NF-κB and its relevance to arthritis and inflammation

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Short title: NF-κB, inflammation, and arthritis
Abstract

In the synovial cells of patients with rheumatoid arthritis (RA), activation of the NF-κB pathway results in the transactivation of a multitude of responsive genes that contribute to the inflammatory phenotype, including TNFα from macrophages, matrix metalloproteinases from synovial fibroblasts and chemokines that recruit immune cells to the inflamed pannus. This is largely a consequence of activation of the ‘canonical’ NF-κB pathway that involves heterodimers of p50/p65. Whilst much information on the role of NF-κB in inflammation has been gleaned from genetic deficiency of the respective genes in mice, important differences exist in the signalling networks between human and murine immune cells and immortalised cell lines. Despite these differences at the molecular level, the importance of NF-κB in inflammation is undisputed and inhibition of the pathway is widely believed to have great potential as a therapeutic target in RA. Commercial effort has gone into developing inhibitors of NF-κB activation. However, inhibition of the NF-κB activation can result in an exacerbation of inflammation if TNFα production by macrophages is not controlled. It will be important that such inhibitors are carefully monitored before their long term use in chronic inflammatory conditions such as RA.

Introduction

The nuclear factor-κB (NF-κB) family of transcription factors are amongst the most intensively studied in vertebrate biology. Since their discovery in the eighties, they have been shown to be involved in many different pathways, including inflammation and also cell survival, proliferation and differentiation. As will be described in detail in this review, activation of the NF-κBs is normally pro-inflammatory. However, they are also anti-apoptotic (pro-survival) and a delicate balance between the two functions must be tightly regulated (1, 2). The importance of the NF-κBs in human physiology is emphasised by the common instances of disease where their activity is dysregulated.

The NF-κB family consists of five members (Table 1); p105 (constitutively processed to p50), p100 (processed to p52 under tightly regulated conditions), p65 (also known as rela), RelB and c-Rel. These NF-κB subunits form homodimers and heterodimers to produce NF-κB transcription factors. They bind to the NF-κB consensus sequence (5'-GGGRNYYYCC-3’) and then activate (or, in some cases, repress) target gene transcription (3, 4). The most common activating form [also present in activated macrophages (5, 6)] is a heterodimer of p50 and p65. In unstimulated cells, NF-κB transcription factors are found in the cytosol, rendered inactive by an Inhibitor of κB (IκB) molecule. Activation of the IκB kinase (IKK) complex targets IκB for degradation, thus releasing NF-κB.

In recent years there has been an explosion of publications describing the complexities of the pathways of NF-κB activation in response to different cellular stimuli, in different disease states and in different cell types. Despite this, there is still much to learn and new observations and discoveries in this field are a regular event. This review will attempt to bring the reader up to date with models of NF-κB activation that are relevant to inflammation in RA. But first, let us examine the evidence that supports a role for NF-κB in the pathogenesis of RA.
NF-κB is activated in rheumatoid arthritis

The joints of patients with RA are characterised by an infiltration of immune cells into the synovium, leading to chronic inflammation, pannus formation and subsequent irreversible joint and cartilage damage (7). The RA synovium is known to comprise largely of macrophages (30-40%), T cells (~30%) and synovial fibroblasts, but also of B cells, dendritic cells, other immune cells and synovial cells such as endothelium (7, 8). RA synovial fluid has been shown to contain a wide range of effector molecules including pro-inflammatory cytokines (such as IL-1β, IL-6, TNFα and IL-18), chemokines (such as IL-8, IP-10, MCP-1, MIP-1, and RANTES), matrix metalloproteinases (MMPs, such as MMP-1, -3, -9 and -13) and metabolic proteins (such as Cox-1, Cox-2 and iNOS). These interact with one another in a complex manner that is thought to cause a vicious cycle of proinflammatory signals resulting in chronic and persistent inflammation (7), see Figure 1. TNFα in particular is the prime inflammatory mediator and also induces apoptosis. Importantly, the genes encoding TNFα and many of the other factors mentioned above are now known to be under the control of NF-κB transcription factors (4), suggesting that NF-κB could be one of the master regulators of inflammatory cytokine production in RA. Indeed, the presence of activated NF-κB transcription factors have been demonstrated in cultured synovial fibroblasts (9-11), human arthritic joints (11-17) and the joints of animals with experimentally induced RA (18, 19). Immunohistochemistry has demonstrated the presence of both p50 and p65 in the nuclei cells lining the synovial membrane and macrophages (16-18). Furthermore, nuclear extracts of cells have demonstrated an ability to bind to the NF-κB consensus sequence (10, 16). New techniques such as in vivo imaging have also been used to demonstrate the activity of NF-κB in a mouse model that mimicked RA-like chronic inflammation. By placing the luciferase gene under the control of NF-κB, increased luminescence was observed in the joints of live mice (12).

These findings are supported by a study that investigated experimentally induced arthritis in mice that carried knockouts of the genes for the NF-κB family members p50 or c-Rel. The two experimental models used were collagen induced arthritis (CIA; a model of chronic RA where disease development involves both T and B cells) and an acute/destructive model induced by methylated BSA and IL-1 (involving exclusively T cells and not B cells). Lack of c-Rel had no influence on the acute model and, whilst reducing the incidence of CIA, did not prevent a severe immunohistopathology in affected joints. In addition, c-Rel could not be found in the nuclei of cells explanted from the arthritic joints of wild-type mice, suggesting that this subunit of NF-κB is of limited importance in RA (20). In contrast, lack of p50 caused a complete loss of a humoral response, severely impeded T cell proliferation and conferred resistance to both forms of arthritis (20). This clearly demonstrates a central role for p50 (presumably p50/p65 heterodimers) in the inflammation that underlies RA.

Core principles of the ‘canonical’ NF-κB pathway

The molecular events that lead to activation of NF-κB transcription factors in the RA synovium are clearly of great interest and involve the so-called ‘classical’ or ‘canonical’ pathway. The three main players in the pathway, the IKK complex, IκBs and the NF-κB transcription factors will be discussed in turn.
The IKK complex
The high molecular weight IKK complex plays an extremely important role in the activation of NF-κB since it represents a convergence point for the signals that are transmitted from many different cellular stimuli, such as the bacterial endotoxin lipopolysaccharide (LPS) or cytokines such as TNFα and IL-1. The function of the IKK complex in the canonical pathway is to phosphorylate IκBα and IκBβ and target them for degradation by the ubiquitin/proteasome pathway. The canonical IKK complex consists of at least three subunits; IKK1 (also known as IKKα), IKK2 (also known as IKKβ) and NF-κB essential modulator (NEMO, also known as IKKγ). Additional, as yet unidentified, subunits are likely to be discovered. Both IKK1 and IKK2 have catalytic activity and IKK2 is generally considered to be the most relevant to RA, since it is indispensable for phosphorylation of IκBα by the IKK complex (4). The role of IKK1 is less clear, but recent evidence points towards a negative regulatory role, acting as a ‘checkpoint’ in NF-κB activation to prevent uncontrolled stimulation of cells (5, 21). NEMO does not have kinase activity but is necessary for phosphorylation of IκBα/IκBβ by the IKK complex (22, 23).

IκBα, IκBβ and IκBe
IκBα is the prototypical member of the seven member IκB family (Table 2) and was identified by its ability to render the common NF-κB p65/p50 dimer inactive in the cytosol of unstimulated cells. Both IκBα and IκBβ bind to NF-κB and mask the nuclear localisation sequence on the p50/p65 heterodimer thus inhibiting its entry into the nucleus. Following IκBα phosphorylation by the IKK complex and degradation, the nuclear localisation signal is no longer masked and this causes translocation of the active dimer to the nucleus.

One of the unique features of the canonical NF-κB pathway is its rapid yet transient activation, which prevents a persistent response that could result in pathological changes in affected cells. Down-regulation of NF-κB activity coincides with the reappearance of IκBα, which requires new protein synthesis. Indeed, the IκBα gene promoter contains 11 NF-κB consensus sequences making it extremely responsive to NF-κB activation. Newly synthesised IκBα enters the nucleus, binds NF-κB dimers and returns them to the cytosol, thus dampening the response. If the stimulus is still present, these are again degraded and NF-κB activity rises again. Following LPS exposure, this results in a phenomenon known as ‘rapid oscillatory activation’ where the response gradually becomes dampened over time (24). The NF-κB response is also negatively regulated by IκBe, which is a target of NF-κB and is synthesised in anti-phase compared to IκBα (25). In contrast to IκBα and IκBe, IκBβ is not a genetic target of NF-κB and it is not rapidly resynthesised following NF-κB activation. Therefore, situations in which IκBβ predominates have the potential to result in prolonged NF-κB activation (4). However, the relevance of both IκBβ and IκBe to RA is unclear, since IκBα is so dominant in the inactivation of NF-κB.

The NF-κB family of transcription factors
A crucial aspect of the NF-κB response is the make-up of the dimers that are bound to and inhibited by the IκBs. There is considerable variation in the combinations that have been observed (3). The subunits that are present in the dimers influence their biological activity because the subunits have different functional domains. As mentioned above, all five members of the NF-κB transcription factor family (Table 1) contain a Rel-homology domain (RHD) that binds to DNA. In contrast, only three of the family (p65, RelB and c-Rel)
contain transactivation domains (TADs) that interact with general transcription factors and co-activators, whereas p50 and p52 do not. This difference can influence whether a specific dimer has the potential to act as an activator or a repressor. For instance the common heterodimer of p50 and p65 is able to activate gene transcription due to the presence of a TAD in p65. Conversely, homodimers of p50 contain no TAD and they can therefore act as transcriptional repressors by competing for p50/p65 binding to the NF-κB consensus sequence. In addition, subtle differences in NF-κB consensus sequences have now been shown to demonstrate preferential binding to different NF-κB dimers (26). This is exemplified by the -863 C/A polymorphism in the human TNFα promoter. Here, the C allele can bind both p50/50 and p50/p65 dimers, whereas the A allele can bind only the inhibitory p50 homodimer (27) suggesting that the A allele should demonstrate a dampened TNFα response following NF-κB activation. Indeed, this polymorphism may influence the incidence of RA (28).

Once activated, the ability of NF-κB to induce transcription can be further enhanced by post-translational phosphorylation and acetylation of the subunits (3, 26). For instance, serine phosphorylation of p65 can occur at different residues and is stimulus specific. Phosphorylated p65 can then be acetylated and this molecule has maximum activity. Acetylation of p65 is performed by CBP and p300, transcriptional coactivators that also recruit the transcriptional machinery. In addition, they have histone acetyltransferase activity, which helps to ‘relax’ the chromatin environment surrounding the activated genes and increase the efficiency of transactivation. Histone modification by NF-κB can lead to epigenic control of gene transcription, reviewed elsewhere (29).

The role of NF-κB – evidence from genetic knockouts

The experimental approach of murine genetic knockout has been used extensively to examine the NF-κB pathway. Overall, the results from these studies support the contention that NF-κB is primarily involved in dynamic responses to the cellular environment. Some of the results that impact inflammation and RA will be summarised here, but the interested reader is referred to one of several more detailed reviews (30-32).

Some knockout mice have a milder phenotype than might have been predicted. For instance, mice with genetic deficiency of the genes encoding p50, p52, c-Rel and RelB develop normally and the mice appear healthy. However, they do have abnormal immune cell responses such as in B and T cell proliferation, antigen presentation, isotype switching, and cytokine production. Furthermore, mice in which the coding regions of IκBβ replaced those of IκBα are normal and have no inflammatory disease, emphasising the functional similarity of these two homologous proteins (33). Mice that lack IκBβ have not been published in detail, however one study found that the effects of intrapulmonary LPS on wild-type and IκBβ-deficient mice were identical (34).

In contrast, genetic deficiency of other pathway members results in very severe phenotypes. Mice lacking IκBα die 7-10 days after birth due to severe inflammatory dermatitis and granulocytosis, alongside elevated expression of proinflammatory cytokines, emphasising the importance of IκBα in regulating the immune response (30). Furthermore, p65, NEMO and IKK2 knockouts die during late embryonic development or at
birth due to TNFα-dependent hepatocyte apoptosis (31, 35-37). This clearly demonstrates an anti-apoptotic role for the NF-κB pathway, and the genes upregulated by NF-κB following TNFα exposure. The role of inappropriate TNFα signalling was confirmed when the phenotype of p65-deficient mice was rescued by crossing them with mice deficient in the TNF receptor. However, these mice frequently died, probably due to increased susceptibility to infection (35). This highlights the delicate balance that exists between inflammation and apoptosis in vertebrate physiology.

Recently, the fatalities in IKK2 and NEMO knockout mice have been bypassed in order to study their role in inflammatory disease. These studies utilised conditional knockouts, where the ‘cre/lox’ system inactivates the genes in specific cell types. NF-κB activation was prevented in different cells by removing IKK2 from keratinocytes (38, 39) or IKK1/IKK2 or NEMO from intestinal epithelial cells (40, 41). Somewhat counterintuitively, this resulted in chronic inflammation in the affected tissues leading to phenotypes that resembled psoriasis and colitis, respectively. In both models, disease was accompanied by a massive influx of immune cells expressing proinflammatory cytokines (particularly macrophages expressing TNFα) and an increase in apoptosis. As above, the phenotypes could be normalised by crossing the animals with TNF receptor knockout mice (39, 41). This suggested that macrophage produced TNFα was a key driver of disease in inflammatory conditions.

The role of the canonical pathway in RA - primary human cell studies

The studies described above have been extremely important in establishing the molecular events that can occur in the canonical NF-κB pathway. However, their relevance to the activation of NF-κB seen in RA cannot be assumed. Important differences in immune cell function exist between humans and mice, and between transformed and non-transformed cells (dealt with in detail below). Research in primary human cells was hampered for many years because these non-dividing cells are resistant to conventional transfection techniques. Recently, this technological challenge was overcome by the use of adenoviral systems that efficiently infect primary cells and deliver exogenous expression constructs. Here, dominant negative (dn) variants of canonical pathway signalling components were expressed in cells that are relevant to RA, including primary synovial cell cultures (containing a mixture of cells) from patients undergoing knee replacement surgery, synovial fibroblasts derived from them, and primary M-CSF differentiated macrophages from normal human blood donors.

In such studies, dnIKK1 was found not influence spontaneous cytokine production from primary synovial cell cultures, whereas dnIκBα and dnIKK2 profoundly inhibited IL-6, IL-8 and VEGF production (42). Somewhat surprisingly dnIKK2 did not significantly inhibit spontaneous TNFα production. However, these findings generally support the hypothesis of an important role for the canonical pathway in RA and that IKK2 is the dominant kinase in the IKK complex. To extend these studies, the dn proteins have also been tested in the different cells types present in the synovial cell cultures. Here, dnIKK2 was found to inhibit cytokine production from both TNFα and IL-1β stimulated macrophages and RA synovial fibroblasts. This same molecule could also block IL-6 and IL-8 production in LPS stimulated RA synovial fibroblasts. However, in stark contrast to findings in murine cells, it is interesting to note that dnIKK2 did not affect TNFα, IL-6 or IL-8 production following LPS stimulation of human macrophages (42). This could have suggested that the
canonical pathway is of low importance in LPS stimulated macrophages. However, dnIκBα effectively blocks expression of TNFα, IL-1β, IL-8 and IL-6 production in response to LPS (42, 43). This suggests that other (unidentified) IκB phosphorylating kinase(s) are present in these cells. It might also explain why the dnIKK2 could not affect spontaneous TNFα production from the synovial cell cultures, since the main source of TNFα here is macrophages. IκBα also has differential effects on the spontaneous production of different cytokines in primary RA synovial cultures. While IL-1β, IL-6, IL-8, MMP-1, -3 and –13 were all IκBα-dependent as expected, TNFα production was not affected (42).

These studies serve to highlight the complexities of the role that the NF-κB pathway plays in RA. Whilst the pathways activating NF-κB can be described in a straightforward way, in reality there is enormous variation in the molecular events that can occur between different cell types, in response to different cellular stimuli and for different genes that respond to NF-κB activation.

The ‘non-canonical’ pathway of NF-κB activation

An ‘alternative’ or ‘non-canonical’ pathway of NF-κB activation has been described that occurs specifically in B cells in response to small subset of stimuli (44). Here p100 itself, rather than an IκB, acts to sequester RelB in the cytosol. The processing of p100 is tightly regulated and virtually absent in unactivated cells. B cell stimulation with lymphotoxic results in p100 phosphorylation by a complex of IKK1 and NF-κB Inducing Kinase (NIK). It then undergoes limited proteolysis by the proteasome, giving rise to p52, and p52/RelB dimers are than able to activate transcription. Both NIK and IKK1 are indispensable for this activity. Recently p100 was shown to be a bona fide member of the IκB family and designated IκBδ (45). However, as NIK is not required for NF-κB activation following TNFα or IL-1α stimulation in primary human macrophages or, fibroblasts, neither is it involved in the spontaneous TNFα production by RA synovial cell cultures (46) it will not be considered further here.

Diverse functions of the IκB family

In addition to IκBα and IκBβ, the IκB family contains several other members that were assigned on the basis of sequence homology (Table 2). All include a series of ankyrin repeats that are important for binding to the Rel proteins, but some of the family play a quite different, yet important, role in the NF-κB pathway.

Bcl-3, IκBNS and IκBζ differ from the other IκBα and IκBβ, in that they are found not in the cytoplasm but in the nucleus and act as direct co-activators or co-repressors of p50 and/or p52 homodimer-dependent DNA binding (47-51). In mice, both Bcl-3 and IκBNS can induce the nuclear localisation of p50 homodimers and act as transcriptional co-repressors. However, the activity of the two IκBs differs, for whereas IκBNS specifically inhibits late responsive genes such as IL-6, without affecting the expression of early genes such as TNFα (50, 51), Bcl-3 inhibits TNFα without affecting IL-6 (52, 53), and this can be explained by the specific recruitment to the respective promoters. Both of these genes can be induced by the anti-inflammatory cytokine IL-10 and this may help to explain the resistance to inflammation of colonic lamia
propria macrophages, which constitutively express IL-10 as well as Bcl-3 and IκBNS (50). However, it should be noted that Bcl-3 can also act as a transcriptional coactivator in some cases (54-56).

The role of IκBξ seems to be primarily as a transactivator of a subset of inflammatory genes including IL-6, IL-12p40 and GM-CSF (57), although it can also inhibit NF-κB activity when it is overexpressed. IκBξ is strongly and transiently induced following stimulation of cells with IL-1β or LPS but not by TNFα and interacts with the p50 subunit in p50/p65 heterodimers. The difference in IκBξ induction seems to be due to mRNA stability. Whereas TNFα stimulation does not lead to IκBξ induction on its own, this can be supplemented by the non-NF-κB activating cytokine IL-17, which is known to stabilise mRNA (49).

**Pathological triggers of the NF-κB signalling cascade in RA**

Recently there has been much interest in teasing out the molecular triggers of chronic inflammation in RA, but the factors that cause this remain obscure. Indeed, the spectrum of signalling molecules that can result in NF-κB activation is large (58). Signalling via the TNFα and IL-1 receptors are strong contenders for mediating the chronic inflammation, since both of the cytokines are abundant in RA synovial fluid. In addition, molecules in the Toll-like receptor (TLR) family are potential candidates for receiving and transmitting the triggering event in RA. The TLRs have emerged in recent years as important initiators of the innate immune response, recognising specific components of pathogenic organisms by means of pathogen-associated molecular patterns (PAMPs). They exert tightly regulated and exquisitely specific responses that have many features in common with signalling via the IL-1R (26, 59). TLR signalling in primary human macrophages differs in some respects to other cells, since adenoviruses expressing dn forms of the TLR adaptors MyD88, Mal and TRAM did not significantly reduce pro-inflammatory cytokine production in response to the TLR4 ligand LPS (60, 61).

The TLRs have become of interest to RA because, whilst no infectious organism has ever been isolated from the joints of RA patients, bacterial products such DNA (TLR9 ligand) and peptidoglycan (TLR2 ligand) (62) have been detected. These agents are very strong inducers of the type of NF-κB activity observed in rheumatoid tissue. This suggests that unresolved infection, resulting in TLR activation, could be one of the causes of RA. In addition, other so-called 'endogenous' danger signals (compared to the exogenous PAMPs) have been shown to stimulate TLR4 (63-68) and others (TLR2, TLR3 and TLR9). TLR2, TLR3, TLR4 and TLR7 are all strongly upregulated in rheumatoid compared to healthy synovial tissue (69-73).

Other pathways of NF-κB activation in RA may include cellular stimuli such as cytokine activated T cells (Tcs) that resemble T cells enriched from RA synovial tissue (74). Tcs can induce cytokine production from monocytes in a contact- and IκBα- dependent manner. Cells that remain in synovial culture after T cell removal rapidly lose their spontaneous TNFα production suggesting that the contact-dependent ("cognate") stimulation is driven by NF-κB (74, 75).
Disparity in NF-κB responses between humans and mice, primary cells and transformed cell lines

A key outcome of the hundreds of papers that are published annually concerning the NF-κB pathway is that different stimuli result in different responses, and that these are tuned to precise physiological outcomes required for that stimulus. For instance, viral infection results in a different spectrum of cytokines to bacteria, in line with the characteristics of these different types of infections. In addition, different cell types can also respond differently to the same stimuli, in line with their physiological role. These differences are a reflection of evolutionary pressure and this could explain the observed differences between the human and murine immune systems. Since the lifespan of humans is far longer than of mice, our lifetime exposure to infection is far greater and our immune systems have evolved to cope with this. One example of this phenomenon is that whereas, in mice, TLR3 stimulation results in the activation of NF-κB and the production of NF-κB-responsive cytokines, this does not occur in primary human myeloid-lineage cells (Lundberg et al, manuscript submitted).

In the field of inflammation, many laboratories have studied the NF-κB pathway in primary murine cells (immune cells or embryonic fibroblasts), in human or murine transformed immune cell lines or even using overexpression in non-disease relevant transformed cell lines. The former has obviously made very important contributions to our knowledge of NF-κB, by facilitating genetic experiments, such as gene knockouts. However, it is also now well recognised that cell lines and primary cells show very significant differences in the signalling pathways in response to inflammatory cytokines and bacterial products. In many respects, this is not surprising since proliferation is inherent to the cells lines, and macrophages do not divide. This is exemplified by a microarray analysis study, in which the expression of many different genes was examined in concert. The differential expression of genes that are expressed in response to LPS were assessed in primary human PBMC-derived macrophages, the monocytic leukaemia cell line THP-1 and the histiocytic lymphoma cell line U-937. Many differences were observed between the primary cells and the cell lines, in particular with the U-937 cells (76). Other studies have shown specific differences in the activity of certain molecules, such as contribution of NIK to the canonical NF-κB pathway. Early results in transformed cells suggested that NIK was involved (77), but later studies in NIK knockout and aly/aly mice, as well as primary human cells disproved this (46, 78, 79). Such possibilities must be borne in mind when considering any data obtained in transformed cell lines.

Potential therapeutic targets in the NF-κB pathway

The weight of evidence gathered from RA patients suggests NF-κB activation is intimately involved in the chronic inflammation of the RA synovium. Consequently, inhibitors of NF-κB activation are potential candidates as therapeutics for RA. The success of clinical trials for biological treatments that directly target the products of NF-κB driven genes, such as TNFα, IL-6 and IL-1 has been a major breakthrough in the treatment of RA patients that do not respond to standard treatment (80). However, these treatments are expensive and are injected rather than orally administered. There is therefore much interest in developing specific, small molecular inhibitors that can be given in tablet form in order to reduce NF-κB activation in RA tissue, leading to attenuated cytokine production.
The canonical IKKs are considered to be amongst the most promising targets in the NF-κB pathway since they are not known to participate in any pathways other than NF-κB activation. The major drive by the pharmaceutical industry has been to try to develop small molecular inhibitors of IKK2 because it is considered to be the functionally important kinase and it also plays a pivotal role in transmitting various different stimuli into an NF-κB response. Most of the inhibitors identified can inhibit both IKK1 and IKK2, often inhibiting IKK2 in the nanomolar range and IKK1 in the micromolar range. These inhibitors are considered to be selective for IKK2 at therapeutic doses. In contrast, no specific inhibitors of IKK1 have been described. The IKK2 inhibitors that have been in development were recently reviewed in (81, 82), but this is potentially a lucrative area of research and new inhibitors are regularly discovered (83-85). Good candidates under development include SPC-839 and SC-514, (ATP-competitive inhibitors), BMS-345541 (non-competitive inhibitor), TPCA-1 and ML120B (81, 82, 86). These have all shown success in in vitro models of NF-κB activation as well as different animal models including endotoxin challenge in rats (SPC-839, SC-514) and CIA (BMS-345541, TPCA-1 and ML120B), although BMS-345541 was only effective if administered before the onset of disease (87). Indeed, using in vivo imaging, ML120B was recently shown to suppress NF-κB and active proteases in live mice with CIA (88).

One potential drawback of the small molecule inhibitor approach is caused by the way that they are tested for target specificity. New inhibitors are routinely tested against large panels of kinases and are considered to be specific if they do not ‘hit’ any other tested kinase. However, the kinome is an order of magnitude larger than these panels, and using proteomic approaches established inhibitors have now been found to be far less specific than thought [for instance (89)]. Biological approaches would circumvent this problem, and these are also being pursued to inhibit the action of IKK2. A gene therapy approach resulting in the expression of dnIKK2 in diseased cells should dampen the NF-κB response. This is attractive because a strategy of intra-articular injection of dnIKK2 expressing adeno-associated virus (AAV) would result in NF-κB inhibition only in the affected joints, rather than a systemic reduction in NF-κB. Preliminary data from a rat model of adjuvant induced arthritis suggests this approach may be viable (90).

Whilst much effort is being exerted to develop IKK2 inhibitors, murine genetic models of IKK complex inactivation give cause for concern regarding their long-term use in patients with RA. In the conditional knockouts, where NF-κB activation was prevented in keratinocytes or epithelial cells, a functional NF-κB system in macrophages resulted in TNFα production, causing uncontrolled inflammation. It will be important to guarantee effective shut down of macrophages in patients receiving IKK2 inhibitors, to ensure that the risk of accompanying inflammatory disease in other organs is avoided. Given that NF-κB activation in primary human M-CSF derived macrophages is only IKK2-dependent in response to IL-1β and TNFα but not LPS (42), this is may prove to be a stumbling block for these therapies.

**Conclusion**

It is now clear that NF-κB is a key player in the pathogenesis of RA, and is central to the production of pro-inflammatory mediators in the inflamed synovium. However, whilst much is known about the signalling
pathways that result in NF-κB activation in transformed cells and in mice, these events often differ in the cells that are relevant to RA, such as primary human myeloid cells and cells in the synovium. These events are only now being fully explored, using new technologies such as adenoviral infection. Cutting edge technologies, such as small inhibiting (si) RNA, will doubtless also give great insights into the functional roles of these proteins in the future. This will be important to help identify new therapeutic targets for the treatment of RA and validate those therapies already under development. The exquisitely specific NF-κB response induced by different stimuli in different cells gives hope that treatments can be developed to specifically target NF-κB activation in the inflamed synovium without detrimental effects on the innate immune system. This could overcome potentially serious problems that may occur as a result of long-term NF-κB inactivation.

**Acknowledgements**

The authors thank Dr Theresa Page and Stefan Drexler for critical reading of the manuscript.
References


Table 1. The NF-κB of transcription factors

<table>
<thead>
<tr>
<th>Protein</th>
<th>Human gene</th>
<th>Function</th>
</tr>
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| p105/p50 | NFKB1 | Canonical pathway.  
p105 constitutively processed to p50  
p50/p65 form activating heterodimers  
p50/p50 form inhibitory homodimers  
Found in RA synovium |
| p100/p52 | NFKB2 | Non-canonical pathway  
p100 processing to p52 tightly regulated |
| p65 | RELA | Canonical pathway.  
p50/p65 activating heterodimers  
Contains TAD  
Found in RA synovium |
| RelB | RELB | Contains TAD |
| c-Rel | REL | Contains TAD |

Abbreviations: RA, rheumatoid arthritis; TAD, transactivation domain. Collated from (3, 4, 31, 44, 58)
### Table 2. The IkB family

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mode of action</th>
<th>Model tested</th>
<th>Ref</th>
</tr>
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<tbody>
<tr>
<td>IkBα</td>
<td>Inhibitor of NF-κB in the canonical pathway&lt;br&gt;Rapid resynthesis following degradation</td>
<td>Many, including primary human cells</td>
<td>(4)</td>
</tr>
<tr>
<td>IkBβ</td>
<td>Inhibitor of NF-κB in the canonical pathway&lt;br&gt;Prolonged NF-κB activation</td>
<td></td>
<td>(4)</td>
</tr>
<tr>
<td>IkBδ (p100)</td>
<td>Inhibitor of RelB in the non-canonical pathway</td>
<td>IkBα, β and ε knockout MEFs</td>
<td>(45)</td>
</tr>
<tr>
<td>IkBε</td>
<td>Inhibitor of NF-κB in the canonical pathway&lt;br&gt;Negative regulation of the NF-κB response</td>
<td>IkBε knockout MEFs</td>
<td>(25)</td>
</tr>
<tr>
<td>Bcl-3</td>
<td>Transcriptional co-repressor, specifically effecting TNFα</td>
<td>Overexpression in RAW264.7 cells&lt;br&gt;Bcl-3 knockout mφ</td>
<td>(52, 53)</td>
</tr>
<tr>
<td>IkBNS</td>
<td>Transcriptional co-repressor, specifically effecting IL-6</td>
<td>Primary murine lamia propria mφ&lt;br&gt;RAW264.7 cells&lt;br&gt;IkBNS knockout mφ</td>
<td>(50) (50) (51)</td>
</tr>
<tr>
<td>IkBζ</td>
<td>Transactivation of IL-6&lt;br&gt;Inhibitor of NF-κB activity</td>
<td>IkBζ knockout mφ</td>
<td>(57)</td>
</tr>
</tbody>
</table>

Abbreviations: MEF, murine embryonic fibroblast; mφ, macrophage, RAW264.7 cells, a murine macrophage-like cell line.
Figure 1. The role of NF-κB in rheumatoid arthritis.

NF-κB is known to be activated in the pathogenesis of RA, and is central to the chronic cycle of inflammation that underlies its pathology. The triggering molecule that starts this process is unknown, but could include molecules on the surface of T cells (T cell receptors, TCRs), endogenous or exogenous ligands for the toll-like receptor family (TLR ligands), or other unknown molecules (?, represented at the top of the figure). These activate resident macrophages in the synovium, leading to phosphorylation of the inhibitor of κB (IκB) by the IκB kinase (IKK) complex that targets it for degradation by the proteasome. This releases NF-κB dimers such as p50/p65 that induce the expression of many pro-inflammatory cytokines and chemokines and leads to inflammation and an infiltration of large numbers of immune cells into the synovium. The inflammatory mediators, particularly TNFα, activate cells in the synovium in an autocrine (macrophages, Mφ) and paracrine (fibroblast-like synoviocytes, FLS) manner, and this is also largely NF-κB-dependent. FLSs synthesise many NF-κB-induced genes in response to TNFα or IL-1, including chemokines that lead to further inflammatory infiltrates, and matrix metalloproteinases (MMPs) that promote joint destruction.