Toll-like Receptors. II. Distribution and Pathways Involved in TLR Signalling

(TLR distribution / MyD88 / IRF / NFκB)

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Abstract. The innate immune system senses invading microorganisms by a phylogenetically conserved family of proteins – TLRs. They are expressed in several types of cells that represent a route of entry of pathogens into the host organism and that can contribute to protection against infection. Except for cells of the immune system, TLRs are present in epithelial cells of the skin, respiratory, intestinal, and genitourinary tracts that form the first protective barrier to invading pathogens. Polarized regulation of TLR expression in epithelial cells explains why pathogenic but not commensal bacteria elicit inflammatory responses. TLR-induced intracellular signalling pathways show remarkable complexity: apart from a common signalling pathway, additional signalling pathways specific for each of the TLRs are responsible for a fine tuning of the immune response, thus securing effective pathogen-directed biological responses.

Human Toll-like receptors (TLRs) represent the earliest surveillance systems for primary infection by pathogens. To date, ten TLRs have been identified in humans and demonstrated to recognize a variety of pathogen-associated molecular patterns (PAMPs) (Sandor and Buc, 2005). TLRs are widely distributed and induce specific gene expression profiles that are suited to ensuring efficient removal and destruction of the invading pathogen. The result of their activation ranges from the induction of synthesis of proinflammatory cytokines and interferons which orchestrate innate immunity to the expression of co-stimulatory molecules which promote T-cell activation and specific immunity.

Expression of TLRs

Several studies have elucidated the expression patterns of TLR family members in human tissues. Most tissues tested expressed at least one TLR and several expressed all (the spleen, peripheral blood leukocytes). Professional phagocytes express the greatest variety of TLRs. Neutrophils were found to express all the TLRs except TLR3. The agonists of all TLRs expressed in neutrophils triggered cytokine release, superoxide generation, L-selectin shedding and increased phagocytosis of opsonised latex beads (Hayashi et al., 2003). Eosinophils constitutively express TLR1, 2, 4, 6, 7, 9, and 10 mRNAs (Nagase et al., 2003). Basophils express TLR2 and TLR4 but not CD14 (Sabroe et al., 2002). Mast cells express mRNA for TLR1, TLR2, and TLR6 but not TLR4. Apart from polymorphonuclear cells, monocytes and macrophages play a pivotal role in innate as well as adaptive immune responses. Monocytes/macrophages express mRNA for all TLRs except TLR3 (Muzio et al., 2000; Hornung et al., 2002). Human blood dendritic cells (DCs) are divided into two subsets: myeloid (mDCs) and plasmacytoid (pDCs). mDCs express all TLRs except TLR7 and TLR9, respectively; these are expressed almost exclusively in pDCs, although some data support TLR7 expression in mDCs as well (Jarrossay et al., 2001; Ito et al., 2002). Immature DCs enter the maturation process when stimulated by various microbial components and display distinct patterns of TLR expression during this process. Expression of TLR1, 2, 4, 5 decreases as DCs mature (Visintin et al., 2001). In contrast, TLR3 is expressed in mature DCs only (Muzio et al., 2000). As for other mononuclear cells, B cells express high levels of TLR1, 6, 9, 10, and low levels of TLR2, 4, 7 (Hornung et al., 2002). NK cells are major players in the antiviral immune response and express TLR3 and are activated directly in response to poly(I:C). NK cells also seem to be able to respond directly to other viral TLR stimuli, such as CpG DNA. Therefore, activation of NK cells might not be a unique feature of TLR3 but might similarly apply to TLR9 (Schröder and Bowie, 2005). The latest reports also indicate that NK

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Abbreviations: ATF – activating transcription factor, BLPs – bacterial lipopolysacharides, CMV – cytomegalovirus, Cox-2 – cyclooxygenase-2, DCs – dendritic cells, ds – double stranded, ICSBP – IFN consensus sequence-binding protein, IKK – IκB kinase, IRAK – IL-1-receptor-associated kinase, IRF – interferon regulatory factor, Mal – MyD88-adaptor-like, PGE2 – prostaglandin E2, R – receptor, STAT – signal transducers and activators of transcription, TICAM – Toll-interleukin 1 receptor domain containing adaptor molecule, TIR – Toll-IL-1-resistance, TIRAP – TIR domain-containing adaptor protein, TLR(s) – Toll-like receptor(s), Tollip – Toll-interacting protein, TRAF – TNF-receptor-activated factor, TRAM – TIR domain-containing adaptor-inducing IFN-β-related adaptor molecule, TRIF – TIR domain-containing adaptor-inducing IFN-β, WT – wild type.

cells in stimulation with TLR2 and TLR5 ligands induce IFN- production and enhance secretion of -defensins (Chalifour et al., 2004).

In addition to immune cells, TLRs are expressed in several other types of cells that may represent a route of entry of pathogens into the host organism and that can contribute to protection against infection. Namely, the skin, respiratory, intestinal, and genitourinary tracts are covered with epithelial cells, forming a protective barrier to invading pathogens. Keratinocytes were found to constitutively express TLR1, 2, 3, 4, and 5 but not TLR6, 7, 8 or 10; however, the data on TLR4 expression are somehow conflicting (Pivarcsi et al., 2003). mRNAs for TLR2 and TLR4 were detected in nasal mucosa, tonsilar tissue and adenoids (Claeys et al., 2003).

Immunohistochemical labelling of TLR2 in normal human airways revealed TLR2 expression throughout the epithelium, with an apparently higher level of expression on non-columnar basal epithelial cells (Hertz et al., 2003). Pulmonary epithelial cells were found to express TLR4 (Guillot et al., 2004), whereas TLR2 was expressed mainly in type II alveolar epithelial cells (Droemann et al., 2003). Experiments on TLR2- and TLR4-deficient mice underlined the essential role of these receptors in mediating an effective immune response to *Haemophilus influenzae, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Mycobacterium tuberculosis* in intrapulmonary infection models (Abel et al., 2002; Wang et al., 2002; Branger et al., 2004). Intestinal cells are constantly exposed to bacteria on their apical surface, yet this does not result in induction of inflammation. Only those pathogenic bacteria that invade the basolateral compartment side elicit inflammatory response in these cells. Accordingly, TLR5, the receptor for flagellin, is exclusively expressed on the basolateral, but not the apical surface of the intestinal epithelial cells (Gewirtz et al., 2001). Moreover, low TLR4 expression in intestinal epithelial cells explains hyporesponsiveness of these cells to LPS (Abreu et al., 2001). This fine polarized regulation of TLR expression in epithelial cells may provide explanation why pathogenic Gram-negative bacteria, but not commensal bacteria, elicit inflammatory responses in the intestine. Renal inflammation evoked by ischaemia markedly enhanced synthesis of TLR2 and TLR4 mRNA followed by significant elevation of protein expression in the distal tubular epithelium, the thin limb of Henle's loop and collecting ducts. This phenomenon was shown to be completely dependent on the action of IFN- and TNF. Thus, TLR expression in renal cells may contribute to the detection of a bacterial invasion into the lumen of the tubules and initiation of inflammatory response (Wolfs et al., 2002). Investigating the presence of TLRs in the mucosal epithelial cells of the lower female genital tract, it was found that epithelial cells derived from normal human vagina, ectocervix and endocervix expressed mRNA for TLR1, 2, 3, 5, and 6. However, they failed to express TLR4. Consistent with this, endocervical epithelial cells were unresponsive to purified LPS from *Neisseria gonorrhoeae* and *Escherichia coli*. However, stimulation with whole Gram-negative bacteria and bacterial lysates resulted in proinflammatory cytokine production (Fichorova et al., 2002). In contrast, corneal epithelial cells express TLR4, which seems to contribute to inflammatory responses leading to river blindness after infection with parasitic filarial nematodes (Saint Andre et al., 2002). Apart from detecting the invading pathogens at the sites of possible entry, the host organism possesses mechanisms of detecting pathogens once they have already invaded the circulation. Besides TLR5, human endothelial cells express predominantly TLR4 and weakly TLR2. Preincubation with TNF or IFN- significantly induces endothelial TLR2 expression. When stimulated with LPS, endothelial cells were found to activate the NF- B transcription factor (Faure et al., 2000). Thus, endothelial cells may have a role in detection of pathogens.

Signalling Pathways Induced by TLR Ligands

The host defence against microorganisms mediated by TLRs relies mainly on **signalling pathways** induced by a common Toll-interleukin 1 receptor (TIR) domain and shows a high homology to that of the IL-1R (IL-1 receptor) family. Both TLRs and IL-1R interact with the intracellular adaptor protein **MyD88** (myeloid differentiation factor). The MyD88 possesses two domains, C-terminal which is similar to the TIR-domain and N-terminal death-domain – DD (Fig. 1) (Medzhitov et al., 1998). TLRs and IL-1R associate intracellularly with the MyD88 adaptor protein via interaction between the respective TIR domains. MyD88 afterwards recruits the serine/threonine kinase IRAK. This, similarly to MyD88, contains DD and upon recruitment to MyD88 via DD-domain interactions is phosphorylated and subsequently associated with TRAF-6. Its activation in turn leads to initiation of two distinct downstream pathways resulting in activation of NF- B and AP-1 transcription factors (Muzio et al., 1997; 1998).

In the process of analysing the MyD88-independent activation of the LPS-TLR4 signalling a novel adaptor protein named TIRAP or Mal was discovered (Fitzgerald et al. 2001; Horng et al., 2001). The **TIRAP/Mal** has the C-terminal TIR; however, it lacks the N-terminal DD. It associates with TLR4 through interaction between their respective TIR domains. TIRAP/Mal forms dimers with MyD88 followed by association with IRAK and activation of NF- B (Fig. 1). Further data suggest that TIRAP/Mal is not specific to TLR4 signalling and that it takes part in the TLR2 signalling as well; however, it does not participate in the MyD88-independent pathway (Yamamoto et al., 2002).

Tollip is an adaptor molecule that was first identified in the context of IL-1 signalling. Before IL-1 treatment, Tollip is present in a complex with IRAK. Following the IL-1 stimulation, the recruitment of Tollip-IRAK complexes to the activated IL-1R occurs through the association of Tollip with IL-1R. Co-recruited MyD88 then triggers IRAK autophosphorylation, which in turn leads to rapid dissociation of IRAK from Tollip (Burns et al., 2000). The inhibition of NF- B activation in response to IL-1, as well as TLR2 and TLR4 ligands (Fig. 1), is mediated through the ability of Tollip to suppress the activity of IRAK. Negative regulation of TLR signalling by Tollip may therefore serve to limit the production of proinflammatory mediators during inflammation and infection (Zhang et al., 2002).

MyD88 was shown to be crucial for inflammatory cytokine production in stimulation with a variety of microbial ligands. However, in the MyD88-deficient macrophages LPS is still able to induce activation of NF- B and AP-1 transcription factors, but with delayed kinetics (Kawai et al., 1999), which suggests that there also exists a **MyD88-independent pathway**. Accumulating evidence indicates that the LPS-induced DC maturation depends on the MyD88-independent pathway: LPS stimulation of DCs from MyD88-deficient mice induced their functional maturation, including up-regulation of co-stimulatory molecules and enhancement of antigen-presenting activity (Kaisho et al., 2001). According to subsequent studies in the MyD88-deficient mice, LPS exposure, but not TLR2 ligands, still initiated a variety of responses: activation of IFN-regulatory factor 3 (IRF-3) and induction of several IFN-inducible genes such as *IP-10* (Kawai et al., 2001). Similarly to LPS, the dsRNA stimulation of TLR3 in the MyD88-deficient cells induced activation of NF- B, but no production of inflammatory cytokines (Alexopoulou et al., 2001). The dsRNA and viral infection are also known to activate IRF-3, thereby inducing the *IFN- /* and IFN-regulated genes (Iwamura et al., 2001). Mice lacking the transcription factor IRF-3 are more vulnerable to virus infection as virus-induced *IFN- /* gene expression levels are reduced. Moreover, IRF-3 is also essential for the LPS-mediated *IFN-* gene induction (Sato et al., 2000; Sakaguchi et al., 2003). Thus, viral infection or dsRNA stimulation via TLR3 as well as LPS stimulation via TLR4 induces phosphorylation and subsequent activation of IRF-3 and its translocation to the nucleus, thereby leading to the *IFN-* gene expression. Based on this data it is supposed that TLR3 and TLR4 have evolutionarily diverged from other TLRs to activate IRF-3, which mediates a specific gene programme responsible for innate antiviral responses (Doyle et al., 2002). Similar to the LPS response, TLR9 signalling can also induce expression of *IFN-* and IFN-inducible genes, and up-regulation of CD40. However, all these effects are MyD88-dependent. Thus in TLR4 signalling, *IFN- /* expression can be induced either by the MyD88-dependent or MyD88-independent pathways, whereas the TLR9 signalling is dependent on the MyD88 only (Hoshino et al., 2002; Kawai and Akira, 2005).

As stated above, accumulating evidence demonstrates the existence of the MyD88-independent pathway, which may explain unique biological responses of individual TLRs, particularly TLR3 and TLR4. By searching for adaptors, a novel TIR domain-containing molecule, named **TRIF** or **TICAM-1** was identified (Fig. 2a). Over-expression of TRIF, but neither MyD88 nor TIRAP, activated the *IFN-* promoter. Dominant-negative TRIF inhibited TLR3-dependent activation of both the NF- B-dependent and *IFN-* promoters. TRIF physically associates with TLR3 and IRF-3 in response to poly(I:C). Thus, dsRNA-TLR3-dependent production of IFN- is mediated mainly by TRIF/TICAM-1 (Oshiumi et al., 2003a, b; Schröder and Bowie, 2005). In next experiments a germline mutation, called Lps2, was generated in mice, which abolishes cytokine responses to dsRNA and severely impairs responses to LPS. Lps2 homozygous mice were found to be resistant to the toxic effects of LPS, and were hyper-susceptible to mouse CMV, failing to produce type I interferons when infected. Positional cloning revealed that the TRIF had been structurally altered (Hoebe et al., 2003). Likewise, TRIF-deficient mice were defective in both TLR3- and TLR4-mediated expression of *IFN-* and activation of IRF-3 (Yamamoto et al., 2003a). These data suggest that TRIF is essential for TLR3- and TLR4-mediated signalling pathways facilitating mammalian antiviral host defence. However, some data indicate that TRIF is involved in NF- B but not IRF-3 activation. In these experiments, TRIF was found to recruit TRAF-6 to TLR3 through its TRAF6-binding site. Furthermore, mutation of the TRAF6-binding site of TRIF only abolished its ability to activate NF- B but not IRF3, suggesting that TLR3-mediated activation of NF- B and IRF3 might bifurcate at TRIF (Jiang et al., 2004).

TRAM (or TICAM-2) is the fourth TIR domain-containing adaptor protein described that participates in Toll receptor signalling. Restricted to the TLR4 pathway, TRAM activates IRF-3, IRF-7, and NF- B-dependent signalling pathways in MyD88-independent manner (Figs. 1, 2a). TRAM interacts with TRIF, TIRAP, and TLR4 but not with TLR3 (Fitzgerald et al., 2003). TRAM-deficient mice showed defects in cytokine production in response to the TLR4 ligand, but not to other TLR ligands. Moreover, TLR4- but not TLR3-mediated MyD88-independent IFN- production and activation of signalling cascades were abolished in TRAM-deficient cells (Yamamoto et al., 2003b). According to the latest data, TRAM physically bridges TLR4 and TRIF and functionally transmits LPS-TLR4 signalling to TRIF, which in turn activates IRF-3 (Fig. 1). Hence, in LPS signalling, the TLR4 recruits two types of adaptors, TIRAP and TRAM, to its cytoplasmic domain that are indirectly connected to two effec-

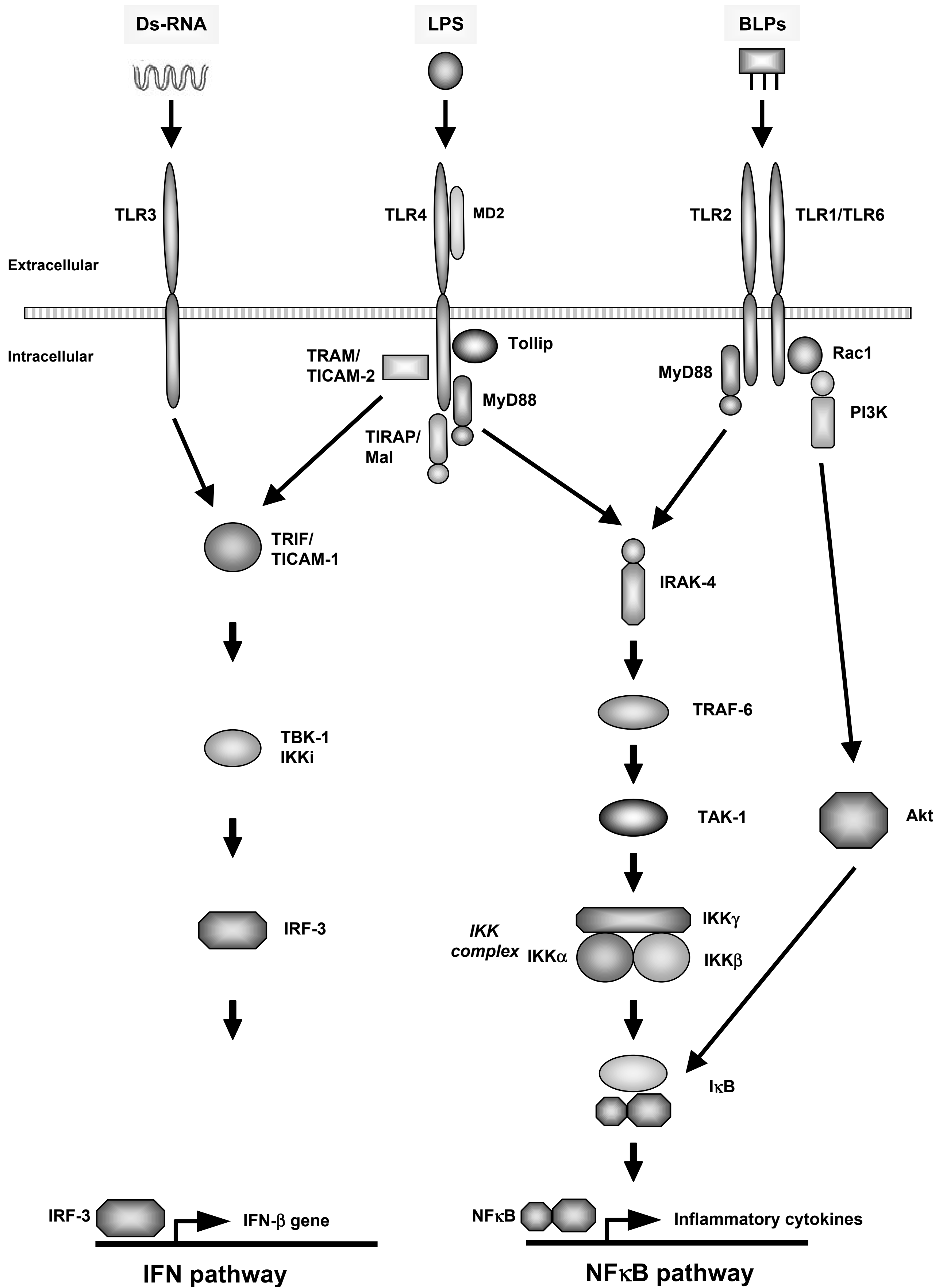

Fig. 1. Adaptors and transcription factors in TLR signalling pathways

tive adaptors, MyD88 and TRIF, respectively. It is concluded that for LPS-TLR4-mediated activation of IFN- , the adaptor complex of TRAM and TRIF plays a crucial role (Oshiumi et al., 2003b).

Recently, two IKK-related kinases, inducible I B kinase (IKK-i) and TANK-binding kinase 1 (TBK1), were suggested to act as **IRF-3 kinases** and to be involved in IFN- production in TLR signalling and viral infection (Fig. 1, Fig. 2b). dsRNA-induced induction of *IFN-* and IFN-inducible genes were completely abolished in IKK-i/TBK1 doubly deficient cells. These data indicate that TBK1 as well as, albeit to a lesser extent, IKK-i play a crucial role in the induction of *IFN-* and IFN-inducible genes in both TLR-stimulated and virus-infected cells (Hemmi et al., 2004; Moynagh, 2005).

Intensive research regarding adaptor molecules suggests that in addition to the common MyD88 signalling pathway, each of TLRs may possess its unique signalling pathway. In the TLR2-mediated signalling, stimulation of human monocytes by *S. aureus* induces a fast and transient activation of the Rho GTPases Rac1 and Cdc42 followed by the recruitment of active Rac1 and phosphatidylinositol-3 kinase (PI3K) to the TLR2 cytosolic domain. This in turn activates Akt kinase that induces activation and translocation of the p65 subunit of NF- B into the cell nucleus in a process that is independent of I B degradation (Fig. 1) (Arbibe et al., 2000). Further experiments demonstrated that the PI3K-Akt axis also occupies a central role in TLR2-induced activation of neutrophils (Strassheim et al., 2004). The latest data indicate that multiple GTPase-regulated pathways emerge from stimulated Toll receptors, controlling different aspects of NF- B-mediated gene transcription (Teusch et al., 2004).

Most studies so far have analysed DC activation by single microbial compounds. However, pathogens express several TLR agonists that may engage different TLRs at different times and in distinct cellular compartments. Therefore, a synergic effect of TLR signalling pathways could be supposed. Indeed, recently it was found that agonists of TLR3 and TLR4 had acted in synergy with agonists of TLR8 in inducing IL-12, IL-23 and Delta-4 in amounts that were 50- to 100-fold higher than those induced by optimal concentrations of single agonists leading to enhanced and sustained T_H1-polarizing capacity (Napolitani et al., 2005).

In summary, the existence of individual pathways using specific adaptor proteins and protein kinases for each TLR could explain the distinct biological responses evoked by different TLR ligands.

Transcription factors involved in TLR-mediated signalling are numerous. Initially, **NF- B** (nuclear factor-kappa B) was found to be essential for the transcription of immunoglobulin light chain genes in B cells. Subsequent experiments confirmed that the NF- B family of transcription factors is evolutionarily conserved and that they are expressed in a great variety of cells. In mammals five family members are known: RelA (p65), RelB, c-Rel,

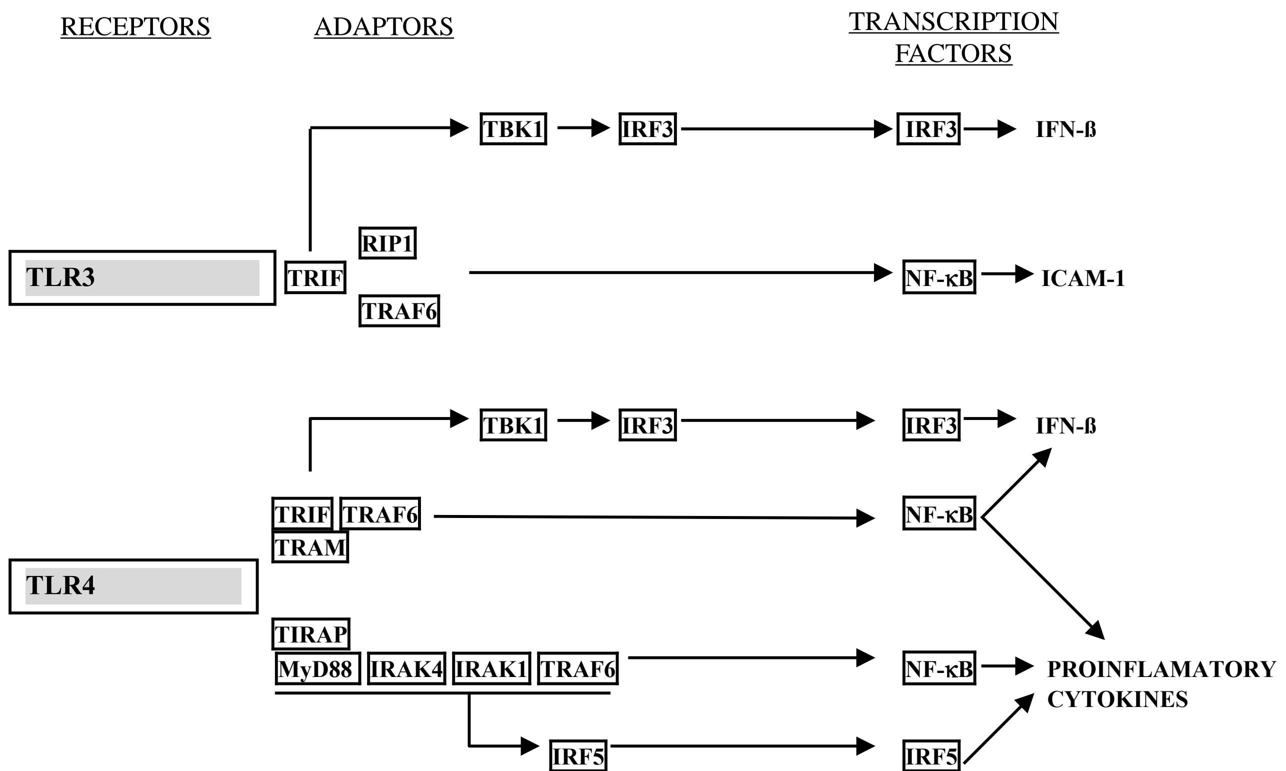

Fig. 2a. Schematic representation of TLR signalling pathways, I (adapted from Kawai and Akira, 2005) (for explanation – see the text)

p100/p52, p105/50 (Ghosh et al., 1998). Each member of this family plays an important role in TLR-mediated signalling. Mice deficient in individual NF- B subunits showed increased susceptibility to microbial infections. Mice lacking p50 were unable to effectively clear *Listeria monocytogenes*, were more susceptible to infection with *Streptococcus pneumoniae* and exhibited multifocal defects in immune responses involving B cells (Sha et al., 1995). Mice deficient in p52 revealed impaired ability to generate antibodies to T-dependent antigens, which was consistent with the absence of B-cell follicles and an inability to form germinal centres (Franzoso et al., 1998). RelB-deficient mice challenged with *Toxoplasma gondii* produced negligible levels of IFN- and had reduced NK-cell activity compared with wild-type (WT) mice (Caamano et al., 1999). Moreover, maturation of DCs was also defective in doubly deficient p65 and p50 as well as in RelB-deficient mice. They displayed an impaired cellular immunity and impaired antigen-presenting function (Burkly et al., 1995). In contrast, DCs from p50 and c-Rel doubly deficient mice developed normally – no significant impairment in MHC and co-stimulatory molecule expression was observed – yet IL-12 production was abolished (Ouaaz et al., 2002). These data suggest that NF- B subunits orchestrate the development and function of DCs in a very fine manner. Apart from microbial components, cells that die in a necrotic, but not apoptotic way, are able to induce inflammation (Cvetanovic et al., 2004). As already stated, following necrotic cell death heat-shock proteins exit mammalian cells and they exert expression of inflammatory genes and trigger maturation of DCs via TLR2 and TLR4, respectively (Beg, 2002).

AP-1 (activating protein-1) is a collective term referring to dimeric transcription factors composed of Jun, Fos or ATF subunits that bind to a common, the AP-1, DNA-binding site. The activation of the AP-1 is induced through phosphorylation by the MAP kinases such as JNK and ERK, respectively, that are activated by means of TLR or IL-1R engagement (Karin, 1995). TLR4 ligand LPS and various TLR2 ligands rapidly activated JNK kinases, which was followed by activation of the AP-1 transcription factor (Mackman et al., 1991; Gupta et al., 1999). Not just a bacterial, but also a viral infection or dsRNA induce JNK kinase, leading to stimulation of AP-1 (Chu et al., 1999). Accordingly, in neonatal rat ventricular myocytes anti-TLR2 antibody inhibited nuclear translocation of AP-1 and NF- B (Frantz et al., 2001).

NF-IL6 (nuclear factor-IL6) specifically binds to an IL-1-responsive element in the *IL-6* gene. The NF-IL6 mRNA was induced by the stimulation with LPS, IL-1 or IL-6. Interestingly, NF-IL6 was shown to bind to regulatory regions of various acute-phase protein and several other cytokine genes such as *TNF*, *IL-8* and

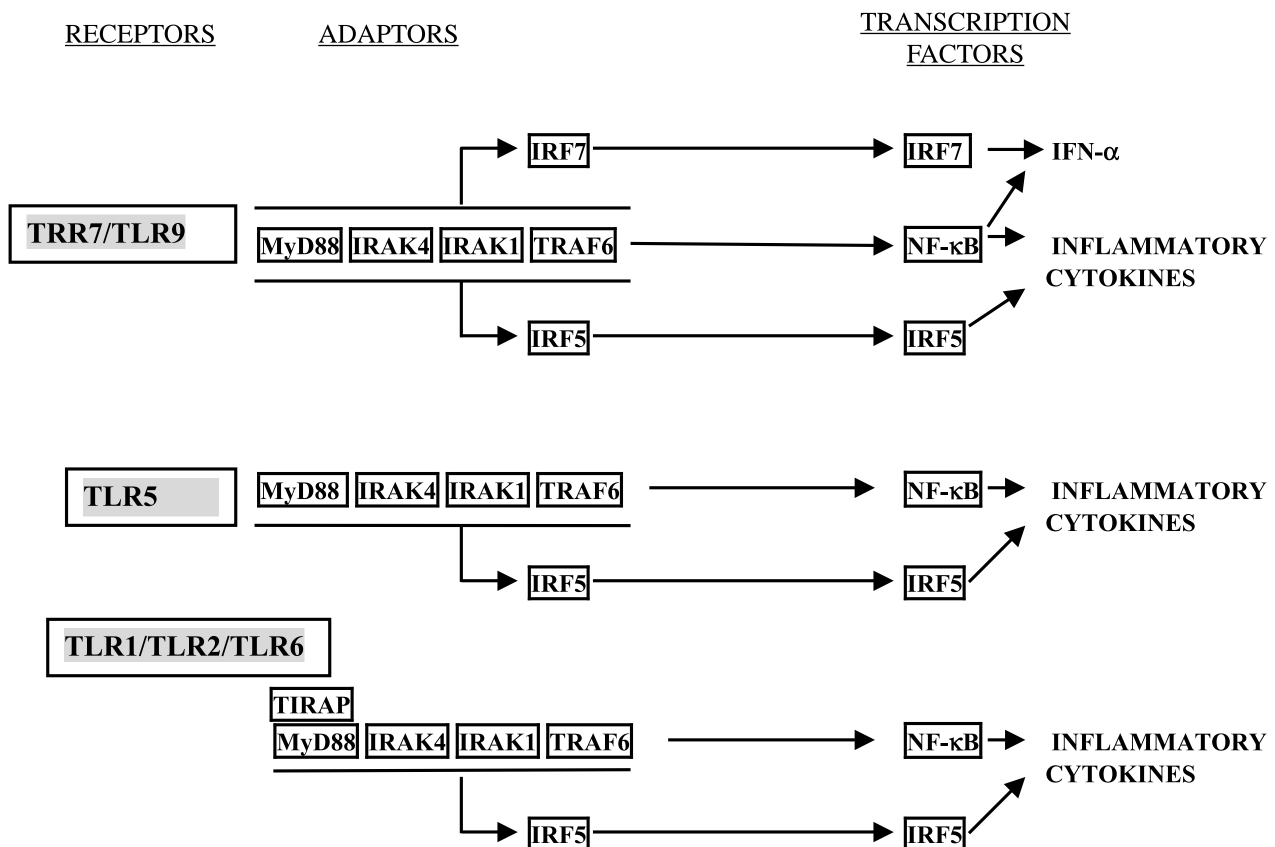

Fig. 2b. Schematic representation of TLR signalling pathways, II (adapted from Kawai and Akira, 2005) (for explanation – see the text)

G-CSF, implying that NF-IL6 has a role in the regulation not only of the *IL-6* gene, but also of several other genes involved in acute-phase reaction, inflammation and haematopoiesis (Akira et al., 1990). LPS or inflammatory cytokine stimulation of macrophages and hepatocytes activated MAP kinases that in turn phosphorylated NF-IL6, enhancing its transcriptional efficacy (Trautwein et al., 1993). The NF-IL6 deficient mice were highly susceptible to infection with *Listeria monocytogenes*; moreover NF-IL6 was essential for the induction of G-CSF in macrophages (Tanaka et al., 1995). NF-IL6-deficient murine macrophages in stimulation with LPS displayed impaired expression of membrane-bound glutathione-dependent PGE2 synthase or Cox-2 (Uematsu et al., 2002). These data indicate that the presence of NF-IL6 is essential for LPS-induced gene expression in macrophages and defines NF-IL6 as an important transcription factor in the TLR-mediated signalling pathway.

Interferon regulatory factors (IRFs) constitute a family of transcription factors that commonly possess a helix-turn-helix DNA-binding motif and are essential to the regulation of innate immune responses. To this date, altogether nine members of IRFs have been identified (Taniguchi et al., 2001). Mice deficient for **IRF-1** showed impaired resistance to infection with encephalomyocarditis virus (Kimura et al., 1994). Macrophages from these mice when stimulated with LPS or IFN- failed to produce NO and synthesized barely detectable levels of inducible NO synthase (iNOS) mRNA; furthermore, infection with *Mycobacterium bovis* was more severe in IRF-1-deficient mice than in WT-mice (Kamijo et al., 1994). T cells from mice lacking the transcription factor IRF-1 fail to mount T_H1 responses and instead exclusively undergo T_H2 differentiation *in vitro*. Compromised T_H1 differentiation is found to be associated with defects in multiple cell types, namely impaired production of IL-12 by macrophages, hypo-responsiveness of $CD4^+$ T cells to IL-12, and defective development of NK cells (Taki et al., 1997). As described above, reliable data exist about the involvement of **IRF-3** in the MyD88-independent signalling pathway used by TLR3 and TLR4, respectively (Moynagh 2005). Activated by TLR3 and TLR4, IRF-3 binds the interferon-sensitive response element (ISRE) in DNA and induces expression of the *IFN-* gene. The p65 subunit of NF- B was detected in the LPS-activated but not poly(I:C)-activated ISRE-binding complex. The results therefore indicate that IRF-3-mediated activation of the ISRE by TLR4 requires the p65 subunit of NF- B (Wietek et al., 2003). **IRF-7** is also induced by viral infection and is critically involved in the system of *IFN- /* gene induction (Taniguchi et al., 2002, Honda et al., 2005). Experiments in **IRF-8/ICSBP** (interferon consensus sequence-binding protein)-deficient animals revealed impaired T_H1 responses due to a major deficiency in the expression of both IFN- and IL-12 when infected with *Toxoplasma (T.) gondii*. In the IRF-8-deficient mice, *Leishmania (L.) major* induced an antibody pattern that was indicative of T_H2, not T_H1 cell type-driven response. Consequently, IRF-8-deficient mice were highly susceptible to infection with *T. gondii* and *L. major* (Giese et al., 1997; Scharton-Kersten et al., 1997). In addition, DCs from the IRF-8-deficient mice were unresponsive to CpG DNA and failed to induce TNF and IL-6 as well as to activate NF- B. This was due to the selective inability of DCs to activate IkB kinase that is required for NF- B in response to CpG DNA. Reintroduction of IRF-8 fully restored the CpG DNA activation of NF- B and cytokine induction. These data suggest that TLR9 signalling in DCs depends uniquely on the function of IRF-8 (Tsujimura et al., 2004).

IL-1 and LPS stimulation induce activation of the STAT family of transcription factors, which consists of seven members. STAT proteins represent substrates for Jak tyrosine kinase (Tsukada et al., 1996). Bacterial infection of macrophages or treatment with LPS resulted in rapid STAT1 serine phosphorylation, independent of concomitant tyrosine phosphorylation (Kovarik et al., 1998). LPS-induced expression of IRF-1, iNOS and IFN-inducible genes such as *IP-10* was severely impaired in STAT1-deficient murine macrophages, suggesting that STAT1 may play a role in the response to LPS (Hoshino et al., 2002). Thus, STAT1 knockout mice are defective in IFN-mediated functions. The STAT4 and STAT6 knockout mice showed defective responses to IL-12 and IL-4, respectively. Conditional knockout study of STAT3 demonstrated its critical roles in cytokine-mediated functions in several tissues, including T cells, macrophages, skin, and mammary gland (Takeda et al., 2000). Other studies proved that LPS stimulation of macrophages using the MyD88-independent pathway and IRF-3-induced expression of *IFN-* , which then induced expression of IFN-regulated genes through activation of STAT1 (Gao et al., 1998; Toshchakov et al., 2002). These results indicate that indirect activation of STAT1 participates in promoting optimal expression of LPS-inducible genes and suggests that STAT1 may play a critical role in innate immunity against Gram-negative bacterial infections.

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