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REVIEW

Negative regulation of NF-κB and its involvement in rheumatoid arthritis

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Abstract

The transcription factor NF-κB plays crucial roles in the regulation of inflammation and immune responses, and inappropriate NF-κB activity has been linked with many autoimmune and inflammatory diseases, including rheumatoid arthritis. Cells employ a multilayered control system to keep NF-κB signalling in check, including a repertoire of negative feedback regulators ensuring termination of NF-κB responses. Here we will review various negative regulatory mechanisms that have evolved to control NF-κB signalling and which have been implicated in the pathogenesis of rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis, affecting up to 1% of the adult population. RA is characterised by a symmetrical polyarthritis in which chronic inflammation of joints is associated with a progressive destruction of cartilage and bone, leading to functional decline and disability. Infiltration of cells of the innate and adaptive immune system into the joint space drives the local production of proinflammatory T-helper type 1 and T-helper type 17 cytokines, chemokines, and matrix metalloproteinases by infiltrating monocytes and synovial cells. Proliferation of synovial fibroblasts leads to the formation of pannus tissue, which invades and degrades articular cartilage and subchondral bone.

The aetiology of RA is still not understood, but it is well accepted that activation of NF-κB-dependent gene expression plays a key role in the development of RA and many other autoimmune diseases. NF-κB represents a family of structurally related and evolutionarily conserved proteins (p100 or NF-κB2, p105 or NF-κB1, p65 or RelA, RelB, c-Rel) that function as homodimers or heterodimers [1], and that regulate the expression of a large number of genes – such as TNF, IL-1, IL-6, cyclooxygenase-2, chemokines, inducible nitric oxide synthase, and matrix metalloproteinases – that are involved in RA. In addition, TNF and IL-1 are themselves very potent activators of NF-κB (reviewed in [2,3]).

NF-κB activation can be detected in cultured synovial fibroblasts and synovial tissue from RA patients, and animal models of inflammatory arthritis also demonstrate the active role of NF-κB in the development and progression of RA (reviewed in [4]). The time course of NF-κB activation appears to precede the onset of disease, and blockade of NF-κB by different means decreases disease severity [5,6].

Next to its role in proinflammatory gene expression, NF-κB is also essential for osteoclastogenesis, mainly by mediating the effects of receptor activator of NF-κB ligand (RANKL). Defects in the regulation of osteoclastogenesis are the major cause of bone erosion in osteolytic diseases such as RA [7].

Finally, recent discoveries revealing a genetic association with several genes relevant to NF-κB signalling, including *CD40*, *TRAF1*, *TNFAIP3*, and *c-REL*, further highlight the importance of NF-κB activation in RA pathogenesis [8].

Pathological triggers of NF-κB signalling in RA

Since NF-κB is central to the process of inflammation in RA, much research deals with the identification of the molecular triggers that activate NF-κB in RA. It is well accepted that proinflammatory cytokines such as TNF and IL-1 play an important role, and administration of TNF antagonists is an effective treatment for severe RA (reviewed in [9]). TNF and IL-1 are both very potent activators of NF-κB and it can be expected that NF-κB activation by these cytokines mediates most of their proinflammatory activities in RA (reviewed in [3]).

NF-κB activation by receptor activator of NF-κB (RANK), a TNF receptor family member, is important for osteoclastogenesis, and defects in proper RANK–NF-κB

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signalling are likely to be involved in RA pathology and other diseases associated with bone loss [7]. CD40 is another TNF receptor family member that is functionally expressed on a variety of cell types, including smooth muscle fibroblasts from normal and RA patients and RA synovial cells, B cells, macrophages, and dendritic cells, and can be upregulated by proinflammatory cytokines including TNF [10]. Binding of the CD40 ligand (CD154), which is transiently expressed on the surface of activated $CD4^+$ T cells, triggers NF-κB activation resulting in fibroblast proliferation and secretion of proinflammatory cytokines and chemokines, which contributes to joint destruction. However, studies with antagonistic anti-CD40 or anti-CD154 antibodies led to the conclusion that CD40 signals may be important at the initial stages of arthritis induction, but are not required once disease is established and pathogenic antibodies are already present [11,12]. Enhanced expression of the TNF receptor family member B-cell activating factor (BAFF), allowing the survival of autoantibody-producing B lymphocytes, is also characteristic for RA, and antagonists of BAFF have been developed to counter RA [13]. Finally, lymphotoxin β receptor signalling has been implicated in tertiary lymphoid organ formation at sites of chronic inflammation including RA [13].

Toll-like receptors (TLRs) have been implicated in a variety of autoimmune diseases and are potential candidates for inducing NF-κB-dependent inflammation in RA. In addition to microbial ligands, an increasing number of endogenous ligands – a group of proteins derived from host tissues and cells – have been reported as candidate activators of TLRs inducing so-called sterile inflammation (reviewed in [14,15]). TLRs are expressed in RA synovial tissues and various endogenous ligands are present within the inflamed joints of RA patients. Moreover, animal models using TLR knockout mice or strategies to block TLR signalling clearly identify TLR-dependent inflammation as being important in the pathogenesis of the disease.

High mobility group box chromosomal protein 1 (HMGB1), a highly conserved chromatin component that can be actively secreted by macrophages or passively released by necrotic cells, is one of the most putative endogenous TLR4 ligands involved in RA pathology. HMGB1 is increased in RA synovial tissue and HMGB1 neutralising antibodies or the antagonistic BoxA domain of HMGB1 protect against collagen-induced arthritis in mice [16]. Myeloid-related protein 8 and myeloid-related protein 14, damage-associated molecular pattern molecules belonging to the S100 family of calcium-binding proteins, are also abundantly present in RA synovial fluid, and have been suggested to be involved in TLR4-induced chronic inflammation in RA [17,18]. Other endogenous TLR ligands that may be involved in RA pathology are extracellular matrix components such as fibrinogen, fibronectin, biglycan, tenascin C, and hyaluronic acid fragments (reviewed in [14,15]). Together, these studies suggest that several TLR ligands in the inflamed joint tissue may contribute to NF-κB activation and inflammatory gene expression in RA.

Basic principles of NF-κB signalling

NF-κB proteins are sequestered in the cytoplasm as latent complexes by inhibitory proteins referred to as inhibitor of NF-κB (IκB) proteins, which prevent NF-κB nuclear translocation and DNA binding [19]. Whereas the majority of IκBs (IκBα, IκBβ, IκBε, p105 (also known as NF-κB1), p100 (also known as NF-κB2)) serve as inhibitors of NF-κB, IκBξ and Bcl-3 instead potentiate NF-κB transactivation in the nucleus. p100 and p105 are precursors of the p52 and p50 NF-κB subunits, respectively. There are two unique NF-κB signalling pathways, termed canonical (or classical) and noncanonical (or alternative) NF-κB pathways.

The canonical NF-κB pathway plays a major role in innate and adaptive immunity, and is triggered by many stimuli including proinflammatory cytokines (for example, TNF, IL-1), antigens, RANKL, and TLR ligands. NF-κB signalling initiated by different receptors requires the formation of proximal protein–protein interactions that are often receptor specific, but ultimately converge in the activation of the IκB kinase (IKK) complex, which mediates phosphorylation of the inhibitory IκB protein leading to its K48-polyubiquitination and degradation by the proteasome [20]. The IKK complex is comprised of the two catalytic subunits IKK1 and IKK2 (also known as IKKα and IKKβ) and the regulatory subunit NF-κB essential modulator (NEMO – also known as IKKγ) (Figure 1). Gene targeting experiments showed that IKK2 and NEMO, but not IKK1, are required for canonical NF-κB activation [21].

One of the best studied NF-κB signalling pathways is the TNF pathway. TNF stimulation results in the recruitment of TNF receptor-1-associated death domain (TRADD) protein and of receptor interacting protein 1 (RIP1), which function as adaptor proteins for the E3 ubiquitin ligases TNF receptor-associated factor (TRAF) 2 and TRAF5, which in turn bind the E3 ubiquitin ligases cellular inhibitor of apoptosis (cIAP) 1 and cIAP2 (Figure 1). On TNF stimulation, TNF-receptor bound RIP1 is rapidly modified by K63-linked polyubiquitin chains. TRAF2/5 and cIAP1/2 are good candidates for RIP1 ubiquitination, but the specific role of each is still unclear. The polyubiquitin chains on RIP1 are believed to create a scaffold to recruit the IKK and TAK1 complex via the ubiquitin-binding proteins NEMO and TAB1/2, respectively. The recent identification of a distinct E2/E3 enzyme complex that modifies NEMO with linear

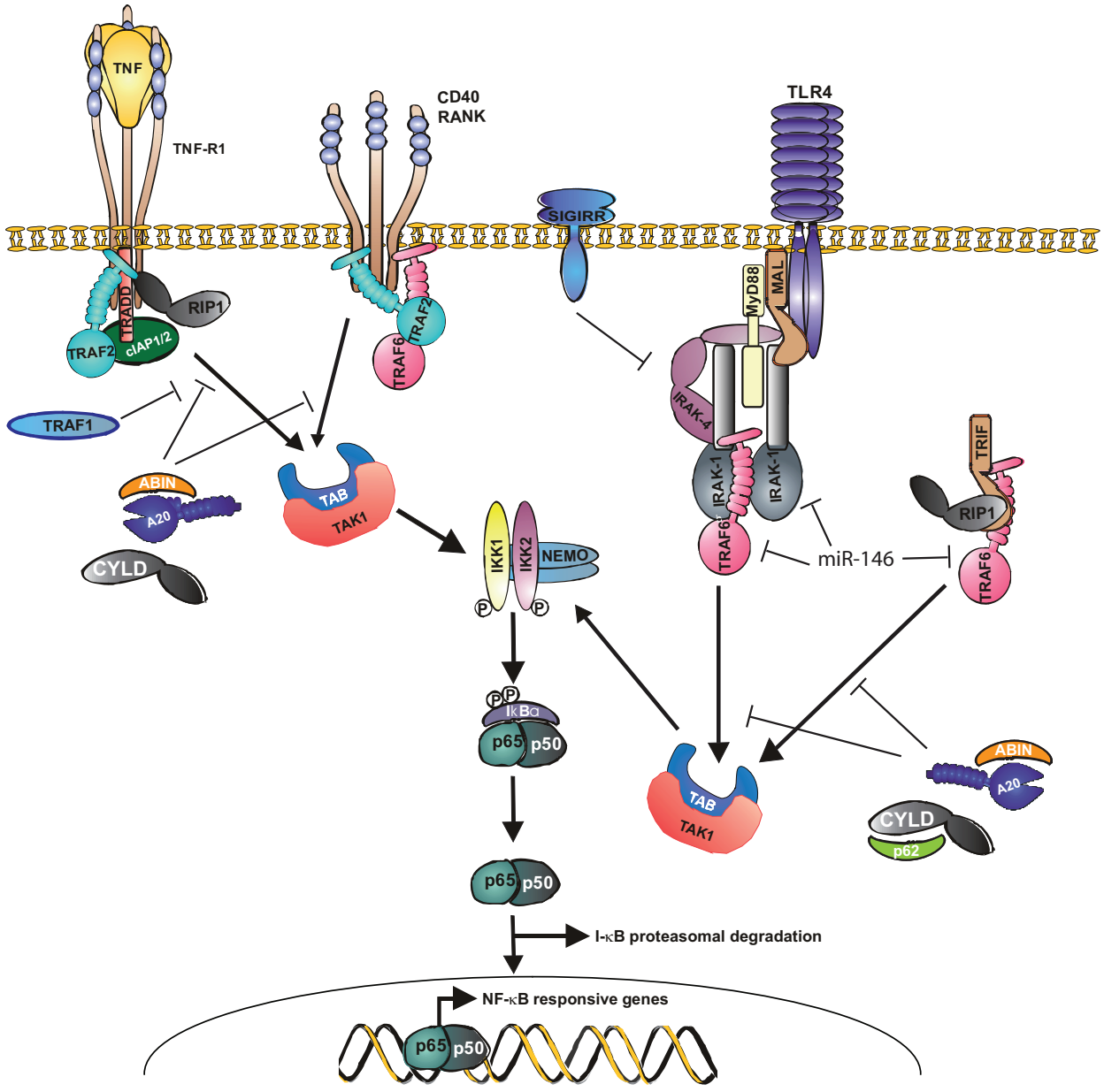

Figure 1. Canonical NF-κB signalling and negative regulators. Ligand engagement of specific membrane receptors such as TNFR1, CD40, RANK, and TLR4 trigger the recruitment of specific adaptor proteins (TNF receptor 1-associated death domain protein (TRADD), MyD88, MAL, TIR domain-containing adaptor-inducing IFNβ (TRIF)), kinases (RIP1, IRAK1, IRAK4), and ubiquitin ligases (TRAF2, TRAF6, cIAP1, cIAP2) to the receptor. K63-linked polyubiquitination of TRAFs, RIP1 and IRAK1, is recognised by NEMO and TAB proteins, resulting in the recruitment and activation of respectively IKK2 and TAK1. TAK1 then phosphorylates and activates IKK2, which in turn phosphorylates IκBα, triggering its K48-linked ubiquitination and proteasomal degradation. This allows NF-κB (here shown as a heterodimer of p65 and p50) to translocate to the nucleus and promote target gene expression. TRAF1, which has no ubiquitin ligase activity, can negatively regulate NF-κB activation, most probably by competing with other TRAFs. A20 and CYLD are deubiquitinating enzymes that control NF-κB activation by targeting specific signalling proteins including RIP1 and TRAF6, to which they are recruited using specific ubiquitin-binding adaptor proteins such as ABIN-1 and p62. miR-146 is thought to negatively regulate TLR signalling by inhibiting expression of IRAK1 and TRAF6. Finally, TLR signalling can also be inhibited by the transmembrane protein SIGIRR, which has been proposed to compete with TLR4 for binding to IRAK1 and TRAF6. The expression of many of these negative regulatory molecules is NF-κB dependent, implicating them in the negative feedback regulation of NF-κB activation. ABIN, A20-binding inhibitor of NF-κB; cIAP, cellular inhibitor of apoptosis; CYLD, cylindromatosis; IKK, IκB kinase; IκB, inhibitor of NF-κB; IRAK, IL-1R-associated kinase; MyD88, myeloid diff erentiation primary response gene 88; NEMO, NF-κB essential modulator; NF, nuclear factor; RANK, receptor activator of NF-κB; RIP1, receptor interacting protein 1; SIGIRR, single-immunoglobulinIL-1 receptor-related; TIR, Toll-like receptor/IL-1R; TRAF, TNF receptor-associated factor; TLR, Toll-like receptor; TNF, tumour necrosis factor.

polyubiquitin chains and is essential for TNF-activated NF-κB signalling adds further complexity [22]. The exact role of protein-anchored polyubiquitin chains remains unclear, as it was recently suggested that unanchored polyubiquitin chains can directly activate the TAK1 complex [23].

Similar signalling principles apply to other receptors. For example, TLR4 stimulation by lipopolysaccharide induces the recruitment of Toll/IL-1 receptor adaptor protein (also referred to as Mal) and TRIF-related adaptor molecule (TRAM), which most probably serve as bridging factors to recruit myeloid differentiation primary response gene 88 (MyD88) and TIR domain-containing adaptor-inducing IFNβ (TRIF), respectively. MyD88 in turn recruits members of the IL-1R-associated kinase (IRAK) family and TRAF6, leading to oligomerisation and self-ubiquitination of TRAF6 [24]. TRIF also recruits TRAF6 [25] and RIP1 [26] via a direct interaction. Both pathways then activate TAK1 and IKK in a ubiquitination-dependent manner similar to the TNF pathway (Figure 1).

The noncanonical NF-κB pathway can be activated by the lymphotoxin β receptor, BAFF receptor, CD40, and RANK (Figure 2). In this pathway, p100 is processed by the proteasome to p52, which together with the RelB NF-κB subunit regulates a distinct set of target genes that control B-cell development, secondary lymphoid organ development, and osteoclastogenesis [27] The noncanonical NF-κB pathway is strictly dependent on IKK1, which is activated upon phosphorylation by NF-κB inducing kinase (NIK). NIK is predominantly regulated at the post-translational level and is present at extremely low levels in most cell types. In unstimulated cells, NIK occurs in a cytoplasmic complex with TRAF2, TRAF3, and cIAP1/2, which K48-polyubiquitinates NIK, leading to its continuous degradation by the proteasome. Receptor ligation has been shown not only to remove TRAF3 from this complex by recruiting it to the receptor, but also to attract TRAF2 and cIAP1/2, which are essential for subsequent TRAF3 degradation. All this contributes to releasing NIK from its constitutive degradation, resulting in NIK accumulation and IKK1 phosphorylation [28,29] (Figure 2).

It should be mentioned that CD40, lymphotoxin β receptor and RANK mediate the activation of both canonical and noncanonical NF-κB signalling pathways. Upon binding of their ligand, CD40 and RANK interact with several TRAF members, including TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6, and this leads to the proteolysis of both TRAF2 and TRAF3, which represents an important step in the activation of the noncanonical pathway as described above. Specific TRAF molecules are associated with overlapping and distinct CD40-mediated functions. For example, in B cells TRAF6 is required for CD40-mediated JNK activation and IL-6 production, while TRAF2 is required for activation of NF-κB, and TRAF3 serves as a negative regulator of CD40 signalling [30,31].

Negative regulation of NF-κB signalling in RA

Since NF-κB activation is so crucial to many cellular processes, a tight regulation of the NF-κB signalling pathway and the genes it induces is an absolute requirement to fine-tune the inflammatory response. Moreover, terminating an NF-κB response is essential to prevent persistent NF-κB activation that may lead to chronic inflammation and/or tumorigenesis. To achieve this, cells employ different mechanisms, including the expression of inhibitory proteins that downregulate NF-κB signalling [32]. Below we give an overview of a number of proteins that are involved in the dampening or termination of the NF-κB response, some of them under the control of NF-κB itself and thus acting in a negative feedback loop. In addition, we discuss the potential role of these NF-κB inhibitory factors in the immunopathology of RA. Several other proteins involved in the negative regulation of NF-κB-dependent inflammatory responses, such as MyD88s, IRAK-M, and TOLLIP, have been described (reviewed in [33]). These proteins are not discussed here, since a link with RA pathology has not yet been reported.

Although IKK1 is a critical component of the noncanonical NF-κB pathway, it should be mentioned that this kinase also plays a prominent role in the negative regulation of both canonical and noncanonical NF-κB pathways. Macrophages from IKK1-deficient mice or knockin mice expressing inactive IKK1 show increased production of proinflammatory cytokines as a result of enhanced IKK2 activation and IκBα degradation [34]. IKK1 has also been shown to inhibit nuclear NF-κB and to downregulate proinflammatory signalling by phosphorylating STAT1 [35]. Interestingly, a recent study has demonstrated that IKK1 phosphorylates NIK in negative feedback regulation of the noncanonical NF-κB pathway [36], supporting the idea that IKK1 plays important roles in terminating both canonical and noncanonical NF-κB pathways with possible implications for chronic inflammatory diseases like RA.

A20 protein

A20 (also known as TNFAIP3) is a ubiquitin-editing protein that negatively regulates NF-κB-dependent gene expression in response to different immune-activating stimuli, including TNF, IL-1 and antigens, and the triggering of TLRs and the nucleotide-binding oligomerisation domain-containing 2 receptor (reviewed in [37]). A20 is believed to inhibit NF-κB function by deubiquitinating specific NF-κB signalling molecules, such as RIP1, RIP2, TRAF6 and MALT1, which disrupts specific protein–protein interactions [38-41] (Figure 1). Recently, the disruption of interactions between E2

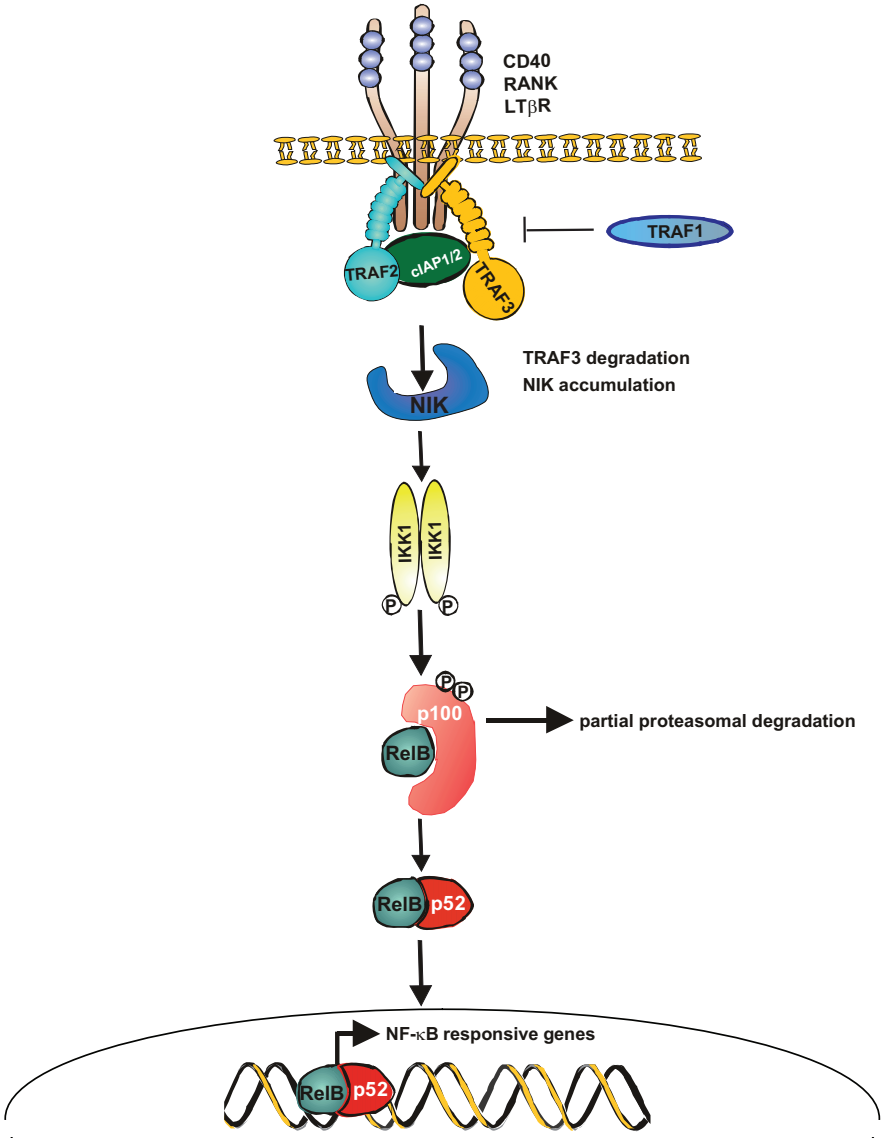

Figure 2. Noncanonical NF-κB signalling. CD40 and RANK can activate the noncanonical NF-κB pathway that is dependent on NF-κB inducing kinase (NIK) expression levels. In unstimulated cells NIK forms a cytosolic complex with the ubiquitin ligases TRAF2, TRAF3 and cIAP1/2, which facilitates the K48-linked polyubiquitination and proteasomal degradation of NIK, keeping NIK levels low. Upon ligand binding, TRAF3 is recruited to the receptor, where TRAF2 directs nondegradative K63-linked polyubiquitination of cIAP1/2, resulting in their activation. Subsequently cIAP1/2 directs its K48-linked polyubiquitination to TRAF3, rather than NIK. As a result, TRAF3 is degraded and NIK is stabilised, resulting in increased NIK levels in the cell. NIK then phosphorylates and activates IKK1, which mediates NF-κB p100 phosphorylation. This is followed by K48-linked polyubiquitination and partial proteasomal degradation of p100 to p52, which forms a heterodimer with RelB to activate transcription. Next to TRAF3, TRAF1 has also been identified as a negative regulator of this pathway, most probably by competing with other TNF receptor-associated factors. cIAP, cellular inhibitor of apoptosis; IKK, IκB kinase; NF, nuclear factor; RANK, receptor activator of NF-κB; TRAF, TNF receptor-associated factor; TNF, tumour necrosis factor.

ubiquitin conjugating enzymes (Ubc13 and Ubc5hc) and E3 ubiquitin ligases (TRAF6, TRAF2 and cIAP1/2) were described as another important mechanism used by A20 to downregulate NF-κB signalling [42]. The association of A20 with its targets requires specific ubiquitin-binding adaptor proteins, including A20-binding inhibitor of NF-κB (ABIN) 1 and Tax1-binding protein 1 [43-46].

Next to its role in suppressing NF-κB activation, A20 is also a strong inhibitor of apoptosis, at least in some cell types. The mechanisms by which A20 regulates apoptotic signalling, however, are still elusive. A20-deficient mice spontaneously develop multiorgan inflammation and cachexia and die within 2 weeks of birth, illustrating the potent anti-inflammatory function of this molecule [46].

A20-deficient cells are also more susceptible to TNF-mediated apoptosis, confirming its role as an antiapoptotic protein. We recently showed that mice specifically lacking A20 in intestinal epithelial cells exhibit increased susceptibility to experimental colitis due to the hypersensitivity of their intestinal epithelial cells to TNF-induced apoptosis, confirming A20 as a major antiapoptotic protein in the intestinal epithelium [47]. Two independent studies showed that mice lacking A20 in B cells develop autoimmunity due to hyperactive NF-κB responses in B cells leading to unrestricted B-cell survival [48,49].

A20 expression has been observed in several cell types that play important roles in the pathophysiology of RA, such as fibroblasts, synoviocytes and lymphocytes. Interestingly, A20 expression is itself regulated by NF-κB [50], implicating A20 in the negative feedback regulation of NF-κB signalling. Recently, intra-articular injection of an A20-expressing adenovirus was shown to reduce the severity of synovial inflammation and joint destruction in a mouse model of collagen-induced arthritis, even in untreated joints, in both a prophylactic and therapeutic setting. A20 expression in synovial tissue was associated with inhibition of NF-κB activity and decreased levels of TNF, IL-1β, IL-6, soluble RANKL, monocyte chemoattractant protein 1, and IL-17, suggesting that A20 induces a protective effect in collagen-induced arthritis mice through suppression of NF-κB activation and NF-κB-dependent gene expression. [51]. Because TNF and IL-1β are known to mediate synovitis, pannus formation, and erosion of cartilage and bone in RA, the decreased serum levels of TNF and IL-1β in A20-transduced mice might explain the beneficial effects in the clinical, pathological, and radiological findings. This study also demonstrated that A20 overexpression leads to a decrease in the number of activated osteoclasts in joint tissue. A20 might therefore minimise joint destruction through decreasing the osteoclast number and activity. It will be interesting to analyse in future the susceptibility of conditional knockout mice that lack A20 in specific cell types such as synovial fibroblasts, macrophages, dendritic cells, B cells or T cells.

Importantly, several SNPs in the human *A20* locus have been associated with increased susceptibility to development of autoimmune pathologies (reviewed in [52]). Several genome-wide association studies also revealed a clear association between mutations in the A20 locus in the 6q23 chromosome and susceptibility to RA [52]. Although the identified variants are not located in a gene, they are thought to influence *A20* as its nearest gene (~150 kb downstream of *A20*), probably by the presence of potential regulatory DNA elements in this region. As A20 is required for termination of TNF-induced signals, and TNF is the primary inflammatory cytokine in RA, these findings reveal *A20* as a candidate susceptibility locus for RA. How these variants affect normal A20 activity and how they cause RA, however, remain unclear. Recently, Elsby and colleagues functionally evaluated *in vitro* the regulatory ability of RA-associated SNP variants on *A20* promoter activity, and could show repressed *A20* transcription for some of the SNPs investigated [53]. It will be of interest to identify the actual causal variants and to elucidate the functional consequences of these variants. In this context, knockin mice for the corresponding A20 SNPs, combined with mouse models for RA, will be very valuable tools.

A20-binding inhibitors of NF-κB

ABIN-1, ABIN-2 and ABIN-3 (also known as TNIP-1, TNIP-2, and TNIP-3) were identified as ubiquitously expressed A20 interacting proteins and were shown to inhibit NF-κB activation by TNF and several other inflammatory stimuli upon overexpression. Because ABINs contain a specific ubiquitin-binding motif, they have been proposed to target A20 to polyubiquitinated substrates [43,44]. Similar to A20, ABIN-1 and ABIN-3 expression is NF-κB dependent, implicating a potential role for the A20/ABIN complex in the negative feedback regulation of NF-κB activation (reviewed in [54]). Unexpectedly, both ABIN-1-deficient and ABIN-2-deficient mice exhibit only slightly increased or normal NF-κB responses, respectively, possibly reflecting redundant NF-κB inhibitory activities of multiple ABINs [55,56]. Functional ABIN-3 is expressed in humans, but mice only express a truncated and inactive form lacking the crucial ubiquitin-binding domain [57]. As for A20, ABIN-1 also strongly inhibits TNF-induced apoptosis [58], and ABIN-1 deficient mice die embryonically due to TNF-dependent foetal liver apoptosis [56].

Using oligonucleotide microarray analysis, ABIN-1 was identified among TNF-induced genes in human synoviocytes, and high levels of ABIN-1 mRNA were detected in RA tissue biopsies, indicating a potential role for ABIN-1, together with A20, in the negative feedback regulation of NF-κB signalling and the pathogenesis of RA [59]. In a recent study, inhibition of TLR responses by immunoreceptor tyrosine-based activation motif (ITAM)-coupled receptors was shown to depend on the expression of the NF-κB inhibitory proteins ABIN-3 and A20. Moreover, this protective effect of the ITAM was strongly suppressed in inflammatory arthritis synovial macrophages [60]. Interestingly, ABIN-1 polymorphisms have been associated with psoriasis and systemic lupus erythematosus in humans [61,62]. A high degree of overlap between systemic lupus erythematosus and RA susceptibility loci might be expected as the two diseases show some clinical overlap in joint involvement, autoantibody production, systemic features and response to treatments such as B-cell depletion (rituximab). It will therefore be interesting

to investigate whether SNPs that have been reported to be associated with systemic lupus erythematosus are also associated with RA.

Cylindromatosis protein

Cylindromatosis (CYLD) protein was originally identified as a tumour suppressor involved in familial cylindromatosis [63]. CYLD is also involved, however, in diverse physiological processes ranging from immunity and inflammation to cell cycle progression, spermatogenesis, and osteoclastogenesis (reviewed in [64]). CYLD is a deubiquitinating enzyme that negatively regulates NF-κB signalling initiated by TNFR, RANK and T-cell receptor stimulation (reviewed in [64]) (Figure 1), by deubiquitinating several NF-κB signalling proteins including NEMO, TRAF2, TRAF6, TRAF7, RIP1 and TAK1. Many of these are also targeted by A20 and it is still not clear why the cell needs A20 as well as CYLD to control NF-κB activation. As A20 is only expressed in many cell types upon stimulation, it has been suggested that this protein mainly regulates later phases of NF-κB signalling, whereas CYLD would regulate constitutive and early signalling. In addition, their relative activity might also be cell-type dependent [64].

Interestingly, CYLD has been shown to negatively regulate RANK signalling and osteoclastogenesis in mice [65]. Mice with a genetic deficiency of CYLD have aberrant osteoclast differentiation and develop severe osteoporosis. Osteoclast precursors of these mice are hyper-responsive to RANKL-induced differentiation and produce more and larger osteoclasts. CYLD expression is markedly upregulated under conditions of RANKL-induced osteoclastogenesis and is recruited to ubiquitinated TRAF6 via the ubiquitin-binding adaptor protein p62 (also known as sequestosome 1) [65], followed by the CYLD-mediated deubiquitination of TRAF6. In this context, it is worth mentioning that transgenic mice expressing a mutated form of p62 also display abnormal osteoclastogenesis and develop progressive bone loss [66]. These findings suggest that CYLD-mediated inhibition of RANK-induced NF-κB signalling plays a key role in the negative regulation of osteoclastogenesis and indicate CYLD as a potential genetic factor involved in the pathology of bone disorders such as RA.

Single-immunoglobulin IL-1 receptor-related protein

Single-immunoglobulin IL-1 receptor-related (SIGIRR) protein, also known as TIR-8, is a member of the TLR/IL-1R family that has been extensively characterised as an inhibitor of IL-1R and TLR signalling, probably through direct interaction with these receptors, MyD88, IRAK1 or TRAF6 [67] (Figure 1). Given the important role of IL-1R and TLR signalling in the chronic inflammation observed in RA [68], a regulatory role for SIGIRR in RA is not unlikely. SIGIRR has a very restricted expression pattern, being expressed in epithelial cells, monocytes and immature dendritic cells, but not in mature macrophages [69,70]. Recently, SIGIRR overexpression was shown to inhibit the spontaneous release of inflammatory cytokines by human RA synovial cells. This inhibitory function of SIGIRR was further confirmed *in vivo*, since SIGIRR-deficient mice developed a more severe disease in zymosan-induced arthritis, as well as collagen-induced arthritis mouse models [70]. It will be interesting to compare the expression of SIGIRR in RA patients with its expression in control patients, or to investigate whether the function of SIGIRR is compromised in RA patients. Because of its restricted expression pattern, SIGIRR may also be an interesting therapeutic target in RA.

TNF receptor-associated factor 1

TRAF1 is a unique member of the TRAF protein family because it lacks a RING finger domain and therefore lacks ubiquitin ligase activity. Accumulating data support a role for TRAF1 as both a negative and a positive modulator of NF-κB signalling by certain TNF family receptors, possibly in a cell-type-dependent manner [71,72]. Expression of TRAF1 is inducible by TNF and overexpression of TRAF1 inhibits TNF-induced NF-κB activation. TRAF1-deficient T cells are hyper-responsive to TNF, with enhanced proliferation and activation of the NF-κB signalling pathway. TRAF1 also functions as a negative regulator of CD40-induced NF-κB activation. TRAF1-deficient dendritic cells, however, show attenuated responses to secondary stimulation by TRAF2-dependent factors, suggesting a positive regulatory role in these cells. The mechanism by which TRAF1 modulates NF-κB activation is still unclear. Most probably, TRAF1 competes with TRAF family members for binding to the receptor or other signalling proteins. Alternatively, TRAF1 might recruit A20 with which it can physically interact [73]. A genome-wide association study examining more than 300,000 SNPs among approximately 1,500 autoantibody-positive RA cases and 1,800 controls identified a genetic variation at the TRAF1–complement component 5 locus as an important RA risk locus [74]. Subsequent work indicates that TRAF1 is more likely to be the causative locus [75]. Recent work in a Korean population also demonstrates genetic association of the TRAF1 region with RA [76].

microRNAs

miRNAs are recently discovered regulators of gene expression and represent a class of noncoding RNA molecules essential in many cellular and developmental processes, including immune responses and inflammation. They bind the 3'-UTR of target mRNAs leading to the repression of

protein expression and the promotion of target mRNA degradation [77].

miR-146a/b and miR-155 are of particular interest for inflammatory signalling to NF-κB, since these miRNAs can be induced by inflammatory stimuli such as IL-1β, TNF and TLRs [78,79]. In addition, miR-146a is an NF-κB-dependent gene, and the NF-κB signalling molecules *IRAK1* and *TRAF6* were identified as target genes of miR-146a [78] (Figure 1). Similarly, miR-155 was shown to target transcripts for the NF-κB signalling molecules IKKε and RIP1 [79,80]. Notably, both miR-146 and miR-155 are expressed at higher levels in RA synovial fibroblasts and synovial tissue [81,82], as well as in peripheral blood mononuclear cells of RA patients [83]. miR-146a is also overexpressed in $CD4^+$ and IL-17-producing T cells from RA patients [84,85]. Interestingly, a polymorphism in the 3'-UTR of the miR-146a target gene was recently shown to be associated with RA susceptibility [86]. miR-155 overexpression in synovial fibroblasts was able to prevent the TLR and cytokine-inducible expression of specific matrix metalloproteinases that mediate tissue destruction in RA [81]. Moreover, miR-155 was shown to promote TNF production, a key process in the pathogenesis of RA [87]. miR-146 and miR-155 may therefore be important negative regulators of inflammation in RA and their potential for the development of new treatments is substantial. In addition, their increased expression in RA patients is potentially useful as a marker for disease diagnosis, progression, or treatment efficacy [88], but this will require confirmation using a large and well-defined cohort.

Besides miR-146 and miR-155, a number of other miRNAs with a potential role in the control of NF-κB-dependent inflammatory responses in RA pathology were recently identified. In this context, miR-124a – a key regulator of the chemokine monocyte chemoattractant protein 1 – was shown to be decreased in synoviocytes from RA patients [89]. Similarly, elevated levels of miR-203 – leading to increased secretion of matrix metalloproteinase-1 and IL-6 – were detected in RA synovial fibroblasts [90]. Finally, miR-16, miR-132, and miR-223 were also shown to have an altered expression in RA patients, indicating their potential as diagnostic biomarkers for pathogenesis [83,88,91].

Conclusions

The NF-κB family of transcription factors plays crucial roles in the inflammatory processes in RA leading to cartilage and bone destruction. Keeping NF-κB activation under control can thus be very important for the design of specific therapeutics. The existence of multiple negative regulators ensuring a tight regulation of the NF-κB pathway, however, raises the question of the specific role of each of these regulators and the relationship between them. In addition, given the number of miRNAs in humans and the multiple mRNAs they target, intense complexity can be expected. How all these regulatory signals are themselves regulated will be an important question in order to clarify how NF-κB signalling is organised, and, more importantly, how this knowledge may lead to new treatments for inflammatory diseases such as RA.

Abbreviations

ABIN, A20-binding inhibitor of NF-κB; BAFF, B-cell activating factor; cIAP, cellular inhibitor of apoptosis; CYLD, cylindromatosis; HMGB1, high mobility group box chromosomal protein 1; IFN, interferon; IKK, IκB kinase; IκB, inhibitor of NF-κB; IL, interleukin; IRAK, IL-1R-associated kinase ; ITAM, immunoreceptor tyrosine-based activation motif; miRNA, microRNA; MyD88, myeloid differentiation primary response gene 88; NEMO, NF-κB essential modulator; NF, nuclear factor; NIK, NF-κB inducing kinase; RA, rheumatoid arthritis; RANK, receptor activator of NF-κB; RANKL, receptor activator of NF-κB ligand; RIP1, receptor interacting protein 1; SIGIRR, single-immunoglobulin IL-1 receptor-related; SNP, single nucleotide polymorphism; TIR, Toll-like receptor/IL-1R; TRAF, TNF receptor-associated factor; TLR, Toll-like receptor; TNF, tumour necrosis factor; TRIF, TIR domain-containing adaptor-inducing IFNβ; UTR, untranslated region.

Competing interests

The authors declare that they have no competing interests.

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