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ROLE OF TOLL LIKE RECEPTORS IN RHEUMATOID ARTHRITIS

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Abstract

An increasing body of data supports the role of the innate immune system in the pathogenesis of rheumatoid arthritis (RA). Toll-like receptors (TLRs) are expressed by cells within the RA joint and a variety of endogenous TLR ligands are present within the inflamed joints of patients with RA. Further, a variety of animal models suggest that TLR signaling is important in the pathogenesis of disease. Overall, the data suggest that activation by endogenous TLR ligands may contribute to the persistent expression of pro-inflammatory cytokines by macrophages and the joint damage to cartilage and bone that occurs in RA. The data supports a potential role for suppression of TLR signaling as a novel therapeutic approach in patients with RA.

INTRODUCTION

When exposed to an immunogenic stimulus, such as a microbial pathogen, the initiation of the inflammatory and immune response is mediated by Toll-like receptors (TLRs) which result in the activation of cells of the innate immune system including monocytes, macrophages and dendritic cells. Activation of TLRs results in the rapid expression of pro-inflammatory cytokines such as TNFα, and chemokines such as IL-8(CXCL8) and MCP-1 (CCL2). These mediators orchestrate an immune response that recruits neutrophils, monocytes and lymphocytes. Macrophages and dendritic cells take up and process the pathogen and migrate to peripheral lymphoid tissue where antigen presenting cells initiate the generation of adaptive immunity resulting in the generation of cellular immunity and antibodies. Under normal circumstances, the inflammatory response mediated by the innate immune system, together with the adaptive immune response, eliminates the invading pathogen, which results in resolution of the inflammatory response.

In contrast, in rheumatoid arthritis (RA), all aspects of the innate and adaptive immune response persist. As opposed to a microbial infection that can be eliminated resulting in resolution of inflammation, RA becomes chronic, with the persistence of pro-inflammatory mediators Supporting the importance of the innate immune system in RA, macrophage numbers and TNFα in the synovial tissue of patients with RA are good predictors of the ultimate clinical course of the disease (1) and anti-cytokine therapy is effective. The mechanisms responsible for the persistent activation of macrophages in the rheumatoid joint are not clear, however, a role for TLRs is possible since the NF-κB-mediated expression of pro-inflammatory cytokines is mediated by TLRs.

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Toll Like Receptors

At least 12 mammalian Toll Like Receptor (TLR) family members have been identified. TLRs are pattern recognition receptors (PRRs) that may be found in a variety of cells and tissues (2), which recognize Pathogen Associated Molecular Patterns (PAMPs) expressed on microbial pathogens or Danger Associated Molecular Patterns (DAMPs), that may be expressed by cells under stress. DAMPs may include proteins such as heat shock proteins or other molecules such as ATP and monosodium uric acid crystals. The best characterized role for TLRs involves monocytes, macrophages, and dendritic cells, which contribute to the initial recognition of microbial pathogens. TLR1, -2, -4, -5 and -6 are on the cell surface and they interact with components found on the surface of pathogens, and TLR2 may heterodimerize with TLR1 or TLR 6. In contrast, TLR3 and -7, -8 and -9 - are located intracellularly on endosomal membranes, and their ligands must be taken up into the endosome to result in activation. The microbial PAMPs identified for each TLR member are listed in Table 1 (2). Upon ligand binding, TLRs interact with their corresponding adaptors resulting in the activation MyD88-dependent and MyD88-independent pathways (Figure 1). The MyD88-dependent pathway leads to the activation of NF-κB and results in cytokine gene expression. In addition mitogen activated protein kinases (MAPKs), including JNK, p38 and ERK may also be activated. Activation of TLR3 and TLR4 through the MyD88-independent TRIF signaling pathway results in the expression of type I interferons α and β (IFN α/β), mediated by activation of Interferon Response Factor-3 (IRF-3), which may also result in the delayed activation of NF-κB and MAPKs mediated by release of TNFα (2).

TLR expression is increased in RA

TLRs are highly expressed in patients with RA. Increased expression of TLR2 and TLR4 on peripheral blood monocytes from patients with RA has been demonstrated (3,4). Utilizing immunohistochemistry, both TLR2 and TLR4, as well as endosomal TLR3 and TLR7 were expressed in RA synovial tissues (3,5,6), and TLR3 and TLR4 were highly expressed in early as well as in longstanding RA (7). Further, employing RT-PCR and *in situ* hybridization, TLR2 was expressed in the rheumatoid joint, including in the pannus, and was up-regulated in RA synovial fibroblasts by TNFα and IL-1β (8). Isolated RA synovial fibroblasts expressed TLR1 to -6, but TLR7 to -10 were not detected (7). Since TLR7 was detected in RA synovial tissue by others (6), it is possible that it is expressed on other cell types such as macrophages or dendritic cells. Our recently published data demonstrated the increased expression of TLR2 and TLR4 on RA synovial fluid macrophages compared with control in vitro differentiated macrophages (9). Additionally, increased TLR2 and TLR4 were detected on RA synovial fibroblasts compared to those from patients with osteoarthritis (10).

TLR Ligands in RA

A number of studies have identified the presence of potential endogenous TLR ligands in the synovial tissue of patients with RA, including fibrinogen, HSP 60 and 70, and EDA fibronectin (reviewed in (11)). Recently, HSP22 was identified as another potential endogenous TLR4 ligand expressed in the joints of patients with RA (12). Further, low molecular weight fragments of hyaluronic acid, as may be found in inflammatory synovial fluid, activated myeloid dendritic cells through TLR4 (13). High Mobility Group Box chromosomal protein 1 (HMGB-1), a highly conserved nuclear protein that stabilizes nucleosome formation, is increased in RA synovial fluid, and is highly expressed in RA tissue macrophages (14). HMGB-1 induced NF-κB activation may be mediated through TLR2 and TLR4 (15) as well as RAGE (14). The extracellular matrix component biglycan, a member of the family of small leucine-rich protoglycans, has been shown to activate through TLR2 and 4 (16), and is expressed in RA synovial tissue fibroblasts (11). Additionally, serum amyloid A, an acute phase reactant elevated in the peripheral blood of

patients with RA and expressed in RA synovial tissue, was identified recently as a TLR2 ligand (17).

Although most of these potential endogenous TLR2 and TLR4 ligands may be expressed in the RA joint, it is not clear that they each reach the extracellular space, which is necessary to activate cell surface TLR2 and TLR4. In RA, the extracellular space may be sampled by examining the synovial fluid. In RA synovial fluid we did not detect HSP60, HSP70 or biglycan by immunoblot analysis, and while fibronectin was highly expressed, the fragment with the EDA domain exposed, which may be a TLR ligand, represented only a small fraction of the fibronectin ((11) and data not shown). In contrast, we identified that the endoplasmic reticulum stress response protein gp96 was highly expressed in RA synovial fluid and synovial tissue compared to patients with osteoarthritis and other forms of inflammatory arthritis (11). Further, we demonstrated that gp96 bound to the extracellular domain of TLR2 and activated macrophages primarily through TLR2, supporting the importance of gp96 an endogenous TLR ligand in RA.

Additional studies have documented the presence of functional TLR ligands in RA synovial fluid. A recent study demonstrated that RA synovial fluids activated HEK293 cells expressing TLR4, suggesting that RA-synovial fluids contain TLR4 ligands (18). We have observed that RA synovial fluids activate normal macrophages through TLR2 and TLR4 (unpublished studies). Further, conditioned media from RA synovial membrane cultures stimulated normal macrophages in a MyD88, Mal/TIRAP-dependent manner, consistent with activation through TLR2 or TLR4 (19). Mal/TIRAP is an adaptor for MyD88 in the TLR2 and TLR4 pathways. Together, these observations suggest the presence of functional extracellular TLR4 and TLR2 ligands within the joints of patients with RA.

Response of cells from Patients with RA to TLR ligands

Microbial TLR ligands—A variety of microbial TLR ligands have been shown to activate cells obtained from patients with RA. The TLR2 ligand bacterial peptidoglycan, but not the TLR9 ligand CpG, induced the expression of IL-6 and CXCL8 by RA synovial fibroblasts (20,21,22). The lack of activation by CpG is consistent with the lack of TLR9 in RA synovial fibroblasts (7). The TLR3 ligand poly IC (a mimetic for viral dsRNA) induced the expression of IL-1β mRNA in RA synovial fibroblasts, although IL-1β protein was not secreted (23). Pre-treatment of RA synovial fibroblasts with IFNα or IFNβ induced TLR7 expression and response to the TLR7 agonist loxoribine (23). A recent study dissected the TLR2, -3, and -4 pathways leading to JNK activation in synovial fibroblasts (24). LPS and peptidoglycan-induced JNK activation was mediated by MKK7 primarily, similar to the results observed with TNFα, resulting in the activation of cJun. However activation with the poly IC was mediated by both MKK4 and MKK7 which resulted in both c-Jun and IRF3 activation (24). Overall these studies document that RA synovial fibroblasts are responsive to a variety of microbial TLR ligands, particularly TLR2, -3 and -4.

Employing macrophages isolated from the synovial fluid of patients with RA, we observed increased responsiveness to both peptidoglycan and LPS (9). While TLR2 and TLR4 were both increased on the RA synovial macrophages compared with in vitro monocyte differentiated macrophages, the increased response to the microbial TLR ligands did not correlate with the cell surface TLR expression. In contrast employing normal macrophages, the expression of TLR2 correlated with activation by the TLR2 ligand peptidoglycan (9), suggesting that the increased response may be related to other factors, such as the decreased expression of IL-10 following activation of TLR2 or TLR4 or by increased IFNγ induced by RA synovial macrophages, each of which may increase TLR signaling (25). We recently demonstrated the increased expression of IFNγ by RA synovial fluid macrophages following TLR2 activation (26). Further supporting the role of TLR signaling, peripheral blood

mononuclear cells from patients with recent onset RA demonstrated increased responsiveness to the microbial TLR4 ligand LPS (27). These observations demonstrate that RA synovial macrophages and peripheral blood monocytes, express TLR2 and TLR4 and are more responsive to TLR ligation compared to controls.

Endogenous TLR ligands—Several studies employed whole synovial membrane cultures to determine the potential role of endogenous TLR ligation in the pathogenesis of RA. A TLR4 antagonist suppressed the spontaneous secretion of IL1β and TNFα by ex vivo RA synovial tissues cultures (18), while inhibition of MyD88 and Mal/TIRAP, which mediate TLR2 and TLR4 signaling (19), suppressed the spontaneous secretion of inflammatory cytokines from RA synovial tissue membranes, suggesting the presence of endogenous TLR ligands in RA synovial tissue. To examine the endosomal TLR pathways, RA synovial tissue cultures incubated with mianserin or chloroquine to suppress TLR3, -7, -8 and -9 signaling, as well as a more specific TLR8 inhibitor, resulted in suppression of TNFα and IL-6, while either a TLR8 or a TLR3 agonist increased TNFα secretion (28). These observations support the potential role of TLR8, and possibly TLR3, in the persistent cytokine production observed in the RA joint. Since ss and dsRNA viruses are the known ligands for these TLRs, these observations suggest a viral infection or activation by an as yet to be characterized endogenous TLR ligand might contribute to the pathogenesis of RA. One possible endogenous TLR ligand may be necrotic cells, since necrotic synovial fluid cells induced the expression of CXCL10, CCL5 and IL-6 in a TLR3-dependent fashion, by RA synovial fibroblasts (29). TLR3 was recently shown to be a sensor for tissue necrosis employing TLR3−/− mice (30). These observations suggest that the presence of endogenous TLR ligands for TLR3 and TLR8 may be contributing to the ongoing inflammatory process.

A potentially relevant endogenous TLR ligand has been employed to determine its ability to activate cells from patients with RA. We recently identified that the stress response protein gp96 activates normal macrophages primarily through TLR2. Supporting this observation, gp96 strongly activated HEK-TLR2, but not HEK-TLR4 cells. Additionally, the activation of RA synovial fluid macrophages by gp96 was significantly greater than observed with control macrophages. Also gp96 induced the expression of TLR2 on macrophages, and the level of expression of TLR2 on synovial fluid macrophages strongly correlated with the level of gp96 in the RA synovial fluid. These observations support the potential role of gp96 as an endogenous TLR ligand which may contribute to the pathogenesis of RA (Figure 2).

New insights into the regulation of TLR signaling

TLR ligation is known to activate NF-κB, MAPKs and IRF3 and recent studies have provided novel insights that may have therapeutic implications. RANTES, highly expressed in the RA joint, is induced by TLR ligation and is well documented to promote leukocyte migration. We recently demonstrated that RANTES also has an anti-inflammatory effect, suppressing TLR4 signaling, mediated by the induction of IL-10 (31). Therefore, inhibition of RANTES, in addition to suppressing chemotaxis may promote inflammation by reducing the level of IL-10. In contrast, low concentrations of IFNγ found in the RA joint may enhance TLR signaling by suppressing IL-10 (25). In addition, cross-regulation by molecules expressed within the RA joint may also promote inflammation mediated by TLR signaling. Macrophages in the synovial lining in RA express the death receptor Fas and its ligand FasL. Even though these macrophages are in intimate contact with each other in the synovial lining, they are protected from apoptosis due to the increased expression of the anti-apoptotic molecule FLIP (32). However, when Fas and FasL on the surface of macrophages interact, the adapter molecule FADD is recruited to the Fas receptor and it is no longer available to interact with MyD88. The interaction of FADD with MyD88 suppresses TLR and IL-1R signaling. These observations suggest that in the RA synovial

lining, the intimate contact between synovial macrophages serves as a rheostat to increase the sensitivity of local macrophages to respond to endogenous TLR ligands (32). Additionally, cross regulation mediated through immunoreceptor tyrosine-based activation motives, such as Fcγ receptors by immune complexes which are present in the RA joint, may enhance or suppress TLR signaling, depending upon the intensity of the signaling (33). Thus cross-regulation of TLR activation, which occurs within the RA joint, offers potential novel mechanisms to modulate TLR signaling.

TLR activation has also been shown to induce a number of pathways that suppress TLR activation, which may also be harnessed to suppress TLR signaling. These pathways include the induction of A20 which suppresses NF-κB activation (34), ATF3 which attenuates activation by NF-κB and C/EBPδ (35), and induction of the microRNA miR-9 which is induced by TLR2 and TLR7/8 agonists and provides feedback suppression of NF-κB activation (36). A20 is particularly interesting since mice deficient in A20 develop a lethal inflammatory response that is mediated by the inability to suppress the inflammation induced by commensal microbial flora (34). Further studies will be required to determine if the expression of A20 in the RA joint is reduced, and if this might contribute to the increased response of RA synovial macrophages to TLR2 and TLR4 ligands (9). Therefore, TLR signaling, not only generates pro-inflammatory mediators, but at the same time generates self-regulating signals to suppress activation, which may potentially be excellent therapeutic targets for RA.

Overview of the role of TLRs in RA

Endogenous TLR ligands are expressed and released as a result of the inflammation in early RA and may contribute to persistent, destructive disease (Figure 2). In shared epitope positive individuals, the initial insult may be the result of immune complexes containing anti-cyclic citrullinated peptides. The pathogenic immune complexes providing the initial danger signal, together with the released endogenous TLR ligands, such as gp96, may result in a self-perpetuating inflammatory process, driven by the persistent expression of macrophage-related cytokines such as TNFα and IL-6. Non-apoptotic Fas-FasL signaling may lower the threshold for the activation of synovial macrophages, and possibly synovial fibroblasts, sensitizing them to activation by the endogenous TLR ligands, thereby promoting the development of chronic, persistent disease. The release of low levels IFNγ may further sensitize the synovial macrophages to activation by endogenous TLR ligands. Thus, the local environment of the RA joint may provide the milieu for the perfect storm of chronic inflammation.

TLRs contribute to the pathogenesis of experimental models of RA

A variety of experimental models of RA have been employed which identify the potential role of TLRs in the pathogenesis of RA.

Streptococcal cell wall model—To examine the role of TLR ligands in experimental arthritis, streptococcal cell wall (SCW) induced arthritis was examined. A single SCW injection into murine joints resulted in joint inflammation mediated through TLR2 and MyD88 (37). Repeated intraarticular SCW injections resulted in chronic destructive arthritis mediated in the late stages through TLR4 (38). TLR4 was important in the destructive phase, and it contributed to matrix metalloproteinase-mediated cartilage damage and osteoclast formation (39). Therefore, in this model of chronic arthritis, TLR2 necessary initially and TLR4 was important later in the disease, suggesting that endogenous TLR4 ligands may contribute to the destructive phase.

Collagen induced arthritis—Collagen induced arthritis (CIA) is induced in susceptible mice following immunization with type II collagen and is dependent on T cells and the induction of autoantibodies to collagen. Inhibition of TLR4 suppressed CIA clinically and histologically, without suppressing adaptive immunity to collagen (40). Further, the addition of the TLR4 ligand LPS enhances anti-collagen antibody-mediated arthritis. Also supporting the role of TLRs in CIA, RP105 deficient mice demonstrated increased severity of arthritis (41). RP105 is a TLR homolog and a negative regulator of TLR4 signaling (42). These observations suggest that an endogenous TLR4 ligand might contribute to the pathogenesis of CIA, however, this interpretation must be tempered by the fact that the adjuvant employed to induce the initial immune response to collagen may activate TLR4.

Serum transfer model—The role of TLRs was also examined following the transfer of serum from K/BxN mice which express antibodies to glucose-6-phosphate isomerase (GPI). Transfer of these antibodies to naïve mice results in an immune complex-mediated arthritis due to the anti-GPI antibodies that localize within the joints. The injection of microbial TLR2 or TLR4 ligands into the peritoneum significantly enhanced the arthritis induced by anti-GPI serum transfer (43). Further, the course of anti-GPI-induced arthritis was not sustained in TLR4 deficient mice (44). The local expression of immune complexes within the joint may result in increased Fcγ receptor expression mediated through TLR4 which may promote further joint damage (45). Together these observations support the potential role of TLR ligands in promoting joint inflammation and destruction which is mediated by immune complexes.

IL-1 receptor antagonist deficient mice—Another model of RA is the IL-1 receptor antagonist deficient (IL-1Ra −/−) mouse that develops spontaneous arthritis. However, when the IL-1Ra −/− mice were raised in a germ free environment the arthritis did not develop, suggesting that gut flora may contribute to the arthritis (18). When these mice were crossed with mice deficient in TLR2, the arthritis was worse and this was associated with an increase of IFNγ and a decrease of FoxP3 and TGFβ, suggesting that the exacerbation may be due to reduced T regulatory cells. In contrast, when IL-1Ra−/− mice were crossed with TLR4 −/− mice the arthritis was reduced. Consistent with these observations a TLR4 antagonist suppressed the spontaneous arthritis observed in IL-1Ra −/− mice (40). Therefore, TLR4 is important in the arthritis of IL-1Ra −/− mice, however, in this model the TLR4 ligand appears to be coming from commensal flora. (18)

Other models—The data for the role of endosomal TLRs, TLR3, 7 and 9, in experimental models of RA is less clear, and may depend on the mode of activation. Motifs of CpG, a TLR9 ligand injected directly in the joints of mice resulted in mild arthritis (45). In contrast, CpG oligodeoxynucleotides administered systemically suppressed the serum transfer K/BxN model of RA, which was mediated by the induction of IL-12 from CD8+ dendritic cells, which induced IFNγ from NK cells, which suppressed neutrophil recruitment (43). The injection of viral dsRNA or a mimetic, poly IC, into the joints of mice, resulted in an arthritis that was associated with the accumulation of macrophages and their cytokines (46). Unexpectedly TLR3 deficient mice were not protected. This suggests that another pathway such as the retinoic-acid-inducible gene I or the melanoma-differentiation-associated gene 5 pathway, which are capable of activating interferon regulatory factor and NF-κB (47), might be contributing. In contrast to the local effects of poly IC, the systemic administration of poly IC suppressed the immune complex mediated arthritis induced by the transfer of anti-GPI serum or collagen antibody induced arthritis (48). Supporting the role of type 1 interferon, IFNα or poly IC administered systemically also resulted in the local reduction of inflammatory cytokines and chemokines with the joints. There fore, although TLR3 and TLR9 ligands induced a mild arthritis when injected locally, when administered

systemically they suppressed experimental arthritis. This contrasts with TLR2 and TLR4 ligands, which when administered systemically, enhanced anti-GPI and anti-collagen antibody-induced arthritis.

Recently, a novel approach was taken to examine the potential role of TLR7 in chronic inflammation, taking advantage of the observation that low dose TLR activation may result in tolerance or a decreased response to repeated application. Mice injected with low doses of a synthetic TLR7 agonist resulted in tolerance to TLR2, -7 and -9 (49), and suppression of the arthritis induced by the transfer of anti-GPI serum (49), suggesting that TLR7 tolerance may be an alternative approach to suppressing inflammation. In summary, the available data strongly support the role of TLR2 and TLR4 in the SCW model of experimental models of arthritis and TLR4 in CIA and IL1Ra−/− models. The data suggests a potential role TLR3, TLR7 and TLR9 in experimental models of RA, however, further studies are required to clearly define the potential mechanisms.

New Methods to Inhibit TLR signaling

The targeting of TLRs to treat human disease shows great promise. The use of TLR agonists as vaccine adjuvant is being developed and in some areas are already in use (50). These include TLR3, -4, -7 and -9 agonists which are being used to develop effective and safe immune responses to a variety of infectious diseases including Hepatitis B and C, HIV, human papilloma virus, influenza and anthrax (50). Imiquimod is a chemical TLR7 agonist currently approved as a cream to treat papillona-induced warts, actinic keritosis and basal cell carcinoma. TLR agonists are also being examined to treat asthma and allergic rhinitis which are designed to shift the TH2/TH1 balance and in a variety of cancers by promoting the development of immune responses to recognize tumor antigens. TLR antagonists are less well developed clinically. Two TLR4 antagonists have been studied to treat severe sepsis. In preclinical development, TLR7 and 9 inhibiting ODNs have been synthesized, which are effective in vitro at suppressing the expression of IFNα by pathogenic immune complexes. In RA, chaperonin 10, a small molecular weight heat shock protein, was well tolerated and clinically effective in a small study of patients with RA (51), providing proof of principal that the TLR pathway is important in the pathogenesis of RA. As mentioned above choroquine and hydeoxychloroquine are inhibitors of endosomal TLR activation, although it is not know if this is the mechanism responsible for the clinical activity.

Conclusion

The available studies in patients and experimental animals document a potentially important role for innate immunity, particularly the TLR signaling pathway in the pathogenesis of RA. While a role for microbial TLR ligands is possible, abundant data supports the role for endogenous TLR ligands in the perpetuation and progression of RA. The targeting of the TLR signaling pathway is expanding into clinical practice, and holds great promise in RA.

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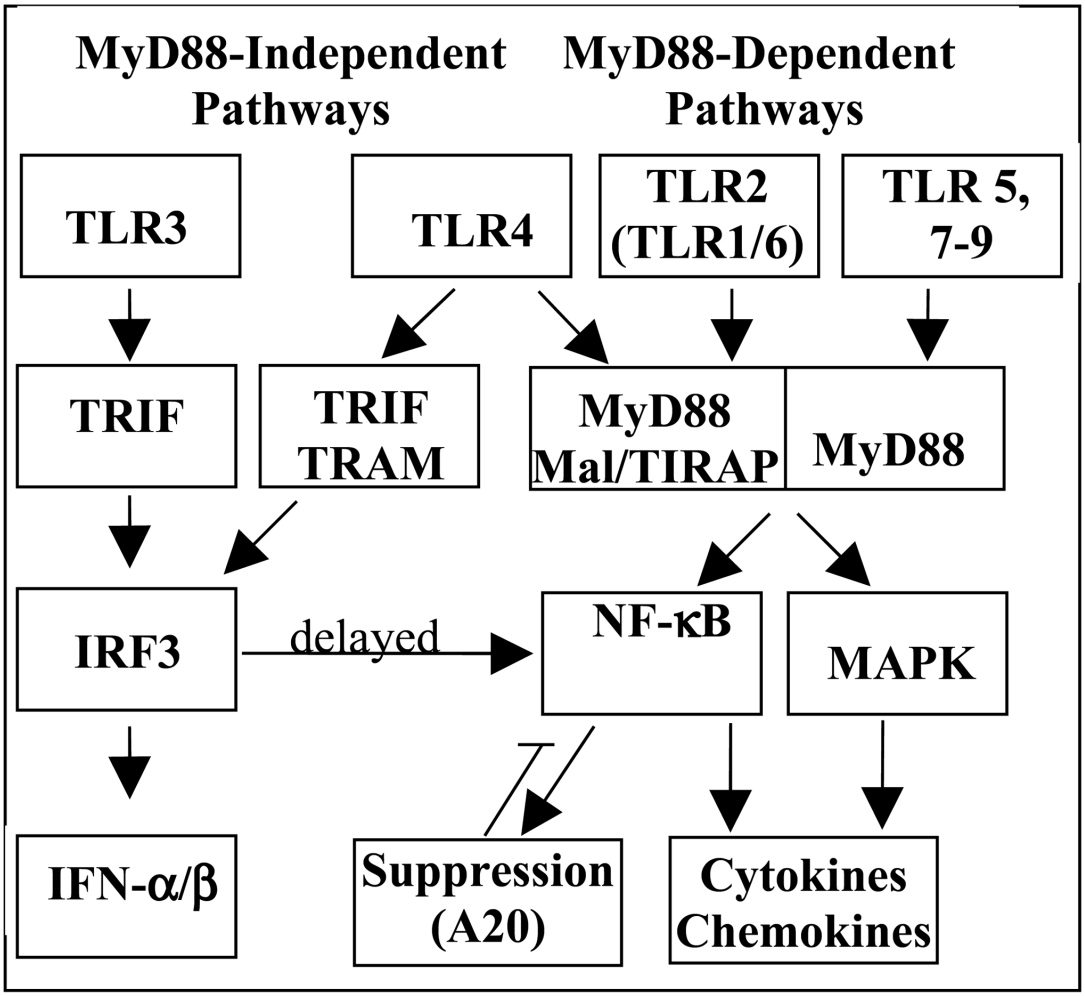

Figure 1. Overview of TLR signal pathway

Upon ligand binding, TLRs interact with its corresponding adaptors, which result in MyD88-dependent and MyD88-independent pathways. Each of the TLRs except TLR3 transmits signals through the MyD88-mediated pathway, which leads to the activation of NF-κB and MAPKs, resulting in the expression of pro-inflammatory cytokines and chemokines. Activation of TLR3 and TLR4 through the MyD88-independent pathway is mediated through TRIF, resulting in the expression of type I interferons, IFNα and β, and the delayed activation of NF-κB and MAPKs, which is mediated through TNFα. The activation of NF-κB also results in the induction of A20, which suppresses NF-κB activation.

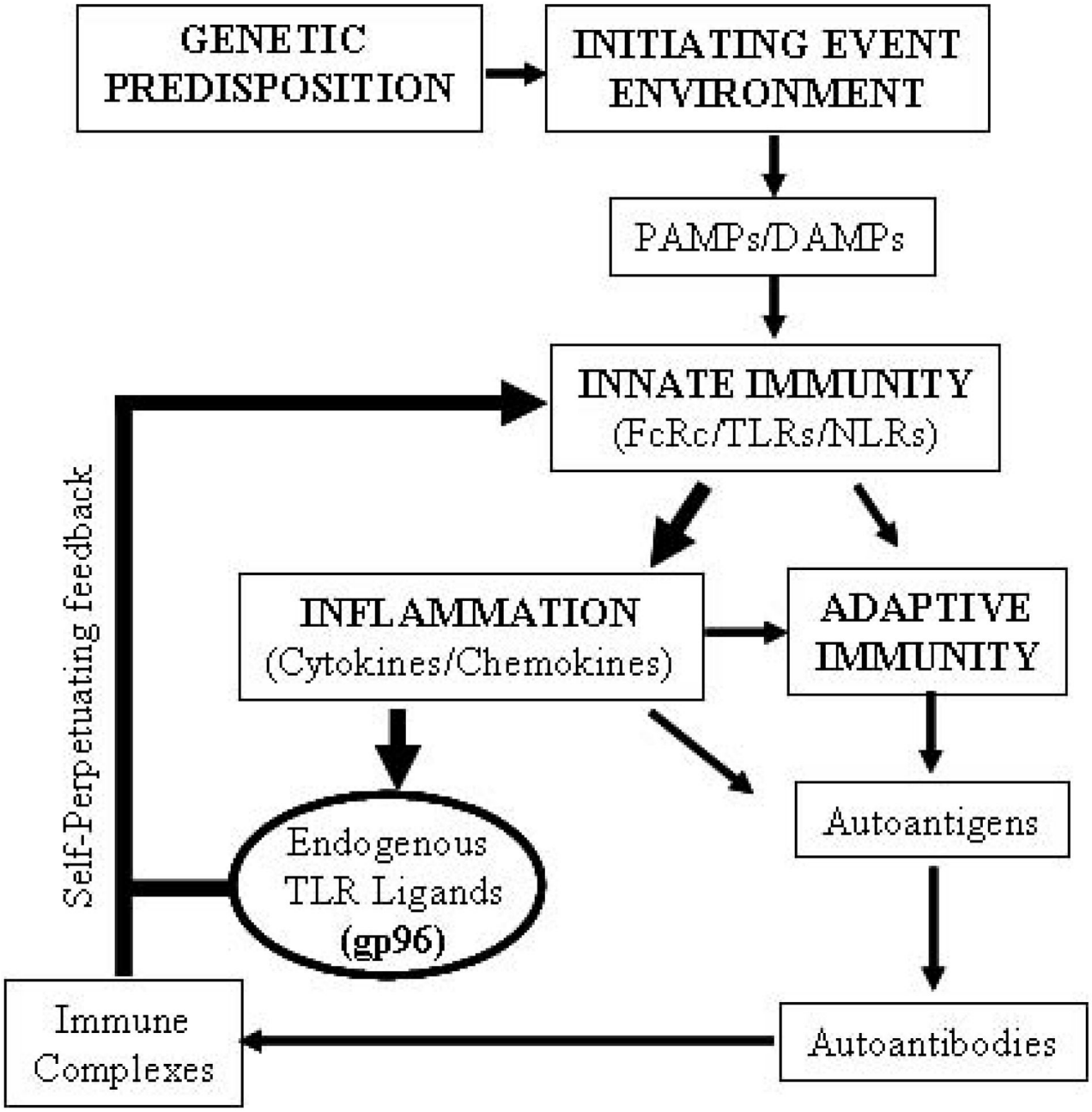

Figure 2. Unifying hypothesis of endogenous TLR ligands in the pathogenesis of RA

The exposure of genetically susceptible individuals into initial environmental stimulus might result in the activation of the innate and adoptive immune systems followed by inflammation. Endogenous TLR ligands (such as gp96) are expressed and released as a result of the inflammation. Other components released may also serve as autoantigens resulting in the formation of both pathogenic immune complexes or endogenous TLR ligands, capable of inducing a self-perpetuating inflammatory process, driving the persistent expression of macrophage-related cytokines and chemokines, which play an important role in the pathogenesis of RA.

Table 1

Toll-like receptors and their corresponding ligands

Type of TLR	Microbial Pathogen Associated Molecular Patterns (PAMPs)(1)	Potential endogenous TLR ligands (DAMPs) in RA	Defined source in RA (synovial tissue, cells or fluid)
TLR2 (dimerization with TLR1or 6)	Lipoglycans (Mycobacterium) Lipoteichoic Acids (Gram-positive bacteria) Peptidoglycan (Gram-positive bacteria) Zymosan (Yeast)	HSP 60	Macrophage(2), synovial tissue*
		HSP70	fibroblast(2), macrophage(2) synovial tissue
		gp96	fibroblast(2), macrophage(2), synovial tissue and synovial fluid(2)
		HMGB-1	synovial tissue macrophages, synovial fluid
		Biglycan	fibroblast(2)
		Serum amyloid A	serum, synovial tissue
TLR4	LPS (Gram-negative bacteria) Mannan (Candida) Envelope protein (Virus)	HSP22	synovial tissue, fibroblast
		HSP 60	Macrophage(2), synovial tissue
		HSP70	fibroblast(2), macrophage(2) synovial tissue
		EDA fibronectin	synovial fibroblast, synovial fluid
		fibrinogen	Synovial tissue, synovial fluid
		low molecular weight hyaluronic acid	synovial fluid
		HMGB-1	synovial tissue macrophages, synovial fluid
		Biglycan	fibroblast
TLR5	Flagellin (Gram-negative bacteria)		
TLR3	ds RNA (virus)	Undetermined	necrotic synovial fluid cells
TLR7	ss RNA(virus)		
TLR8	ss RNA(virus)	Undetermined	necrotic synovial fluid cells
TLR9	CpG motif (bacteria, virus)	immunostimulatory CpG motifs	serum

* Present is synovial tissue, the precise cell-type not identified