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THE TOLL-LIKE RECEPTOR-NUCLEAR FACTOR κB PATHWAY IN RHEUMATOID ARTHRITIS

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1. ABSTRACT

The study of the role cytokines play in the pathogenesis of rheumatoid arthritis (RA) has provided a whole new range of targets for drug development. Many of them (e.g. TNF, IL-1, IL-6, IL-15 and IL-18) are already being targeted in the clinic with success using neutralizing monoclonal antibodies or soluble cytokine receptors. Targeting TNF, in particular, has shown great efficacy in controlling both the inflammation and structural damage of the joints, setting a new gold standard for the treatment of RA. However, what triggers the production of inflammatory cytokines such as TNF in RA remains to be determined. In this article, we review evidence suggesting that the transcription factor Nuclear Factor κB (NF-κB) is essential for the expression of both inflammatory cytokines and tissue destructive enzymes in RA. Also, we discuss whether Toll-like receptors (TLRs), major receptors involved in pathogen recognition and potent activators of the NF-κB pathway, are involved in triggering the inflammatory and joint destructive process in RA and whether they constitute sensible targets for monoclonal antibodies/soluble receptors and small molecule inhibitors. We conclude that although the TLR- NF-κB pathway offers ample opportunities for therapeutic intervention, future drugs to be approved will need to match or exceed the efficacy and safety of anti-TNF agents, with safety the most difficult aspect to predict.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a multifactorial disease characterized by chronic inflammation of the joints. Both genetic and environmental factors are involved leading to joint destruction and ultimately disability and premature death in more severe cases. The disease is believed to be initiated through the activation of CD4+ T cells that inappropriately recognize autoantigens and respond by triggering an immune response. For not yet understood reasons, this immune response becomes autoantigen-independent and sustained, and drives the disease unremittingly with a role for B lymphocytes which is not yet understood. Only in rare cases RA resolves spontaneously, and this occurs only very early during the disease process. In most cases, the disease progresses through exacerbations and remissions to progressive destruction of multiple joints and damage of other organs.

By the time RA patients seek medical attention and health professionals methods of treatment, the disease has already progressed to the self perpetuating stage. This is characterized by synovial hyperplasia, infiltration of various immune cells such as macrophages, T cells, B cells and neutrophils, and angiogenesis. At this stage, almost all known cytokines can be detected in the synovium, with the notable exception of IL-4. These include TNF, IL-1, IL-6, IL-8, IL-10, IL-12, IL-15 and IL-18 and are considered therapeutic targets (1, 2). Novel therapies for RA based on anti-cytokine monoclonal antibodies or soluble cytokine receptors that target specific cytokines are being developed, with several already approved, and used in the clinic. The first to be approved and the most efficacious biological agents at present have been TNF inhibitory ones that were shown to block not only the inflammatory part of the

disease and relieve patients from joint swelling and pain, but also to halt bone resorption (and in some cases even induce repair) and protect patients from disability (2, 3). Anti-TNF agents already approved are infliximab (Remicade®), FDA approved for RA in 1999 but the first to be tried in clinical trials, etanercept (Enbrel®), FDA approved in 1998, and adalimumab (Humira®) FDA approved in 2002. Other biologicals that have shown efficacy in the clinic are IL-1ra (Anakinra®) and MRA, a humanized anti-IL-6 receptor monoclonal antibody (4). Anakinra received FDA approval in 2001 for its use in RA, whereas MRA is beginning Phase III clinical trials.

Despite the success of anti-cytokine biological agents in treating RA, none of them cure the condition, as the disease returns upon cessation of therapy. This indicates that current anti-cytokine-based approaches target the effector arm of the disease and its symptoms but not the cause. Thus, novel therapies that get closer to the cause of the disease are being sought. In this article, we discuss the role of the Toll-like receptor (TLR) and Nuclear Factor κB (NF-κB) pathways in RA. We review previous work on the involvement of the transcription factor NF-κB in regulating the expression of inflammatory and tissue destructive genes in RA, and the potential of the pattern recognition receptors, TLRs, in triggering NF-κB activation in RA in response to infections or damaged self tissue.

3. NUCLEAR FACTOR κB AND TOLL-LIKE RECEPTORS IN INFLAMMATION AND IMMUNITY

3.1. Nuclear Factor κB

NF-κB is a pleiotropic transcription factor that is found in almost every cell type known and that plays an essential role in development, inflammation and immunity (5). In mammals, NF-κB exists as a homo- or heterodimeric complex formed by combinations of five distinct DNA-binding subunits, p65/RelA, RelB, c-Rel, p50 and p52, all of which share the Rel homology domain that allows their dimerisation and translocation into the nucleus. RelB, p65 and c-Rel, which are synthesized as mature proteins, also contain a transactivation domain that is subject to modulatory phosphorylations that enhance the transcriptional activity of NF-κB. In contrast, p50 and p52, which do not contain a transactivation domain, are synthesized as p105 and p100 precursors, respectively, and undergo proteolytic processing. NF-κB dimers are all bound to inhibitors of NF-κB proteins (IκB) such as IκBα and IκBβ, which retain NF-κB in the cytosol.

The rate-limiting step in NF-κB activation is the phosphorylation, ubiquitination and degradation of IκB proteins that release NF-κB from the cytosol to rapidly translocate into the nucleus to regulate gene expression, a process also referred to as the 'canonical' or 'classical' pathway of NF-κB activation (reviewed in 5, 6). This can occur in response to various stimuli that include physical and chemical stress (e.g. UV light or hypoxia), viral and microbial products (e.g. LPS), and inflammatory cytokines (e.g. IL-1 and TNF). IκBα phosphorylation is catalyzed by an IκB kinase (IKK) complex that consists of three identified proteins, IKK1/α, IKK2/β and IKKγ/NEMO. IKKγ is essential for the activity of the IKK complex but does not phosphorylate IκB, whereas IKK2 is required for the phosphorylation of IκBα and IκBβ proteins (5, 6).

In addition to that, an alternative pathway leading to NF-κB activation also exists that controls the proteolytic processing of p105 and p100 to p50 and p52, respectively, that translocate into the nucleus to regulate gene expression. This pathway is termed the 'non-canonical' or 'alternative' pathway of NF-κB activation and occurs constitutively for p105 processing to p50, whereas for p100 processing to p52 the process is inducible and under the control of NF-κB -inducing kinase (NIK) and IKK1 (5). In response to stimuli that include $LT\alpha_1\beta_2$, CD40 ligand and Blys, NIK has been shown to activate IKK1 and induce subsequent release of p52 independently of the canonical complex. It has been proposed that the non-canonical pathway of NF-κB activation plays a major role in signaling cascades that take place during development, organization and function of the lymphoid tissue (7).

Once activated, NF-κB binds to the promoter regions of genes where it acts in concert with other transcription factors and proteins to form multicomponent complexes to regulate the timing, level and duration of gene expression (8). A huge variety of chiefly pro-inflammatory genes that include cytokines (TNF, IL-1, IL-2, IL-6, IL-12, IL-18 etc), chemokines (IL-8, RANTES, MIP-1α/β etc), adhesion and costimulatory molecules (ICAM, VCAM, E-selectin, CD40, CD80, CD86), MHC antigen presenting molecules (class I and class II), enzymes (COX-2, iNOS, matrix metalloproteinases, cathepsins) and anti-apoptotic proteins (c-IAP-1, c-IAP-2, Bcl-X_L, Fas ligand, cyclin D1 etc.) are regulated by NF-κB (9), thus explaining the major role this transcription factor plays in immunity and inflammation. Indeed, NF-κB and its upstream activator IKK2 have been shown to be essential for efficient antigen presentation and induction of immunity or tolerance (10-13), processes of importance to the pathogenesis of autoimmune or inflammatory diseases.

3.2. Toll-like receptors

Although NF-κB can be activated by multiple and very diverse stimuli, a major pathway of NF-κB activation involves the triggering of TLRs. TLRs are a family of phylogenetically conserved proteins recently identified as the receptors recognizing numerous molecular structures of microbial or viral pathogens, thus distinguishing 'self' from 'foreign' and triggering an immune response against them. For instance, TLR2 is required for the response to microbial lipoproteins and yeast carbohydrates (14, 15), TLR3 for the response to viral double-stranded RNA (16), TLR4 for the response to bacterial LPS (17, 18) and respiratory syncytial virus (19), TLR5 for the response to bacterial flagellin (20), TLR8 for the response to viral single-stranded RNA in humans but TLR7 in mice (21), TLR9 for the response to unmethylated CpG motifs found in bacterial DNA (22) and TLR11 for the response to uropathogenic bacteria (Table 1) (23). The

Table 1. Exogenous and endogenous Toll-like receptor ligands

TLR family member	Exogenous ligands	Endogenous ligands
TLR1	Lipopeptides (bacteria, mycobacteria) Soluble factors (neisseria meningitides)	-
TLR2	Lipoproteins/lipopeptides (various pathogens) Peptidoglycan (gram-positive bacteria) Lipoteichoic acid (gram-positive bacteria) Lipoarabinomannan (mycobacteria) A phenol-soluble modulin (staphylococcus epidermidis) Glycoinositolphospholipids (trypanosoma cruzi) Glycolipids (treponema maltophilum) Porins (neisseria) Zymosan (fungi) Atypical LPS (leptospira interrogans) Atypical LPS (porphyromonas gingivalis)	hsp 60 hsp70 hsp 96
TLR3	Double-stranded RNA (virus)	mRNA
TLR4	LPS (gram-negative bacteria) Taxol (plant) Fusion protein of RSV (virus) Envelope proteins of MMTV (virus) Hsp60 (chlamydia pneumoniae)	hsp60 hsp70 hsp 96 Fibronectin EDA domain Oligosaccharides of hyaluronic acid Polysaccharide fragments of heparan sulfate Fibrinogen
TLR5	Flagellin (bacteria)	-
TLR6	Di-acyl lipopeptides (mycoplasma) Heat-labile soluble factor (group B streptococcus) Phenol-soluble modulin (staphylococcus)	-
TLR7	?	-
TLR8 (human, murine TLR7)	Single-stranded RNA (viruses) Imiquimod (synthetic compound) Loxoribine (synthetic compound) Bropirimine (synthetic compound) R-848/Resiquimod (synthetic compound, activates both TLR7 and TLR8)	-
TLR9	Unmethylated CpG DNA (bacteria)	Chromatin-IgG complexes
TLR10	?	-
TLR11 (murine)	Factors from uropathogenic bacteria	-

Adapted from 26

specificity of TLRs can be further tuned through the formation of functional pairs of TLRs that can change their specificity or influence their signaling ability. Thus, although TLR2 alone recognizes lipopeptide of gram-positive bacteria, when complexed with TLR6 it recognizes peptidoglycan (24). Moreover, when TLR2 forms pairs with TLR1, it recognizes certain soluble factors released by Neisseria meningitis (25).

More recently, TLRs have been shown to recognize certain endogenous or 'self' ligands (Table 1). Thus, TLR4 was suggested to be involved in the recognition of self heat shock proteins (hsp) 60, 70 and Gp96 (26-29), extra domain A (EDA)-containing fibronectin fragments (30), hyaluronan fragments (31), fibrinogen (32), β-defensins (33), oxidized low density lipoprotein (LDL) (34) and heparan sulphate (35). TLR2 was also suggested to play a role in the recognition of hsp60 (27) as well as unidentified factors from necrotic cells (36). In addition, TLR3 was shown to recognize self RNA (37), and TLR9 self-DNA (IgG-chromatin) complexes (38). Genomic DNA released from dying cells has also been reported to activate human DCs but whether TLR9 or another TLR is involved in that process has not yet been demonstrated (39). With respect to chronic inflammatory and autoimmune responses, this finding is particularly interesting as it suggests that at sites of chronic inflammation such as RA joints where extensive tissue destruction is taking place, TLRs are able to promote inflammation in the same way they do when recognizing pathogens.

Although most cell types can express TLRs under certain conditions, the highest levels of TLRs are mainly found in cell types involved in the first line of defence such as macrophages, dendritic cells, neutrophils, mast cells, mucosal epithelial cells, endothelial cells and fibroblasts. Distinct subsets of these cells also express different TLRs that enable them to shape the immune response to one type or another according to the invading pathogens. Because of

that, TLRs are considered to be the receptors bridging innate and adaptive immunity, as they also control the activation of dendritic cells, macrophages and B cells, the three types of 'professional' antigen-presenting cells. Thus, in response to LPS and other TLR ligands, DCs up-regulate the expression of costimulatory and antigen-presenting molecules and the production of cytokines such as IL-2, IL-12, IL-15 and IL-18, although the patterns of cytokines and chemokines induced and the type of T cell responses initiated may differ (40). Human monocytes and macrophages also up-regulate the expression of cytokines, chemokines and costimulatory molecules as well as their antigen presenting function in response to TLR ligands, although these processes have not been studied in detail (41). Similarly, many TLR ligands activate B cells and induce their activation and isotype switching to mature antibody-producing cells (41).

3.3 TLR downstream signaling pathway and activation of NF-κB

Soon after their discovery, it was suggested that TLRs mediate their downstream effects by using a framework of signaling molecules analogous to that used by the IL-1 receptor (IL-1R). This was due to the observation that in addition to an extracellular domain containing leucine-rich motif repeats characteristic of pattern recognition receptors, TLRs have a cytoplasmic domain that is homologous to the intracellular signaling domain of the IL-1R, termed as the Toll/IL-1 receptor (IL-1R) homology (TIR) domain. The TIR domain is found in the cytoplasmic portion of all TLR and IL-1 receptor family members such as IL-1RI, IL-1RacP, IL-18R, IL-18RacP, T1/ST-2, IL-1Rrp2 and IL-1RAPL (41-43). Upon ligand-binding, both TLR and IL-1R family members recruit the adaptor molecule myeloid differentiation protein 88 (MyD88) through homotypic interactions with a TIR domain that MyD88 contains in its C-terminus (44, 45). MyD88 then recruits IL-1R-associated kinase (IRAK), IRAK-2 and IRAK-4 which induces their phosphorylation and activation (44, 45). Toll-interacting protein (Tollip) negatively regulates this step as in resting cells it forms a complex with IRAK and prevents its phosphorylation (46). The pathway subsequently bifurcates and leads to the activation of the TNF receptor-associated factor 6 (TRAF-6) and mitogen activated protein kinases (MAPK) (47). TRAF6 activation is again negatively regulated by IRAK-M, another IRAK homolog that prevents the dissociation of IRAK and IRAK-4 from MyD88 and the formation of IRAK-TRAF6 complexes (48). TRAF-6, in turn, activates NF-κB through a process that involves the IκB kinase (IKK) complex (49, 50) and probably the kinases TAB-1 and TAK-1 (51), or MEKK-1 (9). Activation of NF-κB is essential for the expression of genes such as the cytokines and chemokines TNF, IL-6, IL-12, IL-18, MIP-1 and RANTES, and the adhesion molecules ICAM-1, VCAM-1 and E-selectin (52).

However, it became apparent that important differences between signaling through TLRs and IL-1R or even between individual TLR members also exist (49). For example, although MyD88 is essential for MAPK and NF-κB activation in response to IL-1 as well as TLR2, TLR5, TLR7, TLR8 and TLR9 ligands, it is only minimally required for the activation of MAPK and NF-κB in response to the TLR4 ligand LPS. Four more adaptor molecules sharing homology with MyD88 were identified and suggested to account for this difference, MyD88-adaptor like/TIR domain-containing adaptor protein (Mal/TIRAP) (53), TIR domain-containing adaptor inducing IFN-beta/TIR-containing adaptor molecule-1 (TRIF/TICAM-1) (54, 55), TRIF-related adaptor molecule/TIR-containing adaptor molecule-2 (TRAM/TICAM-2) (53, 56) and sterile alpha and HEAT-Armadillo motifs (SARM) (57). According to evidence from mice deficient in these molecules, MyD88 and Mal/TIRAP appear to be involved in the early phase of NF-κB activation in response to LPS whereas TRIF and TRAM appear to be involved in the later phase of NF-κB activation (54).

Another important difference between signaling through IL-1R and different TLR members is their ability to activate the interferon regulated factors (IRF) 3 and 7 of the IRF family of transcription factors. Thus, it was demonstrated that signaling through TLR3 and TLR4 but not IL-1R or other TLR members leads to the activation of IRF3 and IRF7, and the induction of a distinct set of genes involved in antiviral responses such as IFNβ, IP-10 and RANTES (54). This pathway requires the adaptor molecule TRIF/TICAM-1 and the kinases IKKε and TBK1 (53, 56, 58). It is worth noting that IL-1R/TLR signaling can further differ between different cell types and tissues. In humans for example, although MyD88 and Mal/TIRAP are essential for LPS-induced NF-κB activation and cytokine production in human non-myeloid cells, both molecules are redundant for that process in human myeloid cells such as macrophages and dendritic cells (59). This is in agreement with other studies demonstrating that non-myeloid cells such as embryonic fibroblasts and B cells from mice deficient in MyD88 have the LPS downstream signaling pathway completely abrogated (60). It is such similarities and differences in the signaling pathways between IL-1R and individual TLR members, inducing both unique and common sets of genes, that explain the distinct but also overlapping biological effects these receptors have.

4. NUCLEAR FACTOR κB IN RHEUMATOID ARTHRITIS

In RA, activated NF-κB has been detected in human synovial tissue from both early and later stage patients in both macrophage- and fibroblast-like synoviocytes (61-63). In particular, macrophage-like synoviocytes that localise in the synovial lining layer and the vascular endothelium have been shown to contain both p65 and p50 NF-κB subunits in their nucleus, indicative of functional NF-κB (62).

This did not come as a surprise as the RA synovium is a site of active inflammation and several other transcription factors such as AP-1 (64-66), JNK (67) and STAT-1 (68) are also elevated and localized in the nucleus of cells in RA joints. What came as a surprise, however,

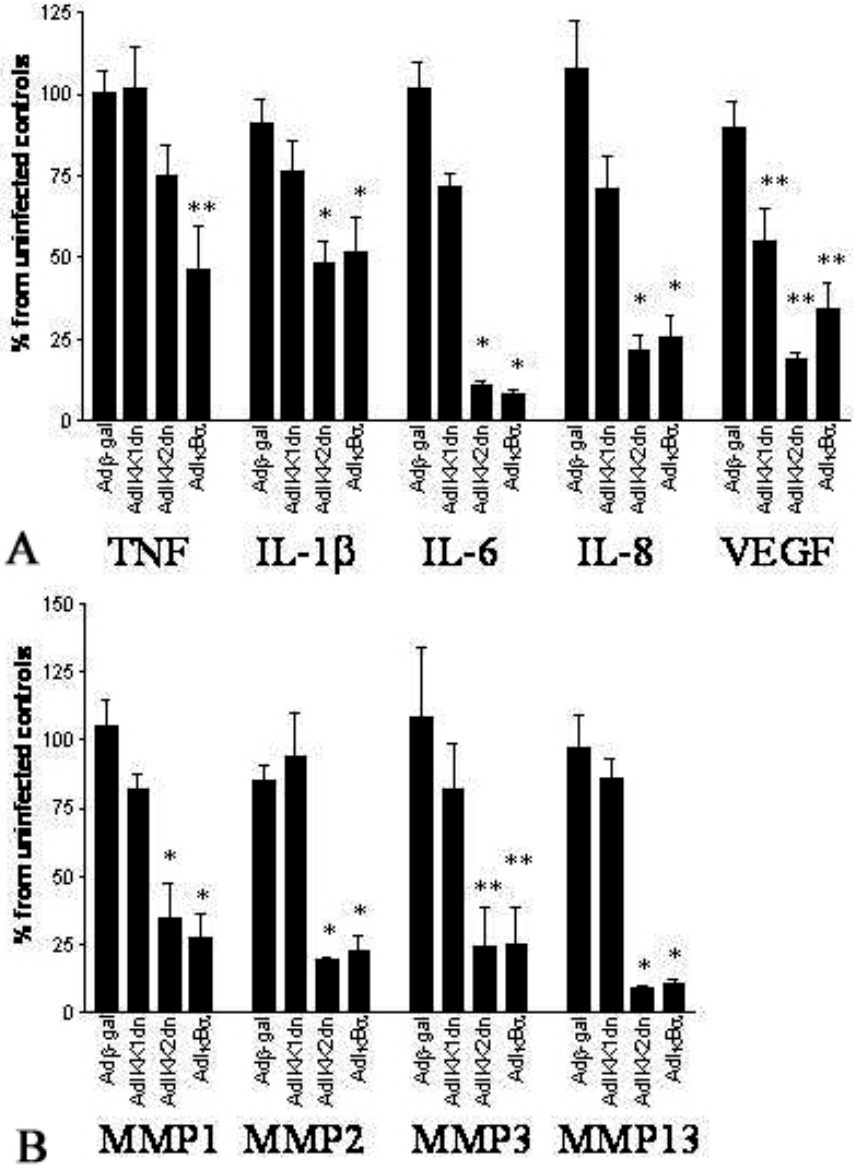

Figure 1. NF-κB regulates the expression of cytokines and MMPs in RA. RA synovial membrane cells were left uninfected, or infected with Adβ-gal, AdIKK1dn, AdIKK2dn and AdIκBα. Supernatants were collected after 48h and assayed for the presence of TNF, IL-1β, IL-6, IL-8, VEGF, MMP-1, MMP-2, MMP-3 and MMP-13 by ELISA. Percentage (%) cytokine production from uninfected cells (±SEM) of triplicate cultures from 7 unrelated patients is shown. For the statistical analysis of these parametric data, a *repeated measures ANOVA test with the Dunnett's comparison post-test* was used to compare uninfected control cells with recombinant adenovirus-infected cells (*$p<0.05$, **$p<0.01$).

was the rate-limiting role NF-κB was found to play in the expression of several pro-inflammatory and tissue destructive enzymes. Thus, in studies in dissociated synovial membrane cultures from RA patients with active disease, overexpression of IκBα, the natural inhibitor of NF-κB, significantly reduced the production of TNF and IL-1β, as well as that of other pro-inflammatory cytokines such as IL-6 and IL-8 (69-70). Interestingly, the expression of anti-inflammatory mediators such as IL-1ra, IL-10, IL-11 and the shedding of soluble TNF receptors was only marginally affected (70). In addition, overexpression of IκBα blocked the secretion of the tissue degrading enzymes matrix metalloproteinases (MMPs) 1,3 and 13 (Figure 1) without affecting the expression of the tissue inhibitor of MMPs (TIMP) 1 (70). Similar effects on the production of most pro-inflammatory cytokines and MMPs was also observed when IKK2, one of the upstream NF-κB activators was targeted. Overexpression of a kinase-defective dominant negative form of IKK2 (IKK2dn) resulted in a significant inhibition of IL-6, IL-8 and MMPs

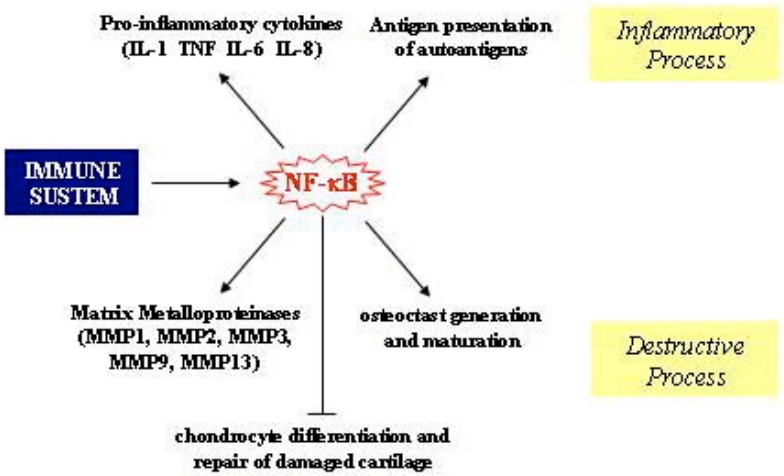

Figure 2. NF-κB affects multiple aspects of the disease process of RA. NF-κB activation promotes both the inflammatory and destructive processes in RA by increasing DC autoantigen presentation, by up-regulating the expression of inflammatory cytokines and MMPs, by inducing osteoctast generation and maturation, and by inhibiting at the same time chondrocyte differentiation and repair of damaged cartilage tissue.

1, 2, 3 and 13 but only marginally affected the expression of TNF (Fig.1), suggesting that there is heterogeneity in the mechanisms controlling NF-κB-dependent gene expression in RA (49).

The essential role of NF-κB in RA has also been backed by studies in animal models of arthritis. Thus, the genetic inactivation of c-Rel and NF-κB1 in *rel*$^{-/-}$ and *nfkb1*$^{-/-}$ deficient mice protected the animals from the development of collagen-induced arthritis (CIA) (71), as did the transgenic expression of serine 32 and 36 mutated (superepressor) IκBα in cells of the T cell lineage (72). Similarly, the administration of NF-κB decoy oligonucleotides suppressed the severity of already established disease in both the CIA and streptococcal cell wall-induced (SCW) models of rat arthritis by inhibiting the production of TNF and IL-1 within the joints and by reducing paw swelling and disease activity (73, 74). A proteosome inhibitor that prevents IκB degradation also protected rats from SCW-induced arthritis (75).

NF-κB and its upstream activator IKK2 may also influence several other processes of relevance to RA pathogenesis by promoting inappropriate antigen presentation of autoantigens (11), by inducing osteoctast generation, maturation and increased bone-resorbing activity (76, 77), and by inhibiting chondrocyte differentiation and repair of damaged cartilage tissue (78, 79). These studies support NF-κB and its upstream activators as therapeutic targets in RA, as blocking NF-κB may reduce the inflammatory response and restore the cytokine equilibrium in the rheumatoid joint by reducing at the same time cartilage and bone damage (Figure 2).

5. TOLL-LIKE RECEPTORS IN RHEUMATOID ARTHRITIS

TLRs are candidates for the regulation of the pathological process of RA. First, TLRs recognize viral and bacterial pathogens as herpes virus and mycoplasma which have long been suspected to be associated with RA (80), as well as several endogenous proteins found in abundance in the RA joint such as heat shock proteins, necrotic cells, fibronectin fragments, hyaluronan oligosaccharides and IgG-chromatin complexes (Table 1). Second, TLRs are able to initiate or promote strong immune and inflammatory responses characterized by the recruitment and activation of neutrophils, macrophages and T cells, an inflammatory infiltrate reminiscent of that seen in RA. Finally, TLRs are able to potently activate NF-κB and induce the production of various cytokines and chemokines including TNF and IL-1, two validated targets for the treatment of RA (2).

Although preliminary, there is evidence that some but not all TLRs are expressed in RA joints. Thus, mRNA for TLR2 was shown to be predominantly expressed in fibroblast-like synoviocytes (FLS) and infiltrating lymphocytes as was mRNA for TLR9 (81, 82). At the protein level, TLR2 was present in both the synovial lining layer of rheumatoid joints and at sites of attachment and invasion of the synovial tissue into cartilage and bone, as well as the areas around small vessels and infiltrating lymphocytes (81, 82). Synovial macrophages were also shown to express TLR2 at the protein level (83). One study has recently demonstrated that in fibroblast-like synoviocytes peptidoglycan (a TLR2 ligand) but not unmethylated CpG (a TLR9 ligand) up-regulates the expression of IL-6, IL-8, chemokines and MMPs 1, 3 and 13 (81, 82, 84), raising the possibility that signaling through TLR2 expressed in synovial fibroblasts may promote inflammatory and tissue destructive processes in the joint.

The role of TLR signaling has also been investigated in SCW-induced arthritis. It was found that mice deficient in the TLR adaptor protein MyD88 were unable to develop inflammation and joint swelling (85).

The expression of proinflammatory cytokines and chemokines in synovial joints was strongly reduced, the infiltration of inflammatory cells was abrogated and the cartilage matrix proteoglycan loss was absent. TLR-2-deficient mice were also protected from SCW-induced arthritis albeit to a lesser extent. Thus, there was still an influx of inflammatory cells into the joint cavity, although the number of cells was markedly reduced. In addition, cartilage matrix proteoglycan loss was completely absent in MyD88 knockout mice (85). These findings suggest that MyD88 and TLR2 are key components of disease development in this animal model of arthritis.

Thus, it is quite probable that TLRs are involved with RA as they can affect multiple stages of the disease process that include its induction, perpetuation, severity of inflammation or even tissue damage. Interestingly, three recent studies have examined the association of the rare Asp299Gly TLR4 polymorphism that attenuates receptor signaling with RA susceptibility, severity and outcome. One found that this polymorphism was indeed associated with disease susceptibility but not its severity and outcome (86), whereas two others found no association with either of the two (87, 88). In addition, another study examined the relation of the Arg753Gln TLR2 polymorphism with RA but no association was again found (89).

6. PERSPECTIVE

Despite the rapid progress in the understanding of the pathology of RA and the control of its symptoms with the development of anti-cytokine agents, progress in the understanding of its etiology has been slow. The association of RA disease and RA exacerbations with bacterial and viral infections has been suspected, but conclusive evidence for that has been lacking. Here, we have reconsidered that hypothesis.

By reviewing recent literature in the field, it becomes apparent that the prolonged up-regulation of inflammatory cytokines (such as TNF) and tissue destructive enzymes is likely to be due to the inappropriate activation of the NF-κB pathway, a major response of cells in response to infections or cell damage that leads to the development of inflammation and the pathogen clearance. Indeed, overexpression of IκBα or kinase-defective IKK2 blocks the expression of most pro-inflammatory cytokines or MMPs but not anti-inflammatory cytokines studied, although there are some differences in the extend this is achieved by each NF-κB inhibitor. These findings suggest that targeting the NF-κB pathway and the mechanism triggering NF-κB activation is of therapeutic potential.

Potent activations of NF-κB and TNF production are the TLRs, a phylogenetically conserved family of receptors recently proposed to act as major receptors recognizing invading pathogens. Although it is early days and reagents are still lacking, it appears that some but not all TLRs are expressed in RA and thus may be involved in promoting inflammatory cytokine and MMP production in RA joints. As TLRs recognize pathogens as well as various 'self' molecules from damaged tissue, this may provide not only an explanation between the link of RA and infections, but also an explanation of how inflammation may be perpetuated in the absence of pathogens, through the triggering of TLRs by damaged 'self' tissue. Thus, studying the TLR-NF-κB pathway and developing the most effective ways of specifically blocking it, is indeed worth pursuing

With respect to drug development, the TLR-NF-κB pathway offers ample opportunities for therapeutic intervention. Kinases involved in the phosphorylation of IκBα such as IKK2 and TAK1, and the processing of p100 such as IKK1 and NIK, as well as enzymes involved in IκBα degradation such as ubiquitinases, can be targeted by small molecule inhibitors. In addition, TLRs can be targeted by humanized monoclonal antibodies or even soluble TLR receptors. It is even likely that small molecules can be used to target TLRs too, as TLR7 and TLR8 recognize with high affinity imidazoquinolines, synthetic compounds used as anti-viral compounds in the clinic. No matter how the TLR-NF-κB pathway is targeted for therapeutic success, it will have to match or exceed in efficacy and safety the anti-TNF agents. This may not be an easy task to achieve, especially on the safety front.

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