[Frontiers in Bioscience 13, 62-70, January 1, 2008]

The role of toll-like receptors in macrophages

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1. ABSTRACT

Macrophages play diverse roles including but not limited to homeostatic clearance, detection of invading pathogens and induction of the adaptive immune response. Since their discovery, it has become clear that Toll-like receptors (TLRs) can play a major role in these macrophage functions. This review will describe the TLRs, their signalling pathways and their role in macrophage function.

2. INTRODUCTION

Macrophages are the scavenger cell of the immune system. Due to their heterogeneity, macrophages have the ability to reside in many different tissues of the body, particularly in lymphoid organs and in the portal entrances for micro-organisms, such as the lungs and in the lamina propria of the gut. Their job is to 'reside' in these tissues and play a broad homeostatic role in the clearance of apoptotic and necrotic cells as well as any invading pathogens (1). To do this, macrophages contain a plethora of receptors such as the scavenger receptors (SR), mannose receptors and β-glucan receptors which can bind to apoptotic cells and invading pathogens and internalise them in a process known as phagocytosis (2). In addition to this, it is crucially important that in response to pathogens macrophages mount an appropriate immune response to further aid in the fight against infection. The macrophage immune response can be divided into innate and adaptive components where they have the ability to detect and engulf invading pathogens (innate) but also function as antigen-presenting cells (APCs), thus initiating T cell responses (adaptive) (3, 4, 5). The major players in the detection of invading pathogens are the recently identified Toll-like receptors (TLRs). The success of TLRs to function as major sensors of invading pathogens is their ability to identify a range of conserved microbial motifs

termed 'pathogen-associated molecular patterns' (PAMPs) (6, 7, 8). Innate recognition of PAMPs by TLRs can initiate a cascade of signalling pathways that eventually culminate in the induction of a wide range of immune and inflammatory genes. The most important products of these genes include chemokines and adhesion molecules which result in the recruitment of circulating monocytes from the bloodstream and the production of inflammatory cytokines such as tumour necrosis factor (TNF), interleukin-1 (IL-1) and interferon (IFN) which mount an inflammatory immune response (9). As well as their initiation of the innate immune response, there is increasing evidence to suggest that TLRs can also play a role in other macrophage functions such as phagocytosis, antigen processing and presentation and initiation of the adaptive immune response.

3. THE DISCOVERY AND CHARACTERISATION OF TOLL-LIKE RECEPTORS

TLRs are a family of type I transmembrane receptors, which are characterised by an extracellular leucine rich repeat (LRR) domain and an intracellular Toll/IL-1 receptor resistance (TIR) domain (10, 11, 12). The identification of TLRs came about when the cytoplasmic domain of the Toll receptor in *Drosophilia* was found to be analogous to the intracellular portion of the type I IL-1 receptor (IL-1RI) in mammals. Although Toll was originally identified as playing a role in the establishment of dorsal ventral polarity in the developing *Drosophilia* embryo, it was later discovered that it could also play a role in host defense against fungal infections. Because the IL-1RI was known to play a role in innate immune responses, this drove the search for human homologs of the *Drosophilia* Toll. This resulted in the identification of the first human Toll, TLR4 (11).

3.1. TLR4

Out of the ten human TLRs that have been identified to date, TLR4 is the most characterised. Lipopolysaccharide (LPS), an integral component of Gram-negative bacteria, was identified as the ligand for TLR4 when positional cloning of the *Lps* gene in the LPS-non-responsive C3H/HeJ mouse strain, was shown to encode TLR4 (13, 14). C3H/HeJ mice are unresponsive to LPS due to a point mutation in the TIR domain of TLR4, which abrogates downstream signalling (15). The generation of *Tlr4*$^{-/-}$ mice demonstrated a similar non-responsiveness to LPS, confirming that LPS is indeed the ligand for TLR4 (16).

TLR4 alone is not sufficient to induce LPS signalling and it requires several accessory molecules. LPS must first bind to a serum protein, LPS-binding protein (LBP) (17). This association accelerates the binding of LPS to a soluble form of a glycosylphosphatidylinositol (GPI)-anchored form of the cell surface glycoprotein, CD14 (18). In addition the TLR4 complex requires the presence of MD-2, which is expressed on the cell surface in association with the ectodomain of TLR4 (19). In addition to LPS, TLR4 can recognise other ligands such as lipoteichoic acid (LTA) (20) and a heat sensitive factor derived from *Mycobacterium tuberculosis* (21). Interestingly, even before the identification of TLR4, LPS was known to cause potent activation of macrophages, which is characterised by the production of inflammatory cytokines such as TNFα, IL-1 and IL-6, and inflammatory effector substances such as prostanoids, leukotrienes and nitric oxide (22).

Expression of inflammatory cytokines downstream of TLR4 is dependent on the transcription factor nuclear factor-κB (NF-κB). Signalling events leading to the activation of NF-κB occurs when LPS binds to TLR4 homodimers resulting in the recruitment of the adaptor molecules myeloid differentiation factor 88 (MyD88) and MyD88 adaptor-like molecule (Mal) (Figure 1) (23, 24). The presence of a death domain on MyD88 allows it to interact with members of the IL-1 receptor associated kinase (IRAK) family. Phosphorylation of IRAK-1 results in its association with TNF-receptor associated-factor 6 (TRAF6). Subsequent ubiquitination of TRAF6 activates inhibitor of kB (IκB) kinase (IKK) -α and IKKβ via a TAK1-TAB1-TAB2 kinase complex. It is the IKKs which are responsible for the phosphorylation and subsequent degradation of the NF-κB inhibitory molecule, IκB. NF-κB is then free to translocate to the nucleus thereby initiating genes with κB promoter elements. In response to LPS, this MyD88 dependent signalling pathway also gives rise to the activation of mitogen-activated protein kinases (MAPK) such as p38 and JNK, which in addition to NF-κB finally results in gene expression of inflammatory cytokines (25, 8).

In addition to activating the transcription factor NF-κB and MAPK signalling components, LPS can also activate the transcription factor IFN-regulatory factor (IRF) 3, leading to the expression of type I interferons (IFN-α/β) and other IFN-inducible genes. This pathway is MyD88 and Mal independent and occurs when the two adaptors TIR domain-containing adaptor inducing IFN-beta (TRIF) and TRIF-related adaptor molecule (TRAM) are recruited to TLR4 (Figure 1). The N-terminus of TRIF contains binding sites for the non-canonical kinases, tank binding kinase (TBK)-1 and IKKi, and it is these kinases which are responsible for the phosphorylation and activation of IRF3. This results in IRF3 dimerisation and translocation to the nucleus where it binds to interferon stimulated response element (ISRE) motifs in the promoters of IFN-inducible genes (26, 27, 28, 29, 25). Interestingly, TRIF and TRAM can also induce a late phase of NF-κB activation through the recruitment of TRAF6 and receptor interacting protein (RIP)-1, which was discovered when MyD88 knockout mice were still responsive to LPS in terms of NF-κB activation, albeit with delayed kinetics (30).

3.2. TLR2

TLR2 detects components of Gram-positive bacteria such as *Staphlococcus aureus,* mycobacteria and fungi (31, 32). However, TLR2 also has the ability to heterodimerise with either TLR1 or TLR6, thereby increasing its range of detection (31, 32). For example, the association of TLR2 with TLR1 permits recognition of triacyl lipopeptides whereas TLR2/TLR6 heterodimers

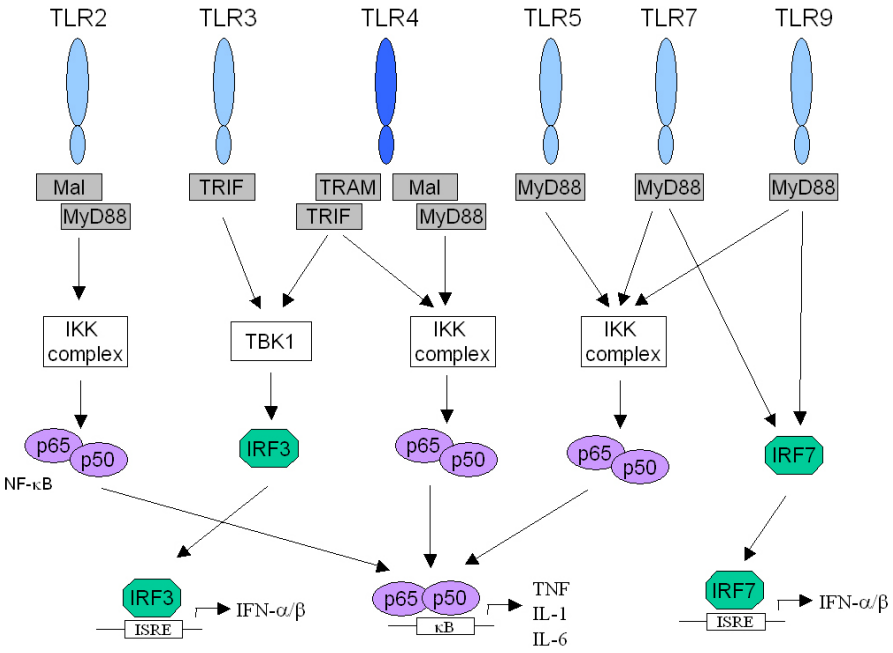

Figure1. TLR signalling pathways. With the exception of TLR3, all TLR pathways signal through the adaptor molecule myeloid differentiation factor 88 (MyD88). In addition to MyD88, TLR2 and TLR4 also recruit a MyD88-like adaptor molecule Mal, resulting in the activation of NF-kB via an IkB kinase (IKK) complex. NF-kB can then translocate to the nucleus and bind to kB promoter elements resulting in the expression of inflammatory cytokines such as TNF, IL-1 and IL-6. TIR-domain containing-adaptor inducing interferon-b (TRIF) is the sole adaptor used by TLR3, leading to activation of interferon regulatory factor (IRF) 3. IRF3 dimerises and enters the nucleus where it binds to interferon-sensitive response element (ISRE) motifs and induces the expression of type I interferons, IFN-a/b. TLR4 also utilises the adaptor molecule TRIF along with a TRIF-related adaptor molecule (TRAM) for the activation of IRF3 and the delayed activation of NF-kB. To date, signalling downstream of TLR5, TLR7 and TLR9 is known to be solely dependent on MyD88, resulting in the activation of NF-kB. TLR7 and TLR9 can also signal to IRF3 related molecules such as IRF7, leading to the expression of type I IFN-α/β.

recognise diacyl lipopeptides such as MALP-2 (33, 34). TLR2 signalling is also MyD88 dependent, resulting in the activation of NF-κB and induction of inflammatory cytokines (Figure 1). Although TLR2 and TLR4 share the same downstream signalling pathways, and induce some of the same cytokines such as IL-1 and TNFα, there is evidence to suggest that their signalling pathways diverge to induce distinct sets of inflammatory genes. For example, activation of TLR4 potently induces IL-12p40, IL-6 and IFN-γ mRNA expression, whereas TLR2 fails to induce these same genes, favouring induction of IL-12p35 over IL-12p40 (35). These differences may be attributed to differences in strength of signal mediated by TLR2 and TLR4 or may be due to the fact that TLR2 can heterodimerise with different TLRs.

3.3 TLR3

TLR3 is located within the endosomal membrane and recognises double-stranded (ds) RNA which is produced by many viruses at some point of their replication cycle. A common dsRNA mimic used for TLR3 activation is polyI:C. Signalling downstream of TLR3 is solely dependent on the adaptor molecule TRIF, resulting in the activation of IRF3 via TBK1 and IKKi and the activation of NF-κB via TRAF6 (Figure 1). Viral stimulation of TLR3 results in the potent production of the type I IFN-α/β.

3.4 .TLR5

TLR5 recognises bacterial flagella from both Gram positive and Gram negative bacteria. TLR5 signals through the MyD88 dependent signalling pathway leading to NF-κB activation and the production of inflammatory cytokines (36). There is also evidence of a TLR5/TLR4 heterodimeric complex, which would enhance the diversity of the TLR5 response, most likely through the engagement of MyD88 independent adaptors (37, 38).

3.5. TLR9, TLR8, TLR7

Like TLR3, TLR9, TLR8 and TLR7 are all localised within the endosomal membrane. TLR9 recognises unmethylated CpG motifs in bacterial and viral DNA whereas TLR8 and TLR7 both confer responsiveness to viral single-stranded (ss) RNA and the anti-viral compound R848 (39). Signalling downstream of TLR7 and TLR9 is similar in that they can both activate IRF-3 related transcription factors such as IRF-7, resulting in the production of type I IFN-α/β. In addition they can activate the transcription factor NF-κB to induce inflammatory cytokines. Interestingly, TLR7 and TLR9 signalling relies solely on MyD88 which is in contrast to TLR3 and TLR4 signalling. It is not entirely resolved how MyD88 can signal to both IRF-7 and NF-κB but it is thought that MyD88 may form a complex with IRAK-1 and IRF-7 (40, 25).

3.6. TLR expression on macrophages

Evidence has shown that most of the ten TLRs are expressed on macrophages. In an early study where the mRNA expression of TLRs 1-5 was analysed in a fresh human leukocyte population containing monocytes, T lymphocytes, natural killer (NK) cells, dendritic cells (DC) and polymorphonuclear (PMN) cells, TLR1 was found to be ubiquitiously expressed, whereas TLR2, TLR4 and TLR5 were found on monocytes, DCs and PMNs and the expression of TLR3 appeared to be exclusively expressed on DCs (41, 42). Although macrophages were not analysed in this study, it is important to note that the expression of TLRs on monocytes can induce their activation so that they differentiate into either macrophages or DCs (43). Further analysis has revealed that TLR6, TLR7 and TLR8 are also expressed on freshly isolated human monocytes, whereas TLR9 and TLR10 have been shown to be expressed on certain subsets of human DCs (43, 44). To add to the complexity, TLR expression appears to differ between mouse and human. For example, human TLR3 appears to be exclusively expressed on DCs, whereas it is expressed and strongly induced in macrophages from mice. TLR4 is expressed strongly on monocytes and macrophages in both species, however TLR4 mRNA expression increases upon LPS stimulation in human macrophages, whereas TLR4 mRNA is down-regulated in response to LPS in murine macrophages (45). In addition, TLR9 appears to be almost exclusively expressed on plasmacytoid DCs in both humans and mice, however in response to LPS, TLR9 expression can be up-regulated in murine macrophages (43, 46). Mice fail to express TLR10, however they express additional TLRs such as TLR11, TLR12 and TLR13 which are absent in humans (47).

4. ROLE OF TLRS IN PHAGOCYTOSIS

Phagocytosis plays a crucial role in macrophage function. Macrophages use phagocytosis for the removal of apoptotic cells and necrotic cells, thus giving them the description as 'scavenger cells' of the immune system. In addition, they use phagocytosis for the ingestion and processing of invading pathogens, resulting in the death of the pathogen or the presentation of antigen peptides on MHC class I or class II molecules on the cell surface in order to trigger an adaptive immune response. Phagocytosis involves a series of definitive steps: initially the apoptotic cell or pathogen is bound to the cell surface by specific phagocytic receptors; actin polymerisation leads to pseudopod extension around the cell or pathogen after which it is completely engulfed by the cell; lastly the actin is depolymerised allowing further phagosome maturation through membrane transport events (48). Phagocytosis of both microbial and apoptotic cells rely on the same cellular machinery and is performed by an array of receptors such as the SRs including SR-A, MARCO and mannose receptors, Fc-receptors, complement receptors and various integrins (3, 4, 49). Phagocytosis of pathogens is often accompanied by inflammatory responses. As the inflammatory response is driven by TLRs, this suggests that phagocytosis and TLR activation may be functionally linked (Figure 2) (5, 50). This theory was first addressed by Underhill and co-workers (51).

4.1. Role of TLRs in the phagasome

Underhill and co-workers provided evidence that TLRs may play a role in phagocytosis when they showed that HA-tagged TLR2 expressed in Chinese hamster ovary (CHO) cells was specifically recruited to macrophage phagosomes containing yeast and that this enhancement of TLR2 was present at all stages of phagocytosis (51). In a similar study, both TLR1 and TLR6 in addition to TLR2 were recruited to phagosomes that contained the components of Gram-positive and Gram-negative bacteria. However, they were also recruited to phagosomes containing IgG-opsonized erythrocytes that do not display any microbial components, thus suggesting that TLRs are recruited to all phagosomes irrespective of the contents and once in position they can sample the contents and identify the nature of the pathogen (Figure 2A) (32). Although TLRs are recruited to phagosomes, it is however evident that they do not have the ability to act as phagocytic receptors. For example, a point mutation in TLR2 abrogated TNF-α production in response to yeast and Gram-positive bacteria, however phagocytosis was unaffected by this mutation (51). In addition, *Tlr2*$^{-/-}$ and *Myd88*$^{-/-}$ macrophages were still able to internalise the TLR2 ligand zymosan, indicating that phagocytosis and cytokine production are separate processes (52).

4.2. Role of TLRs in the formation of pseudopods

Rearrangement of the actin cytoskeleton is required for the formation of pseudopods and the internalisation of microbes (Figure 2B). Interestingly, some TLRs have been shown to associate and activate signalling molecules such as phosphoinoside 3-kinase (PI3-K) and Rho GTPases, molecules which are already known to play a role in cytoskeletal rearrangements during phagocytosis (53, 49). For example, in response to *S.aureus*, the cytosolic domain of TLR2 was shown to form a complex with the Rho GTPase, Rac1 and the p85 subunit of PI3-K (54). In another study, dominant negative mutants of MyD88, IRAK-1 and p85 could all block protein kinase B (PKB) activity, a kinase found downstream of PI3-K, thus suggesting a role for TLRs in PI3-K signalling cascades (55). More recently, TLRs were shown to play a role in the early stages of phagocytosis internalisation. Bone marrow-derived dendritic cells (BMDCs) that had been stimulated with a range of TLR ligands showed an increase in their ability to uptake the endocytosis marker fluorescein isothiocyanate (FITC)-dextran, at the early time points of 30 and 45 minutes. In addition, this acute stimulation was also shown to increase antigen presentation and trigger actin cytoskeletal rearrangements (56).

4.3. Role of TLRs in phagosome maturation

Further studies began to analyse whether TLRs play a role in phagosome maturation. This can be analysed through the identification and the rate at which certain markers appear on early endosomes, late endosomes and lysosomes. In one study where *Myd88*$^{-/-}$, *Tlr2*$^{-/-}$ and *Tlr4*$^{-/-}$ macrophages were employed, the acquisition of lysosomal markers in response to bacteria was severely impaired in the absence of TLR2 and TLR4 signalling. In contrast phagosomes containing apoptotic cells were shown to acquire lysosomal markers at equivalent rates in both wild-

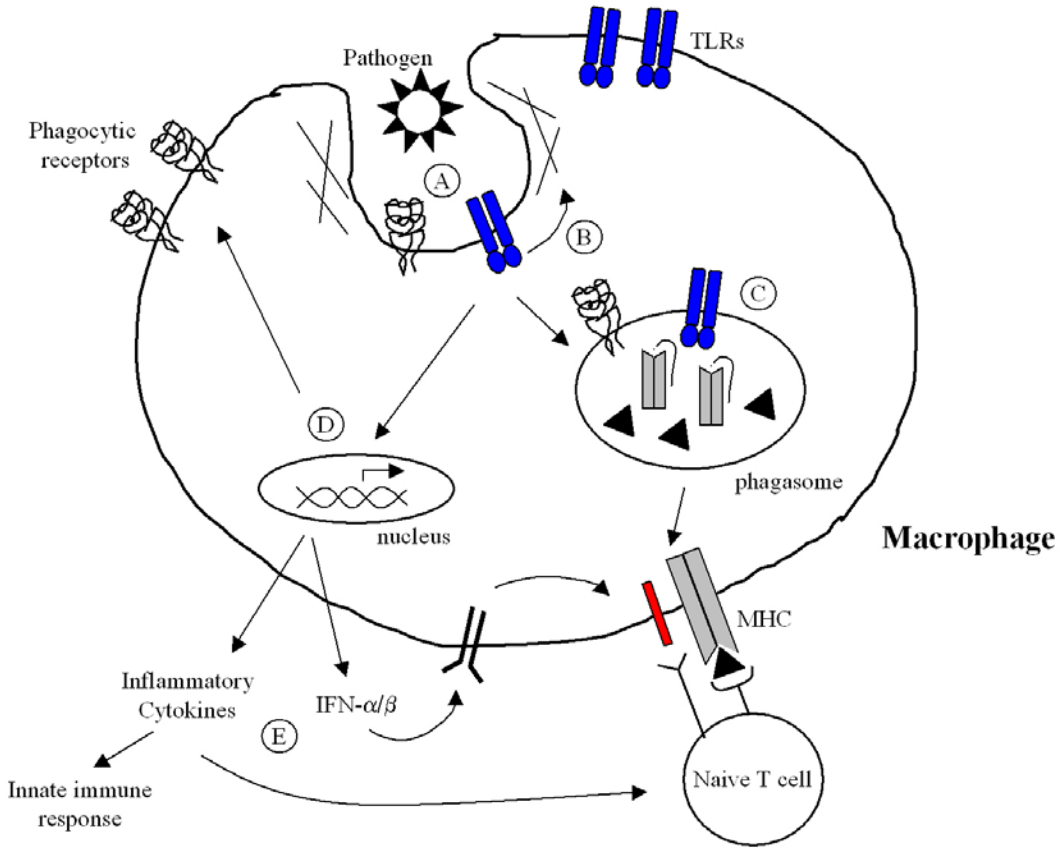

Figure 2. The role of TLRs in macrophage phagocytosis and adaptive immunity. (A) Phagocytosis is initiated when phagocytic receptors bind to invading pathogens resulting in pathogen internalisation. Although TLRs cannot act as phagocytic receptors, they have been shown to locate at the phagosome, suggesting that they may play a role in phagocytosis. (B) TLR signalling may play a role in actin rearrangements required for the formation of pseudopods by activating signalling molecules such as phosphoinisitide 3-kinase (PI3-K) and the Rho GTPase, Rac1. (C) Evidence has shown that the presence of TLRs within the phagasome can enhance phagosome maturation as well as triggering efficient antigen presentation and MHC class II presentation. (D) TLR signalling within the macrophage has been shown to upregulate genes required for all stages of phagocytosis especially those required for the expression of phagocyte receptors, SR-A and MARCO. (E) In addition, TLR signalling initiates the expression of inflammatory cytokines and type I interferons (IFN). Cytokines such as IL-12 and IL-10 can aid the adaptive immune response by promoting differentiation of naïve T cells, whereas IFN-a/b signalling through the IFNA receptor has been shown to result in the upregulation of costimmulatory molecules.

type and *Tlr2*$^{-/-}$ and *Tlr4*$^{-/-}$ double knockout macrophages (57). However, another study using non-coated IgG silica particles or silica particles coated with LPS or PAM_3CSK_4 (a TLR2 ligand) to induce phagocytosis in wild-type bone marrow derived macrophages (BMDMs), demonstrated that phagosome and lysosome fusion actually occurred at the same rate with or without TLR activation (58, 59). These discrepancies may be explained by the fact that the latter study used knockout macrophages which regardless of stimulation may already have defective abilities to phagocytose due to these mutations, however the authors do claim that although there are many genes downregulated in *Myd88*$^{-/-}$ macrophages such as those required for inflammatory cytokines and chemokines, no intrinsic differences in expression were seen in genes encoding Rab, SNARE, ARF or other trafficking proteins (60, 61). The discrepancies may therefore be simply due to experimental design and interpretation of results.

4.4 Role of TLRs in the expression of genes required for phagocytosis

TLR signalling has been shown to positively enhance phagocytosis through the induction of genes required for phagocytosis (Figure 2D). In one study, microarray analysis was used to observe the changes in gene expression which occurred when macrophages were stimulated with LPS. Over 50 genes that participate at all stages of phagocytosis were found to be up-regulated, including genes involved in microbial recognition, actin cytoskeletal dynamics, membrane trafficking, ion transport and antigen presentation (62, 50).

A more recent study illustrated that TLRs can directly promote phagocytosis through the up-regulation of phagocytic receptors on the macrophage cell surface (Figure 2D). Microarray analysis of BMDMs which had been stimulated with TLR ligands such as CpG, lipid A and

polyI:C discovered that genes required for the expression of scavenger receptors MARCO, SR-A and LOX-1 were up-regulated (63). In addition, the expression of these genes was found to be reliant on MyD88 signalling through IRAK4 and p38. This is supported by earlier data, where *Myd88*$^{-/-}$ mice show drastically reduced expression of MARCO (60). In addition genes such as *CD36*, which encodes a receptor thought to be involved in apoptotic phagocytosis and *Fc*γ*R*, a gene involved in opsonin-dependent phagocytosis were found to be up-regulated by TLRs (63).

6. ROLE OF TLRS IN ANTIGEN PROCESSING AND PRESENTATION

In addition to their important role in the detection and engulfment of pathogens by phagocytosis, macrophages also function as antigen presenting cells (APCs) where they present peptide antigens on their cell surface to initiate an adaptive T cell response. Once a phagosome has matured it has the ability to process and present pathogenic peptides from the internalised cargo. In an recent set of elegant experiments it was shown that only cargo containing TLR ligands could trigger efficient antigen processing and MHC class II presentation (Figure 2C) (64). For example, phagocytosis of microbial cells resulted in the transport of MHC II to the cell membrane of BMDCs, whereas phagosomes that contain apoptotic cells, do not enclose any TLR ligands and MHC II was found to remain intracellular. Further experiments revealed that only phagosomes containing microbial cells or microspheres adsorbed in LPS could effectively present antigen and subsequently activate $CD4^+$ T cells derived from T cell receptor (TCR) transgenic mice. It was also observed that the MHC II-associated invariant chain (Ii) that occupies the MHC peptide binding groove was consistently degraded in these phagosomes. Ii degradation results in the formation of an N-terminal fragment called CLIP which can then be exchanged for antigenic peptides derived from cargo proteins. This data suggests that phagosomes containing TLR ligands can trigger the processing of Ii whereas phagosomes that do not engage TLR signalling fail to process Ii (61, 64).

This data was further supported by *in vivo* work carried out by Yarovinsky *et al* who have been studying the functional characteristics of the newly identified TLR11 found only in mice (65). They showed that when a fluorescently labelled TLR11 ligand, profilin from the *Toxoplasma gondii* parasite was injected into wild-type, *Myd88*$^{-/-}$ or *Tlr11*$^{-/-}$ mice, it could be taken up by wild-type DCs but not by DCs lacking TLR11 or MyD88. In addition only the wild-type DC populations could stimulate an adaptive $CD4^+$ T cell response (66). This data further demonstrates a role for TLR signalling in promoting antigen uptake, antigen presentation and activation of T cell responses. Although each of the above experiments have concentrated on the effects of antigen presentation in DCs, it is hard not to value this information as a process that may also occur in macrophages considering that phagocytosis and antigen presentation is also such an integral part of macrophage function.

5. ROLE OF TLRS IN THE ADAPTIVE RESPONSE

In order to elicit an appropriate adaptive immune response, a macrophage relies on the ability of its MHC molecule loaded with foreign peptide to interact with specific T cell receptors. However it is also crucially dependent on the expression of co-stimulatory molecules and inflammatory cytokines to maintain and sustain this response. TLR signalling plays an important role in each of these processes. For example, activation of TLR signalling in response to invading pathogens results in the expression of a milieu of cytokines such as TNF-α, IL-1 and IL-6 which play an important role in the recruitment of antigen specific T cells. Expression of IL-12 plays an important role in the differentiation of naïve T cells into Th1 effector cells, whereas IL-10 and IL-4 induce Th2 differentiation (11, 67). However it is the expression of type I IFN-α/β which have been shown to play a key role in supporting the adaptive immune system (68, 69) (Figure 2E).

Type I IFN-α/β are potently induced after viral stimulation of TLR3 and bacterial stimulation of TLR4 and TLR9 and have been shown to aid the adaptive immune response by promoting $CD8^+$ T cell proliferation and survival as well inducing effective B cell isotype switching and differentiation (70, 43). However there is also sufficient evidence to suggest that the IFN-α/β produced by TLR signalling plays a crucial role in the expression of co-stimulatory molecules CD40, CD80 and CD86. This was first demonstrated when *Myd88*$^{-/-}$ BMDCs failed to up-regulate co-stimulatory molecules CD80 and CD86 in response to CpG, but not LPS and polyI:C, suggesting that MyD88 is an important requirement for the expression of these molecules in response to TLR9 activation but not TLR4 or TLR3 (71, 72). However a role for TLR4 and TLR3 was quickly established when *Trif*$^{-/-}$ macrophages were analysed for their ability to up-regulate co-stimulatory molecules and MHC in response to LPS and polyI:C. This demonstrated that the up-regulation of CD40, CD80 and CD86 along with the up-regulation of MHC class II were completely abolished in response to LPS and was moderately diminished in response to dsRNA (73). This was very exciting and established a role for TLR signalling in the up-regulation of co-stimulatory molecules which are required for the adaptive immune response. Therefore it was interesting that the up-regulation of CD40, CD80 and CD86 in *Trif*$^{-/-}$ macrophages could be rescued in response to IFN-α/β, suggesting that the up-regulation of co-stimulatory molecules is a secondary effect mediated by IFN-α/β (73).

The up-regulation of co-stimulatory molecules is thought to occur when IFN-α/β produced by TLR signalling feeds-back on the cell and binds to its type I IFNA receptor (IFNAR) (Figure 2E). For example macrophages from *Ifnar*$^{-/-}$ mice fail to up-regulate co-stimulatory molecules in response to LPS and dsRNA (73). It has been speculated that the binding of IFN-α/β to the type I IFNAR initiates a signalling cascade resulting in the expression of IRF-1, a protein which is involved in the regulation of many IFN-inducible genes (68, 69). For

example, analysis of the promoter regions of CD40, CD80 and CD86 illustrate that they contain IRF-E promoter sequences, a motif which allows IRF-1 to bind. Indeed, *Irf-1*$^{-/-}$ macrophages were shown to have reduced levels of CD40 expression in response to LPS (74). Further analysis of *Irf-1*$^{-/-}$ macrophages revealed that IRF-1 may play a role in positive feedback by upregulating the expression of TLR3, TLR6 and TLR9 in response to LPS (75). Interestingly, IFN-α/β have also been shown to function as part of a positive feedback loop where they can up-regulate the expression of TLR1, TLR2, TLR3 and TLR7 in response to viral infections, thus re-iterating the important role of IFN-α/β in linking the innate and adaptive immune responses (76).

The fact that many of the ten TLRs are expressed on macrophages confirms how important these receptors are for the functioning of an efficient macrophage. Not only are they essential for the innate detection of invading pathogens thereby initiating an inflammatory response but they are also important for each of the stages of phagocytosis, ranging from engulfment of invading pathogens to antigen processing and presentation of antigenic peptides. In addition TLR signalling is also required for the up-regulation of cytokines, MHC molecules and co-stimulatory molecules that are needed to mount an appropriate adaptive T cell response.

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Key Words: TLR, Macrophage, Phagocytosis, Phasasome, Antigen Presentation, Review

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