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Review

The critical role of toll-like receptors — From microbial recognition to autoimmunity: A comprehensive review

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ABSTRACT

Toll-like receptors (TLRs) constitute an important mechanism in the activation of innate immune cells including monocytes, macrophages and dendritic cells. Macrophage activation by TLRs is pivotal in the initiation of the rapid expression of pro-inflammatory cytokines TNF, IL-1β and IL-6 while promoting Th17 responses, all of which play critical roles in autoimmunity. Surprisingly, in inflammatory arthritis, activation of specific TLRs can not only induce but also inhibit cellular processes associated with bone destruction. The intercellular and intracellular orchestration of signals from different TLRs, their endogenous or microbial ligands and accessory molecules determine the activating or inhibitory responses. Herein, we review the TLR-mediated activation of innate immune cells in their activation and differentiation to osteoclasts and the capacity of these signals to contribute to bone destruction in arthritis. Detailed understanding of the opposing mechanisms of TLRs in the induction and suppression of cellular processes in arthritis may pave the way to develop novel therapies to treat autoimmunity.

Contents

Abbreviations: TLRs, Toll-like receptors; RA, Rheumatoid arthritis; PRRs, Pattern Recognition Receptors; ED, Ectodomain; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PAMPs, Pathogen Associated Molecular Patterns; HMGB-1, high mobility group box-1; CD14, cluster of differentiation 14; CD36, cluster of differentiation 36; UNC93B1, unc-93 homolog B1; TRAF-6, tumor necrosis-factor-receptor-associated-factor 6; oxLDL, oxidized LDL; MyD88, myeloid differentiation factor 88; TRIF, toll/interferon response factor.

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1. Introduction

Macrophage activation is an integral part of the immune response against infection and a variety of receptors and co-stimulatory molecules are involved in the regulation of the duration, magnitude and the nature of the immune response. The diverse stimuli in different tissues further differentiate and activate tissue resident macrophages and those cells acquire specialized phenotypes such as Kupffer cells in the liver, microglia in the brain, and synovial macrophages and osteoclasts in the joints. The enormous variety of macrophage activating signals is generalized in the concept of classic and alternative activation of macrophages (also termed M1 and M2) which falls short of expectations in elucidating pathogenic mechanisms in inflammatory arthritis and calls for reassessment [1]. Synovial macrophages are activated during infection and tissue injury not only through Toll-like receptors (TLRs), but also through nucleotide-binding and oligomerization domain (NOD)-like receptors, retinoid acid-inducible gene-I (RIG-I)-like receptors, C-type lectin receptors and immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors which exhibit a dual capacity as orchestrators of a cytokine mediated pro-inflammatory response and differentiation to osteoclasts [2]. There has been extensive discussion of toll-like receptors and, in particular, their interrelationships with innate immunity and a variety of signaling pathways in multiple models involving loss of tolerance [3–11]. In this review we focus on the recent advances in structural and molecular biology of TLR signaling and identify unique elements that may enhance our understanding of the pathogenesis of autoimmune models of arthritis and place the data in the context of microbial recognition.

2. Structure and function/recognition of ligands by TLRs

Toll-like receptors (TLRs) are type I transmembrane glycoproteins that play a key role in the immune response against microbes. Ten human TLRs have been identified to date and a subset of TLRs recognizes forms of nucleic acids, including double-stranded RNA, single-stranded RNA, and DNA. TLRs localize in the cell surface such as the case of TLRs 1, 2, 4, 5, 6, 10 or have an endosomal localization as TLRs 3, 7, 8, 9 [12]. All ten TLRs are expressed in human macrophages and mice express TLRs 11, 12, and 13 [13], none of which is represented in humans.

TLRs are composed of an extracellular or ectodomain, a single-path transmembrane domain and an intracellular domain and are classified as Pattern Recognition Receptors (PRRs) as they recognize conserved molecular structures in microbes termed Pathogen Associated Molecular Patterns (PAMPs) [13–15]. The ectodomain is involved in the recognition of ligands, which induce the dimerization of the intracellular domain, termed TIR (Toll/IL-1 resistance) domain and the activation of the signaling pathways. Recent crystal structures of ligands-TLR ectodomains have shed lights to the way that these recognitions take place. The ectodomains of TLRs are composed of an N-terminal cap, a leucine-rich repeat domain (LRR domain), and a cysteine rich domain [13]. The most important ligands for human TLRs are summarized in Table 1.

2.1. The surface TLR receptors

2.1.1. TLR1/2/6/10

TLR2 recognize the broadest range of ligands among TLRs due to its association with other TLRs (TLR1 and TLR6) [15–17]. Crystal structure of TLR2/TLR1 in complex with a triacyl-lipopeptide and TLR2 in complex with a diacyl-lipopeptide showed that the hydrophobic pocket of TLR2 formed by the central LRRs (LRR9 to LRR12) binds the diacylglycerol acyl chains while TLR1 interacts with the N-acyl chains of the ligands (Fig. 1A) [16]. Furthermore, TLR2 also recognize glycolipids such as lipoteichoic acid from Gram-positive bacteria [17,18], lipoarabinomannan from mycobacteria [17,19], and GPI anchor structures from *Trypanosome Cruzi* [20]. We have recently reported that the single hydrophobic pocket of human TLR2 ectodomain is also responsible for binding microbial glycolipids and other lipopeptides [17]. Based on the TLR1/TLR2 and TLR2/TLR6 complex structures, homology models of hTLR10 show a ligand binding pocket similar to TLR2 [21].

Table 1
TLRs and their corresponding endogenous and microbial ligands.

Type of TLR	Microbial ligands (PAMPs)	Potential endogenous TLR ligands (DAMPs)
TLR2 (in association with TLR1 or 6)	Lipomannan (Mycobacterium), Lipoteichoic Acids (Gram-positive bacteria), di-acylated and try-acylated bacterial lipopeptides	HSP 60, HSP70, HSP 96, HMGB-1, gp96, Biglycan, SP-D,
TLR4	LPS (Gram-negative bacteria)	Biglycan, HSP 60, HSP 70, HSP 96, fibrinogen, fibronectin, hyaluronic acid, HMGB-1, OxLDL(in association with TLR6), beta amyloid (in association with TLR6)
TLR5	Flagellin (Gram-negative bacteria)	Undetermined
TLR3	dsRNA (virus)	mRNA (necrotic cells)
TLR7	ssRNA(virus)	ssRNA, imiquimod
TLR8	ssRNA(virus)	ssRNA, microRNAs
TLR9	CpG motif (bacteria, virus)	Self-DNA

2.1.2. TLR4/MD-2

TLR4 requires the association with MD-2 to recognize the lipopolysaccharides (LPS) [22]. MD2 is a 160 amino acid glycosylated soluble protein that associates with the extracellular domain of TLR4 and is required for TLR4 surface expression [22]. The crystal structure of TLR4/MD-2 LPS showed that MD-2 binds to the concave face of TLR4, five acyl chains of LPS binds to MD-2 and the six acyl chain interacts with a hydrophobic patch in TLR4 [23] (Fig. 1B).

2.1.3. TLR 5

TLR5 recognizes bacterial flagellin [24] and has a basolateral localization in intestinal epithelium to respond to flagellin of pathogenic invasive bacteria [25]. TLR5 is also involved in the transport of the pathogenic *Salmonella typhimurium* from intestinal tract to mesenteric lymph nodes [26]. Recently the crystal structure of flagellin TLR5 was solved showing that the first nine N-terminal LRRs of TLR5 are involved in the recognition of the D1 domain of flagellin [27] (Fig. 1C).

2.2. The endosomal TLRs

2.2.1. TLR3

TLR3 recognizes the double stranded RNA (dsRNA) formed during the replication of positive stranded RNA virus [28]. TLR3 has an important role in encephalitis mediated by West Nile virus [29] and herpes simplex virus [30] and participate in the pathogenesis of influenza virus [31]. The N-terminal and C-terminal LRRs of TLR3 are involved in the recognition of dsRNA [32]. The crystal structure of the complex hTLR3ED/dsRNA showed that TLR3 recognizes the phosphate backbone of dsRNA, but not the nitrogenous bases of dsRNA [32] (Fig. 1D).

2.2.2. TLR7, TLR8, TLR9

TLR9 recognize bacterial DNA, which is rich in unmethylated CpG motifs [33] while TLR7 and TLR8 recognize viral single stranded RNA (ssRNA) [34]. The recent crystal structure of TLR8 with ssRNA and degradation products shows that TLR8 expressed as a dimer in the unligated form, has two ligand binding sites, one situated in the heterodimerization domain and one in the concave face and that upon ligand binding the C-terminal LRR domain come close to each other [35,36]. Likewise TLR8, TLR9 has also an insertion between LRR14 and

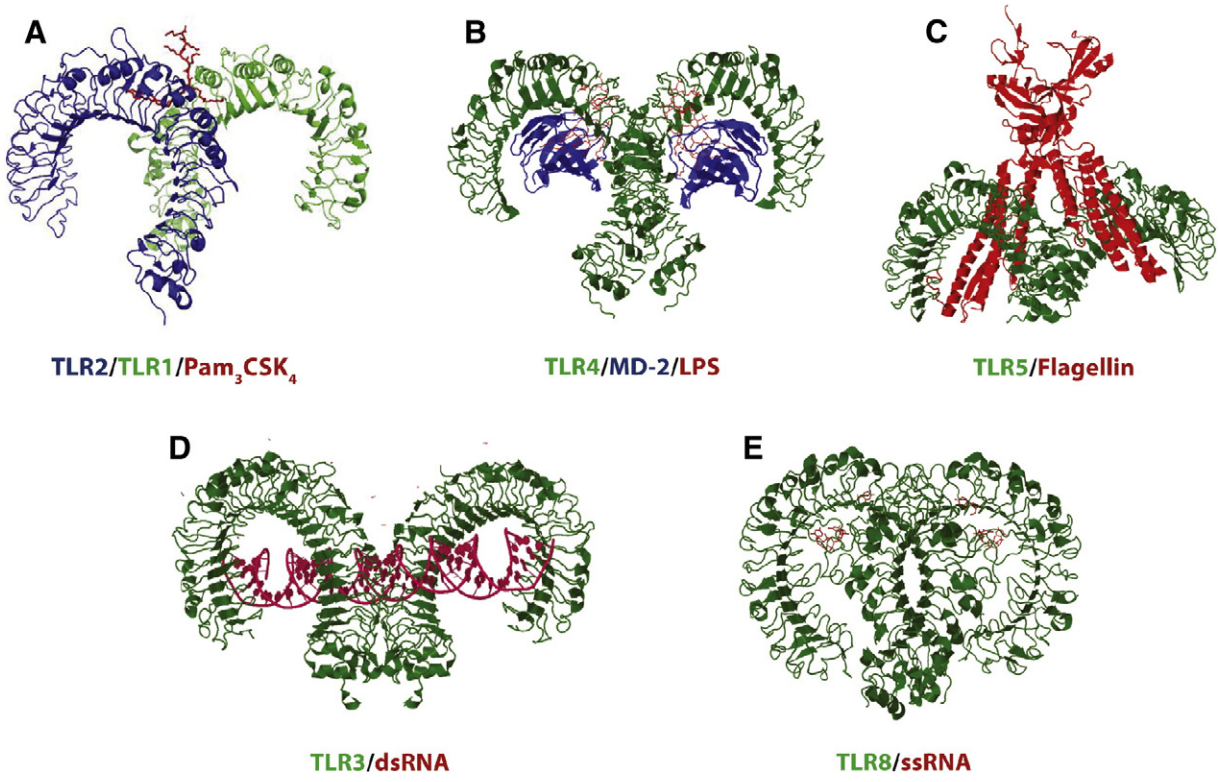

Fig. 1. Crystal structure of TLR receptors. A) Crystal structure of hTLR2ED/hTLR1ED (human TLR2 ectodomain/human TLR1 ectodomain) in complex with Pam3CSK4. hTLR2ED is shown in blue, hTLR1ED in green. Pam3CSK4 is in red. This figure was generated from PDB code 2Z7X [16]. B) Crystal structure of hTLR4ED/hMD-2 in complex with LPS. hTLR4ED is shown in green hMD-2 in blue, LPS is in red. This figure was generated from PDB code 3FXI [23] C) Crystal structure of zebrafish TLR5ED (zTLR5ED) in complex with flagellin. zTLR5ED is depicted in green and flagellin in red. This figure was generated from PDB code 3v47 [27]. D) Crystal structure of mouse TLR3ED (mTLR3ED) in complex with dsRNA. mTLR3ED is depicted in green while dsRNA is in red. This figure was generated from PDB code 3CIY [23]. E) Crystal structure of hTLR8ED ssRNA40. hTLR8ED is depicted in green while ssRNA 40 is in red. This figure was generated from PDB code 4R08 [27]. All the figures were generated from The Research Collaboratory for Structural Bioinformatics PDB website: http://www.rcsb.org/pdb/ [109].

LRR 15 termed Z loops [35,36] (Fig. 1E). The crystal structure of TLR9 with un-methylated CpG DNA, will be required to determine if its ligands recognition is similar to TLR8.

3. Recognition of DAMPs by TLRs in inflammatory arthritis

The recognition of microbial structures by TLRs is in agreement with the theory of Janeway that the innate immune system has evolved to detect foreign structures [37]. For instance, mammals do not synthesize lipoteichoic acids and LPS, and therefore the immune system can recognize these microbial cell wall structures as foreign ones. Nevertheless, there are an increasing number of reports that TLRs can also recognize endogenous molecules. This recognition agrees with Matzinger's theory that the immune system has evolved as a surveillance mechanism designed to detect and respond to endogenous danger signals [38]. Among the danger molecular patterns (DAMPs) recognized by TLRs are extracellular matrix proteins such as hyaluronic acid and molecules derived from necrotic and apoptotic cells [39,40]. The contribution of TLR activation in arthritis is clearly documented by the numerous cases of infectious arthritis and septic arthritis [41,42].

Activation of TLRs in the synovial fluid macrophages present within the arthritic joint may be mediated by PAMPs and DAMPs from necrotic cells present in the synovial fluid, or even from constituents of the inflammatory synovial fluid. Synovial fluid is a dialysate of plasma containing very high amounts of hyaluronic acid, along with other glycoproteins, albumin and small quantities of larger proteins, which is modified by factors secreted mainly by synovial fibroblasts in joint tissues. Normal synovial fluid has a high content of high and low molecular weight hyaluronic acid and it is largely void of any cell infiltrate. On the contrary synovial fluid from inflamed joints such as RA has a high content of low molecular weight hyaluronic acid and contains numerous neutrophil polymorphs, macrophages and lymphocytes. Cells that leaked within the synovial fluid are not connected to vasculature and become apoptotic releasing their RNA. Low molecular weight hyaluronic acid and RNA recognition by TLRs can lead to autoimmune inflammation [43]. The inflamed joint is a unique environment that provides additional pro-inflammatory signals and may activate co-stimulatory pathways in the synovial and synovial fluid macrophages. Interestingly, synovial fluid macrophages have the capacity to differentiate to bone resorbing osteoclasts in the presence of pro-inflammatory cytokines [44]. Moreover, the inflammatory synovial fluid itself is able to stimulate osteoclast formation in macrophages and dendritic cells [45,46]. Collectively, RNA from necrotic cells, low molecular HA and pro-inflammatory cytokines provide multiple pathways for the activation of innate immune cells and the differentiation of synovial fluid macrophages to osteoclasts in inflammatory arthritis.

Other DAMPs reported to be recognized by TLRs include heat shock proteins [47], surfactant protein A (SP-A) [48], high mobility group box-1 (HMGB-1) protein [49], beta amyloid [50], oxidized LDL (oxLDL) [51] and endogenous nucleic acids [52]. The activation of TLRs by the DAMPs produce a "sterile" inflammation, that could be useful to repair the damaged tissue but could also contribute to the pathogenesis of cancer, autoimmune diseases and atherosclerosis [53,54]. However, there is still some controversy that the activity of the endogenous ligands is mainly due to PAMPs contaminants [55]. Crystal structures of endogenous ligands with TLRs will be relevant to determine if they are true ligands of TLRs and the molecular mechanisms of recognition of the endogenous ligands by TLRs.

4. Accessory molecules involved in the activation of TLRs

Several accessory molecules are necessary for the activation of TLRs. The protein unc-93 homolog B1 (UNC93B1), the cluster of differentiation

14 (CD14) and the cluster of differentiation 36 (CD36), are among the most important accessory molecules of TLRs. UNC93B1 is a multimembrane protein that is essential for the trafficking of endosomal TLRs (TLR3, TLR7, TLR9) from the endoplasmic reticulum to the endolysosomes [56]. Interestingly, mice harboring a point mutation in UNC93B1 showed TLR7-dependent, systemic lethal inflammation through an increase of $CD11b^+$ cells and subsequent regulation of Th1 and Th17 differentiation [57]. These data clearly point out a relevant role of UNC93B1 in controlling autoimmunity. LL37 is a cationic antimicrobial peptide that binds self-nucleic acids enhancing the activation of TLR9 [58], TLR7, TLR8 [59], and also contributes to the activation of TLR3 by dsRNA [60]. Progranullin and granullin peptides facilitate the interaction of CpG DNA with TLR9 in the endolysosomal compartment [61,62].

CD14 is a 375 amino acid leucine-rich repeat glycoprotein that is present in soluble form in the blood or as a glycosylphosphatidylinositol (GPI)-anchored membrane protein on myeloid cells and contributes to both TLR2 and TLR4 ligand recognition [63,64]. CD36 has been involved in the activation of TLR2 [65,66] by negatively charged microbial ligands and TLR4/TLR6 by endogenous ligands involved in atherosclerosis (OxLDL) [67] and neurodegenerative diseases (beta amyloid) [68]. Recently, we proposed a model in which CD36 binds negatively charge ligands (lipoteichoic acids, mycoplasmal lipopeptides and mycobacterial lipomannanan), transfer it to CD14, which loads them into TLR2/TLR6 (lipoteichoic acid and mycoplasmal lipopeptides) or into TLR1/TLR2 (lipomannan) [65]. Our results about the preferences of CD36 to bind negatively charged diacylglycerol ligands has also been supported by the recent crystal structure of a member of the CD36 family [69]. Furthermore, although crystal structure of CD14 revealed the presence of an N-terminal hydrophobic pocket in its N-terminal [70], crystal structures of CD14 and CD36 complexes with microbial glycolipids and lipopeptides would greatly enhance our understanding of their role as co-receptors of TLRs. CD14 delivers LPS into TLR4/MD2 [70] complex and also controls the lipopolysaccharide (LPS)-induced endocytosis of TLR4 via the recruitment of Syk and PLCγ2 [71]. Therefore, CD14 could have an important role in the progression of RA as LPS promotes the production of pro-inflammatory cytokines in macrophages and the survival of osteoclasts [72]. In the next section we will look into the signaling pathways of TLR receptors and how these pathways may overlap with macrophage differentiation and osteoclastogenesis in autoimmunity.

5. TLR signaling pathways in inflammatory arthritis

The recognition of PAMPs by TLRs results in the formation of an M shape structure in the ectodomain (Fig. 1), and induces the dimerization of the TLR intracellular domains termed TIR domains. This event leads to the recruitment of two main adaptor molecules termed myeloid differentiation factor 88 (MyD88) and/or toll/interferon response factor (TRIF) [12] depending on the TLR, which is activated. The signaling pathway, which results from recruitment of the MyD88 adaptor, is termed the MyD88 pathway, while the pathway that results from recruitment of TRIF is termed the MyD88 independent pathway or TRIF pathway. TLR2 complexes with TLR1 or TLR6, TLR5, TLR7, TLR8, and TLR9 signal through the MyD88 signaling pathway exclusively, while TLR3 signals through the TRIF signaling pathway. TLR4 can signal through both MyD88 and TRIF pathways. The interactions of TLR2 and TLR4 with MyD88 are indirect and are mediated by an extra adaptor called (MAL)/TIR domain-containing adaptor protein (TIRAP), while TLR3 interacts with TRIF or so called TICAM-1 (TIR domain containing adaptor inducing (INF-β/TRIF) TIR domain containing molecule-1). TLR4 uses an extra adaptor protein termed TRAM (TRIF-related adaptor molecule) for the MyD88 independent pathway [73] (Fig. 2).

MyD88 consists of two domains: a TIR domain, which interacts with the TIR domain of toll-like receptors and a death domain. The death domain of MyD88 recruits IRAK (interleukin-1 receptor-associated kinase) proteins. IRAK proteins consist of an N-terminal death domain and a central serine/threonine-kinase domain. IRAK1 is recruited to the complex and phosphorylated by IRAK4. TRAF-6 is also recruited to the receptor complex, by associating with phosphorylated IRAK1. Phosphorylated IRAK1 and TRAF6 then dissociate from the receptor and form a complex with TAK1 (transforming growth factor β activating kinase), TAB1 (TAK1 binding protein 1) and TAB2 (TAK1 binding protein) at the plasma membrane, which induces the phosphorylation of TAB2 and TAK1. IRAK1 is degraded at the plasma membrane, and the remaining complex (consisting of TRAF6, TAK1, TAB1 and TAB2) translocate to the cytosol leading to the ubiquitination of TRAF6, and activation of TAK1. Activated TAK1 modulates the IκB kinase (IKK) complex, which is composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit termed IKKγ or NEMO (NFκB essential modulator). In resting cells, IκB is bound to NFκB avoiding the translocation of NFκB to the nucleus. Activation of IKK leads to phosphorylation of IκBs, which trigger their poly-ubiquitination and proteosomal degradation and subsequently, releasing NFκB to translocate to the nucleus. TAK1 also activates the MAP kinase kinase MKK3/6-p38 signaling cascade, leading to cAMP response element binding (CREB) nuclear transcription factor activation and the MKK4/7-Jun N-terminal kinase (JNK) mediated activation of the transcription factor activator protein-1 (AP-1) (Fig. 2). AP-1 in concert with NFκB, activate the expression of pro-inflammatory cytokines, chemokines and MHC co-stimulatory molecules, which play pivotal roles in inflammatory arthritis.

In the MYD88-independent or TRIF pathway, activation of TLR4 recruits TRAM and TRIF to the TIR domain of TLR4. Subsequently IKKε (also known as inducible IKK (IKKi)) and TBK1 (TANK binding kinase 1) and TRAF3 are recruited to the TRAM/TRIF/TIR complex. RIP1 (receptor interacting protein 1) mediates the NFκB, activation induced by the carboxy-terminal region of TRIF. TBK1 phosphorylates IRF3, which activates, in complex with p300 and CBP (CREB binding protein), the expression of interferon inducible genes, IP-10 and RANTES. TRIF can bind TRAF6 and activate a late production of inflammatory cytokines through NFκB activation. TLR7, TLR8 and TLR9 are endosomal receptors, which utilize the MyD88 dependent pathway.

Activation of the MyD88 pathway, induces the expression of several pro-inflammatory cytokines including IL-1, IL-6, INF-α, INF-β, and also stimulates the expression of CD40, B7.1 (CD80), B7.2 (CD86) and MHC class II in immature antigen presenting cells (APCs). The activated APC's migrate to the secondary lymph nodes and upon encountering a naïve $CD4^+$ T cell, differentiate it toward Th1, Th2 or Th17. The elicited T helper response depends on the cytokine repertoire that the APC generates. The repertoire is strongly influenced by the particular TLR and the ligand that produced the activation [74]. In contrast, the activation of the MyD88 independent pathway by TLR3 in myeloid dendritic cells, and MyD88 dependent pathway by TLR7 and TLR8 in plasmocytoid dendritic cells lead to the activation of IRF-3 and IRF-7 [75], which up-regulate the synthesis, and secretion of chemokines such as RANTES (regulated upon activation normally T-cell expressed and secreted) and interferon-inducible protein 10 (IP-10) and type I interferons (INF-β and INF-α). INF-β and INF-α are responsible for generating strong Th1 immune response against viral infections [76]. Thus, TLRs can regulate the type of immune responses and influence the bone destruction process in inflammatory arthritis via T cell regulation of the osteoclast. Specifically, TLRs and Th1 responses negatively regulate osteoclastogenesis by inhibiting the expression of receptor activator for NFκB Ligand (RANK), a receptor that belongs to TNF receptor family, in early osteoclast precursors [77]. The signaling pathway of RANK, activated by its ligand RANKL in myeloid cells is critical, for osteoclastogenesis. Interestingly, although TLRs in this mechanism inhibit osteoclastogenesis in early precursors, the activation of NFκB by TLRs in mature osteoclasts stimulates their resorbing activity and may potentially counteract the negative effects on early precursors [77,78].

On the contrary, in RA patients, TLR3 activation has been associated with increased levels of RANKL expression in synoviocytes suggesting a

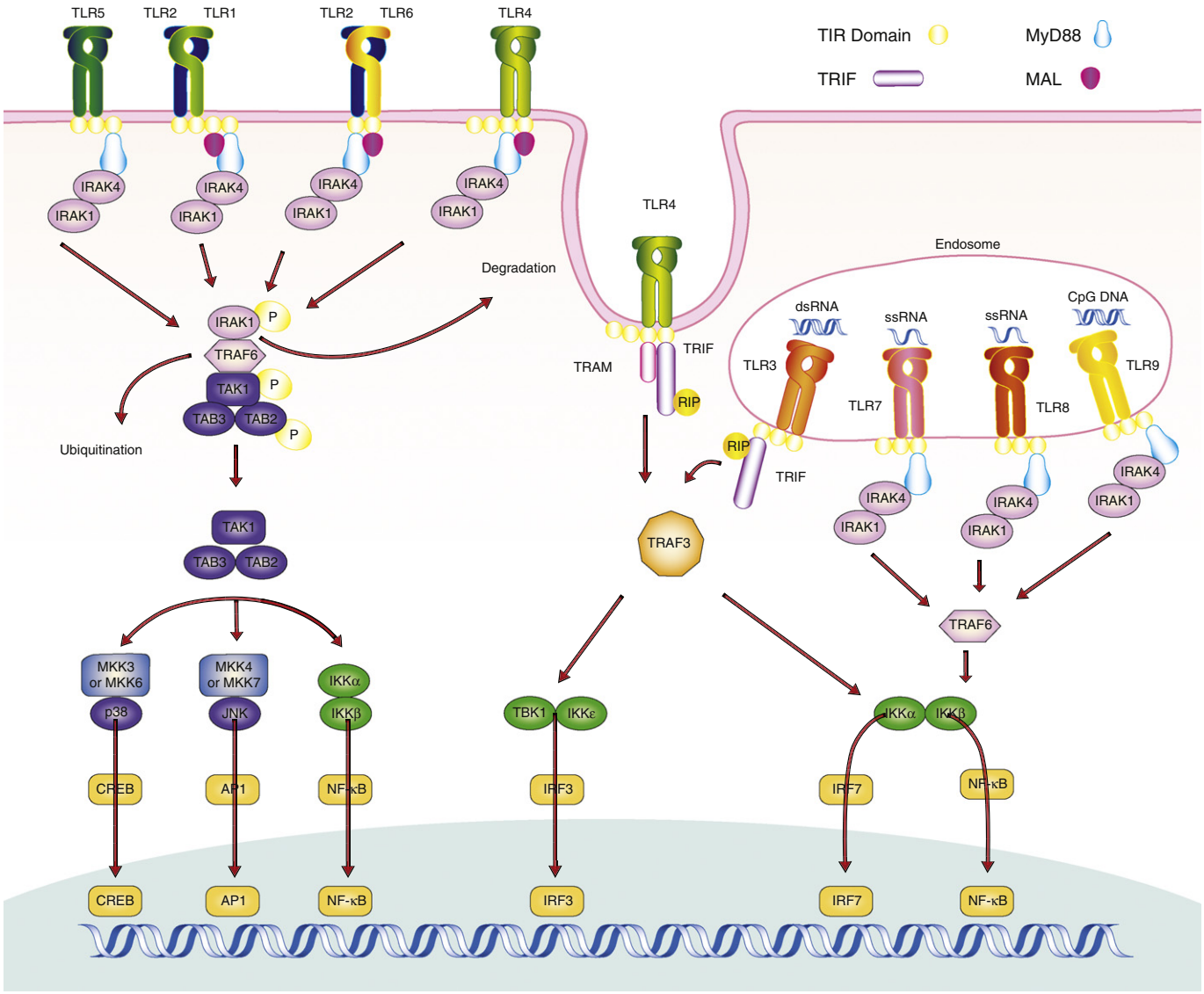

Fig. 2. TLR signaling pathways. TLRs signal through MyD88-dependent and MyD88-independent pathways.1) *MyD88 signaling pathway*. After ligand binding at the plasma membrane (TLR2/1, TLR2/6, TLR4/MD-2 and TLR5) or endocytic vesicles (TLR7, TLR8, TLR9), TLRs recruit the adaptor molecule MyD88 to their TIR domains. TLR2/1, TLR2/6, TLR4/MD-2 also recruit the MyD88 adapter like protein (MAL) or also called TIR domain-containing adaptor protein (TIRAP). MyD88 consists of two domains: a TIR domain, which interacts with the TIR domain of toll-like receptors and a death domain. The death domain of MyD88 recruits IRAK proteins 1, 2, 4, which recruit TRAF6 to the receptor complex. Phosphorylated IRAK1 and TRAF6 then dissociate from the receptor and form a complex with TAK1, TAB1 and TAB2 at the plasma membrane, which induces the phosphorylation of TAB2 and TAK1. Following TAK1 and TAB1 phosphorylation, IRAK1 is degraded at the plasma membrane, and the remaining complex (consisting of TRAF6, TAK1, TAB1 and TAB2) translocate to the cytosol leading to the ubiquitination of TRAF6, and activation of TAK1. Activated TAK1 modulates the IκB kinase (IKK) complex, which is composed of two kinase subunits (IKKα and IKKβ) and a regulatory subunit termed IKKγ or NEMO (NFκB essential modulator). In resting cells, IκB is bound to NFκB avoiding the translocation of NFκB to the nucleus. Activation of IKK leads to phosphorylation of IκBs, which trigger their poly-ubiquitination and proteosomal degradation and subsequently, releasing NFκB to translocate to the nucleus. AK1 also activates the MAP kinase kinase MKK3/6-p38 signaling cascade, leading to cAMP response element binding (CREB) nuclear transcription factor activation and the MKK4/7-Jun N-terminal kinase (JNK) mediated activation of the transcription factor activator protein-1 (AP-1). AP-1 in concert with NFκB activates the expression of pro-inflammatory cytokines, chemokines and MHC co-stimulatory molecules. 2) *TRIF signaling pathway*. After activation of TLR4 and TLR3, TRIF are recruited to their TIR domains. TRAM (TRIF-adaptor molecule) is another adaptor recruited to the TIR domain of TLR4. Subsequently IKKε (also known as inducible IKK (IKKi)) and TBK1 and TRAF3 are recruited to the TRIF/TIR or TRAM/TRIF/TIR complexes. TBK1 phosphorylates IRF3 and IRF7, which activate, in complex with p300 and CBP (CREB binding protein), the expression of interferon inducible genes, IP-10 and RANTES. TRIF can also bind TRAF6 and activate a late production of pro-inflammatory cytokines through NFκB activation.

potential increase in osteoclast differentiation [79]. Although it is generally appreciated that *in vitro* RANKL induced osteoclast differentiation is inhibited by TLR signaling [78] due to these contradicting observations regarding attenuation of RANK and up-regulation of RANKL in synoviocytes, there is no clear indication as to whether TLR signaling should be strictly considered inhibitory [80]. Moreover as pro-inflammatory cytokines such as TNF, IL-23 and IL-17 also induce osteoclastogenesis through RANKL-dependent and RANKL-independent mechanisms, it is highly possible that TLR activation of macrophages may be osteoclastogenic, at least in certain patients [2]. Furthermore, both TLRs and RANKL-RANK activate NFκB pathways through recruitment and activation of TRAF6 but it is unclear if this overlap can lead to a synergism between both pathways and increased osteoclast differentiation and activation [81,82]. In this respect, it is important to understand the different mechanisms that fine-tune the activation of TLRs.

The activation of TLR pathways is a tightly regulated process and in order to avoid aberrant TLR signaling, several molecules are involved in its inhibition at different steps [83]. Secreted isoforms of TLR2 and TLR4 ectodomains in saliva, plasma and breast milk have been described as negative regulators of the immune response [84,85]. Recently, we showed that soluble human TLR2 ectodomain (hTLR2ED) binds a broad range of microbial glycolipids and lipopeptides independent of

the presence of TLR6 or TLR1 [17]. Therefore, the hTLR2ED could down-regulate the activation of TLR2/TLR6 and TLR2/TLR1 by competing for ligand binding with the TLR2 transmembrane receptor [17]. TLR10, which express preferentially in B cells, has been reported to bind TLR2 ligands, and shown to be a negative regulator of TLR2 [86]. Furthermore, activation of TLR10 up-regulates IL-1R antagonist (IL-1Ra) which could inhibit the generation of Th17. Thus, the activation of TLR10 could preclude an effective immune response against certain pathogens [87] or retard the progression of autoimmune responses [88]. Another negative regulator of the MyD88-dependent signaling pathway is MyD88s, a splice variant of MyD88, which has a TIR domain but lacks the death domain. MyD88s binds to the TIR domain of TLRs and blocks the recruitment of IRAK4 [13,83]. Other negative regulators of the TLR pathways are SOCS (suppressor of cytokine-signaling-1), IRAK-M, A20 and SARMs [13,83]. A20 promotes the ubiquitination and posterior degradation of TRAF6 while SOCS-1 mediates degradation of TIRAP. IRAK-M inhibits the release of IRAK1 and IRAK4 from MyD88. SARM (Two sterile alpha motifs and heat armadillo repeats) has a TIR domain and was reported to inhibit activation by TRIF pathway but not by the MyD88 pathway. Recently, an increasing number of micro RNAs, a class of small non-coding RNA, have been reported to down-regulate the activation of TLRs [89–91] or activate TLR8 signaling pathway [92] and modulate the activation of TLRs during cancer and chronic inflammatory diseases.

6. TLRs in activation of auto-reactive B cells and Th17 cells

TLRs play an important role in direct and indirect activation of t cells in autoimmunity as recently reviewed and also contribute to the activation of auto-reactive B cells [93]. Cross-linking of rheumatoid factor (RF) surface receptor with complex DNA-immunoglobulin has been shown to be necessary in the activation of auto-reactive B cells [52]. However, a second signal due to the activation of TLR9 by un-methylated CpG motifs leads to auto-reactive B cell activation and RF antibody secretion [94]. Similarly, an increased production of autoantibodies in mice harboring a duplicated TLR7 gene is observed [95]. The BCR/TLR two-signal mechanism explains the high prevalence of autoantibodies against nuclear proteins in autoimmune diseases like systemic lupus erythematosus (SLE) [96]. Furthermore, M2 macrophages produced pro-inflammatory cytokines in the presence of IgG-TLR ligands, increasing the pro-inflammatory cytokine secretion and the polarization toward Th17, which are critical in the pathology of RA [97,98]. Th17 is also involved in bone destruction via osteoclastogenesis [99]. Therefore, the activation of TLRs have multiple roles in exacerbating the progression of various inflammatory arthritides and their relevance have been highlighted by the increasing number polymorphism in TLRs associated with rheumatoid arthritis and psoriatic arthritis [100–102].

The current traditional therapies for RA, based on targeting TNF (eg. infliximab, etarnecep), are, not completely effective, very expensive and come with very undesirable side effects. Alternative therapies are also needed as the anti TNF therapies also induce drug resistance One interesting novel approach would be the employment of synthetic ligands inhibitors which bind but not activate the TLR2, TLR4 to decrease the inflammation in RA [17,41,103]. Synthetic oligodeoxynucleotides with immuno-regulatory sequences (IRS) that blocks signaling via TLR7 and/or via TLR9 could also be employed to inhibit auto-antibody production [104]. Furthermore, chemical inhibitors that bind the BB loop of the TIR domain of MyD88 have been developed [105]. Peptides inhibitors of TRAF6, which inhibit osteoclastogenesis by blocking both the TLR (MyD88 dependent) and RANKL-RANK signaling pathways have been used with some relative success [106–108].

7. Conclusions

Activation of TLRs by DAMPs and PAMPs increase not only the pro-inflammatory response but also the progression of bone destruction in inflammatory arthritis patients. The developments of novel treatments, based on small molecule inhibitors of the TLR pathways, are very promising therapies to retard the progression of many devastating autoimmune diseases including RA and psoriatic arthritis.

Take-home messages

- In inflammatory arthritis, activation of specific TLRs can not only induce but also inhibit cellular processes associated with bone destruction.
- TLR modulation of NFκB activation in inflammatory arthritis is directly linked with osteoclastogenesis and bone destruction.
- TLRs play pivotal role in the activation of auto-reactive B cells and Th17 cells.
- TLR activation is regulated by PAMPs and DAMPs in the inflamed joint

Disclosures

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Proinflammatory microenvironments within the intestine regulate the differentiation of tissue-resident CD8+ T cells responding to infection

Bergsbaken et al. (Nature Immunol 2015;16:406) report that oral infection with Yersinia pseudotuberculosis results in the development of two distinct populations of pathogen-specific CD8+ tissue-resident memory T cells (TRM cells) in the lamina propria. CD103− T cells did not require transforming growth factor-β (TGF-β) signaling but were true resident memory cells. Unlike CD103+CD8+ T cells, which were TGF-β dependent and were scattered in the tissue, CD103−CD8+ T cells clustered with CD4+ T cells and CX3CR1+macrophages and/or dendritic cells around areas of bacterial infection. CXCR3-dependent recruitment of cells to inflamed areas was critical for development of the CD103− population and pathogen clearance. These studies have identified the 'preferential' development of CD103− TRMcells in inflammatory microenvironments within the lamina propria and suggest that this subset has a critical role in controlling infection.