

Review

NF- κ B signaling and inflammation – drug repurposing to treat inflammatory disorders?

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Simple Summary: Since its first description 35 years ago, the transcription factor NF- κ B (Nuclear Factor κ -light-chain-enhancer of activated B-cells) has been shown to be a key mediator of immune cell responses to inflammatory mediators, oxidative stress and genotoxic injury. Dysregulated NF- κ B signalling drives inflammation in inflammatory disorders such as multiple sclerosis, rheumatoid arthritis or inflammatory bowel disease. Thus, re-establishing the appropriate regulation of NF- κ B activity seems like a promising approach to treat inflammatory diseases. Current anti-inflammatory drugs have many- often serious- side effects. Thus, there is an unmet clinical need for safe and effective anti-inflammatory medicines that both decrease inflammatory mediator production and enhance endogenous anti-inflammatory and pro-repair pathways. So far, traditional *de novo* drug discovery has fallen short of satisfying this need. Drug repurposing is a cost- and time-effective alternative to *de-novo* drug development for the identification of novel and has already resulted in the identification of effective anti-inflammatories in the ongoing COVID-19 pandemic. In this review we critically review NF- κ B as a potential target for the development of anti-inflammatory drugs with an emphasis on drug repurposing as a strategy to identify new approaches to treat inflammatory diseases.

Abstract: NF- κ B is a central mediator of inflammation, response to DNA damage and oxidative stress. As a result of its central role in so many important cellular processes, NF- κ B dysregulation has been implicated in the pathology of important human diseases. NF- κ B activation causes inappropriate inflammatory responses in diseases including rheumatoid arthritis (RA) and multiple sclerosis (MS). Thus, modulation of NF- κ B signaling is being widely investigated as an approach to treat chronic inflammatory diseases, autoimmunity and cancer. The emergence of COVID-19 in late 2019, the subsequent pandemic and the huge clinical burden of patients with life-threatening SARS-CoV-2 pneumonia led to a massive scramble to repurpose existing medicines to treat lung inflammation in a wide range of healthcare systems. These efforts continue and these efforts continue to be controversial. Drug repurposing strategies are a promising alternative to *de-novo* drug development, as they minimize drug development timelines and reduce the risk of failure due to unexpected side effects. Different experimental approaches have been applied to identify existing medicines which inhibit NF- κ B that could be repurposed as anti-inflammatory drugs.

Keywords: Inflammation; NF- κ B; drug repurposing; drug development; autoimmunity; COVID-19; multiple sclerosis; rheumatoid arthritis

NF-κB signaling in inflammation

A brief history of NF-κB signaling in inflammatory diseases

The transcription factor NF-κB (Nuclear Factor κ-light-chain-enhancer of activated B-cells) is named for its 1986 discovery in B cells, in which it was found to bind to the enhancer element of the κ-IgG chain gene [1]. In a broader context, NF-κB is expressed in almost all cell types [2] and is involved in essential cellular processes like apoptosis and cell cycle progression [3]. In immune cells, NF-κB is key in the response of innate cells to viral or bacterial antigens and other stimuli such as cytokines during inflammation [4]. Despite its name, NF-κB signaling is an important regulator of the transcription of genes such as cytokines, chemokines or interferon-stimulated genes (ISGs) in innate immune cells [5]. As a result of its central role in many cellular processes, NF-κB dysregulation has been implicated in the pathology of numerous diseases. In several cancer types, NF-κB is constitutively activated, resulting in unregulated proliferation, thus making it an important therapeutic target in many cancers, such as breast cancer, lung cancer, gastric and colorectal cancer as well as hematologic malignancies [2, 6-11]. As a central mediator of inflammation, NF-κB activity causes inappropriate inflammatory responses in rheumatoid arthritis (RA), inflammatory bowel disease (IBD), multiple sclerosis (MS) and atherosclerosis [12, 13]. Thus, modulation of NF-κB signaling is being widely investigated as an approach to treat such diseases.

NF-κB signaling in inflammatory diseases

In mammals, the NF-κB family consists of the five structurally related transcription factors p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), c-Rel and RelB [14, 15]. There are three distinct pathways through which NF-κB signaling can occur: the canonical (or classical) pathway, the non-canonical (also non-classical or alternative) pathway and the atypical signaling pathway [16], and these are classified by their different activating mechanisms (see Table 1).

Table 1. Stimuli and receptors triggering NF-κB activation

Stimulus	Receptor	NF-κB pathway
LPS	TLR4	Canonical
TNF-α	TNFR1	Canonical
IL-1	IL-1R	Canonical
BAFF	BAFFR	Non-canonical
CD40L	CD40	Non-canonical
RANKL	RANK	Non-canonical
LTβ	LTβR	Non-canonical
TNF	TNFR2	Canonical/Non-canonical
TWEAK	Fn14	Canonical/Non-canonical
EGF	EGFR	Atypical
UV	CK2	Atypical

[3-5]

The canonical NF-κB pathway can be activated by diverse stimuli such as TNF-α, IL-1 or LPS (Fig.1) [16]. Upon recognition of these ligands by their receptor, the IKK2 complex, consisting of IKKβ and NEMO (NF-κB essential modulator), is phosphorylated [13, 20]. Subsequently, IκBα is phosphorylated [21-23], causing the ligation of ubiquitin chains to IκB, thereby tagging the inhibitor for proteasomal degradation [24]. Upon degradation of IκB, the nuclear localization sequences become unmasked and the p65:p50 heterodimer can translocate from the cytoplasm into the nucleus, where the transcription factor binds to

the promoter of primary response inflammatory genes including TNF or IL1 β and initiates their transcription [5, 16, 25, 26].

In contrast to the canonical pathway, the non-canonical pathway is I κ B-independent [8] and is activated by a subset of members of the TNF cytokine family [13, 27, 28] (Fig.1). Under normal conditions, NF- κ B inducing kinase (NIK) is constantly ubiquitinated and degraded. Upon ligand binding, NIK is stabilized and consequently phosphorylates and activates IKK α . IKK α phosphorylates NF- κ B subunit p100, which is subsequently ubiquitinated and cleaved to form p52 [29-32]. p52 proceeds to form a heterodimer with RelB, which translocates to the nucleus and binds DNA to induce transcription of target genes.

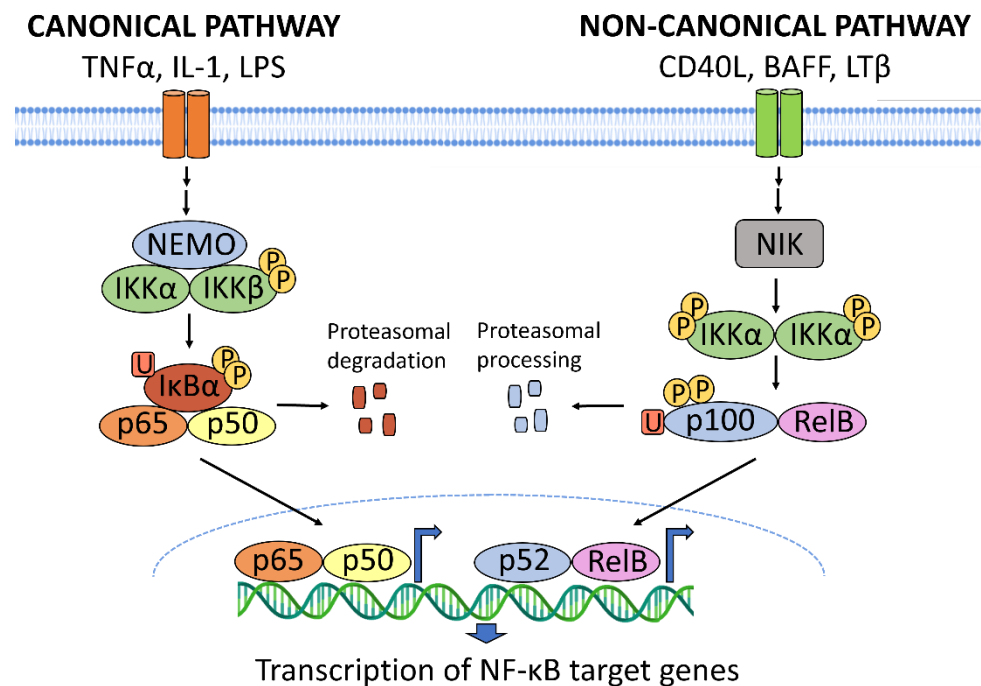


Figure 1. Activation of the canonical and non-canonical NF- κ B signaling pathway.

The canonical pathway is initiated by ligand binding to cytokine receptors such as TNF-receptor or the IL-1 receptor and results in the activation of the IKK complex, consisting of IKK α , IKK β and NEMO. This causes the phosphorylation (P) and the ubiquitination (U) of I κ B α , targeting it for degradation by the 26S proteasome. The NF- κ B heterodimer translocates to the nucleus, where it activates the transcription of NF- κ B target genes. Binding of ligands to a subset of TNF receptor family members, such as the CD40, BAFF or the LT β receptor, activates the non-canonical NF- κ B pathway. Following ubiquitination of TRAF2/3 by cIAP1/2 at the receptor and subsequent degradation, NIK is stabilized. Activated NIK accumulates and phosphorylates IKK α , which in turn phosphorylates p100, causing it to be proteolytically processed to p52. RelB and p52 form a heterodimer, which translocates to the nucleus to induce the transcription of target genes. This figure was generated summarizing experimental findings reviewed in [5, 13, 16].

Finally, atypical NF- κ B signaling pathways are those which cannot be classified into either canonical or non-canonical signaling. Although each pathway is unique to the stimulus, atypical signaling is largely induced by genotoxic stress, such as UV damage or exposure to ROS. This signaling pathway can also be activated by casein kinase 2 or tyrosine kinases such as EGFR [17, 33-36].

NF- κ B activity as a druggable target in inflammatory diseases

NF- κ B signaling can be modulated at different stages between receptor activation and the initiation of gene transcription [37]. Strategies for NF- κ B inhibition include targeting the receptors, receptor adaptor proteins (e.g., BTK, IRAK, PI3K/AKT or c-IAP), the IKK complex or the ubiquitin-protease system to prevent the degradation of RelB. Further,

interfering with nuclear translocation, DNA binding or the initiation of transcription of NF- κ B target genes are all attractive strategies to inhibit NF- κ B signaling (Fig.2) [37, 38].

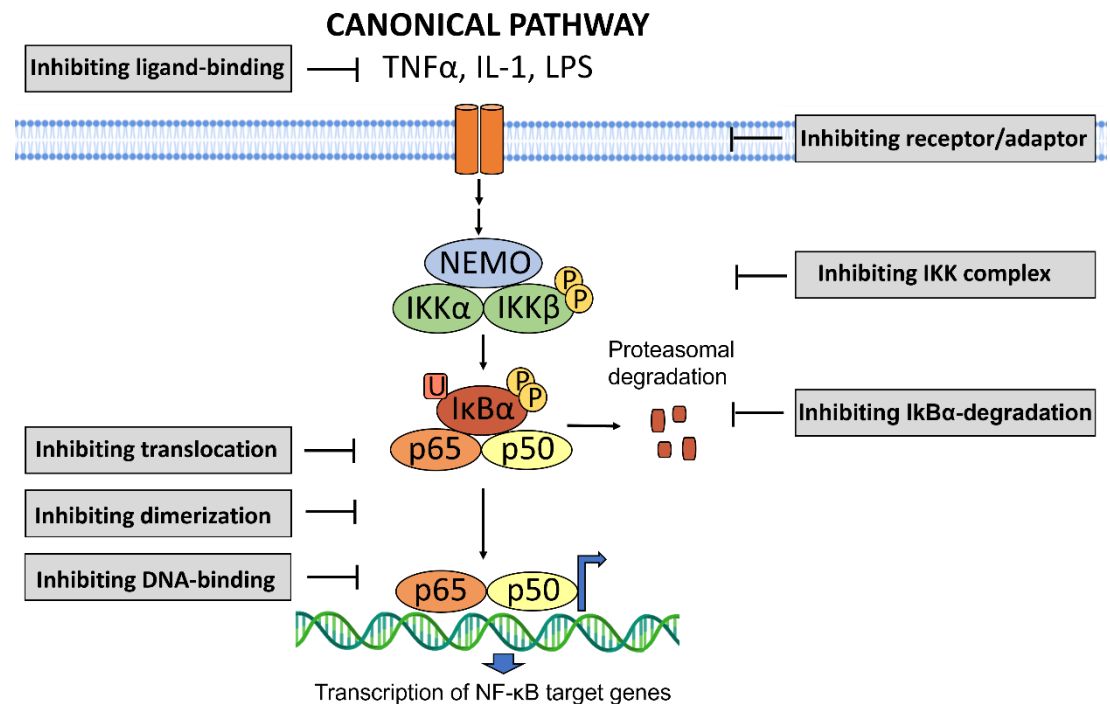


Figure 2. Strategies to inhibit NF- κ B signaling

NF- κ B signaling can be inhibited by preventing the activation of receptors triggering NF- κ B activation by using monoclonal antibodies or receptor antagonists. Targeting IKK α or IKK β inhibits I κ B α phosphorylation and ubiquitination. I κ B α can be targeted directly, which can increase its expression. By inhibiting the proteasome, inhibitors can prevent the degradation of I κ B α and the subsequent translocation of the p65/p50 dimer into the nucleus. Finally, inhibitors can interfere with nuclear translocation directly, as well as with DNA-binding or NF- κ B target gene transcription.

Given the importance of NF- κ B activity for the pathology of many human diseases, drug development efforts have identified a number of NF- κ B inhibitors [39, 40], which can be broadly categorized into recombinant proteins, peptides, natural products and synthetic compounds [38]. Despite hundreds of NF- κ B inhibitors having been reported to date, few have found clinical application [37]. Therefore, this review aims to investigate drug repurposing as an alternative strategy to identify novel NF- κ B inhibitors with anti-inflammatory properties.

Drug repurposing to identify NF- κ B inhibitors

Why do we need new anti-inflammatory drugs?

Many common anti-inflammatory drugs have potentially serious side effects [41, 42]. Disease-modifying-anti-rheumatic drugs (DMARDs) require close monitoring due to the increased risk of infection and hepatotoxicity. Glucocorticoid treatment often results in glucocorticoid resistance and therefore is limited for long-term treatment [43, 44]. TNF-blockers, a widely used intervention for autoimmune diseases like MS or RA, can exacerbate MS symptoms as well as the frequency and the severity of MS attacks [45, 46]. Further, RA patients treated with TNF inhibitors can develop demyelinating lesions in the CNS or MS [47]. Finally, current anti-inflammatory drugs have been selected to

reduce inflammatory mediator production and not necessarily selected for their ability to enhance tissue repair processes.

Drug repurposing (also referred to as drug repositioning, drug reprofiling or drug re-tasking) seeks to identify new uses for existing drugs or compounds outside the scope of their original indication [48]. Increasing costs of de novo drug discovery combined with long development timelines are major challenges in drug development. Bringing a new drug to the market has been estimated to take 15 years and to cost an average of US\$2 billion to US\$3 billion [49, 50]. In contrast, drug repurposing is estimated to cost only 10 % that of de novo drug development, and with an average timescale of 6.5 years, the process is much more time efficient [51]. Moreover, 90 % of drug candidates fail in clinical trials due to safety and efficacy concerns. Because of extensive safety testing in pre-clinical animal models and in clinical trials [52, 53], drug repurposing minimizes this risk of failure. Furthermore, drug repurposing offers the opportunity to rescue compounds that have undergone clinical testing and have good pharmacokinetic and safety profiles but have previously failed to achieve clinical approval due to lack of efficacy in their original indications.

Approaches to drug repurposing

Many approaches to drug repurposing exist, including biological, experimental or computational approaches as well as combined approaches.

Initially, drug repurposing occurred when medicines were observed to have consistent unexpected off-target side effects in patients. This was the case with sildenafil, a drug developed to treat angina [57], which has since been successfully marketed by Pfizer to combat erectile dysfunction. After growing evidence highlighted the benefits of sildenafil treatment of pulmonary hypertension, the drug received approval to be further re-purposed for the treatment of PAH [54]. Rare, serendipitous observations continue to be exploited but this strategy has become a less reliable approach to drug repurposing, leading systematic approaches to dominate in recent years. In the next section of this review different approaches to drug repurposing will be discussed and specific examples of how they have been utilized to target NF- κ B will be outlined.

Computer based drug repurposing strategies

Computational drug repurposing strategies are screening approaches that are capable of testing thousands of candidate compounds at a rapid rate. Typically, these screens investigate libraries of drugs that have chemical structures or molecular targets similar to those of drugs already known to be active in the desired context. Molecular docking can predict previously unreported interactions of existing drugs with therapeutically relevant targets. Alternatively, screens can be performed to identify diseases with shared molecular targets and thus shared treatment options. Many successful drug repurposing efforts combine drug- and disease-based approaches [55, 56].

A recent study identified thioridazinehydrochloride (TDZ) as a novel IKK β inhibitor from a panel of FDA approved drugs [57]. The drug repurposing strategy took a drug-based computational approach. Since de novo drug development has not resulted in the approval of any IKK β inhibitors [58-61], the study aimed to repurpose existing drugs as IKK β inhibitors by developing a computer-assisted structure-based drug repurposing strategy. A virtual screen using a subset of the ZINC database of FDA-approved drugs and a crystal structure of inhibitor-bound IKK β revealed thioridazinehydrochloride (TDZ) as a potential IKK β inhibitor. To validate the

repurposing approach, TDZ, a current Schizophrenia therapeutic, was tested both in vitro and in vivo. TDZ was shown to inhibit IKK β phosphorylation and I κ B α degradation, thereby inhibiting NF- κ B activity and resulting in the attenuation of inflammation in a mouse model of endotoxemia [57]. These findings validate the computer-aided drug repurposing approach to identify novel NF- κ B inhibitors with anti-inflammatory properties, which can be further investigated for clinical benefit in NF- κ B dependent inflammatory diseases [57].

Artificial intelligence-aided drug repurposing

Recently, drug repurposing strategies have been developed that use artificial intelligence (AI) to identify novel indications for existing drugs. For example, known drug-target interactions can be used to predict new interactions via an AI method called deepDTnet, which contains a heterogenous network of drugs, genes and diseases, including chemical, phenotypic and genomic data [62]. The method was trained using a library of FDA-approved small molecules and was shown to identify novel targets of a known drug using deep learning algorithms. This approach was successfully used to identify drugs that interact with ROR- γ t, which is linked to autoimmune diseases such as MS [62]. The authors identified the FDA-approved topoisomerase inhibitor topotecan as a promising repurposing candidate, which was validated in the EAE mouse model in vivo [62]. The network-based arbitrary-order proximity embedded deep forest approach (AOEDF) is based on deepDTnet and can accelerate target-based drug repurposing. Similar to deepDTnet, it integrates drug, disease and target data to identify new targets but seems more effective in predicting novel drug-target interactions [63].

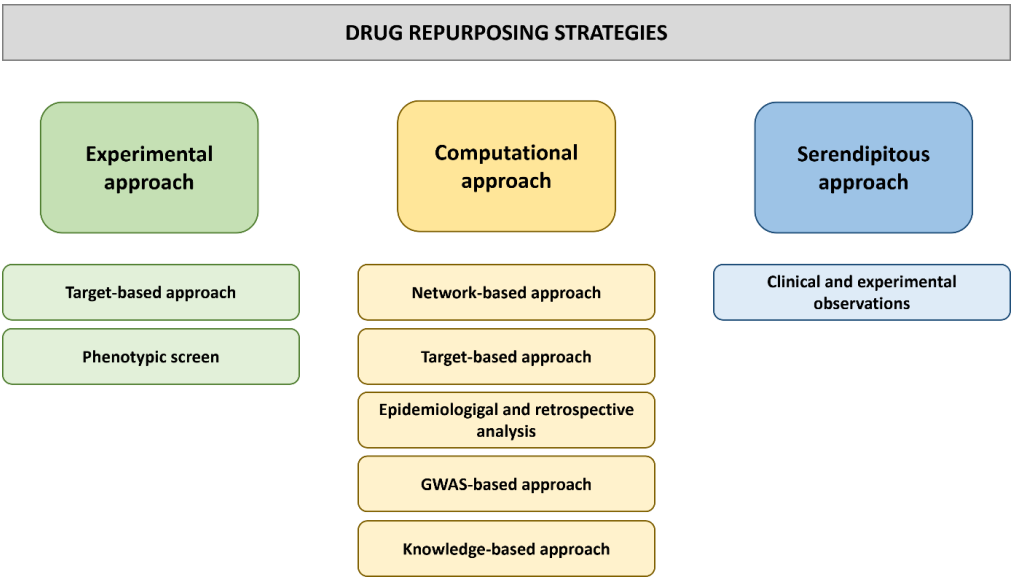


Figure 3. Drug repurposing approaches
Different experimental, computational and serendipitous strategies can be employed to identify promising drug repurposing candidates among existing drugs.

Experimental approaches to drug repurposing

Experiment-based drug repurposing approaches can be divided into target-based strategies and phenotypic screens. Target-based drug repurposing requires knowledge of the molecular target of candidate drugs, whereas phenotypic screens do not rely on extensive scientific knowledge of the mode of action of a drug or the molecular pathology of a disease [64-67].

Target-based approaches to drug repurposing

Given the role that tyrosine kinases such as CSFR-1, KIT, Lck and DDR play in RA pathology, multiple studies have investigated the effect of tyrosine kinase inhibitors (TKIs) in models of arthritis [68-71]. Dasatinib, a second-generation TKI used to treat chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia [72, 73], has been identified as a promising new therapeutic option for the treatment of RA. In one study, Guo et al. [71] investigated the effect of dasatinib on RA pathology due to its similar target profile to other TKIs imatinib and nilotinib, which were previously found to be effective in collagen induced arthritis (CIA) animal models [74, 75]. Dasatinib reduced disease severity by attenuating the production of pro-inflammatory cytokines IL-1 β , TNF- α and IL-6 in mice with CIA, while increasing anti-inflammatory IL-10 [71]. Moreover, dasatinib inhibited the migration and proliferation of human fibroblast-like synoviocytes (FLS), which in their activated state promote bone erosion based on their ability to secrete receptor activator of nuclear factor κ B ligand (RANKL), therefore inducing osteoclast differentiation and bone destruction [71]. These findings validate dasatinib as an anti-inflammatory drug in a pre-clinical model that has the potential to be repurposed as an RA treatment.

Phenotypic screening approaches to drug repurposing in cell lines and model organisms

Pre-clinical drug identification and development traditionally relies on cell-based assays to identify and optimize promising lead compounds. With nine out of ten drugs entering clinical trials failing to achieve FDA approval [76, 77], there is a need for reliable assays to test the safety and effectiveness of drugs in early drug development stages.

In order to identify FDA-approved drugs that promote remyelination in MS, Mei et al. developed a high-throughput functional screening assay using micropillar arrays, which allow for the detection and quantification of myelin wrapping [78]. The screen identified clemastine fumarate, a H1-antihistamine that is used to treat allergic reactions. Clemastine promotes oligodendrocyte precursor cell differentiation in animal models and human cells [78-80]. As only differentiated oligodendrocytes can produce myelin [81], this differentiation process induced by clemastine was linked to an increase in remyelination in a variety of animal models [79, 80, 82-85], which was confirmed to be specifically due to increased oligodendrocyte differentiation [80]. Furthermore, it inhibited the production of pro-inflammatory cytokines, microglial M1-like activation and astrocyte loss in mice with depression-like symptoms and a mouse model of ALS [86, 87]. Studies have linked the anti-inflammatory activity of clemastine to its ability to inhibit NF- κ B [88, 89]. A phase II clinical trial recently demonstrated the ability of clemastine to promote myelin repair in patients with relapsing MS [90].

Although many cell-based assays allow for high throughput screening, results obtained from in vitro testing on human cells or tissues have limited reliability in terms of the effect of the drug on a whole organism. Therefore, automated, high-throughput, quantitative in vivo screens have been developed, with Danio zebrafish becoming an increasingly popular model organism [91] due to their increased throughput screening capacity in comparison to mice and the resemblance of their immune system to that of humans [70, 71]. Several zebrafish inflammation models have been developed, which have been successfully used to identify and study drugs with anti-inflammatory properties [91]. Hall et al. demonstrated the potential of using in vivo zebrafish neutrophil migration assay in screening for novel anti-inflammatories [90]. The assay, which assesses the recruitment of neutrophils to tail fin injury as a model of acute inflammation, was applied to identify previously unknown anti-inflammatory properties of approved drugs in a high-throughput screen. The anti-inflammatory activity of the ten most potent repurposing candidates was subsequently tested in a mouse model of atopic dermatitis, in which they potentially inhibited dermatitis-related inflammation [92].

Zebrafish embryos are also a useful model organism in drug development screens. Their innate immune system develops early in embryogenesis, and as early as 26 hours after fertilization, phagocytosis and ROS production can be detected in embryonic macrophages [93, 94]. Not until later stages of development does the adaptive immune system mature, therefore making it possible to study both arms of the immune response [95, 96].

Furthermore, in zebrafish (*Danio rerio*), the blood brain barrier (BBB) is not developed until 3-10 days post fertilization, with tight junctions forming after day 5. Therefore, drugs added into water can cross the BBB, allowing modulatory effects on zebrafish behavior to be studied [97]. In the first study investigating the behavioral profile of zebrafish, Rihel et al. were able to classify drugs in a high-throughput functional screen by analyzing the rest/wake cycle of fish [98]. For example, anti-inflammatory drugs including glucocorticoids and NSAIDs co-clustered by promoting a unique sleep/wake behavioral fingerprint. There is exciting potential to use behavioral fingerprint to identify anti-inflammatory activity of existing drugs.

NF- κ B as a potential target for drug development in CNS inflammation

Multiple sclerosis is an autoimmune disorder that causes chronic inflammation in the central nervous system (CNS). It is the most widespread disabling neurological disease in young adults and results in physical or cognitive disorders [99]. MS pathology is the result of immune cells infiltrating the CNS, releasing cytokines and other inflammatory mediators leading to the destruction of myelin sheath, the reduction of oligodendrocyte numbers and finally axon degeneration [100]. Canonical and non-canonical NF- κ B signaling play an important role in MS pathology in both innate and adaptive immune cells (Fig. 4). Genome wide association studies (GWAS) have correlated central components of the NF- κ B pathway with an increased risk of developing MS [101-103].

NF- κ B drives the expression of pro-inflammatory mediators, which induces the differentiation of naïve CD4⁺ T cells towards pro-inflammatory Th1 and Th17 cells and the differentiation towards regulatory T cells, as NF- κ B activity is required for both [102, 104, 105]. In addition, NF- κ B activity was observed to increase the expression of adhesion molecules, enabling infiltrating inflammatory T cells to cross the BBB [106-109]. Deficiency in central NF- κ B signaling components, such as NIK, p50, IKK2, RelA or c-Rel decrease T-cell differentiation or activation and protect from EAE (experimental autoimmune encephalitis) [110-116].

NF- κ B activation in macrophages or microglia in MS and EAE exacerbates inflammation by promoting the production of pro-inflammatory mediators. Consequently, additional macrophages/microglia are activated, further enhancing inflammation [117, 118].

In the CNS, NF- κ B signaling seems to have a pro-inflammatory effect. However, it was impossible to determine the contribution of the individual non-microglial CNS cell types to the pro-inflammatory function [119].

NF- κ B signaling is an important driver of the pro-inflammatory activity of astrocytes in EAE and promotes myelin loss in mice with cuprizone-induced inflammation [120-125]. In contrast to astrocytes, NF- κ B activation has a protective effect on oligodendrocytes in multiple models of MS. The overexpression of the NF- κ B repressor I κ B α in oligodendrocytes increased oligodendrocyte death and hypomyelination in mice expressing IFN- γ in the CNS and in the cuprizone model. NF- κ B inhibition also increased the susceptibility of the I κ B α Δ N mice to EAE [126]. Also, mice with a conditional knockout of RelB in oligodendrocytes display enhanced p65 NF- κ B activity and survival of mature oligodendrocytes, resulting in reduced EAE severity [127]. Similarly, NF- κ B seems to have a neuroprotective effect on neurons in EAE [128]. However, Lee and colleagues were unable to detect any effect of NF- κ B activity on neurodegeneration in EAE, as the conditional overexpression of I κ B α in neurons did not influence disease pathology [129].

In summary, NF- κ B activity in inflammatory T cells, macrophages and microglia as well as astrocytes has pro-inflammatory effects and aggravates MS and EAE pathology. However, some data also indicates that NF- κ B signaling might have an anti-inflammatory effect in oligodendrocytes and neurons and possibly in macrophages in some cases, which can protect against neurodegeneration. The role of NF- κ B in MS and EAE seems to be highly dependent on the specific cell type.

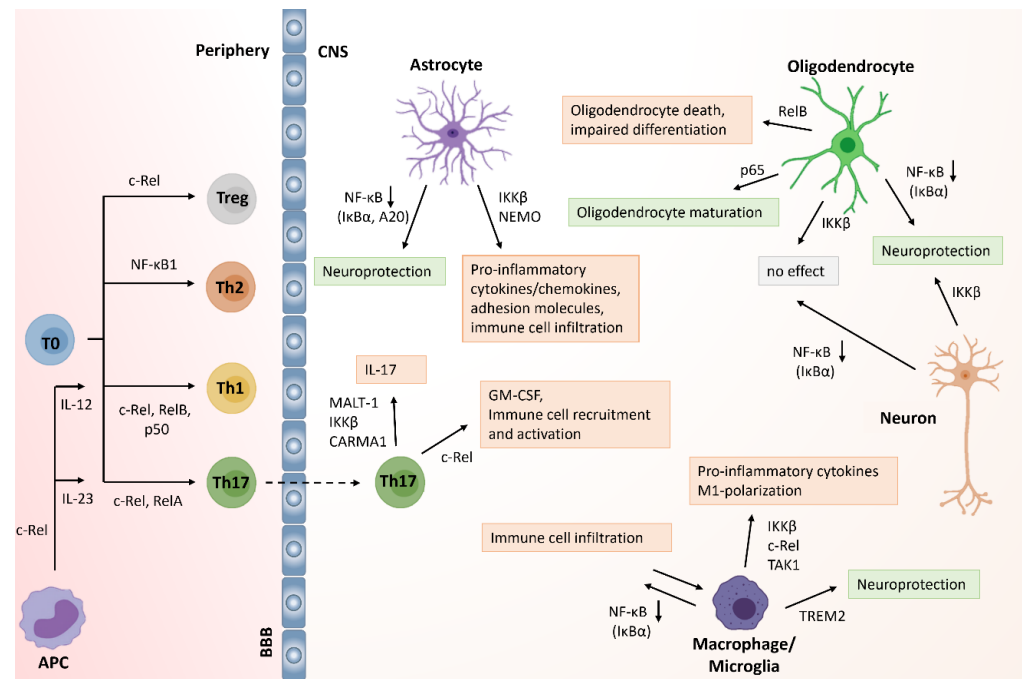


Figure 5. Cell-type specific role of NF-κB signaling in MS and EAE

NF-κB activity plays a key role in the development and progression of inflammation in MS and EAE. It causes Th17 cells, macrophages and microglia and astrocytes to produce an increased amount of pro-inflammatory cytokines, chemokines and adhesion molecules. Further, the transcription factor leads to the recruitment of more immune cells, thereby exacerbating neuroinflammation. Conversely, NF-κB activity can also have a neuroprotective effect in MS and EAE, depending on the cell type. Therefore, the role of NF-κB in MS strongly on the cell type, which needs to be considered when developing treatment strategies. Elements from this figure were adapted from [59, 130].

Repurposing NF-κB inhibitors to treat CNS inflammation

The anti-inflammatory effect of many FDA-approved drugs used to treat MS is thought to be linked- in part- to their ability to inhibit NF-κB signaling [117, 131]. These drugs were not developed as specific NF-κB inhibitors, however, they were found to interfere with NF-κB activation at different stages of the pathway. Therefore, finding approved drugs with the ability to inhibit NF-κB activity for drug repurposing in MS seems like a promising strategy to identify new treatment options (see Table 2).

Imatinib mesylate is a tyrosine kinase inhibitor targeting Bcr-Abl, first approved for the treatment of chronic myeloid leukemia (CML) in 2003. Since then, imatinib was shown to also be a potent inhibitor of NF-κB signaling and inflammation in vivo and in vitro [132, 133]. This effect was linked to a reduction of IκB phosphorylation as well as DNA binding of NF-κB in human myeloid cells [134]. Imatinib is currently being tested in a phase II clinical trial to compare the effects of the drug to those of standard treatment in patients with relapsing multiple sclerosis (Trial number NCT03674099).

While repurposing NF-κB inhibitors to treat MS seems like a promising strategy, certain limitations of this approach have to be considered: A variety of studies highlight the important role of NF-κB activity in the development and progression of MS and EAE. In immune cells, such as macrophages, microglia, T-cells, NF-κB signaling promotes the production of pro-inflammatory cytokines, which enhances inflammation and contributes to tissue damage and disease progression. However, the protective role of NF-κB activation in oligodendrocytes and neurons has also been demonstrated [114]. NF-κB plays different roles in different cell types in MS. Therefore, broad inhibition of

NF- κ B activity does not seem like an ideal therapy strategy for MS. Tight regulation of NF- κ B signaling in a cell-type specific manner will be necessary to avoid toxic side effects or the impairment of general immune function.

Table 2. Drug repurposing candidates for inflammatory diseases targeting NF- κ B signaling

Drug	Original implication	Effect on NF- κ B signaling	Effect on inflammation	References
Imatinib mesylate	Cancer (CML, ALL, GIST, HES, CEL)	Inhibits I κ B phosphorylation and DNA binding of NF- κ B	Attenuates inflammation and enhances BBB integrity in EAE, Phase II clinical trial for MS	[132-134]
Clemastine	Relief of allergy symptoms	Decreases NF- κ B activity and TLR4 expression	Promotes oligodendrocyte differentiation and re-myelination in EAE/MS, inhibits inflammation and microglial M1-like activation	[78-80, 82-85, 90]
Ibuprofen	Asthma, stroke	Inhibits NF- κ B activity (possibly by preventing nuclear translocation)	Reduces inflammation in rats with chronic cerebral reperfusion and MS patients, Phase II clinical trial for MS	[135-138]
Topotecan	Cancer (ovarian cancer, lung cancer, SCLC)	Inhibits IKK β and thus I κ B α degradation	Attenuates inflammation in EAE	[62]
Ibrutinib	Cancer (MCL, CLL, WM)	Inhibits NF- κ B nuclear translocation	Anti-inflammatory effects in models of RA, sepsis and diabetes	[139-142]
Bortezomib	Cancer (MM, MCL)	Proteasome inhibitor, prevents degradation of I κ B α	Anti-inflammatory effects in models of MS, RA, lupus erythematosus and colitis, promotes osteoblast activation and RA pathogenesis, Phase II clinical trial for RA	[143-148] [149, 150]
TDZ	Schizophrenia, psychosis	Inhibits IKK β phosphorylation and I κ B α degradation	Attenuates inflammation in endotoxemia model	[57]
Dasatinib	Cancer (CML, ALL)	Inhibits phosphorylation of IKK α , p65/p100/p105 and c-Rel	Inhibits inflammation and bone erosion in CIA and human FLS, increases IL-10 in CIA	[72, 151-153]
Dexamethasone	Inflammatory conditions (RA, asthma, allergies etc.)	Induces the expression of I κ B α	Reduces mortality in later-stage COVID-19 patients	[154-157]
Anakinra	Relief of RA symptoms	Prevents activation of IL-1R	Reduces hyper-inflammation and mortality and improves clinical signs of COVID-19	[158-163]

List of drug repurposing candidates targeting NF- κ B signaling for the treatment of MS (section 1), RA (section 2) and COVID-19 (section 3)

NF- κ B as a potential target for drug development in joint inflammation

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder, in which the lining of the synovial joints is degraded due to immune cell infiltration and inflammation [164, 165]. As the disease progresses, it results in the destruction of cartilage and bone, leading to disability. Systemic inflammation associated with RA can cause premature death, often due to cardiovascular disease, with 0.5 to 1 % of the population being affected in 2002 [164, 166]. There are currently no drugs available to cure RA. To decrease disease activity and treat joint stiffness and pain, non-steroidal anti-inflammatory drugs

and corticosteroids are prescribed. However, these treatments do not affect disease progression [164].

NF- κ B has been identified as a key player in RA in both humans and animal models (Fig. 4) [167-169]. Multiple studies have found increased NF- κ B activity in inflamed synovial tissue in human patients with RA [167-169]. More specifically, p50 and p65 were detected in CD14⁺ macrophages in synovial tissue from RA patients, which highlights the contribution of NF- κ B activation in macrophages and macrophage-derived cytokines to RA pathology [170].

In animal models of RA, NF- κ B is activated in the synovium [171]. NF- κ B inhibition reduces mediated pro-inflammatory gene transcription, resulting in the attenuation of inflammation [171-173]. Interestingly, NF- κ B activation can be detected before the onset of clinical symptoms. As the disease progresses, NF- κ B activity increases [174]. These findings demonstrate that inhibiting the NF- κ B pathway could be a promising target to treat RA.

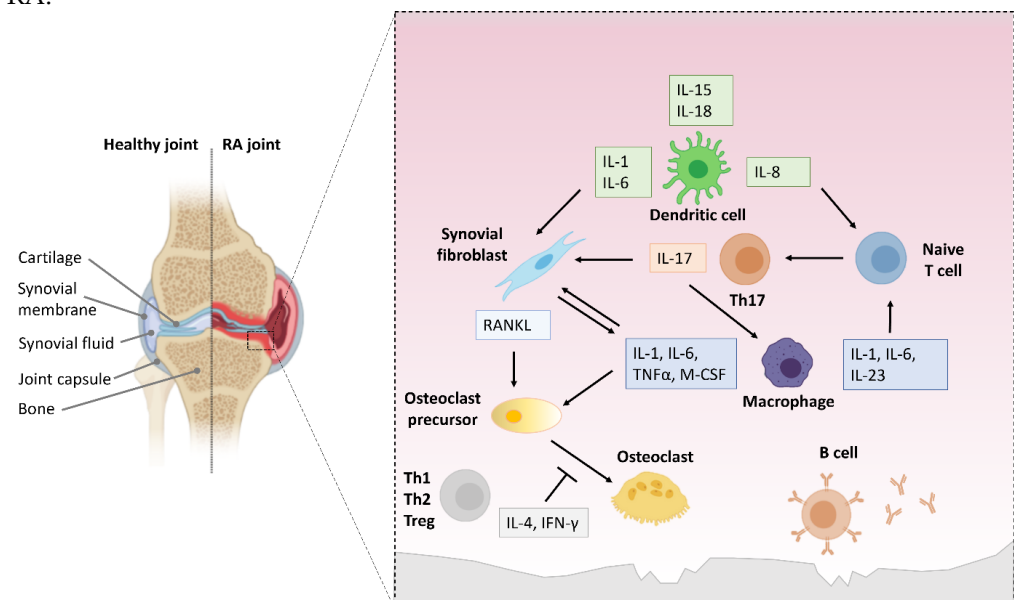


Figure 4. The role of NF- κ B activity in the pathology of RA

Bone destruction in RA is mediated by the interplay of macrophages, fibroblasts, DCs, B cells and infiltrating T cells in the synovium [175-177]. NF- κ B activation in those cells can trigger pro-inflammatory responses, exacerbating disease pathology. In the synovial membrane, NF- κ B activation causes pro-inflammatory T cells, macrophages and synovial fibroblasts to produce pro-inflammatory mediators, creating positive feedback loops. This results in the progression of inflammation and bone erosion. Arrows indicate the effect of cytokines/proteins up-regulated upon chronic NF- κ B activation on other cells, thus exacerbating bone destruction in RA. Elements of this figure were adapted from [176, 178] and summarize experimental findings from many studies.

Repurposing NF- κ B inhibitors to treat joint inflammation

Drug repurposing efforts have identified medicines that have anti-inflammatory effects in RA animal models by inhibiting NF- κ B signaling, making them promising candidates for further studies.

Bruton's tyrosine kinase (BTK) has emerged in recent years as a therapeutic target for the treatment of inflammatory disease. Originally discovered for its critical role in B cell development, and notable as the cause of the primary immunodeficiency X-linked

agammaglobulinemia (XLA), in which patients harbor a loss-of-function mutation, BTK is also highly expressed in monocytes, macrophages and neutrophils [179]. In these myeloid cells, BTK has been demonstrated to play a role in NF- κ B and NLRP3-inflammasome activation [179-182]. The BTK inhibitor ibrutinib demonstrates anti-inflammatory activity in pre-clinical models of RA [139, 140], sepsis [141] and diabetes [142].

An alternative approach to inhibit NF- κ B signaling is to target the proteasome using proteasome inhibitors (PIs). Bortezomib is a proteasome inhibitor that is clinically used to treat multiple myeloma [183]. The drug forms covalent adducts with the threonine residues in the active site of the proteasome and has proven to be an effective anti-inflammatory treatment in autoantibody-mediated immune disease models including MS, RA or colitis [143-147]. In addition to selectively destroying plasma cells in antibody-mediated autoimmune disorders, bortezomib also promotes the differentiation and activation of osteoblasts in multiple myeloma patients [183-186]. In patients with multiple myeloma and RA, bortezomib improved the condition of the joints [146, 148]. In addition, bortezomib prevents the release of cytokines induced by NF- κ B, and promotes apoptosis in T effector cells in RA patients [149].

Drug repurposing for targeting inflammation in COVID-19 pneumonia

The coronavirus disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2, created an urgent need for both novel anti-viral and anti-inflammatory drugs. In severe cases, SARS-CoV-2 induced pneumonia can result in life-threatening acute respiratory distress syndrome (ARDS) [187, 188]. The most prominent cause of death in COVID-19 patients is a hyper-inflammatory immune response characterized by production of pro-inflammatory cytokines, which causes tissue damage, mostly in the lung [188-190]. Severe COVID-19 was linked to hyper-activation of NF- κ B signaling, which causes the increased release of pro-inflammatory molecules like IL-1, IL-6, IL-12, IL-17, IFN- γ or TNF- α by infiltrating immune cells [188, 191, 192].

Several studies have shown that SARS-CoV infection triggers NF- κ B activation. The viral nucleocapsid protein causes dose-dependent activation of NF- κ B in SARS-CoV susceptible Vero E6 cells [193] and the nucleocapsid protein of SARS-CoV-2 was shown to recruit TAK1 and the IKK complex in HEK293T cells [194] to induce NF- κ B signaling. The spike protein of SARS-CoV was found to induce activation and translocation of NF- κ B in human PBMCs and THP-1 cells in vitro, which resulted in a dose-dependent increase in pro-inflammatory gene transcription. This effect was reversed by TPCK, a specific NF- κ B inhibitor [195]. In vivo studies confirmed that the inhibition of NF- κ B in SARS-CoV-infected mice with severe acute respiratory syndrome reduced inflammation and lung pathology and significantly increased survival rates [196].

During coronavirus infection, the NF- κ B pathway gets activated through viral pattern recognition receptors via MyD88, resulting in the induction of transcription of pro-inflammatory mediators [197]. Accordingly, in MyD88-/- mice infected with SARS-CoV, a reduction of infiltrating monocytes and macrophages in early disease stages was observed, along with the persisting absence of cytokine and chemokine production [198]. Further, another study demonstrated that the spike protein of the virus induces NF- κ B activation in a TLR2 and MyD88-dependent manner, resulting in the production of pro-inflammatory cytokines and chemokines by human and murine macrophages [199]. These results strongly support the notion that identifying NF- κ B inhibitors with anti-inflammatory properties could help mitigate hyperinflammation and attenuate disease severity in COVID-19 patients.

Dexamethasone, a glucocorticoid commonly used to treat inflammatory diseases such as RA, is known to inhibit NF- κ B signaling and the production of pro-inflammatory cytokines by promoting the overexpression of I κ B α [154, 155]. The RECOVERY trial found that dexamethasone reduced mortality in hospitalized COVID-19 patients in later but not earlier stages of the disease [156, 157]. The authors concluded that at later stages of the disease, hyperinflammatory events dominate, which may explain why dexamethasone is more effective in these patients [157]. At earlier stages of COVID-19, viral replication needs to be limited by an appropriate anti-viral immune response. Previous studies have shown that glucocorticoid treatment at early stages of the disease dampens the immune response and hence increases the risk of enhanced viral replication, this might explain why dexamethasone treatment might be a more attractive treatment option for patients with severe disease pathology [191, 200]. The impressive therapeutic benefits of dexamethasone in severe COVID-19 have been demonstrated extensively and the scientific literature suggests a strong link between its anti-inflammatory effects and its ability to inhibit NF- κ B signaling. However, its effect has not yet been exclusively linked to NF- κ B inhibition.

Due to their anti-inflammatory properties, other RA drugs and kinase inhibitors were considered as potential candidates to be repurposed for the treatment of COVID-19 [201, 202]. The IL-1 receptor antagonist anakinra reduces pro-inflammatory cytokine production by preventing NF- κ B activation and has been shown to be effective in treating patients who exhibit hyper-inflammation [158]. The effectiveness of anakinra to treat COVID-19 was tested in clinical trials. The drug reduced hyper-inflammation and improved clinical signs of COVID-19 as well as mortality rates [159-163]. These examples of successful drug repurposing demonstrate that this strategy is a time- and cost-efficient way to discover drugs with useful anti-inflammatory properties in a short period of time.

Problems with progressing repurposed drugs to clinical applications

The effectiveness and safety of drugs identified by repurposing still need to be carefully assessed before they can be approved for a new indication. A recent, well-publicized example of rushed approval of a drug repurposing candidate is the use of hydroxychloroquine (HCQ) in COVID-19 infected patients. HCQ is commonly used for the treatment of non-resistant malaria, RA and systemic lupus erythematosus. Its anti-inflammatory properties were attributed to the inhibition of NF- κ B signaling and NLRP3 inflammasome inhibition, reducing the production of pro-inflammatory cytokines and macrophage and neutrophil infiltration in animal models of renal ischemia/reperfusion injury and Immunoglobulin A nephropathy [203-205].

Early in the COVID pandemic, HCQ was suggested to have anti-inflammatory effects on SARS-CoV-2 infection in vitro [206, 207]. A small open-label non-randomized trial associated HCQ with lower viral load in patients hospitalized with COVID-19 [208], causing the FDA to issue an Early Use Authorization (EUA) [209, 210]. However, multiple studies subsequently showed that HCQ had no beneficial effect for COVID-19 patients or when used as pre- or post-exposure prophylaxis [211-222]. On the contrary, studies reported serious cardiac adverse events attributed to HCQ treatment [223, 224], which is of great concern as COVID-19 is associated with cardiac complications [225, 226]. Consequently, the FDA revoked the EUA [227]. The case of HCQ as treatment for COVID-19 demonstrates that extensive high-quality clinical trials, not just small, underpowered non-randomized studies or unreliable observational data, are necessary to investigate the safety and efficacy of a drug before it can receive approval to be repurposed for the treatment of a different disease.

Conclusions and future prospects

The NF- κ B pathway is a key player in many inflammatory diseases. Modulating NF- κ B activity is a promising target for the treatment of inflammation, as many FDA approved drugs or drugs currently in clinical trials inhibit NF- κ B signaling in addition to their originally identified mechanism. In this review, we have shown how different drug repurposing strategies can be used to identify new modes of action for existing drugs as well as indicate new applications for these drugs in inflammatory diseases linked to NF- κ B signaling.

The SARS-CoV-2 pandemic has highlighted the advantages and limitations of drug repurposing: while drug repurposing offers higher success rates and is more time- and cost efficient than de novo drug discovery, it is crucial to carefully assess candidate drugs in well-designed and sufficiently powered clinical trials before they receive approval for any new indication. Even though off-target effects of repurposed drug candidates may be well known, they still need to be carefully monitored, particularly when the molecular target for the new indication differs from that of the current indication.

Further, many NF- κ B inhibitors have been tested only in specific cell-types using a limited number of stimuli, such as LPS or TNF α . Also, concentrations used in many assays that were necessary to achieve sufficient NF- κ B inhibition are often higher than what would be feasible in vivo [228]. It will therefore be necessary to use more pathologically relevant stimuli and to carry out drug screening in whole organisms such as the zebrafish where possible.

Many components of the NF- κ B pathway overlap with other pathways, making the development of specific NF- κ B inhibitors a complex task. This problem could be overcome by using combinations of inhibitors targeting different steps in the NF- κ B pathway in low concentrations [228]. Further, the transcription of specific target genes could be modulated to achieve the desired specificity. One example is the nuclear modification of RelA: Phosphorylation, ubiquitination and acetylation at certain sites can modulate the transactivation activity of the transcription factor, influence its DNA binding ability and/or protein stability [229]. The specificity of NF- κ B signaling is further determined by the interaction of NF- κ B dimers with the DNA and promoters/enhancers [230]. Therefore, interfering with nuclear modification of NF- κ B could be a promising strategy to inhibit a specific set of NF- κ B target genes while minimizing the effect on other signaling pathways. However, NF- κ B has many essential physiological functions, which need to be preserved while its pathological effect is inhibited. Global NF- κ B suppression is associated with severe toxicities in animal models and humans [231-234].

In addition to the cell-type specific role of NF- κ B in diseases like MS, systemic NF- κ B inhibition may result in multiple unwanted side effects, especially if employed as a long-term treatment. Therefore, identifying NF- κ B inhibitors that predominantly target certain cell types over others might lead to a more favorable outcome in inflammatory diseases. Recently, the “sneaking ligand” (SL) approach was proposed for specific NF- κ B inhibition: These ligands consist of an N-terminal domain, which binds to the cell surface, a translocation domain and a C'-terminal effector peptide, which interacts with its cytoplasmic ligand to modulate NF- κ B signaling. This was validated in E-selectin-expressing endothelial cells, in which IKK complex assembly was inhibited in vitro and in vivo, resulting in the reduction of NF- κ B activity specifically in E-selectin expressing cells as well as the attenuation of experimental arthritis (STIA and AIA) in mice [235, 236].

NF- κ B mediates both pro- and anti-inflammatory effects in MS depending on the cell-type. As most currently used or repurposed NF- κ B inhibitors inhibit their target more systemically, their application may be more suitable to treat systemic inflammation (e.g., sepsis) or diseases in which NF- κ B inhibition is more clearly linked only to pro-inflammatory processes (e.g., COVID-19, RA).

Funding

Work in the laboratory of D.R.G. is supported by the British Heart Foundation (BHF) (RG/15/10/3/485). L.C. is the recipient of the BHF studentship (FS/18/63/34/84) and A.R. is supported by a Medical Research Council studentship (DTA MR/N013468/1).

Acknowledgements

We are grateful to the members of the Greaves Lab for their comments and support.

Conflicts of Interest:

The authors declare no conflict of interest.

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