruses. To produce abundant type I IFN, pDCs utilize a TLR pathway not present in macrophages, conventional dendritic cells, or B cells. This MyD88-dependent pathway leads to phosphorylation and activation of IRF7, which induces the transcription of type I IFN genes (Figure 3).

pDCs also produce proinflammatory cytokines in response to TLR7 or TLR9 ligands, using the same MyD88-dependent pathway that operates in macrophages. Type I IFN or proinflammatory cytokines are preferentially produced by pDCs in response to TLR9 activation by one of two types of CpG molecule — A-type CpG-DNA or B-type CpG-DNA. Structurally, A-type CpG-DNA forms aggregates that are retained in the early endosomes of pDCs, whereas B-type CpG-DNA remains monomeric and traffics rapidly through early endosomes into acidified late endosomes or lysosomes. It has now been shown that TLR9 engagement by either type of CpG-DNA in the early endosome leads to IRF7 activation and type I IFN production. In contrast, TLR9 activation in the late endosome results in NF-κB activation and TNF-α production. Whether different viral DNA species are differentially trafficked through endolysosomes, thereby dictating the ratio of type I IFN and proinflammatory cytokines generated, remains unknown.

TLR signaling in pDCs is distinctive not only because of the existence of a special pathway leading to type I IFN production, but also because establishing this pathway and/or the machinery to activate it requires a group of proteins not necessary in other cell types. In addition to the chaperones Gp96, PRAT4A, and UNC93B1, lysosomal sorting proteins of the AP-3, BLOC-1 and BLOC-2 complexes and a twelve-pass transmembrane spanning peptide–proton symporter channel called Slc15a4 are required to permit TLR9 signaling, leading to both type I IFN and proinflammatory cytokine production by pDCs. What function these proteins serve in setting up TLR signaling in pDCs is an open question. Hypotheses include aiding in the transport of pDC-specific components of the TLR signaling pathway to endolysosomes, moderating the acidification of endolysosomes, eliminating inhibitory peptides from endolysosomes, and/or serving as transporters of TLR ligands to endolysosomes.

Aberrant TLR activation is associated with disease

Many diseases are now known to be mediated by cytokines, insofar as specific cytokine blockade can substantially alleviate them. Among these diseases are rheumatoid arthritis, Crohn's disease, ankylosing spondylitis, and psoriasis, for which blockade of TNF-α activity is therapeutic. Although TLRs are not the only receptors capable of initiating TNF-α production, they are likely the receptors most potent in doing so. The intriguing possibility that endogenous ligands may exist for TLRs has prompted the suggestion that proteins, lipids, glycans, and nucleic acids of the host might, under some circumstances, trigger inflammation in the absence of microbial infection, or 'sterile inflammation'. Alternatively, 'sterile inflammatory diseases' might not really be sterile after all, and some or all of these diseases might be microbe driven.

Perhaps the best case for a pathogenic role of TLR signaling in a sterile inflammatory disease may be seen in systemic lupus erythematosus (SLE), modeled in mice with mutations such as the lymphoproliferation (*lpr*) allele of *Fas* (*Cd95*), and the Y-linked accelerator of autoimmunity (*Yaa*). It was demonstrated that B lymphocytes with specificity for immunoglobulin G (IgG) are mitotically activated when the IgG that is recognized engages DNA or ribonucleoprotein. This suggests the existence of an autoamplification loop in which a B-cell clone with specificity for DNA might, if exposed to DNA, internalize a TLR ligand, leading to cell activation and clonal expansion.

The pathogenic role of TLR7 in SLE was demonstrated in the *Fas*^{lpr} model of the disease, in that targeted deletion of *Tlr7* markedly attenuated disease in this model. Consistent with this finding, the *Yaa* locus proved to be a duplication encompassing the *Tlr7* gene within the pseudoautosomal region of the Y chromosome. Hence, an excess of TLR7 signaling accelerates SLE, while abrogation of TLR7 signaling abolishes disease. Similarly, mutation at the *Unc93b1* locus strongly suppressed SLE, as did a *Myd88* mutation. Curiously, *Tlr9* knockout augmented disease, an observation that may indicate competition between TLR7 and TLR9 for transport to the endosome by UNC93B1. These observations indicate that, in SLE, TLR7 (and possibly TLR9) signaling is pathogenically important. These TLRs themselves, and possibly components of the system that supports TLR signaling within the endosome, may be considered targets for therapeutic intervention.

In other inflammatory disease states, notably the severe systemic inflammation and autoimmunity triggered by loss of the protein tyrosine phosphatase Shp1, TLRs are also critically important. Moreover, inflammation is initially triggered by microbes because germ-free animals do not develop disease. MyD88 signaling appears to be important, although not necessarily essential, for the inflammatory disease that results from loss of regulatory T-cell function due to deletion of Foxp3, a master regulator of regulatory T-cell development. Here, too, the importance of microbes might be inferred, in that adaptive immune responses to microbes may be the driving force of disease. As specific TLR inhibitors are developed, whether antibodies or small molecules, we may learn that many inflammatory diseases can be controlled with a high degree of specificity.

Conclusions

Several key concepts define our current understanding of TLR function. First, TLRs are critical activators of the innate immune response, which is characterized by inflammation, induced in large part by inflammatory cytokines produced in a TLR-dependent manner by macrophages. Second, TLR ligands represent all known pathogens and bind by diverse modes to overall structurally similar receptor extracellular domains, leading to the recruitment of adaptors that initiate signaling cascades. Third, the inflammatory response triggered

by TLR activation is a double-edged sword, defending against infection but also potentially harmful to the host, because aberrant activation may lead to autoimmunity and inflammatory disease. Within the framework of this understanding, it is hoped that future studies will permit the development of targeted therapies for both infectious and autoimmune diseases.

Further reading

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Learning to reach for ‘invisible’ visual input

Warrick Roseboom and Derek H. Arnold

Patients who have suffered damage to primary visual cortex can report being blind but display some proficiency when manually interacting with ‘unseen’ objects — a phenomenon known as blindsight [1–4]. There is conflicting evidence about analogous situations in normally sighted people [5–7]; however, to date no study has attempted to assess a *directly* analogous situation, to have normally sighted people *interact* with unseen stimuli. We used a form of binocular masking to suppress awareness of oriented stimuli [8]. Despite initial insensitivity when making verbal judgements, participants who reached as if to grasp perceptually suppressed stimuli displayed increasing proficiency with training and feedback. This was not simply due to practise, as another group did not develop such proficiency when completing a matched number of trials, with feedback, while making verbal responses; however, this same group subsequently developed sensitivity when they too completed training with reaching and feedback. Our data thus reveal a special status for attempts to grasp perceptually suppressed stimuli.

We used presentations of high contrast white noise to one eye to suppress awareness of pairs of oriented lines in the other eye (Figure 1 and see Supplemental

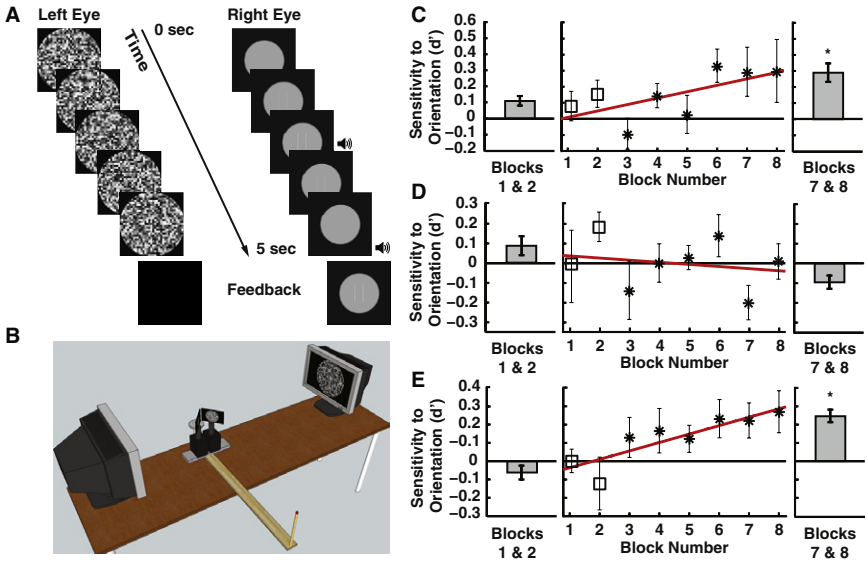

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Figure 1. Example trial sequence, experimental apparatus and data from Experiments 1–3. (A) Depiction of a trial sequence with a vertical target stimulus. Target stimuli reached peak contrast 2.5 seconds into the trial, accompanied by a tonal pip to prompt the participant to respond. A second pip signalled the conclusion of the trial and, on reaching trials, let participants know they could return their hand to a resting position. When feedback was provided, it was presented for one second following the five second trial sequence (Supplemental Movie 1). (B) Depiction of experimental apparatus from the right rear. Participants sat with their head on a chinrest and observed the stimulus presentation via half silvered mirrors. (see Supplemental Information for further details). (C) Participant sensitivity (*d*’) to the target orientation in eight blocks of trials from Experiment 1. In the first two blocks of trials, given by the box data points in the central panel, participants responded verbally and were given no feedback. In the final six blocks of trials (star data points), participants responded by reaching out and pretending to grasp the oriented target and were given trial-by-trial feedback. The red line shows a linear regression to the data. Bar plots show participants’ overall performance during the first (left panel) and last (right panel) two blocks of trials. In these plots a star indicates that this level of sensitivity was significantly different from zero. Error bars show ± 1 SEM. (D) and (E) show the same as (C) for Experiments 2 and 3, respectively.