Review

Toll-like Receptors. I. Structure, Function and Their Ligands

(dendritic cells / Gram-positive and Gram-negative bacteria / lipopolysaccharide / PAMPs / PRRs / TLRs / T_H-cell polarization / viruses)

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Abstract. The innate immune system senses invading microorganisms by a phylogenetically conserved family of proteins PRRs of which TLRs are ones of the most important. There are at least 10 different TLRs in humans and 11 in mice. They have in the course of evolution specialized for the recognition of conserved structures among microorganisms called PAMPs. Activation of TLRs results in induction of innate immunity mechanisms as well in development of antigen-specific adaptive immune responses, thus bridging innate and adaptive immunity.

The role of the immune system is to detect and eliminate invading pathogens by means of discrimination between self and non-self antigens. The immune system in mammals can be divided into innate and adaptive immunity. The phylogenetically newer adaptive immunity detects self and pathogenic antigens using receptors that are expressed on the surface of B and T cells. Through immunoglobulin and T-cell receptor gene rearrangement, B and T cells produce more than 10^{11} unique antigen receptors, respectively. This approach allows the adaptive immune system to respond to an immense variety of different antigens. This elaborate system can be found in vertebrates only and represents a potent mechanism in fighting microbial infections. On the other hand, the innate immune system is present in all multi-cellular organisms and is phylogenetically conserved. In contrast to the system of adaptive immunity, which was in the focus of scientific interest in the past years, less effort was made to study the mechanisms of innate immunity, which remained unclear until recently. The identification of Toll-like receptors (TLRs) has brought more understanding on the mechanisms by which the innate immune system recognizes non-self and how important role TLRs play in detection of invading pathogens. According to the recent evidence, TLRs belong to a family of pattern recognition receptors (PRRs) that recognize conserved parts of microbial components (PAMPs – pathogen-associated molecular patterns).

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Abbreviations: DCs – dendritic cells, ds – double-stranded, IFN – interferon, IL – interleukin, LAM – lipoarabinomannan, LPS – lipopolysaccharide, MHC – major histocompatibility complex, OspA – outer-surface lipoprotein, PAMPs – pathogen-associated molecular patterns, pDCs – plasmacytoid DCs, PRRs – pattern recognition receptors, ss – single-stranded, TLRs – Toll-like receptors.

Toll-like receptors in humans

The first recognized mammalian TLR homologue of the drosophila Toll (Belvin and Anderson, 1996) was TLR4, identified in 1997, just a year after elucidating the role of the drosophila Toll in fighting fungal infection (Medzhitov et al., 1997). In the following years, more proteins structurally and functionally related to the drosophila Toll and recognizing a whole array of microbial structures were discovered, creating a family referred to as the TLRs (Table 1).

Like drosophila Toll, human TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic carboxy-terminal Toll-interleukin 1 receptor (TIR) domain (Fig. 1). Based on the chromosomal localization, genomic structure and amino acid sequences, the human TLRs can be divided into five subfamilies: TLR2, TLR3, TLR4, TLR5, and TLR9. The **TLR2 subfamily** consists of TLR1, TLR2, TLR6, and TLR10, the **TLR9 subfamily** is composed of TLR7, TLR8, and TLR9. TLR3, TLR4, and TLR5 are represented only by one family member, respectively. TLR1 and TLR6 genes are located closely to 4p14, TLR2 maps to 4q32, while TLR3 is located near TLR2, at 4q35. TLR4 resides on 9q33-35, whereas TLR5 is at 1q33.3 (Rock et al., 1998; Takeuchi et al., 1999). TLR7 and TLR8 are located as a tandem in Xp22, TLR9 maps to 3p21.3 (Du et al., 2000).

Toll-like receptor 4 and its ligands

The first ligand for TLR4 was identified in 1998. It was shown that the mammalian TLR4 protein had been

Table 1. Toll-like receptors and their ligands

TLR	Ligands
TLR1	Soluble factors of *Neisseria meningitidis* cell wall
	Triacylated lipopeptides (G^+, G^- bacteria), 19 kDa lipoprotein (mycobacteria)
	Lipoarabinomannan (mycobacterial cell wall)
	Outer-surface protein – OspA (*Borrelia burgdorferi*)
TLR2	Peptidoglycan, lipoteichoic acids (G^+ bacteria)
	Phenol-soluble modulin (*Staphylococcus epidermidis*)
	Di- and triacylated lipopeptides, lipoproteins (many pathogens)
	Outer-membrane porins (*N. gonorrhoeae, H. pylori*)
	Outer-surface protein – OspA (*Borrelia burgdorferi*)
	Lipoarabinomannan (mycobacterial cell wall glycolipid)
	Zymosan (yeast)
	Protozoan cell membrane glycolipids (*Trypanosoma cruzi*)
	Wild-type H protein (measles virus)
	HSV-1, CMV envelope proteins
	Atypical LSP (*L. interrogans, P. gingivalis*)
	Host HSP70
TLR3	Viral and host double-stranded RNA (dsRNA)
	Polyinosinic-polycytidylic acid (poly(I:C))
TLR4	Lipopolysaccharide (LPS) – G^- bacteria
	Fusion protein of RSV (respiratory syncytial virus)
	Murine mammary tumour virus (MMTV)
	Moloney murine leukaemia virus (MMLV)
	Taxol (plant antitumour agent)
	Extravascular fibrinogen/fibrin (host)
	Oligosaccharide fragments of hyaluronan (host)
	Extra domain A of fibronectin (host)
	Polysaccharide fragments of heparan sulphate (host)
	Heat-shock protein 60 – HSP60 (host, *Ch. pneumoniae*)
	Heat-shock protein 70 – HSP70 (host)
TLR5	Flagellin
TLR6	Diacylated lipopeptides (*Mycoplasma fermentans*)
	Zymosan (yeast)
TLR7	Imidazoquinolines (imiquimod, resiquimod, loxoribine, bropirimine)
TLR8	Viral single-stranded RNA (ssRNA) (influenza virus, vesicular stomatitis virus)
	Guanosine and uridine-rich ssRNA oligonucleotides (HIV-1)
TLR9	Unmethylated CpG oligodeoxynucleotides (bacteria)
	Viral genomic DNA (HSV-2)
TLR10	???
TLR11	Uropathogenic bacteria. *Toxoplasma gondii*

adapted primarily to recognize lipopolysaccharide (LPS), a major cell wall component of Gram-negative bacteria (Poltorak et al., 1998). A constitutively active mutant of human TLR4 transfected into human cell lines could induce the activation of NF-κB transcription factor and the expression of NF-κB-controlled genes for the inflammatory cytokines interleukin (IL)-1, TNF, IL-6 and IL-8, as well as the expression of the co-stimulatory molecules CD40, CD80, CD86 (Medzhitov et al., 1997). The role of TLR4 in LPS recognition was further confirmed by generation of TLR4 knockout mice by gene targeting, proving that TLR4 is essential for LPS recognition (Hoshino et al., 1999). However, TLR4 does not sense LPS directly, but requires LPS-binding protein (LBP). It is an acute phase protein present in the plasma and binds a lipid A portion of the LPS molecule (Tobias et al., 1986). The LBP-LPS complex interacts with **CD14**, a GPI (glycosylphosphatidylinositol)-anchored molecule expressed mainly in cell membranes of monocytes, macrophages, and neutrophils.

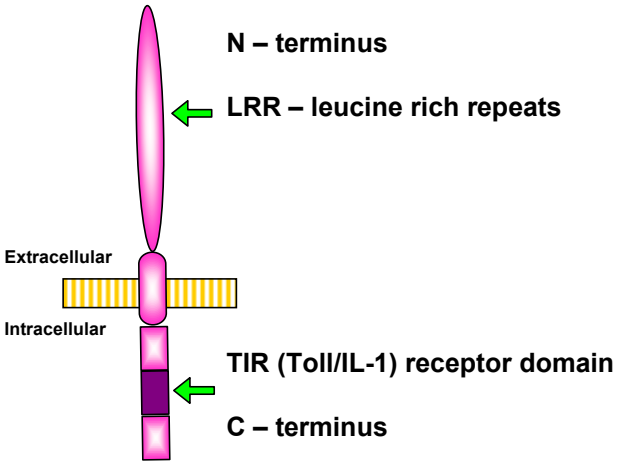

Fig. 1. The structure of a human Toll-like receptor (TLR) Human TLRs are type I transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic, referred to as the Toll-interleukin 1 receptor (TIR) domain, which is homologous to that of the interleukin IL-1 receptor.

Moreover, a novel molecule, **MD-2**, is requisite for LPS signalling of TLR4. It physically associates with the extracellular domain of human TLR4 (Shimazu et al., 1999) and binds the lipid A region of LPS without the need for LBP (Viriyakosol et al., 2000). The latest studies have helped to clarify the earliest events of the TLR4 triggering by LPS. CD14 enhances LPS binding to MD-2, bound to TLR4 on the cell surface, which enables TLR4 binding to LPS and allows formation of stable receptor complexes.

In addition to bacterial LPS, TLR4 senses a certain group of ligands found in viruses. The innate immune response to a fusion protein of respiratory syncytial virus is mediated by TLR4 and CD14 (Kurt-Jones et al., 2000). TLR4 also recognizes some components that are endogenous to the host itself. Extravascular fibrin deposition is an early and persistent hallmark of inflammatory responses. Efforts to define physiologic functions of extravasated fibrinogen have led to the discovery that fibrin stimulates macrophage chemokine secretion by TLR4 (Smiley et al., 2001). Furthermore, TLR4 mediates response to oligosaccharide fragments of hyaluronan, to an extra domain A of FN, and to polysaccharide fragments of heparan sulphate proteoglycan (Termeer et al., 2002; Taylor et al., 2004). Another group of endogenous danger signals recognized by TLR4 are heat-shock proteins (HSP60, HSP70). Their primary role is to chaperone aberrantly folded or nascent proteins; moreover, they have been found to activate macrophages and dendritic cells, which in turn express co-stimulatory molecules and secrete proinflammatory cytokines (Vabulas et al., 2002) (Fig. 2).

Complexity of PAMP recognition by Toll-like receptor 2

TLR2 recognizes a broad spectrum of different microbial components ranging from viruses up to parasites. These include peptidoglycan and lipoteichoic acids that are cell wall components of Gram-positive bacteria (Schwadner et al., 1999; Yoshimura et al., 1999) and modulin from *Staphylococcus epidermidis* (Hajjar et al., 2001). TLR2 is also involved in recognition of lipoproteins and lipopeptides from cell walls of Gram-negative bacteria (Brightbill et al., 1999). Lipoprotein from *N. gonorrhoeae* and porins that constitute the outer membrane of *H. influenzae* and *Neisseria* spp. stimulate cytokine release and NF-κB activation in a TLR2-dependent manner in human monocytes and epithelial cells (Massari et al., 2002; Galdiero et al., 2004). As for spirochetes and mycoplasmas, TLR2 recognizes membrane lipoproteins/lipopeptides from *Treponema pallidum* and *Mycoplasma fermentans* (Lien et al., 1999). TLR2 is also required for innate host defence to *Borrelia burgdorferi* lipoproteins (Hirschfeld et al., 1999). TLR2 is involved even in recognition of mycobacterial cell wall structures, especially lipoarabinomannan (LAM) (Means et al., 1999). Surprisingly, there is evidence that within fungal structures TLR2 senses zymosan, a yeast cell wall component (Underhill et al., 1999). TLR2 is capable to identify some structures present in parasites as well. Protozoan cell membrane glycolipids derived from *Trypanosoma cruzi* trigger potent activation of macrophage TLR2 from both mouse and human origin resulting in IL-12, TNF and NO production (Campos et al., 2001). Finally, TLR2 recognizes even a variety of viral structures. The TLR2 activation of human monocytic cells by measles virus (MV) wild-type H protein results in the induction of proinflammatory cytokines such as IL-6 and the surface expression of CD150, the receptor for all MV strains (Bieback et al., 2002). TLR2 also mediates the inflammatory cytokine response to herpes simplex virus 1 (HSV-1) and CMV envelope protein(s) (Compton et al., 2003; Kurt-Jones et al., 2004).

Interestingly, several atypical kinds of LPS are not recognized by TLR4 but via TLR2. As stated previously, LPS from enterobacteria, such as *E. coli* or *Salmonella*, spp. are recognized by TLR4, but LPS derived from *Leptospira interrogans* and *Porphyromonas gingivalis* is sensed by TLR2 (Werts et al., 2001; Ogawa et al., 2002). It has been proposed recently that the shape of the lipid A component determines the bioactivity of LPS – that with a conical conformation is being more active than that with a cylindrical shape. It has been postulated that the conical-shape LPS (e.g. from *E. coli*) induces cytokine production through TLR4, whereas a more cylindrical LPS (e.g. from *Porphyromonas gingivalis*) induces expression of a different set of cytokines

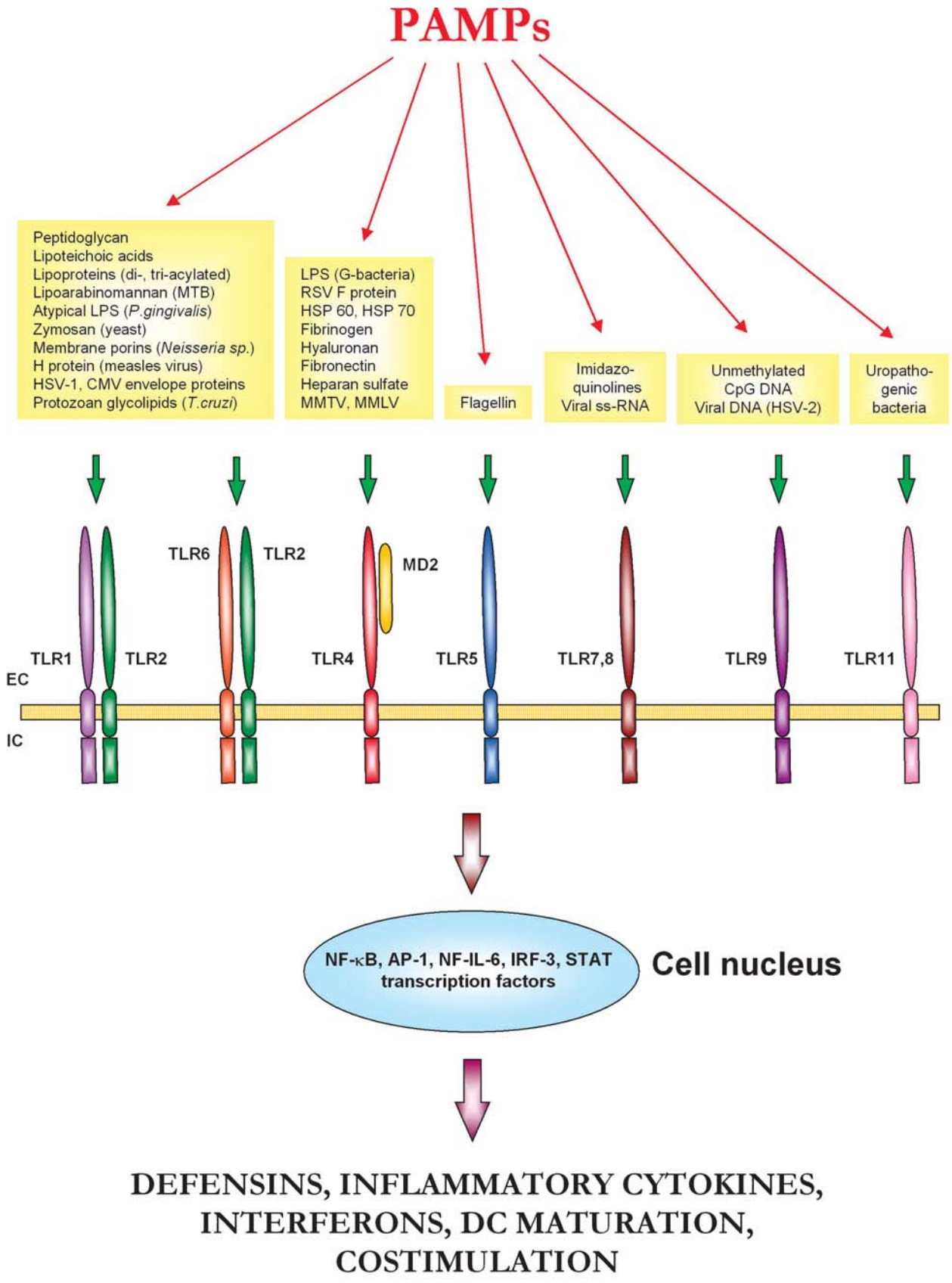

Fig. 2. Mammalian Toll-like receptors and their ligands
A schematic model of known TLRs and their ligands. Following activation of TLRs the signal is propagated into the cell nucleus where various transcription factors modulate the expression of genes responsible for mammalian antimicrobial peptide production (defensins), antiviral response (type I interferons), pro-inflammatory cytokine secretion, co-stimulation and dendritic cell maturation. TRL1, TLR2, TLR4, TLR5, and TLR11 are expressed in cell membranes, TLR3, TLR7, TLR8, and TLR9 intracellularly. EC – extracellular, IC - intracellular

through TLR2. However, the strictly cylindrical LPS molecules (e.g. the lipid A precursor or that from *Rhodobacter sphaeroides*) have antagonistic properties (Netea et al., 2002). The mechanism linking the molecular conformation with the biological activity of LPS has yet to be fully elucidated.

The strikingly numerous varieties of different molecules that have been shown to signal through TLR2 raised suspicions about whether it is the TLR2 only that is solely responsible for NF-κB signal activation and proinflammatory cytokine production. It has been found that TLR2 possesses a unique aspect of ligand recognition where TLR2 cooperates with other TLR family members, especially TLR6 and TLR1 that confer discrimination among different microbial components. As already stated above, a peptidoglycan and the *S. aureus* modulin are identified TLR2 ligands. However, co-expression of a dominant negative form of TLR6 (i.e. a mutant TLR6 receptor which is defective in ligand recognition or intracellular signalling) with wild-type TLR2 results in suppression of TNF production (Hajjar et al., 2001). Accordingly, TLR2 co-immunoprecipitates with TLR6, which suggests a physical interaction between these molecules (Ozinsky et al., 2000). TLR2 has been reported to associate with TLR1 as well (Takeuchi et al., 2002). The experiments indicate that TLR2-TLR1 or TLR2-TLR6 cooperation is responsible for recognizing subtle differences among the lipid moieties of bacterial lipopeptides. TLR2 is absolutely required for both di- and triacylated lipopeptide response. TLR2 in conjunction with TLR1 recognizes triacylated and TLR2 in cooperation with TLR6 recognizes diacylated lipopeptides.

Recent evidence has also shown that in addition to TLR2, TLR1 is involved in recognition of outer-surface lipoprotein (OspA) of *B. burgdorferi*. TLR1- and TLR2-deficient mice did not respond to stimulation with OspA (Alexopoulou et al., 2002). On the basis of extensive testing, the US FDA approved the use of recombinant OspA as a vaccine against Lyme disease in 1998. Protective immunity in humans correlates with the development of antibodies against OspA (Steere et al., 1998); however, some individuals do not develop high titres of anti-OspA antibodies. Based on previous data obtained on murine models it was proposed that these persons might have defects in TLR-mediated lipoprotein signalling. Indeed, the surface expression of TLR1 on macrophages of non-responders was significantly lower. However, factors contributing to an altered TLR1 surface expression are not yet known (Alexopoulou et al., 2002).

Another ligand involved in TLR2 stimulation is glycolipid LAM isolated from mycobacteria. Recent experiments indicate that TLR1 and TLR2 are required for LAM-stimulated cytokine secretion from mononuclear cells and NF-κB activation. Confocal microscopy and co-immunoprecipitation revealed that TLR1 and TLR2 form pre-assembled heterodimeric complexes on the cell surface and in the cytosol. Expression of the extracellular domains along with simultaneous expression of the intracellular domains of both TLRs is necessary to achieve functional signalling; in addition, experiments using dominant negative mutants of TLR1 and TLR2 proteins defined the Toll/IL-1R domains as the area of crucial intracellular TLR1-TLR2 interaction (Sandor et al., 2003).

Surprisingly, bacterial lipopeptides (BLPs) were also found to induce apoptosis in monocytes through the human TLR2. BLPs initiated apoptosis in an epithelial cell line transfected with TLR2. The process of apoptosis was initiated through MyD88 via a pathway involving Fas-associated death domain protein (FADD) and caspase 8. In addition, BLPs stimulated NF-κB and activated the respiratory burst through TLR2. Thus TLR2 also represents a molecular link between microbial products, apoptosis, and host defence mechanisms (Aliprantis et al., 1999). The latest findings point out the time orchestration between inflammation and apoptosis in stimulation with BLPs. Mycoplasmal lipoproteins trigger TLR2- and TLR6-mediated sequential bifurcate responses: NF-κB activation as an early and apoptosis as a later event (Into et al., 2004) (Fig. 2).

Flagellated bacteria are recognized by Toll-like receptor 5

The innate immune system recognizes PAMPs that are expressed on infectious agents, but not on the host. Flagellin is an integral component of bacterial flagella, a highly complex structure that extends out of the outer membrane of motile bacteria. Flagella function as propellers that enable bacteria to move in their water environment. Flagellin is also a virulence factor that is recognized by the innate immune system in organisms as diverse as flies, plants, and mammals. It was shown that **TLR5** is responsible for flagellin recognition from both Gram-positive and Gram-negative bacteria; activation of the receptor mobilizes the nuclear factor NF-κB and stimulates pro-inflammatory cytokine (TNF, IL-8) production (Hayashi et al., 2001). Studies of flagellin from enteroaggregative *E. coli* identified two regions in the conserved D1 domain that are required for TLR5 activation and IL-8 release (Donnelly et al., 2002). More detailed analysis of TLR5 shows that a specific portion of extracellular domain (amino acid residues 386-407) represents a binding site for flagellin. This sequence contains a putative leucine-rich repeat. These studies demonstrate the exquisite ability of the innate immune system to precisely target a conserved site on flagellin that is essential for bacterial motility and provide, for the first time, a molecular basis for the agonist specificity of a TLR (Smith et al., 2003). Further, it has been shown that TLR5 with a stop codon in its allele (TLR5-392STOP) is unable to mediate flagellin sig-

nalling and is associated with susceptibility to pneumonia caused by flagellated *Legionella pneumophila* (Hawn et al., 2003). It is probable that TLR5 has evolved to permit mammals to specifically detect flagellated bacterial pathogens. However, certain bacteria, e.g. *Helicobacter (H.) pylori*, developed unique flagellins of a low activating potential. Such flagellated pathogen escapes the immune clearance, in part, by avoiding detection by TLR5; it may contribute to its long-term persistence in individual hosts where *H. pylori* is able to colonize the stomach for decades unless pharmacologically eradicated (Lee et al., 2003).

In certain compartments of the human body (e.g. the gastrointestinal tract), epithelial cells are exposed to enormous amounts of bacteria, hence the induction of inflammatory response in these tissues would be detrimental to the host. Nature has therefore invented mechanisms to prevent these events. Immunolocalization revealed that TLR5 is expressed exclusively on a basolateral surface of intestinal epithelia, thus providing a molecular basis for the polarity of this innate immune response. When flagellin contacts the apical epithelial surface, e.g. that of commensal *Escherichia coli*, there is no proinflammatory effect; however, when it contacts the basolateral epithelial surfaces, e.g. that of *Salmonella* which translocates its flagellin across epithelia, the proinflammatory response develops (Gewirtz et al., 2001). On the other hand, when bacteria are present in blood stream, it is highly desirable for the organism to prevent the generalization of infection by activation of immune defence mechanisms. Indeed, it was found that microvascular endothelial cells express high constitutive amounts of the TLR5 mRNA and protein (Maaser et al., 2004).

Intracellular Toll-like receptors

TLR3, TLR7, TLR8, and TLR9 contrary to previous TLRs are not located in cell membranes; however, they fulfil their functions intracellularly. **TLR3** is important in the induction of antiviral defence mechanisms. The replicative cycle of viral infection in many cases involves double-stranded RNA (dsRNA) production, where dsRNA is either a by-product generated by symmetrical transcription of DNA viral genomes or is an essential intermediate in viral RNA synthesis. dsRNA is a very potent inducer of type I interferons and other cytokines, which exert potent antiviral and immunostimulatory activities (Bucová, 2002). Some synthetic dsRNAs, such as polyinosinic-polycytidylic acid /poly(I:C)/, have similar activity to that of dsRNA. TLR3 recognizes dsRNA, and activation of TLR3 induces the secretion of type I IFNs and pro-inflammatory cytokines (Alexopoulou et al., 2001). Similarly to TLR4, TLR3 is capable to recognize endogenous ligands as well. Endogenous heterologous mRNA released from necrotic cells stimulates human DCs via TLR3 and induces IFN-α secretion. This suggests that endogenous mRNA, likely through its secondary structure, is a potent host-derived activator of TLR3 (Kariko et al., 2004). Another cell type known to express TLR3 are NK cells. They express TLR3 and up-regulate TLR3 mRNA upon poly(I:C) stimulation, which significantly augments their cytotoxicity. Furthermore, NK cells respond to poly(I:C) by producing pro-inflammatory cytokines such as IL-6 and IL-8, as well as IFN-γ. Thus the ability of NK cells to directly recognize and respond to viral products plays an important role in mounting an effective antiviral response (Schmidt et al., 2004).

Human TLR7, TLR8, and TLR9 belong to the next subfamily of TLRs. Compared with human TLR1–6, they have a higher relative molecular mass, largely as a result of a longer ecto-domain. They also show differential expression patterns: TLR7 is predominantly expressed in the lung, placenta and spleen, TLR8 is more abundant in the lung and peripheral blood leukocytes, and TLR9 is preferentially expressed in immune cell-rich tissues, such as the spleen, lymph nodes, bone marrow and peripheral blood leukocytes (Du et al., 2000).

The first evidence involving **TLR7** and **TLR8** activation came from studies with small antiviral compounds belonging to the group of imidazoquinoline compounds such as imiquimod (R-837), resiquimod (R-848), loxoribine, and bropirimine. It was known that imidazoquinolines possess potent anti-viral and anti-tumour properties and as a result they are already applied or promising for clinical use against viral infections and cancers. However, the mechanism remained unclear until recently when experiments showed that imidazoquinolines activate immune cells via the TLR7 and TLR8 signalling pathways, respectively (Hemmi et al., 2002). Nevertheless, recently, the natural ligand for TLR7 was finally identified. Single-stranded RNA (ssRNA) molecules of viral (e.g. influenza virus and vesicular stomatitis virus genomic RNA) and non-viral origin also induce TLR7-dependent production of inflammatory cytokines and expression of co-stimulatory molecules. These results identify ssRNA as a ligand for TLR7 and suggest that cells of the innate immune system sense ssRNA to detect infection by RNA viruses (Diebold et al., 2004).

Bacterial DNA exerts a stimulatory effect on mammalian immune cells. This stimulatory effect is attributed to unmethylated cytosine-phosphate-guanine (CpG) dinucleotide motifs in bacterial DNA. In contrast, in mammals, where the nucleotides are methylated and the frequency of CpG dinucleotides is low, their DNA does not display the immunostimulatory effect. Moreover, bacterial CpGs induce a very strong T_H1-driving response. The immunostimulatory effect of bacterial CpG is mediated through TLR9 (Hemmi et al., 2000; Bauer et al., 2001). The receptor-ligand interac-

tion site was located to the LRR-repeat motif and lies extracellularly in a domain that is conserved within the evolutionary cluster encompassing TLR7, -8, and -9. Apart from bacterial CpG DNA, several other TLR9 ligands were identified, e.g. DNA of HSV2 (Lund et al., 2003). TLR9 also recognizes non-DNA components. Malaria parasites digest haemoglobin into a hydrophobic haem polymer known as homozoin, which is able to stimulate macrophages and dendritic cells (DCs) to produce cytokines in a TLR9-dependent mechanism (Coban et al., 2005).

Similarly to TLR7 and TLR8, TLR9 also triggers signalling from the endosomal compartment of DCs and macrophages. TLR9 has been found to be located intracellularly, not on the cell surface. Real-time confocal microscopy studies show that CpG DNA moves into early endosomes and is subsequently transported to a tubular lysosomal compartment. Concurrent with the movement of CpG DNA in cells, TLR9 redistributes from the endoplasmic reticulum to CpG DNA-containing structures. Thus internalization and endosomal acidification is conditional for CpG DNA to activate TLR9 (Ahmad-Nejad et al., 2002; Latz et al., 2004).

There are two subsets of human peripheral DCs – myeloid (mDC; CD11c+) and plasmacytoid (pDC; $CD123^{+}$). mDCs express all TLRs except TLR7 and TLR9, which are, however, selectively expressed in pDCs. mDCs and pDCs respond to PAMPs according to their TLR expression. Thus, pDCs were susceptible to stimulation by CpG motifs but not by LPS, while mDCs responded to LPS but not to CpG DNA (Jarrossay et al., 2001). The stimulation of pDCs with CpG DNA supported co-stimulation (CD80, CD86, CD40, MHC class II), chemokine production (IL-8, IP-10), and maturation (CD83). CD40 ligand (CD40L) and CpG DNA synergize to stimulate pDCs to produce IFN-α and IL-12p70. IL-12p70 is the bioactive form of IL-12 and it is a strong T_H1 response-polarizing agent. Thus, T_H1 polarization of developing T cells requires simultaneous activation of pDCs by CD40 ligation and by CpG DNA. As a result, IL-12p70 production by pDCs is under strict control of two signals, an adequate exogenous microbial stimulus such as CpG DNA, and CD40L provided endogenously by activated T cells (Krug et al., 2001). CpG motifs also redirect isotype production of B cells to "T_H1-like" immunoglobulin isotypes (Lin et al., 2004). These results demonstrate that CpG DNA-stimulated pDCs induce plasma cell differentiation in naive and memory B cells in the absence of T-cell help, providing an explanation for the excellent activity of CpG oligodeoxynucleotides as a humoral vaccine adjuvant (Poeck et al., 2004).

The latest discovered Toll-like receptors

TLR 10 is up to this date the latest discovered human Toll-like receptor. Among all the human TLRs, TLR10 is closely related to TLR1 and TLR6, the overall amino acid identity is 50% and 49%, respectively. The TLR10 mRNA is the most highly expressed in lymphoid tissues such as the spleen, lymph nodes, thymus, and tonsils (Chuang et al., 2001). Specifically, analysis of a range of resting and activated leukocyte populations revealed that TLR10 was highly expressed in B cells and weakly in pDCs (Hornung et al., 2002). Expression of TLR10 is rapidly induced following B-cell-receptor triggering (Bernasconi et al., 2003). However, the ligand(s) for TLR10 have not yet been identified. Given the close homology to TLR1 and TLR6 it is highly probable that TLR10 may need another TLR protein (TLR2?) to exert its ligand recognition and subsequent cell activation.

Recently, the latest member of TLR family in mice, **TLR11**, was identified. Its counterpart in humans is represented by a pseudogene only. TLR11 displays a distinct pattern of expression in macrophages, the liver, kidneys, and bladder epithelial cells. Cells expressing TLR11 fail to respond to the known TLR ligands but instead respond specifically to an uropathogenic-bacteria-derived protein. Mice lacking TLR11 are highly susceptible to infection of the kidneys by uropathogenic *E. coli*, indicating a potentially important role for TLR11 in preventing infection of internal organs of the urogenital system (Zhang et al., 2004; Kawai and Akira, 2005). Moreover, a new ligand for TLR11 was identified recently. Yarovinsky et al. (2005) identified that STAg, a soluble extract of the tachyzoite stage of *T. gondii* with a marked sequence homology to profilin proteins present in other protozoa, stimulates DCs by binding to TLR11. This finding proves that TLRs are crucial for the recognition not only of bacteria and viruses, but also of parasites.

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