

REVIEW

Toll-like receptors: their role in allergy and non-allergic inflammatory disease

I. Sabroe, L. C. Parker, A. G. Wilson, M. K. B. Whyte and S. K. Dower

Section of Functional Genomics, Division of Genomic Medicine, University of Sheffield, Sheffield, UK

Summary

The human TLRs comprise an important and interesting group of receptors that regulate pathogen-related responses, and play as yet uncharacterized roles in the amplification of sterile inflammation. Signalling through these receptors, which are powerfully coupled in gene transcription processes, has powerful immunostimulatory and immunomodulatory effects. Exploitation of TLR signalling will probably lead to novel effective therapies for allergic disease, in the first instance through more efficient mechanisms of immunotherapy. The likelihood of adverse consequences of such treatments, though possible, may be minimized by use of conjugated vaccines.

Introduction

Three years ago, few working in the fields of allergy and immunology had heard of Toll-like receptors (TLRs). Now, they seem to be everywhere: and with good reason, since some of the most promising treatments for allergic disease may work via TLRs, and signalling via these receptors may be responsible for driving the maturation of the adult immune system. This review will address the current knowledge of TLR ligands and signalling, their potential roles in allergic inflammation, and their use as novel adjuvant targets to bias immune responses.

TLR 'ligands'

The TLRs are named for their functional and structural homology to a receptor found in the *Drosophila* fruit fly, named Toll (there are now several *Drosophila* Tolls). Toll is essential in activating anti-fungal defence mechanisms in the fly. In humans, TLRs have come to prominence through their unlocking of the secrets of bacterial lipopolysaccharide (LPS) signalling, though it is now clear that they play important roles in the signalling of many pathogen-related molecules and also some endogenous proteins associated with immune activation.

It had been known for some years that CD14 plays an essential role in LPS signalling. However, CD14 has no intracellular signalling face, and can permit LPS responsiveness in soluble form [1], so cannot be solely responsible for LPS signalling in inflammatory cells. Missing components of the LPS response complex were identified when examination of mice resistant to LPS revealed they had defects in a mammalian Toll-like receptor, TLR4 [2].

Then began a slightly confusing story, trying to establish which molecules are required for LPS signalling in humans. The first human TLR linked with signalling in response to LPS was TLR2 [3]. However, other early experiments also showed that, like mice, TLR4 could be involved in the responses of human cells to LPS [4–7]. The explanation for these different results probably lies in the nature of the stimuli tested. The majority of commercial LPS preparations contain small amounts of bacterial lipoproteins (BLPs). These commercial LPS preparations can activate both TLR2 and TLR4. However, repurification of the LPS preparations from many bacteria, including *E. coli* and *Salmonella* sp., leaves a lipoprotein-free LPS preparation that stimulates TLR4 alone [8]. In contrast, synthetic bacterial-type lipoproteins (consisting of palmitoylated small peptides) and purified bacterial lipoproteins, signal via TLR2 but not via TLR4 [9–11]. These data suggest a relatively clean segregation of roles between TLR2 and TLR4, with the former involved in responses to BLPs and the latter to LPS. TLRs need to be dimerized to signal and in the case of TLR4, it seems to signal as a homodimer. In contrast, TLR2 signals only as a Toll-like receptor heterodimer, with either TLR1 [12, 13] or TLR6 [13]. Bacterial lipoproteins signal via a TLR2 heterodimer (though it is not entirely clear which other TLR is involved in this setting [13]) and TLR2/TLR6 is involved in signalling to yeast cell wall products [13], peptidoglycans and various non-LPS bacterial cell wall products [14] and borrelial lipoproteins [15]. TLR2 also has roles in the responses to treponemal glycolipids [16], trypanosomal cell wall products [17], and mycobacterial lipopeptides and cell wall products [15, 18–20].

So in general, TLR2 signals in response to a wide range of lipopeptides, and TLR4 in response to LPS, with CD14 being required as an accessory molecule for efficient LPS signalling. However, other accessory molecules are also involved in LPS responses, in particular MD-2, a transmembrane or soluble protein that is required for efficient LPS responses [21–23]. Interestingly, MD-2 may also confer an ability for TLR2 to respond to purified LPS [22], despite the current general dogma that focuses on TLR4 signals in LPS responses. Other cell surface molecules such as the integrin CD11b/CD18 are also involved in TLR signalling in response to LPS [24, 25] and perhaps to yeast cell wall products [26], although CD14-dependent, CD11b/CD18-independent mechanisms may predominate in the activation processes of human leucocytes by LPS [24].

What about the other TLRs? There are currently 10 described TLRs [27–30] (Fig. 1). Roles for TLRs 1, 2, 4 and 6 are noted above. TLR3 is involved in responses to double-stranded (viral) RNA [31] and TLR5 mediates responses to flagellin [32]. TLR9 mediates responses to bacterial DNA [33–35]. Roles for the other TLRs remain to be described. The responses of man to

Correspondence: Dr Ian Sabroe, Section of Functional Genomics, Division of Genomic Medicine, University of Sheffield, M Floor, Royal Hallamshire Hospital, Sheffield S10 2JF, UK. E-mail: i.sabroe@sheffield.ac.uk

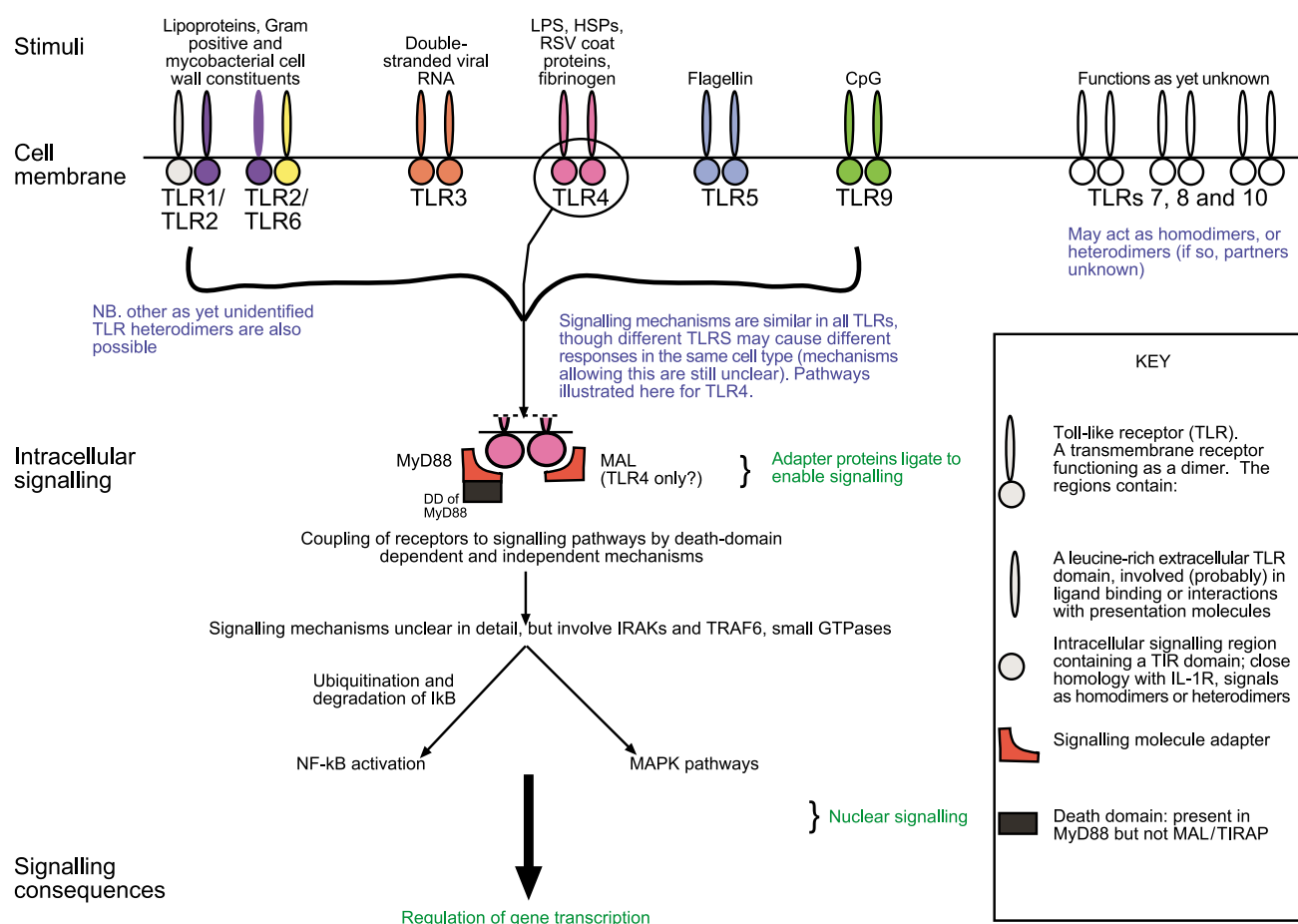


Fig. 1. A brief scheme of TLR stimulating activities and signalling consequences.

bacterial DNA motifs are of particular interest in allergy. Bacterial DNA contains many CG nucleotide pairs, known as 'CpG' motifs. The cytosine in this pair is characteristically unmethylated (whereas cytosines in human DNA are more commonly methylated), and the frequency of this pairing is suppressed in human DNA [36]. These sequences are powerful stimuli of the immune system, acting via TLR9 on monocytes, macrophages and NK cells to cause generation of IL-12 and IFN- γ [36, 37], and they also have significant roles in modulating dendritic cell function [37–41]. Short oligonucleotides (of the order of 20 bases or so) can be readily manufactured that contain these CpG motifs, resulting in B cell, dendritic cell and monocyte stimulatory activities [37–42]. Within the dendritic cell (DC) subset, CpG responses appear confined to dendritic cells of the plasmacytoid lineage, rather than myeloid-derived DC [40, 41]. This is in keeping with TLR expression patterns, since myeloid DCs express TLRs involved in LPS responses whilst plasmacytoid DCs do not express TLR2 and TLR4, do not respond to LPS and peptidoglycan, but do express TLR9 [38, 40, 41]. Interestingly, the DNA sequence around the oligonucleotide containing the CpG motif can affect the kinds of cell stimulated by the sequence, e.g. some sequences are better at stimulating NK cell activation, others at stimulating monocytes and B cells [42]. How these differences are mediated is not clear, but it might be that TLR9 can also heterodimerize with other TLRs in ways analogous to TLR2 described above, resulting in

altered specificity of responses to CpG-containing oligonucleotides.

Surprisingly, TLR4 mediates signalling in response to a range of stimuli other than LPS. These include respiratory syncytial virus coat proteins [43] and bacterial heat shock proteins [44–46]. However, TLR4 also mediates responses to endogenous proteins such as human heat shock protein 60 (HSP60), which is released from damaged cells, and TLR4 also mediates pro-inflammatory responses to fibrinogen [44–48]. TLR4 expression is up-regulated at sites of sterile inflammation [49], and TLR2 has roles in responses of cells to oxidative stress [50]. These data suggest that TLRs, perhaps particularly TLR4, have roles in regulating non-infective inflammatory responses. Additionally, they raise the question about how one receptor, TLR4, can mediate responses to such a diversity of ligands. Perhaps in part this could be because TLR4 might be assembled in a variety of signalling complexes with accessory molecules including CD14, MD-2 and CD11b/CD18. It is possible, though there is no evidence to support this as yet, that TLR4 might heterodimerize with other TLRs, or interact with other accessory molecules. There is evidence for association between CD14/MD-2 and TLR4 when LPS is present [51], but LPS is not easy to use for classical receptor pharmacology studies, and it is not certain that LPS actually binds to TLR4. One recent paper has shown that HSP60, which signals via TLR4, does not directly bind to TLR4 on the cell surface [47].

Thus it is possible that there is some intermediate step between interaction of all these 'ligands' with the cell, and subsequent signalling via TLR4. Such a hypothesis is upheld by studies in *Drosophila*, where activation of Toll requires proteolytic cleavage of a protein, Pro-Spätzle, to generate Spätzle, which is appears to be the true protein ligand for this receptor [52]. In keeping with this, studies in the human monocyte-derived THP-1 cell line have shown that LPS activates a protease that causes activation of TLR4, possibly by generation of a TLR4 ligand [53], or even by proteolysis of TLR4 itself. Therefore, it remains to be established if TLR4 signalling, and perhaps signalling in other human TLRs, is mediated by direct or indirect interactions with their putative ligands.

TLR signalling

The TLRs are members of the IL-1 receptor superfamily, and share with the IL-1R an intracellular signalling domain called the Toll-Interleukin Receptor, or TIR, domain [54, 55]. This couples to adaptor molecules including MyD88 [56], which binds to TLR2, TLR4 and TLR9 [57], and MAL/TIRAP, an MyD88 homologue that binds to TLR4 [58, 59]. A signalling complex is formed including the IL-1 Receptor Associated Kinases (IRAKs), Tollip and TRAF-6, and from this signalling complex, pathways are activated that ultimately activate a classical pro-inflammatory gene transcription regulator, NF- κ B [60–62]. The Toll signalling complex formed in response to TLR2, TLR4 and TLR9 triggering also activates MAP kinases such as p38 MAPK and Jnk, by pathways that remain to be fully characterized [25, 39, 63–65]. It is interesting, however, that activation of different TLRs can result in different consequences within the cell – and indeed different consequences to responses to IL-1, since all these receptors use TIR domains to initiate signalling. For example, TLR2 signalling not only activates pro-inflammatory gene transcription, but can also activate pro-apoptotic pathways resulting in inflammatory cell death [10, 66]. The TIR domain is incredibly powerful, and it may be true that activation of only tens of receptors per cell may result in efficient signalling [67, 68]. The ways in which different TLRs couple to cause various signalling outputs in the cell remain to be fully explained, but are the subject of much active research. A good indication that these pathways are of functional importance is that some viruses have learnt to interfere with TIR-mediated signalling [69], by creating TIR-domain mimicking sequences that interfere with these transduction processes.

TLRs in allergic inflammation

Thus, there are multiple points at which TLR signalling can modulate allergic inflammatory disease. The essence of the 'hygiene hypothesis' [70] is the idea that exposure to specific infections, or perhaps endotoxin, drives the maturing immune system in infancy/childhood towards a Th1 phenotype, and away from the Th2 phenotype associated with atopy. It is an attractive hypothesis, although it still provokes much debate [71, 72]. Since TLRs are implicitly involved in responses to many pathogen-related molecules, they may be critical receptor pathways involved in the maturation of the normal adult

immune system. There is some evidence for this hypothesis, albeit indirect, since polymorphisms in CD14, the coreceptor involved in LPS signalling via TLR4, correlate with IgE levels [73]. Functional polymorphisms in human TLR4 have been described and appear to influence airway reactivity [74], and it will be important to see if such polymorphisms in human TLRs correlate with risks of atopy and atopic disease. Once allergic inflammation is established, endotoxin in inhaled dusts may be as relevant as allergen levels to the allergic asthmatic – a setting in which TLR signalling may worsen inflammation rather than prevent its development [75]. Other allergic diseases such as eczema may be worsened by super-added infection, an effect that could be in part mediated by TLR signalling. A general role for TLRs in amplifying inflammatory responses also seems likely, since as described above, TLRs are involved in signalling in response to endogenous proteins associated with cell damage and inflammation, and oxidative stress.

Perhaps the most interesting aspect of TLR biology with regard to allergic immune disease is the possibility of exploiting the powerful adjuvant properties of TLR signalling in the modulation of allergic inflammation. This is best exemplified by an examination of the roles of CpG DNA motifs. The actions of CpG motifs and their potential roles and therapeutic abilities are extensively reviewed by Wild and Sur [37] and Krieg [36]. CpG motifs trigger the classical antigen-presenting cells monocytes and dendritic cells to make IL-12, with subsequent IFN- γ production from T cells [37]. These immune responses naturally favour the development of a Th1-type response, and the strength of this response is such that the coadministration of CpG motifs and allergen to mice sensitized to develop Th2-type inflammation causes a rapid alteration of the immune bias to a Th1 phenotype [76, 77]. Excitingly, direct conjugation of CpG to allergen reduces the dose of CpG required to cause this response and has been shown to be effective at modifying Th2 responses in a variety of species [78]. This reduction in CpG dose will also reduce the risk of potential adverse effects of CpG administration, such as theoretical risks of induction of auto-immune disease [36, 37] or exacerbation of inflammatory responses [79].

TLRs in non-allergic inflammatory disease

Stimulation of the innate immune system by infectious agents, such as those that activate the TLRs, may contribute to other immunological disorders such as auto-immune diseases and chronic inflammatory responses. An association with systemic bacterial infections, and the presence of bacterial DNA within the joint, has been observed in the development of reactive arthritis [80–83]. Likewise, rheumatoid arthritis (RA) is a classic example of an auto-immune disease which also exhibits a chronic inflammatory response [84, 85] where a role for infectious agents has been proposed. The recent discovery of immunostimulatory agonists for the TLRs, for example bacterial peptidoglycans and DNA, in the synovial tissue of patients with RA [86] may help to elucidate the role of infectious agents in this and other diseases.

Peptidoglycans can be derived from the cell walls of multiple gram-positive bacterial species that are present in mucosal surfaces of the body. These bacterial materials are transported from mucosal surfaces to the joint synovium, by macrophages and dendritic cells, and may persist in macrophages leading to

the chronic stimulation of cytokine production [87,88]. As described earlier, peptidoglycan is thought to signal via TLR2/TLR6 heterodimers [13]. Bacterial DNA represents another mechanism for the involvement of infectious agents in RA, with unmethylated CpG motifs activating the innate immune system via TLR9 [33,35]. The cellular response to bacterial CpG includes B cell proliferation and immunoglobulin secretion [42], dendritic cell maturation [38,40,41] and enhanced APC function, and the stimulation of Th1 inflammatory cytokine production in macrophages with the secondary activation of NK cells, all of which produce severe joint inflammation [89–94]. Finally, it has also been suggested that the TLR4 ligand LPS may play an important role in the reactivation of inflammatory joint diseases, such as RA, in humans [95,96]. Thus, the multiple pathways triggered by the different TLR ligands may result in unregulated or inappropriate host responses to infectious agents and ultimately initiation of RA, or indeed contribute to other mechanisms of auto-immunity or chronic inflammation.

Acknowledgements

Ian Sabroe is funded by an MRC Clinician Scientist Fellowship, Lisa Parker by the Arthritis Research Campaign.

References

- 1 Frey EA, Miller DS, Jahr TG et al. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med* 1992; 176:1665–71.
- 2 Poltorak A, He X, Smirnova I et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998; 282:2085–8.
- 3 Yang RB, Mark MR, Gray A et al. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* 1998; 395:284–8.
- 4 Beutler B. TLR4: central component of the sole mammalian LPS sensor. *Current Opinion Immunol* 2000; 12:20–6.
- 5 Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem* 1999; 274:10689–92.
- 6 Lien E, Means TK, Heine H et al. Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 2000; 105:497–504.
- 7 Tapping RI, Akashi S, Miyake K, Godowski PJ, Tobias PS. Toll-like receptor 4, but not toll-like receptor 2, is a signaling receptor for escherichia and salmonella lipopolysaccharides. *J Immunol* 2000; 165:5780–7.
- 8 Hirschfeld M, Ma Y, Weis JH, Vogel SN, Weis JJ. Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. *J Immunol* 2000; 165:618–22.
- 9 Lien E, Sellati TJ, Yoshimura A et al. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 1999; 274:33419–25.
- 10 Aliprantis AO, Yang RB, Mark MR et al. Cell activation and apoptosis by bacterial lipoproteins through Toll-like receptor-2. *Science* 1999; 285:736–9.
- 11 Hertz CJ, Kiertscher SM, Godowski PJ et al. Microbial lipopeptides stimulate dendritic cell maturation via Toll-like receptor 2. *J Immunol* 2001; 166:2444–50.
- 12 Wyllie DH, Kiss-Toth E, Visintin A et al. Evidence for an accessory protein function for TLR1 in anti-bacterial responses. *J Immunol* 2000; 165:7125–32.
- 13 Ozinsky A, Underhill DM, Fontenot JD et al. The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. *Proc Natl Acad Sci USA* 2000; 97:13766–71.
- 14 Hajjar AM, O'Mahony DS, Ozinsky A et al. Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. *J Immunol* 2001; 166:15–9.
- 15 Bulut Y, Faure E, Thomas L, Equils O, Arditi M. Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and *Borrelia burgdorferi* outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. *J Immunol* 2001; 167:987–94.
- 16 Opitz B, Schroder NW, Spreitzer I et al. Toll-like receptor-2 mediates *Treponema glycolipid* and lipoteichoic acid-induced NF-kappaB translocation. *J Biol Chem* 2001; 276:22041–7.
- 17 Campos MA, Almeida IC, Takeuchi O et al. Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. *J Immunol* 2001; 167:416–23.
- 18 Underhill DM, Ozinsky A, Smith KD, Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci USA* 1999; 96:14459–63.
- 19 Wang T, Lafuse WP, Zwilling BS. Regulation of Toll-like receptor 2 expression by macrophages following *Mycobacterium avium* infection. *J Immunol* 2000; 165:6308–13.
- 20 Noss EH, Pai RK, Sellati TJ et al. Toll-like receptor 2-dependent inhibition of macrophage class II MHC expression and antigen processing by 19-kDa lipoprotein of *Mycobacterium tuberculosis*. *J Immunol* 2001; 167:910–8.
- 21 Shimazu R, Akashi S, Ogata H et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 1999; 189:1777–82.
- 22 Dziarski R, Wang Q, Miyake K, Kirschning CJ, Gupta D. MD-2 enables Toll-like receptor 2 (TLR2)-mediated responses to lipopolysaccharide and enhances TLR2-mediated responses to Gram-positive and Gram-negative bacteria and their cell wall components. *J Immunol* 2001; 166:1938–44.
- 23 Visintin A, Mazzoni A, Spitzer JA, Segal DM. Secreted MD-2 is a large polymeric protein that efficiently confers lipopolysaccharide sensitivity to Toll-like receptor 4. *Proc Natl Acad Sci USA* 2001; 98:12156–61.
- 24 Troelstra A, de Graaf-Miltenburg LA, van Bommel T, Verhoef J, Van Kessel KP, Van Strijp JA. Lipopolysaccharide-coated erythrocytes activate human neutrophils via CD14 while subsequent binding is through CD11b/CD18. *J Immunol* 1999; 162:4220–5.
- 25 Perera PY, Mayadas TN, Takeuchi O et al. CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. *J Immunol* 2001; 166 (1):574–81.
- 26 Au B-A, Williams TJ, Collins PD. Zymosan-induced IL-8 release from human neutrophils involves activation via CD11b/CD18 receptor and endogenous platelet-activating factor as an autocrine modulator. *J Immunol* 1994; 152:5411.
- 27 Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to *Drosophila* Toll. *Proc Natl Acad Sci USA* 1998; 95:588–93.
- 28 Chuang T, Ulevitch RJ. Identification of hTLR10: a novel human Toll-like receptor preferentially expressed in immune cells. *Biochimica Biophysica Acta* 2001; 1518:157–61.

- 29 Chuang TH, Ulevitch RJ. Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. *European Cytokine Network* 2000; 11:372–8.
- 30 Du X, Poltorak A, Wei Y, Beutler B. Three novel mammalian toll-like receptors: gene structure, expression, and evolution. *European Cytokine Network* 2000; 11:362–71.
- 31 Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 2001; 413:732–8.
- 32 Hayashi F, Smith KD, Ozinsky A et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001; 410:1099–103.
- 33 Hemmi H, Takeuchi O, Kawai T et al. A toll-like receptor recognizes bacterial DNA. *Nature* 2000; 408:740–5.
- 34 Bauer S, Kirschning CJ, Hacker H et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci USA* 2001; 98:9237–42.
- 35 Takeshita F, Leifer CA, Gursel I et al. Cutting edge: role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. *J Immunol* 2001; 167:3555–8.
- 36 Krieg AM. The role of CpG motifs in innate immunity. *Current Opinion Immunol* 2000; 12:35–43.
- 37 Wild JS, Sur S. CpG oligonucleotide modulation of allergic inflammation. *Allergy* 2001; 56:365–76.
- 38 Kadowaki N, Antonenko S, Liu YJ. Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c (–) type 2 dendritic cell precursors and CD11c (+) dendritic cells to produce type I IFN. *J Immunol* 2001; 166:2291–5.
- 39 Häcker H, Mischak H, Hacker G et al. Cell type-specific activation of mitogen-activated protein kinases by CpG-DNA controls interleukin-12 release from antigen-presenting cells. *EMBO J* 1999; 18:6973–82.
- 40 Kadowaki N, Ho S, Antonenko S et al. Subsets of human dendritic cell precursors express different Toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001; 194:863–70.
- 41 Bauer M, Redecke V, Ellwart JW et al. Bacterial CpG-DNA triggers activation and maturation of human CD11c–, CD123+ dendritic cells. *J Immunol* 2001; 166:5000–7.
- 42 Verthelyi D, Ishii KJ, Gursel M, Takeshita F, Klinman DM. Human peripheral blood cells differentially recognize and respond to two distinct CpG motifs. *J Immunol* 2001; 166:2372–7.
- 43 Kurt-Jones EA, Popova L, Kwinn L et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nature Immunol* 2000; 1:398–401.
- 44 Kol A, Lichtman AH, Finberg RW, Libby P, Kurt-Jones EA. Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells. *J Immunol* 2000; 164:13–7.
- 45 Vabulas RM, Ahmad-Nejad P, da Costa C et al. Endocytosed HSP60s use Toll-like receptor 2 (TLR2) and TLR4 to activate the Toll/Interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 2001; 276:31332–9.
- 46 Sasu S, LaVerda D, Qureshi N, Golenbock DT, Beasley D. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. *Circulation Res* 2001; 89:244–50.
- 47 Habich C, Baumgart K, Kolb H, Burkart V. The receptor for heat shock protein 60 on macrophages is saturable, specific, and distinct from receptors for other heat shock proteins. *J Immunol* 2002; 168:569–76.
- 48 Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through Toll-like receptor 4. *J Immunol* 2001; 167:2887–94.
- 49 Frantz S, Kobzik L, Kim Y-D et al. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. *J Clin Invest* 1999; 104:271–80.
- 50 Frantz S, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor-kappa B by oxidative stress in cardiac myocytes. *J Biol Chem* 2001; 16:16.
- 51 da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex: transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001; 26:26.
- 52 Levashina EA, Langley E, Green C et al. Constitutive activation of Toll-mediated antifungal defense in serpin-deficient *Drosophila*. *Science* 1999; 285:1917–9.
- 53 Mansell A, Reinicke A, Worrall DM, O'Neill LA. The serine protease inhibitor antithrombin III inhibits LPS-mediated NF-kappaB activation by TLR-4. *FEBS Lett* 2001; 508:313–7.
- 54 Xu Y, Tao X, Shen B et al. Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* 2000; 408:111–5.
- 55 Slack JL, Schooley K, Bonnert TP et al. Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. *J Biol Chem* 2000; 275:4670–8.
- 56 Medzhitov R, Preston-Hurlburt P, Kopp E et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998; 2:253–8.
- 57 Häcker H, Vabulas RM, Takeuchi O, Hoshino K, Akira S, Wagner H. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF) 6. *J Exp Med* 2000; 192:595–600.
- 58 Fitzgerald KA, Palsson-McDermott EM, Bowie AG et al. Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. *Nature* 2001; 413:78–83.
- 59 Horng T, Barton GM, Medzhitov R. TIRAP, an adapter molecule in the Toll signaling pathway. *Nature Immunol* 2001; 2:835–41.
- 60 Zhang FX, Kirschning CJ, Mancinelli R et al. Bacterial lipopolysaccharide activates nuclear factor- κ B through interleukin-1 signaling mediators in cultured human dermal endothelial cells and mononuclear phagocytes. *J Biol Chem* 1999; 274:7611–4.
- 61 Yang RB, Mark MR, Gurney AL, Godowski PJ. Signaling events induced by lipopolysaccharide-activated toll-like receptor 2. *J Immunol* 1999; 163:639–43.
- 62 Muzio M, Polentarutti N, Bosio D, Manoj Kumar PP, Mantovani A. Toll-like receptor family and signalling pathway. *Biochem Soc Transactions* 2000; 28:563–6.
- 63 Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000; 164:966–72.
- 64 Ropert C, Almeida IC, Closel M et al. Requirement of mitogen-activated protein kinases and I kappa B phosphorylation for induction of proinflammatory cytokines synthesis by macrophages indicates functional similarity of receptors triggered by glycosylphosphatidylinositol anchors from parasitic protozoa and bacterial lipopolysaccharide. *J Immunol* 2001; 166:3423–31.
- 65 Arrighi JF, Rebsamen M, Rousset F, Kindler V, Hauser C. A critical role for p38 mitogen-activated Protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF- α , and contact sensitizers. *J Immunol* 2001; 166:3837–45.
- 66 Aliprantis AO, Yang RB, Weiss DS, Godowski P, Zychlinsky A. The apoptotic signaling pathway activated by Toll-like receptor-2. *EMBO J* 2000; 19:3325–36.
- 67 Dower SK, Call SM, Gillis S, Urdal DL. Similarity between the interleukin 1 receptors on a murine T-lymphoma cell line and on a murine fibroblast cell line. *Proc Natl Acad Sci USA* 1986; 83:1060–4.

- 68 Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK, Segal DM. Regulation of Toll-like receptors in human monocytes and dendritic cells. *J Immunol* 2001; 166:249–55.
- 69 Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O'Neill LA. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. *Proc Natl Acad Sci USA* 2000; 97:10162–7.
- 70 Warner JO. Worldwide variations in the prevalence of atopic symptoms: what does it all mean? *Thorax* 1999; 54 (Suppl. 2): S46–51.
- 71 Platts-Mills TA, Woodfolk JA, Sporik RB. Con: the increase in asthma cannot be ascribed to cleanliness. *Am J Respiratory Crit Care Med* 2001; 164:1107–8; discussion 1108–9.
- 72 von Mutius E. Pro: the increase in asthma can be ascribed to cleanliness. *Am J Respiratory Crit Care Med* 2001; 164 (7):1106–7; discussion 1108–9.
- 73 Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respiratory Cell Mol Biol* 1999; 20:976–83.
- 74 Arbour NC, Lorenz E, Schutte BC et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nature Genet* 2000; 25:187–91.
- 75 Michel O, Kips J, Duchateau J et al. Severity of asthma is related to endotoxin in house dust. *Am J Respiratory Crit Care Med* 1996; 154:1641–6.
- 76 Sur S, Wild JS, Choudhury BK, Sur N, Alam R, Klinman DM. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol* 1999; 162:6284–93.
- 77 Broide D, Schwarze J, Tighe H et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol* 1998; 161:7054–62.
- 78 Tighe H, Takabayashi K, Schwartz D et al. Conjugation of immunostimulatory DNA to the short ragweed allergen amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000; 106:124–34.
- 79 Schwartz DA, Quinn TJ, Thorne PS, Sayeed S, Yi AK, Krieg AM. CpG motifs in bacterial DNA cause inflammation in the lower respiratory tract. *J Clin Invest* 1997; 100:68–73.
- 80 Wilbrink B, van der Heijden IM, Schouls LM et al. Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. *Arthritis Rheumatism* 1998; 41:535–43.
- 81 Gerard HC, Wang Z, Wang GF et al. Chromosomal DNA from a variety of bacterial species is present in synovial tissue from patients with various forms of arthritis. *Arthritis Rheumatism* 2001; 44:1689–97.
- 82 Kirveskari J, He Q, Holmstrom T et al. Modulation of peripheral blood mononuclear cell activation status during Salmonella-triggered reactive arthritis. *Arthritis Rheumatism* 1999; 42:2045–54.
- 83 Wuorela M, Granfors K. Infectious agents as triggers of reactive arthritis. *Am J Med Sci* 1998; 316:264–70.
- 84 Thomas R, Lipsky PE. Presentation of self peptides by dendritic cells: possible implications for the pathogenesis of rheumatoid arthritis. *Arthritis Rheumatism* 1996; 39:183–90.
- 85 Thomas R, Lipsky PE. Could endogenous self-peptides presented by dendritic cells initiate rheumatoid arthritis? *Immunol Today* 1996; 17:559–64.
- 86 van der Heijden IM, Wilbrink B, Tchetverikov I et al. Presence of bacterial DNA and bacterial peptidoglycans in joints of patients with rheumatoid arthritis and other arthritides. *Arthritis Rheumatism* 2000; 43:593–8.
- 87 Burmester GR, Stuhlmuller B, Keyszer G, Kinne RW. Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis? *Arthritis Rheumatism* 1997; 40:5–18.
- 88 Salmi M, Rajala P, Jalkanen S. Homing of mucosal leukocytes to joints. Distinct endothelial ligands in synovium mediate leukocyte-subtype specific adhesion. *J Clin Invest* 1997; 99:2165–72.
- 89 Krieg AM, Wagner H. Causing a commotion in the blood: immunotherapy progresses from bacteria to bacterial DNA. *Immunol Today* 2000; 21:521–6.
- 90 Roman M, Martin-Orozco E, Goodman JS et al. Immunostimulatory DNA sequences function as T helper-1-promoting adjuvants. *Nature Med* 1997; 3:849–54.
- 91 Deng GM, Nilsson IM, Verdrengh M, Collins LV, Tarkowski A. Intra-articularly localized bacterial DNA containing CpG motifs induces arthritis. *Nature Med* 1999; 5:702–5.
- 92 Deng GM, Tarkowski A. The features of arthritis induced by CpG motifs in bacterial DNA. *Arthritis Rheumatism* 2000; 43:356–64.
- 93 Arend WP. Cytokines and cellular interactions in inflammatory synovitis. *J Clin Invest* 2001; 107:1081–2.
- 94 Ronaghy A, Prakken BJ, Takabayashi K et al. Immunostimulatory DNA sequences influence the course of adjuvant arthritis. *J Immunol* 2002; 168:51–6.
- 95 Yoshino S, Sasatomi E, Ohsawa M. Bacterial lipopolysaccharide acts as an adjuvant to induce autoimmune arthritis in mice. *Immunology* 2000; 99:607–14.
- 96 Yoshino S, Ohsawa M. The role of lipopolysaccharide injected systemically in the reactivation of collagen-induced arthritis in mice. *Br J Pharmacol* 2000; 129:1309–14.