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Review

TOLL-like receptors linking innate and adaptive immune response

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Abstract

Invading pathogens are controlled by the innate and adaptive arms of the immune system. Adaptive immunity, which is mediated by B and T lymphocytes, recognises pathogens by rearranged high affinity receptors. However, the establishment of adaptive immunity is often not rapid enough to eradicate microorganisms as it involves cell proliferation, gene activation and protein synthesis. More rapid defense mechanisms are provided by innate immunity, which recognises invading pathogens by germ-line-encoded pattern recognition receptors (PRR). Recent evidence shows that this recognition can mainly be attributed to the family of TOLL-like receptors (TLR). Binding of pathogen-associated molecular patterns (PAMP) to TLR induces the production of reactive oxygen and nitrogen intermediates (ROI and RNI), pro-inflammatory cytokines, and up-regulates expression of co-stimulatory molecules, subsequently initiating the adaptive immunity. In this review, we will summarize the discovery and the critical roles of the TLR family in host defense, briefly allude to signaling mechanisms mediating the response to TLR ligands, and will provide an update on current knowledge regarding the ligand specificity of these receptors and their role in immunity of domestic animals, particularly cattle.

Keywords: Innate immunity; TLR; Bovine; Pattern recognition receptor(s)

1. Discovery of the TLR system

The term TOLL originally referred to a cell surface receptor governing dorsal/ventral orientation in the early *Drosophila* larvae (Stein et al., 1991). It was later found to also play a crucial part in antifungal defense (Lemaitre et al., 1996), together with other antimicrobial peptides. Sequencing of the *Drosophila* genome revealed the existence of nine proteins belonging to the TOLL family (Tauszig et al., 2000). Although a function in host defense could so far only be attributed to some family members, it is assumed that each member is involved in the host's defense against pathogens. In the 1990s, the first mammalian proteins structurally related to *Drosophila* TOLL were identified, and are now called human TOLL-like receptor (TLR) 1 and 4 (Medzhitov et al., 1997; Nomura et al., 1994). To date, 10 human and 9 murine transmembrane proteins have been shown to belong to the mammalian TLR family (Akira et al., 2001; Zarember and Godowski, 2002) (Table 1).

TOLL and TLR family proteins are characterised by the presence of an extracellular domain with leucine-rich repeats and an intracytoplasmic region

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Table 1
TLR and their ligands

TLR	Origin of ligand	Ligands	References
TLR1	Gram-positive bacteria	Modulin, lipopeptides	Hajjar et al. (2001); Takeuchi et al. (2002)
TLR2	Gram-positive bacteria	Lipoproteins, peptidoglycan, lipoteichoic acid	Schwandner et al. (1999)
	Pseudomonas aeruginosa	Mannuronic acid polymers	Flo et al. (2002)
	Staphylococcus	Modulin	Hajjar et al. (2001)
	Mycobacteriae, Mycoplasmae	Lipoproteins, lipopeptides, lipoarabinomannan	Aliprantis et al. (1999); Brightbill et al. (1999); Means et al. (2001); Means et al. (1999)
	Listeria	Heat-killed bacteria	Flo et al. (2000)
	Yeast	Zymosan	Underhill et al. (1999)
	Trypanosoma cruzei	GPI anchored proteins	Campos et al. (2001)
	Spirochetae	LPS	Hirschfeld et al. (2001)
TLR3	Virus	dsRNA	Alexopoulou et al. (2001)
TLR4	Gram-negative bacteria	LPS	Poltorak et al. (1998); Poltorak et al. (2000)
	Gram-positive bacteria	Lipoteichoid acid, mannuronic acid polymers	Flo et al. (2002)
	Plant	Taxol	Kawasaki et al. (2000)
	Respiratory syncytial virus	F protein	Haynes et al. (2001); Kurt-Jones et al. (2000)
	Host	Hsp60, Hsp70, Fibronectin EDA domain	Ohashi et al. (2000); Okamura et al. (2001); Vabulas et al. (2001); Vabulas et al. (2002)
TLR5	Gram-negative bacteria Gram-positive bacteria	Flagellin	Gewirtz et al. (2001); Hayashi et al. (2001)
TLR6	Gram-positive bacteria	Modulin, soluble tuberculosis factor STF, *Borrelia burgdorferi* outer surface protein A lipoprotein (OspA−L)	Bulut et al. (2001); Hajjar et al. (2001)
TLR7		Small antiviral compounds	Hemmi et al. (2002)
TLR8		Small antiviral compounds	Jurk et al. (2002)
TLR9	Bacteria	Unmethylated CpG-DNA	Hemmi et al. (2000); Takeshita et al. (2001);Wagner (2002)

Note: some TLR2 ligands are recognised by TLR2-TLR1/TLR6 heterodimers see text.

containing a TOLL/interleukin-1 receptor homology (TIR) domain, critical to both *Drosophila* TOLL and mammalian TLR signalling, indicating that they share homologous signalling components. In fact, for each signalling step, homologous components have been described for the two systems, mammalian TLR and *Drosophila* TOLL (Horng and Medzhitov, 2001) suggesting phylogenetic conservation over time.

2. Critical role of TLR in host defense

A key element in the initiation of an innate immune response against pathogens is the recognition of components commonly found on the pathogen that are not normally found in the host. These have been referred to as pathogen-associated molecular patterns (PAMP) (Medzhitov and Janeway, 1997). Upon infection, antigen-presenting cells (APC), such as macrophages (Mϕ) and dendritic cells (DC), express TLR on their surface, bind these PAMP, and initiate a signalling pathway that stimulates the host defences through the induction of reactive oxygen and nitrogen intermediates (ROI and RNI, respectively). It also initiates adaptive immunity as it activates APC by inducing production of pro-inflammatory cytokines and up-regulating co-stimulatory molecules. Moreover, TLR signalling stimulates the maturation and migration of

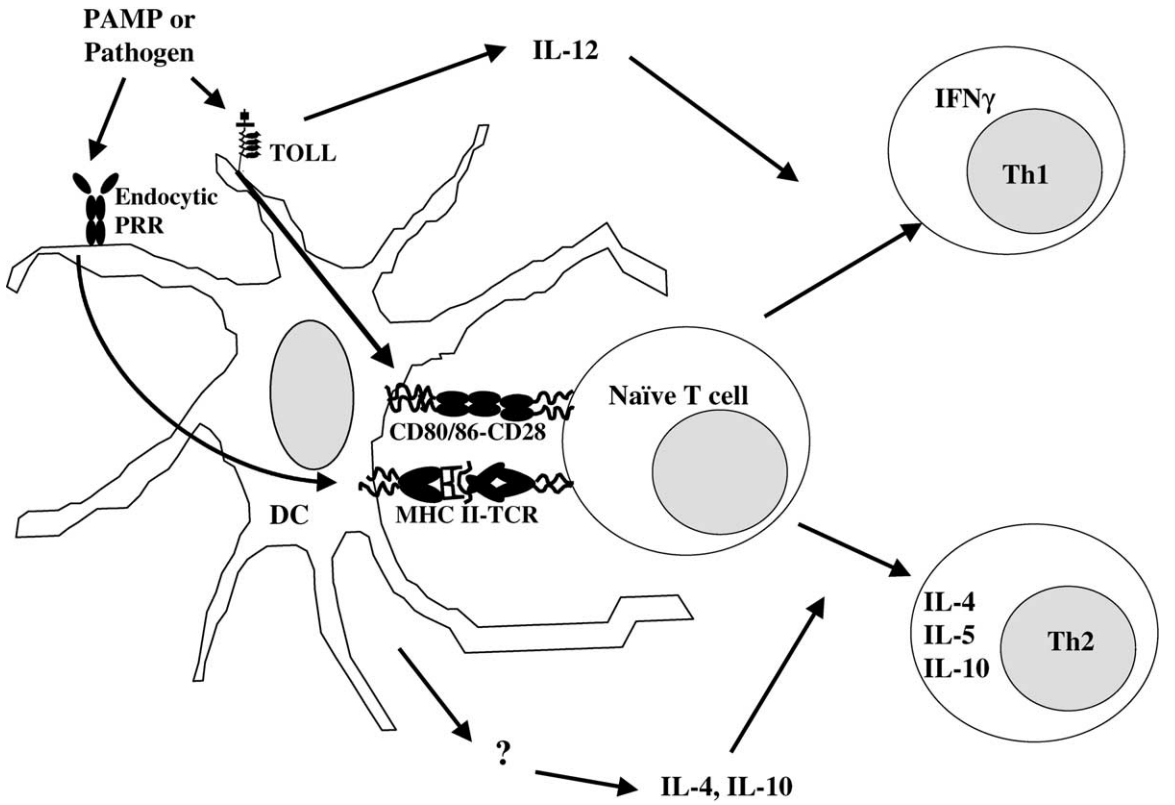

Fig. 1. Activation of adaptive immunity through TLR. Immature DC in the peripheral tissues recognise pathogens by TLR, leading to the up-regulation of cell-surface expression of co-stimulatory (CD80 and CD86) molecules and major histocompatibility complex class II (MHC II) molecules, or captured by endocytosis, subsequently bound to TLR (i.e. TLR9). Concomitantly, captured pathogens are processed and presented to T cells as antigen–MHC complexes. TLR also induce expression of cytokines, such as IL-12, chemokines and their receptors. Induction of CD80/86 on APC by TLRs leads to the activation of T cells specific for pathogens that trigger TLR signalling. IL-12 induced by TLR also contributes to the differentiation of naïve and/or activated T cells into T helper (T_H1) cells. Recently, it has been shown that activation of TLR play a role in the induction of T_H2 responses, too (Dabbagh et al., 2002; Re and Strominger, 2001). Whether this is partially mediated by soluble factors secreted by DC remains to be seen. Thus, establishment of adaptive immunity is greatly influenced by TLR-stimulated DC.

DC to the draining lymph nodes in some species, i.e. mice, whereas this migration seems to be more constitutive in ruminants, but can be modulated (for review see Haig et al., 1999). In mice and humans, cell trafficking is mediated by chemokine receptors expressed by DC following stimulation of TLR (Rescigno et al., 1999, 2000; Sallusto et al., 1999). LPS, a typical PAMP interacting with TLR (see below), downregulates the expression of chemokine receptors such as CCR5, and concomitantly upregulates CCR7 expression on DC. Similarly, cytosine–phosphate–guanosine (CpG) DNA induces trafficking of cells into the regional draining lymph node (Uwiera et al., 2001). In the secondary lymphoid organs, DC stimulate T cells by presentation of MHC molecule-associated peptides, combined with a secondary signal delivered by co-stimulatory molecules such as CD80/86. Concomitantly, DC produce a variety of chemokines thereby recruiting natural killer cells and naïve T cells. In murine models, bacterial infection activates DC via stimulation of TLR and preferentially induces T_H1-inducing cytokines such as IL-12. Therefore, TLR-stimulated DC tend to direct T-cell differentiation towards the T_H1 cell type. Presently, it remains unclear as to whether DC also induce T_H2 cell differentiation by stimulation of

TLR or other PRR upon infection with certain pathogens, but at least for TLR4 this seems to be the case. TLR4-defective mice exposed to an allergen had reductions in airway inflammation accompanied by a reduced T_H2 response (Dabbagh et al., 2002). Thus, TLR4 may be required for the induction of an optimal T_H2 responses to antigen of non-pathogenic sources, suggesting a role for TLR ligands, such as LPS derived from commensal bacteria or endogenously derived ligands, in maturation of the innate immune system before pathogen exposure (Dabbagh et al., 2002). Taken together, these observations show that TLR are crucial not only in the early phase of infection when innate immunity is important, but also links innate and adaptive immunity throughout the entire course of the host defense response (Fig. 1). In the following, it is briefly summarized what is known about the interaction of PAMP with TLR (Table 1).

3. TLR4 recognises bacterial and viral PAMP as well as endogenous molecules

The best-characterized PAMP is LPS, a major component of the outer membrane of Gram-negative bacteria. LPS provokes a variety of immunostimulatory responses, including the production of pro-inflammatory cytokines such as IL 12 and inflammatory effector substances such as NO. A glycosylphosphatidylinositol (GPI)-anchored protein, CD14 expressed by mononuclear phagocytes, was identified to bind LPS. However, CD14 lacks an intracytoplasmic region, suggesting that it is unable to transduce a signal, and that additional membrane protein(s) might be essential for LPS signalling in mononuclear phagocytes (Ingalls et al., 1999). Poltorak et al. (1998) discovered that an LPS signal is transmitted by TLR4. Using positional cloning they showed that LPS tolerant mice had a missense mutant in the TLR4 gene. Similarly, LPS-tolerant C57BL/10ScCr mice had a deletion in the TLR4 gene. Whereas initial transfection studies suggested that TLR2 is a receptor for LPS, this was subsequently found to be due to contaminants of the LPS preparation used that behave as TLR2 agonists (Hirschfeld et al., 2000). Recent data on the activation of TLR4 also indicated that trace amounts of LPS in reagents might stimulate TLR pathways, thus altering the results of the study, especially with respect to cytokine responses and/or antigen presentation studies (Bosisio et al., 2002). Given the importance of trace amounts of LPS in triggering TLR, it is mandatory that all substances used, including media, are checked for low levels of contaminating LPS. In addition to CD14 and TLR4, the MD-2 protein, which is associated with the extracellular portion of TLR4, is necessary for binding of LPS (Akashi et al., 2000; Shimazu et al., 1999). The LPS receptor complex therefore consists of CD14, TLR4 and MD-2. How these molecules interact with LPS is unknown.

Besides LPS, the murine TLR4/MD-2 complex recognises other distantly related substances, including the diterpene taxol, a widely used anticancer drug representing a LPS mimic for murine but not for human Mϕ. Taxol signalling depends on TLR4 (Kawasaki et al., 2000), and MD-2 appears to confer the above-mentioned species specificity.

TLR4 also is involved in viral recognition. For example, the F protein of respiratory syncytial virus (RSV) induces pro-inflammatory cytokines by binding to wildtype TLR4 and CD14 (Haynes et al., 2001; Kurt-Jones et al., 2000) whereas mutant C57BL/10ScCr mice lacking TLR4 on their surface were impaired in their ability to eliminate RSV. However, these mice possess mutations not only in TLR4 but also in IL-12R genes (Poltorak et al., 2001) possibly explaining defective immunity against RSV. This calls for further studies examining the role of TLR4 in pathogenesis of infections by RSV and other viruses.

In addition to these exogenous PAMP, TLR4 also binds endogenous molecules. One of these is heat shock protein (hsp) 60 that induces an inflammatory response in normal mice, but not in C3H/HeJ mice, suggesting the involvement of TLR4 (Ohashi et al., 2000). Collectively, hsp are expressed in bacteria as well as in host cells. As hsp are released from necrotic cells in certain pathological conditions and induce DC maturation (Basu et al., 2000), they could provide the molecular basis for the danger theory of immune activation as proposed by Matzinger. According to this theory, which still awaits confirmation by another laboratory, the immune system does not basically discriminate between self- and non-self, but rather responds to antigens associated with danger signals released from necrotic or stressed cells (Matzinger, 1994).

4. TLR2 recognises various pathogens

Gram-positive bacteria can provoke immune responses similar to those generated by LPS. The cell wall of Gram-positive bacteria contains lipoproteins and lipoteichoic acids (LTA), embedded in a layer of peptidoglycan (PGN). Analysis of TLR2-deficient mice or cells derived from these animals demonstrated that TLR2 is essential for the response to PGN, the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 (MALP-2) (Takeuchi et al., 1999, 2000, 2001) as well as to lipoarabinomannan, a major cell wall glycolipid derived from *Mycobacterium tuberculosis* (Jones et al., 2001). In vitro and transfection studies suggested that lipoteichoic acid and, in one study, the Gram-positive organism, *Listeria monocytogenes*, activate cells via TLR2 (Flo et al., 2000; Kadowaki et al., 2001). According to some authors, the ability of TLR2 to bind such a variety of ligands is based on its ability to form heterodimers with other TLR, mainly TLR6 and TLR1 (Ozinsky et al., 2000; Takeuchi et al., 2001). Experiments involving dominant-negative forms of either TLR showed these to inhibit TNF expression induced by zymosan, Gram-positive bacteria, or PGN, or to be required for recognition of certain factors released by *Neisseria meningitidis* (Ozinsky et al., 2000; Pridmore et al., 2001; Wyllie et al., 2000). Thus, TLR2 appears to broaden its repertoire of specificities by forming at least two distinct types of functional heterodimers with other TLR.

5. TLR5 recognises flagellin

Most pathogenic and commensal bacteria produce flagellin, the structural component of bacterial flagellae. Recently, *Salmonella enteritidis* flagellin (FliC) has been shown to stimulate TNF and RNI production by activating IL-1R-associated kinase (IRAK) in murine and human Mϕ lines (Moors et al., 2001), and this activation depended on myeloid differentiation factor 88 (Myd88) signalling and NF-κB activation (Gewirtz et al., 2001; Hayashi et al., 2001). Thus, flagellin of Gram-negative and Gram-positive bacteria can also be regarded as PAMP, acting via TLR5 (Hayashi et al., 2001). TLR5 is expressed by myelomonocytic (Muzio et al., 2000) and by intestinal epithelial cells, where its expression is polarized to the basolateral side of these cells (Gewirtz et al., 2001).

6. TL3 recognises double-stranded RNA (dsRNA)

Viral replication in infected cells results in generation of dsRNA and induction of type-I interferon. dsRNA can be considered as PAMP as it is not a constituent of host cells. TLR3-deficient mice showed decreased responses to the viral RNA mimic, polyinosinic–polycytidylic acid (poly(I:C)), suggesting that TLR3 is involved in the recognition of dsRNA (Alexopoulou et al., 2001). Viral dsRNA binding to cell surface-expressed TLR3 may be derived from cells destroyed by cytopathic viruses. How a noncytopathic virus, interfering with interferon induction by dsRNA (Schweizer and Peterhans, 2001), interacts with the TLR system is the subject of current investigations. Other molecules such as protein kinase R have been suggested to mediate dsRNA-induced interferon production (Blair et al., 2002; Durbin et al., 2002).

7. TLR9 recognises CpG-DNA

In 1980, Bird and Taggart discovered that in contrast to vertebrates, arthropods and other invertebrates do not possess methylated DNA (Bird and Taggart, 1980). Subsequently, it has been suggested that these differences lead to the evolution of a non-self pattern recognition mechanism in the vertebrate immune system enabling them to recognise invading pathogens (Krieg, 2000). Recently, Hemmi et al. (2000) discovered that TLR9 serves as a PRR for CpG-DNA. Cells from TLR9-deficient mice were unresponsive to CpG-DNA as measured by proliferation of splenocytes, production of inflammatory cytokine by Mϕ, and maturation of DC. In addition to bacterial DNA, oligodeoxynucleotides (ODN) carrying the CpG motif also stimulate lymphocytes and APC of a variety of species, including ruminants (Brown et al., 1999; Pontarollo et al., 2002; Rankin et al., 2001; Shoda et al., 2001a,b; Zhang et al., 2001), pigs (Kamstrup et al., 2001), and carnivores (Rankin et al., 2001). This leads to an enhanced antigen presenting activity and maturation of DC, thereby priming antigen-specific

T_H1 responses (Hartmann et al., 1999; Shirota et al., 2001). CpG-DNA significantly up-regulates the expression of MHC class II, CD40, CD80, CD86, and IL-12 in both murine and human DC. More importantly, the effects of CpG-DNA on DC translate into functional correlates of immunity including increased DC migration (Ban et al., 2000), and enhanced activation of $CD8^+$ T cells (Warren et al., 2000), thus inducing a protective CTL responses against both viral and tumor antigens. This explains why CpG-DNA is such a powerful adjuvant. In humans, but not mice, TLR9 is selectively expressed on plasmacytoid DC, but not on myeloid DC (Jarrossay et al., 2001; Sparwasser et al., 1998, 2000), yet its expression is not restricted to DC. However, it remains to be investigated whether TLR9 is the only receptor for CpG-ODN, as there are conflicting data on the surface expression of TLR9 as well as the ability of DNA-dependent protein kinase catalytic subunit (DNA-PKC) to bind CpG-ODN in the cytoplasm (Ahmad-Nejad et al., 2002; Chu et al., 2000; Chuang et al., 2002).

8. TLR signalling

A variety of extensive reviews has been published on TLR signalling (Hacker, 2000; Kirschning and Bauer, 2001; O'Neill, 2002; Takeuchi and Akira, 2001). Thus, we will only give a short overview on the main events in TLR-mediated signal transduction. Binding of PAMP to a TLR leads to the activation of TIR, forming a signalling complex with MyD88, a cytoplasmic adapter protein, IRAK, and tumor necrosis factor receptor-associated factor 6 (TRAF6) (Fig. 2). This is followed by activation of the mitogen-activated protein kinase (MAPK) cascade and NF-κB. MyD88 is a universal signalling molecule, as MyD88-deficient cells were found to lack activation of NF-κB and MAPK by all TLR, IL-1 and IL-18. In addition, a MyD88-independent pathway exists for stimulation via TLR4, as two major biological effects provoked by LPS, cytokine production and co-stimulatory molecule upregulation, differ in their requirement for MyD88. Since LPS-dependent nuclear translocation of IFN regulatory factor (IRF)-3 is preserved in MyD88-deficient cells (Kawai et al., 2001), IRF-3 activation may contribute to the MyD88-independent pathway (Fig. 2). An adapter protein for TLR4, called TIR domain-containing adapter protein (TIRAP) or MyD88-adapter-like (Mal) associates with TLR4, but not TLR9, and seems to be critical for LPS-induced DC maturation (Fitzgerald et al., 2001; Horng et al., 2001). TIRAP/Mal forms homo- or heterodimers with MyD88, and associates with IRAK-2, thereby leading to NF-κB activation (Fitzgerald et al., 2001). Recently, an additional molecule involved in TLR-mediated signalling was identified as RICK (also referred to as Rip2 or Cardiac) (Dabbagh et al., 2002; Kobayashi et al., 2002). This molecule appears to be a mediator of both innate and adaptive immunity and confers NF-κB activation upon triggering. Although all TLR family members signal via MyD88 and NF-κB (Fig. 2), more recent information point to signalling mechanisms unique to each TLR (O'Neill, 2002).

9. Species-specific responses to TLR ligands

So far, only few publications allow a direct comparison of TLR activation in different species, even between species with a high accessibility of reagents, such as mouse or human. However, several lines of evidence point to species-specificity in binding and/or signalling. For example, human and rodent cells differentially respond to lipid A analogues, a species specificity conferred mainly by TLR4 (Lien et al., 2000; Poltorak et al., 2000). In addition, human TLR4, but not murine TLR4, can recognise a molecular pattern of bacterial adaptation to the host, and this modulation depends on a cross-species hypervariable region (Hajjar et al., 2002). This hypervariable region may also explain why taxol is recognised by the murine TLR4-MD-2 complex, but not by the human counter-part (Kawasaki et al., 2000). Furthermore, several studies have shown species-specific stimulation of immune cells by CpG-ODN. A very detailed study showed species-specific reactions as well as conserved reaction to different CpG-ODN in veterinary and laboratory species (Rankin et al., 2001), including sheep, goats, horses, pigs, dogs, cats and chicken. In addition, cells expressing human TLR9 are stimulated by CpG motifs that are active in primates but not rodents, suggesting that evolutionary divergence between TLR9 molecules underlies species-specific differences in the recognition of bacterial DNA (Chuang et al., 2002; Takeshita et al., 2001).

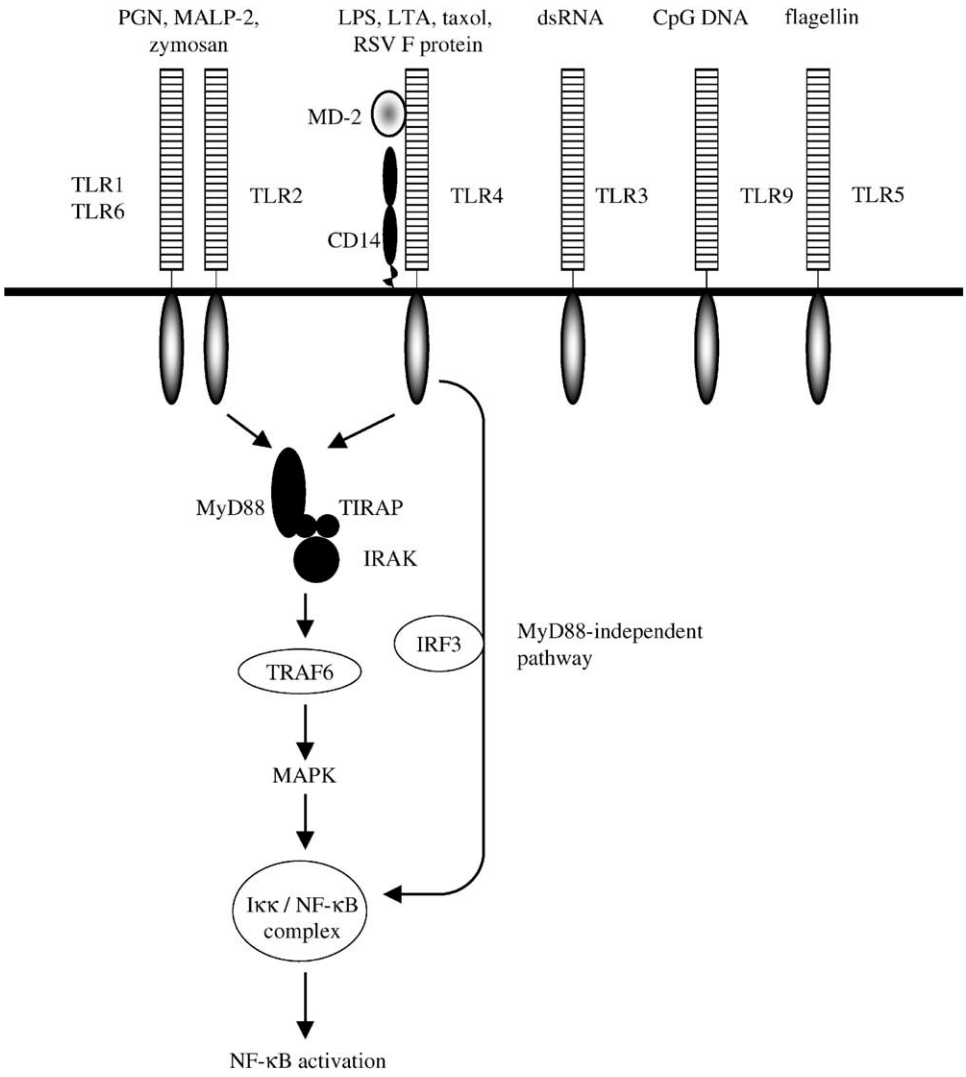

Fig. 2. TLR, their putative ligands, and the intracellular signalling cascade (shown for TLR4 being representative for all TLR family members). Activation of TLR by the ligands shown leads to the association of the TLR-TIR domain with MyD88. MyD88 also possesses the death domain, which mediates the association with the serine–threonine kinase IRAK. Subsequently, TRAF6 is activated and in turn activates MAPK and the Iκκ complex. The Iκκ complex induces phosphorylation of IκB, which renders IκB competent for being ubiquitinated and degraded, liberating NF-κB and allows it to translocate into the nucleus where it can induce target gene expression. In addition, TLR4 can activate NF-κB and MAPK in a MyD88-independent and TIRAP/Mal-dependent manner, leading to the phosphorylation and nuclear translocation of IRF3 that is involved in IFN-inducible gene expression (Akira et al., 2001).

10. TLR variation in domestic animals

Based on the above-mentioned differences in ligand specificity of human and murine TLR, it is necessary to investigate TLR function in domestic animal species. In addition to species-specific responses, data on NO production by Mϕ from chicken with different genetic background showed that the strain used to study TLR expression and/or TLR mediated responses may influence the data obtained as well (Dil and Qureshi, 2002). The recently cloned chicken TLR2, chTLR2 type 2, covers two major microbial PAMP,

LPS and lipoproteins, corresponding to recognition by mammalian TLR2 and TLR4 (Fukui et al., 2001). This points to increasing complexity of the TLR system in the course of evolution, e.g. from avian precursors to mammals. This duplicated TLR in this pattern recognition system may function for host-pathogen discrimination in a manner that is distinct from that in mammals. Interestingly, mammalian TLR but not *Drosophila* TOLL, are PRR, although both have a role in innate immunity; in the latter, PAMP are recognised by humoral TOLL ligands rather than by cellular receptors. Whether cell distribution of TLR shows species variations has not been explored well, and it may be of great importance to study expression of TLR in different tissues of one species first before trying to compare different species, as tissue expression of TLR has recently been recently shown to vary (Zarember and Godowski, 2002).

11. Bovine TLR

So far, only a few sequences are available for non-rodent TLR, most of them derived from TLR4 (canine: GenBank Acc. No. BAB85609; feline: GenBank Acc. No. BAB43947; rat: GenBank Acc. No. AAC13313) and TLR2 sequences (chicken: GenBank Acc. No. BAB16842). However, apart from chicken TLR, no functional data has been published yet. Further details can be obtained from the following web-side: http://www-personal.umich.edu/~ino/List/TOLLRE.htm.

As regards domestic animals, a lot of current work focuses on the TLR system of cattle. Recently, we cloned the bovine homologues of TLR2, TLR4, and MD-2 (GenBank Acc. Nos. AF310951, AF310952 and AF368418), and partial sequences for boTLR3 and boTLR9 are available (AY124007 and AY124008). The similarities at the amino acid level with the human and murine molecules are 77 and 67% for TLR2, 72 and 65% for TLR4, and 64 and 55% for MD-2, respectively. Using RT-PCR, mRNA for boTLR2 and boTLR4 could be detected in monocytes, Mϕ and DC, thus resembling data on human cells (Muzio et al., 2000; Visintin et al., 2001 #7482). In contrast to human cells, no differences in the amount of mRNA transcripts could be detected by a multiplex PCR for these TLR. Monocytes and Mϕ, but not DC, react to TLR2 and 4 ligands, such as LPS, heat-inactivated *Salmonella dublin*, and *L. monocytogenes* with the production of ROI (Werling et al., in preparation). It will be of interest to test whether TLR induce ROI generation by protein-kinase C activation, whether this pathway is expressed in the cells examined, and whether there is a maturation-dependent coupling of TLR signalling pathways to NAD(P)H-dependent oxidase activation. In addition, Mϕ and DC respond by different cytokine patterns to TLR2 and TLR4 ligands, which may differentially direct the adaptive immune response, ensued. Exposure of bovine myeloid DC to poly(I:C) RNA, but not CpG-DNA, stimulated the cells to produce low amounts of type-I interferon, and neither cytokine nor ROI and RNI were detectable (Werling et al., in preparation). Whether CpG-DNA stimulates bovine DC via TLR9, as described for bovine B cells and MΦ (Shoda et al., 2001a), and as described for DC of other species, remains to be seen. Interestingly, bovine DC do respond with the release of IL-12, TNF, and NO, similar to that described for plasmacytoid DC in the human system, despite the fact that only plasmacytoid DC, but neither myeloid DC nor monocytes/Mϕ express transcripts for TLR9 in the human system (Jarrossay et al., 2001; Krug et al., 2001). To clarify whether these differences might be genuine inter-species differences or differences based on different culture systems, the availability of antibodies recognizing bovine TLR would greatly help.

12. Concluding remarks

Over the last decades, antibiotics have proven to be powerful tools in the control of infectious diseases. However, this has been accompanied by the emergence of pathogens with multidrug resistance and an increasing risk of antibiotic residues in animal food products. Thus, the development of novel agents, including vaccines, is expected to contribute to the fight against pathogens. Vaccine formulations often produce undesirable side effects or show poor immunogenicity on their own. The molecular mechanisms underlying adjuvant activity are poorly understood. The discovery and functional analyses of TLR may provide the first step to the development of new adjuvants. PAMP, either co-administered together with safe vaccines or co-expressed with an immunogenic

protein in the same vector, may be formulated that act on or modulate the immune response via TLR. These reagents, either enhancing or inhibiting TLR signalling pathways, can be powerful modulators in the fight against pathogens that have the ability to evade an immune response. The first promising data on the ability to influence the immune response generated in bovine cells using CpG-ODN has been published recently (Brown et al., 1999; Shoda et al., 2001a,b; Stich et al., 1998; Zhang et al., 2001). Whereas in these studies, CpG-ODN was used as an adjuvant, it was shown recently that CpG-ODN coupled with antigens had a similar effect, but also enabled the antigen to be directed to DC (Shirota et al., 2001). Thus, our increasing understanding of the TLR system could provide the molecular basis for preventing or treating a variety of pathological conditions.

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