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Update on Targets of Biologic Therapies for Rheumatoid Arthritis

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Abstract

With the advent of biological therapies, considerable progress has been made in the treatment of rheumatoid arthritis (RA). These revolutionary therapies owe their origin to the role that cytokines play in the pathophysiology of the disease and are best exemplified by the wide use of tumor necrosis factor (TNF) blockade. The identification of additional pro-inflammatory factors and an understanding of their effector function now offer major possibilities for the generation of additional novel biological therapeutics to address unmet clinical needs. Such interventions will ideally fulfill several of the following criteria: control of inflammation, modulation of underlying immune dysfunction by promoting the reestablishment of immune tolerance, protection of targeted tissues such as bone and cartilage, and preservation of host immune capability to avoid profound immune suppression and amelioration of co-morbidity associated with underlying RA. The identification and characterization of the intracellular signaling pathways, in particular, the mitogen-activated protein kinase pathway, the nuclear factor-κB pathway and the cross-talk between these pathways offer several potential therapeutic opportunities. This review will provide an update on cytokine activities and signal transduction pathways that represent, in our opinion, optimal utility as future therapeutic targets.

Keywords

Rheumatoid arthritis; cytokines; signal transduction; biologics

INTRODUCTION

Imbalance in the pro- and anti-inflammatory cytokines leads to the development of chronic synovial inflammation and joint destruction in rheumatoid arthritis (RA). Although the precise cause of RA remains unknown, the importance of inflammatory cytokines in the pathogenesis of RA has been documented in a number of studies [1]. Inflammatory cytokines, that include, interleukin-1 (IL-1), IL-6, IL-17, tumor necrosis factor-α (TNF-α) and the downstream inflammatory mediators produced by activated cells in the arthritic joints are an essential component of the milieu that drives cartilage and bone destruction. The effect of cytokine neutralization as a driving force for inhibition of inflammation was first exemplified by the use of TNF-α antibodies for the treatment of RA [2]. This approach has been subsequently extended to include IL-1 and IL-1 receptor (IL-1R) targeting. Now these agents are also used to treat a variety of autoimmune and chronic inflammatory disorders [3].

In RA, TNF blockade in the clinic is clearly associated with reduced synovial joint inflammation as well as cartilage and subchondral bone damage as well as with improvement

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in quality of life measures. A variety of *in vitro* and *in vivo* models used in the elucidation of TNF-dependent networks in RA synovitis are now being employed to validate the therapeutic potential of other inflammatory cytokines such as IL-6 and IL-17 that have also been implicated in the disease pathogenesis. The success of anti-TNF biologics on one hand, and their short comings on the other, (including lack of efficacy in a significant proportion of patients, loss of efficacy over time, associated risk of infections and high cost) triggered an enormous research effort to identify additional novel potential targets. These targets include components of intercellular communication pathways (especially other members of the pro-inflammatory cytokine network), cell surface receptor systems and components of intracellular signaling pathways such as protein kinases [3]. With the rapid growth in the number of potential new RA targets, this review we will focus on those targets that in our opinion offer the most potential for the treatment of RA. The exclusion from this review of other molecules or pathways involved in RA should in no way be construed as unimportant or irrelevant to RA pathophysiology.

INTERCELLULAR TARGETS

Cytokines are small proteins produced by the cells of the immune system which regulate the inflammatory response. Cytokines and their receptors are either expressed on the cell surface or are secreted into the extracellular environment. This means that they can be inhibited by monoclonal antibodies and/or Ig-fusion proteins that target cytokines soluble receptors. Because of the great degree of pleitropy exhibited by cytokines, the identification of valid targets depends on a thorough understanding of the biology of disease. TNF-α, IL-1 and IL-4 constitute part of a complex effector network which also involves other interesting molecules, particularly, IL-6, IL-15, IL-17 and the receptor activator of nuclear factor-κB ligand (RANKL). This effector network represents the major driving force that enhances the synovial inflammation as well as playing a role in cartilage and bone destruction in an arthritic joint [1]. The concept of cytokine networks in RA that contribute to synovial inflammation and autoimmunity are therefore currently evolving to embrace strategic cytokine inhibition early in disease, with the objective of resolving articular inflammation to prevent tissue destruction and resultant functional decline. In this regard development of anti-TNF-α biologics serves as a useful model for future development of therapies to treat RA and other inflammatory diseases.

CYTOKINES

Interleukin-6 (IL-6)

IL-6 is a 26 kDa pleiotropic cytokine that is involved in numerous biologic processes. IL-6 is expressed by monocytes, T- and B-lymphocytes and fibroblasts and is detectable at elevated levels in RA and psoriatic arthritis patients serum and synovial tissue, together with its receptor components IL-6R (soluble and membrane forms) and gp130 [4,5]. Interaction of IL-6 with the intact IL-6R activates the STAT3-dependent signaling pathway, which induces expression of IL-6 target genes in the nucleus [6,7]. IL-6 can cause the acute phase response by inducing C-reactive protein (CRP) synthesis which, in turn, induces systemic osteoclastic-mediated bone resorption throughout the skeletal system. CRP levels correlate with diseases activity but this quantitative relationship would not predict disease severity [8,9]. This strongly suggests a central role of IL-6 in RA pathogenesis and indicates that blockade of IL-6 may yield beneficial effects. This is supported by studies showing that IL-6 knockout mice were resistant to collagen induced arthritis (CIA) and showed reduced levels of serum TNF-α [3]. IL-6 blockade is therefore an attractive therapy for the treatment of RA.

IL-6 signals through the IL-6R which is a heterodimeric receptor consisting of the IL-6Rα and gp130 subunits. Thus, an understanding of the molecular dynamics involved in the IL-6/IL-6R interaction has led to the development of a soluble IL-6R fusion protein (IL-6RFP), which is

a potent IL-6 inhibitor *in vitro*. Soluble gp130 has an antagonistic effect upon IL-6 whilst soluble IL-6Rα alone acts agonistically [6]. A fusion protein of the two subunits (IL-6RFP), however, exerts strong inhibition of IL-6 activity [10]. The strategy of blocking IL-6 activity in RA patients with the humanized anti-IL-6-receptor antibody tocilizumab was investigated [11]. The published data from phase I and II clinical trials showed that for RA patients who received tocilizumab 78% (versus 11% in the placebo group) reported clinical improvement based on the American College of Rheumatology (ACR) 20 criteria (ACR20), 40% (versus 2% in the placebo group) showed improvements as judged by ACR50 and 16% (versus 0% in the placebo group) by the ACR70 criteria [12]. In addition, treatment with tocilizumab resulted in decreased levels of serum markers of bone resorption and increased levels of serum markers of bone synthesis while also demonstrating a good safety and tolerability profile. Dissecting the mechanisms of cytokine signaling pathways together with the stoichiometry, specificity and stability of such cytokine traps will allow expansion of the IL-6 inhibitor repertoire and the development of more efficacious drugs for the treatment of RA [13].

Interleukin-15 (IL-15)

IL-15 is a cytokine of the innate immune system that is expressed primarily by macrophages but also by fibroblast-like synoviocytes (FLS) and endothelial cells [14]. IL-15 shares a number of biologic activities with other cytokines including the stimulation and proliferation of T-cells. IL-15 was found at elevated levels in the serum and synovial fluid of RA patients relative to osteoarthritis patients [15,16]. In addition, IL-15 produced by synoviocytes in RA joints is considered to be a potent inducer of IL-17 [17] which has been implicated in the RA pathogenesis of disease in a number of studies over recent years [18]. Importantly, administration of a soluble IL-15 receptor (IL-15R) suppressed murine CIA and showed delayed development of collagen specific autoantibodies and reduced cytokine release [19]. More recently, an IL-15-Fcγ2a fusion protein prevented the development of CIA as well as blocking disease progression in this established animal disease model of RA. The fusion protein was a construct containing a point mutation in the IL-15 molecule at glutamine^{169} (codon CAG), and at glutamine^{176} (codon CAA), which were both mutated to aspartate (codon GAG) within the C-terminus of IL-15 [20]. This mutated IL-15 was then fused to the constant region of a murine IgG2a. The IL-15 fusion protein could bind to IL-15R without inducing downstream signaling, thereby blocking IL-15 activity [21]. Daily intraperitoneal injections of IL-15-Fcγ2a in CIA showed reduced clinical score, lower incidence of disease and reduced cartilage erosion relative to an IgG2a control. Further, histological analysis showed that administration of IL-15-Fcγ2a significantly reduced CD4 and CD8 T-cell infiltration into the diseased joints and in a similar fashion reduced levels of joint IL-1β and TNF-α. The Fc portion of the fusion protein was shown to bind to the IL-15R and this ‘tag’ showed that the IL-15R expressing cells included T-cells and macrophages, which are then cleared by the innate immune system. This may account for the low number of joint infiltrating cells as well as for lower levels of inflammatory cytokines. Blockade of IL-15 also resulted in reduced destruction of cartilage and bone in this animal model of RA [21,22]. Studies using the human monoclonal IgG1K anti-IL-15 antibody (AMG 714) have also been shown to induce a beneficial response in RA patients [23]. Thus, in an open-label clinical trial the HUMax IL-15 antibody which targets the epitope of IL-15 that binds to the γc sub-unit of IL-15R, showed substantial improvements in RA disease activity with 63% of the patients showing clinical improvement as measured by the ACR20, whereas 38% met the ACR50 improvement criteria and 25% met the ACR70 improvement criteria, respectively [24]. If these results are reproducible in a placebo-controlled trial, they would be comparable to the effects of TNF-α inhibitors.

Interleukin-17 (IL-17)

IL-17 is produced by a subpopulation of helper T-cells termed Th17 cells which play an important role in host defense mechanisms and in autoimmunity *via* the induction of pro-

inflammatory gene expression, including the expression of TNF-α and IL-6. Thus, IL-17 has been postulated to provide a link between synovial inflammation and cartilage and bone destruction in RA [25-27]. The molecular mechanism by which IL-17 regulates the expression of target genes is not well understood [28] but its role in sustaining inflammation is receiving greater attention [29]. Production of this cytokine is not only induced by IL-15 but also by IL-23, which is produced by activated dendritic cells, macrophages and RA synovial fibroblasts [30]. Thus, IL-17 knock-out mice failed to develop CIA [31], and molecules that blocked IL-17, such as IL-17-specific monoclonal antibodies have also proved effective in ameliorating disease in RA animal models [32-34]. Of note, a recent study using TNF-α deficient mice [35] demonstrated that TNF-α was required for IL-17-induced joint pathology under naïve conditions *in vivo*. The over-expression of IL-17, however, aggravated arthritis in the K/BxN serum transfer model to a similar degree as in TNF-α deficient mice and their wild-type counterparts, indicating that the TNF dependency of IL-17-induced pathology is lost under arthritic conditions [35]. These studies have led to the proposition that IL-17 could be a novel target for treating RA patients that fail to respond to conventional treatments, such as anti-TNF-α biologics.

A novel approach was used recently to block IL-17 by overcoming natural tolerance to IL-17 through vaccinating mice with an IL-17-conjugated virus-like particle. This immunization strategy resulted in high levels of anti-IL-17 antibodies and led to a lower incidence of disease and disease severity in both CIA and the experimental autoimmune encephalomyelitis model of autoimmunity [36].

The data regarding the role of IL-17 in human RA are less clear. IL-17 is expressed in the synovium and synovial fluid of patients with RA contains IL-17, but at low levels [37,38]. What is also not clear is whether inhibition of IL-17 activity *in vivo* will be safe, or whether long-term inhibition (as achieved by vaccination) will increase the risk of infection and/or cancer [39]. The success of this approach in animal models of autoimmune disease, however, clearly warrants further study.

THE B-LYMPHOCYTE STIMULATOR (BLyS) FAMILY OF LIGANDS

An important feature of autoimmune diseases, such as RA is the presence of autoantibodies. This suggests that targeting B-cells may be of value in treating RA. B lymphocyte stimulator protein (BLyS®) (Human Genome Sciences, Rockville, MD) is a recently identified 285-amino acid cytokine that is over expressed in RA [40,41]. Other names given to this protein are TALL-1, BAFF, THANK, TNFSF20 (subsequently renamed TNFSF13B), and zTNF4. APRIL (also called TNFSF13A) is a 250-amino acid member of the TNF ligand superfamily that shares substantial homology with BLyS and binds to 2 of the 3 BLyS protein receptors (BCMA and TACI) [42,43]. APRIL and BLyS are produced by RA synovium and circulating levels of the BLyS protein have been measured in patients with a variety of rheumatic diseases, including systemic lupus erythematosus (SLE), RA and Sjögren's syndrome, and BLys levels have been found to be elevated in a substantial proportion of such patients [43,44].

B-CELL DEPLETION

Rituximab® (Genentech, San Francisco, CA) is a chimeric monoclonal antibody that interacts with the CD20 molecule and is a specific marker for mature B-cells but not on either B precursor cells or plasma cells. When rituximab binds to CD20, the B cell undergoes cytolysis *via* antibody-dependent cytotoxicity related to the constant region of immunoglobulins and *via* complement-dependent cytotoxicity. In an RA clinical trial, B-cell depletion with rituximab showed clinical efficacy [45]. In a similar fashion, the effect of using a decoy receptor transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor: Fc (TACI.FC) was investigated using a human synovium-SCID mouse chimera. The results

of these studies [46,47] suggested that BLyS and APRIL regulated B-cell as well as T-cell function in this RA model. In other studies, blockade of BLyS also prevented disease progression and bone erosion in mice with CIA [48,49]. The use of a BLyS antagonist in a phase II clinical trial involving patients with RA and SLE has shown efficacy and a good safety profile [50]. Thus, these data suggest that the BLyS/APRIL axis may represent an attractive target to treat RA and other inflammatory diseases.

INTRACELLULAR TARGETS

Signal transduction pathways provide an intracellular mechanism by which cells respond and adapt to environmental stress. Generally, ligation of specific cell membrane receptors that sense the extracellular milieu activates a signaling cascade that ultimately alters gene transcription or protein expression [50]. Pro-inflammatory stimuli engage these pathways to direct a response that can either be physiologic, as with exposure to pathogens, or harmful to the host, as in chronic diseases such as RA. Studies dissecting the signal transduction mechanisms have not only led to a greater understanding of the pathogenesis of RA but also have identified potential therapeutic targets [51,52].

Mitogen Activated Protein Kinases

The mitogen-activated protein kinases (MAPK) are members of a highly conserved serine/threonine protein kinase family that regulate gene expression, cell survival, proliferation, cytokine expression, and metalloproteinase production [53]. These kinases phosphorylate serine, threonine, or tyrosine residues on intracellular proteins and are divided into three major classes in mammals, the c-Jun N-terminal kinase (JNK), extracellular signal-related kinases (ERK), and p38 MAPKs [54]. Activation of MAPKs involves a cascade of events that is highly conserved from yeast to mammals [54]. Thus, MAPK activation requires the activation of the upstream MAPK kinase kinase (MAPKKK or MKKK) which phosphorylates a dual specificity MAPKK (or MKK) which then phosphorylates the threonine and tyrosine residues of a conserved T-X-Y motif of the downstream target MAPK [55]. The conserved X residue is different in each class of MAPKs, with glycine in p38-MAPK (T-G-Y), proline in JNK (T-P-Y) and glutamic acid (T-E-Y) in ERK. In turn, activated MAPKs phosphorylate a specific repertoire of cytoplasmic and nuclear proteins that also include transcription factors. Of note, all three classes of MAPKs have been shown to be expressed and activated in the synovial tissue of RA patients [56]. This provided a strong evidence for a role of MAPKs in the pathogenesis of RA.

p38 MAPK

The p38 MAPK pathway has attracted considerable attention as a potential therapeutic target for RA because of its ability to regulate the production of proinflammatory cytokines *in vitro* as well as *in vivo* [57]. Four isoforms of p38-MAPK (α, β, γ, and δ) have been identified, and p38α appears to be a critical factor for cytokine expression and regulation in macrophages and other cell types in the joint [58]. One of its downstream substrates, MAPKAP-2, is another kinase that can phosphorylate the transcription factor ATF-2 which mediates some of the regulatory effects of p38 MAPK on cytokine gene expression [59]. Previous studies showed that p38 MAPK is expressed and activated in the synovium of RA patients and that it is readily activated by a variety of cytokines in cultured FLS derived from RA synovial tissue [56]. Its function is regulated by at least two upstream kinases, MKK3 and MKK6, both of which are also activated in RA synovium and form stable complexes with p38 MAPK in stimulated FLS by IL-1β [58,60]. Abundant preclinical data also suggested that p38 MAPK participated in the pathogenesis of inflammatory arthritis [61]. For example, inhibition of p38 MAPK by p38 MAPK inhibitor such as RWJ-67657 blocked the expression of cyclooxygenase-2 (COX-2), TNF-α, IL-1 and IL-8 in cultured macrophages [62-66]. More recently, the potent p38 MAPK

inhibitor FR167653 prevented CIA from developing in rats and also suppressed joint destruction when treatment was initiated after the onset of arthritis [67]. FR167653 also decreased serum TNF-α and IL-1 as well as synovial fibroblast-induced osteoclast differentiation [68,69]. Inhibition of p38 MAPK activity has also been shown to suppress the IL-1/TNF-α-mediated induction of IL-6 by osteoblasts and chondrocytes [70,71]. Other studies have shown that p38 MAPK inhibitors were effective when prophylactically employed in reducing clinical severity, paw swelling, inflammation, cartilage breakdown and bone erosion in CIA and other animal models of arthritis [72]. As the p38 MAPK inhibitors such as RWJ-67657, BIBB-796, VX-745, VX-702, SCIO-469 etc. were also effective when tested in a therapeutic protocol, these data suggest that p38 MAPK inhibitors may have therapeutic potential in RA patients with established disease. In agreement with these and other studies, a large number of p38 MAPK inhibitors have been developed and tested in clinical trials [73]. However, the efficacy of these compounds appeared to be limited and also exhibited serious side effects such as hepatotoxicity, gastrointestinal toxicity, dental pain and unusual inflammatory conditions of the central nervous system [74]. The observed adverse events may be related to inhibition of key roles played by p38 MAPKs in several vital cellular processes. There are four known isoforms of p38 MAPK and among these p38α and p38γ are expressed in RA synovial tissue [59]. Therefore inhibitors that specifically target inflammation related p38 MAPK isoforms may offer a more potent and safer alternative.

c-Jun N-Terminal Kinase

Although the p38 MAPK signaling pathway is generally considered pivotal for pro-inflammatory cytokine production, JNK is one of the primary MAPKs required for MMP expression and joint destruction in inflammatory arthritis [75]. This is supported by the studies showing that the broad spectrum JNK inhibitor SP600125 suppressed the IL-1 induced MMP-1 expression *in vitro* as well as bone erosion in a mouse model of arthritis *in vivo* [75]. Since JNK-1 is required for the development of bone resorption activity by osteoclasts, the inhibition of bone resorption may be related to JNK-1 inhibition by SP600125 *in vivo*.

The role of the JNK2 isoform, which is the most abundant one expressed in RA FLS, was evaluated in the passive CIA model using JNK2 knockout mice [76]. The absence of JNK2 modestly decreased cartilage destruction but had a minimal effect on clinical signs of inflammation. These data indicate that inhibition of both JNK1 and JNK2 may be required for effectively blocking inflammation and bone resorption in RA. As with p38 MAPK, upstream kinases modulate JNK function in cultured FLS [51]. The primary regulators of JNK in RA appear to be MKK4 and MKK7, which form a stable complex with JNK to facilitate signal transduction [77]. This complex also includes the MAPK kinase kinase, MEKK2 which represents a potent mechanism to amplify cytokine signaling through JNK [51]. Although MEKK2 activates both MKK-4 and MKK7, only MKK7 was found to be required for the activation of AP-1 [78]. AP-1 is a critical transcription factor regulating the expression of MMP-13 [79]. This finding suggested that specific inhibition of MKK7 may be of therapeutic value for suppressing inflammation and bone resorption in RA. Thus, osteoclast differentiation induced by nuclear factor for activated T-cells (NFAT) was abrogated by dominant negative c-Jun (DN-c-Jun) overexpression. This suggested that NF activated T-cell c1 (NFATc1) acts coordinately to regulate osteoclast differentiation [80]. Furthermore, NFATc1 synergistically activated the osteoclast-associated receptor (OSCAR) construct and together with microthalmia (MITF) was found to be critical for osteoclast differentiation [81]. The pivotal role for c-Jun in osteoclast formation is in keeping with the fact that anti-bone resorptive effects of estrogen are substantially mediated by c-Jun repression [80]. RANKL expression is basically a reflection of NFAT partnering with Jun. Thus, RANKL → TRAF6 → MAPK kinase 7 (MKK7) → JNK1 → Jun → NFAT signaling is likely pivotal to the pathogenesis of post menopausal osteoporosis, and inhibition of any of the components could theoretically arrest

or accelerate bone resorption [80]. The therapeutic challenge is how to specifically target these intracellular signaling molecules to osteoclasts.

Extracellular Signal Related Kinases

Like the other MAPKs, ERK is expressed and activated in RA synovium [58]. It appears to participate in the proinflammatory effects of macrophage migration inhibitory factor (MIF) on RA FLS. For instance, MIF induces phosphorylation of ERK, and ERK inhibition blocks the effect of MIF on RA FLS proliferation [82]. Therefore, ERK may play a role in signal transduction mediated by MIF in RA FLS proliferation. Although ERK inhibitors have been available for more than a decade, only limited information is available regarding their therapeutic potential in RA. There are no reports that small molecule ERK inhibitors such as PD184352, PD0325901, etc. reduce inflammation or joint destruction in animal models of arthritis. However, one of these inhibitors, PD98059, was shown to reduce the nociceptive response in arthritic rats indicating that ERK is potentially important in transmitting pain signals in inflammatory arthritis. This compound is a selective noncompetitive inhibitor of ERK kinase (MEK)-1/2 [83,84] by the upstream (MEK)-1/2 enzyme [85]. Clearly more studies are needed before arriving at any conclusions regarding the suitability of ERK inhibitors in RA.

NUCLEAR FACTOR-κB

NF-κB is a transcription factor that participates in immunity and inflammation. NF-κB proteins include RelA (p65), RelB, c-Rel, p50/p105, and p52/p100, which normally bind to IκB in the cytoplasm. Stress, inflammatory cytokines, and microbial products result in IkB phosphorylation and degradation, allowing NF-κB to translocate to the nucleus and regulate gene transcription. IκB kinases (IKK) are the primary enzymes that phosphorylate IκB [86]. NF-κB is expressed in rheumatoid synovium, and the p50 and p65 subunits have been localized to cell nuclei in the synovial intimal lining tissue [87]. Previous studies implicated IKK2 as a central pathway for NF-κB activation in cytokine-stimulated FLS [88]. Thus, blockade of IKK2 *in vitro* with a dominant negative IKK2 adenoviral construct inhibited IL-6, IL-8, and intercellular adhesion molecule 1 (ICAM-1) induction after cytokine stimulation with IL-1 or TNF-α [89]. Additionally, intra-articular gene therapy with the same construct or with decoy oligonucleotides effectively suppressed adjuvant arthritis [90]. Studies using adenoviral dominant negative IKK1, dominant negative IKK2, or IκB constructs in macrophages suggested that IKK2 was not required for lipopolysaccharide (LPS)-induced production of cytokines but that it was essential for CD40 ligand, TNF-α, and IL-1 mediated NF-κB activation [51]. TNF-α production by RA synovial membrane cells does not appear to require IKK2, although IL-1, IL-6, and MMP expression is dependent on this pathway [91]. Additional proof of concept regarding the role of IKK2 comes from studies using small molecule inhibitors of IκB. One of these, SC-514, inhibited IL-1 induced IκB degradation and NF-κB activation in cultured FLS [92]. A selective, orally bioavailable, IKK inhibitor, BMS-345541, was highly effective in modifying murine CIA when both clinical and histologic endpoints were assessed [93]. Another IKK2 inhibitor, SPC839, prevented synovial inflammation and bone destruction in rat adjuvant arthritis model [52]. In addition to IKK1 and IKK2, other IKK-related kinases may also participate in synovial inflammation. Two of these IKK-related kinases known as TANK-binding kinase-1 (TBK-1) and inducible IKK (IKK-i) were identified as kinases that could phosphorylate IκB and potentially activate NF-κB [51]. Additional studies have shown that these proteins play a more important role in host defense through the activation of interferon regulatory factor (IRF)-3 and IRF-7 and subsequent induction of interferon (IFN)-β gene expression after TLR3 ligation [94]. Thus, studies in IKKi knockout murine embryonic fibroblasts demonstrated that IKKi was also a link between NF-κB and CCAAT/enhancer binding protein (C/EBP) [51]. Cells deficient in IKKi normally activate the traditional NF-κB

pathway with induction of C/EBP β and δ mRNA, but fail to induce C/EBP δ-specific DNA binding [93]. IKKi deficiency also resulted in a marked reduction of LPS-induced mRNA expression of TNF-α, IL-1, IL-6, IP-10, RANTES, and COX-2 [95]. The potential relevance of these findings to RA is related to the fact that IKKi is constitutively expressed by RA FLS and also by inflamed synovium and IKKi mRNA levels are further increased by stimulation of RA FLS with IL-1 or TNF-α [96].

A variety of additional biological activities related to NF-κB have been documented in RA. For instance, the novel Ets transcription factor ESE-1 is expressed in RA synovial lining cells [97]. This NF-κB-dependent protein is induced by IL-1 and TNF-α in FLS, monocytes, chondrocytes, and osteoblasts and might also regulate many NF-κB effects in the inflamed joint [98]. NF-κB is also involved in the response to ischemia-reperfusion, and an NF-κB inhibitor blocked the induction of ICAM-1 expression by FLS after hypoxia and reoxygenation [99]. Of note, Bcl-3, a member of the IκB family, was shown *via* microarray analysis and reverse transcription polymerase chain reaction to be an IL-1 responsive gene in a human chondrosarcoma cell line and to be required for induction of MMP-1 expression [100]. However, because NF-κB is ubiquitously expressed and is required for cellular processes associated with both cell survival and cell death, it is unlikely that molecules designed to inhibit the NF-κB pathway are likely to gain a favor as therapeutic targets in RA.

INTERACTIONS BETWEEN NF-κB AND MAPK PATHWAYS

A growing body of evidence suggests that MAPK and NF-κB signaling cascades converge to control inflammation-associated gene expression. Several studies have demonstrated the requirement for both MAP kinase activation and NF-κB activation for the expression of target genes induced by inflammatory stimuli. For example, IL-1 induction of MMP-1 gene expression requires both NF-κB and MAP kinase (primarily ERK) activity, whereas induction of MMP-13 requires p38 MAPK, JNK and NF-κB activity [101,102]. Additionally, IL-17-induced MMP-9 expression in human monocytes and macrophages is blocked by inhibitors of p38 MAPK, ERK and NF-κB [103]. There is also direct evidence for cross-talk between MAP kinase and NF-κB pathways. Thus, MEK kinase 1(MEKK1), an upstream kinase in the JNK pathway, has been shown to phosphorylate and activate both IKKα and IKKβ [104]. Furthermore, dominant negative mutants of MEKK1 inhibit IKK activation [105] and dominant negative IKKβ inhibits MEKK1-stimulated reporter-gene activity [106]. The cross-talk between MAPK pathways and NF-κB, a major mediator of inflammatory conditions, suggests that selective inhibition of MAP kinases or NF-κB signaling pathways may represent future therapeutic targets for the treatment of RA.

CONCLUSIONS

Recent advances in our understanding of cellular and molecular mechanisms in RA and the blockade of TNF-α in RA highlights the feasibility of antagonizing cytokine signaling. Despite these developments, there remains a considerable unmet clinical need in this field. A number of preclinical development programs are ongoing to develop a variety of biologic therapies directed at molecules that are central to immune regulation and extracellu lar matrix destruction in RA. Considerable evidence has been presented to indicate that proinflammatory cytokine networks involving IL-6, IL-15, IL-17 and RANKL, have emerged as suitable therapeutic targets for effectively suppressing immune deregulation as well as the inflammation that are critical regulatory components of RA pathogenesis. In addition, multiple signal transduction pathways and transcription factors have been implicated in RA, and many studies have confirmed their importance *in vitro* as well as in animal models of arthritis. It is clear that MAPKs play a critical role in transducing intracellular signals associated with inflammation and joint destruction in RA. However, there are multiple isoforms of MAPKs and these regulate

a number of essential cellular functions indicating that ablation of p38 MAPK, JNK or ERK activity will likely have serious adverse events and may even be fatal. Therefore, a careful dissection of a targeted pathway is needed so that novel therapeutic interventions designed to specifically block inflammatory signaling may be developed. Further, because these signaling pathways evolved for host defense, questions about the risks of signal transduction blockade using small molecule inhibitors will need to be carefully addressed. Additionally the specificity of these small molecule inhibitors in terms of their interaction with an individual kinase or a transcription factor remains a formidable hurdle to overcome. In the coming years developments in this area are going to be exciting and will influence the therapeutic approaches for the effective suppression of inflammation and joint damage in RA.

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