NF-κB, a Potential Therapeutic Target for the Treatment of Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system (CNS) that afflicts over 2 million people worldwide. On the basis of the temporal course of disease, MS can be subdivided into three clinical groups: relapsing remitting MS (RR-MS), secondary progressive MS and primary progressive MS. There is a high degree of clinical diversity within these subgroups. The pathogenesis of MS in most patients is likely to result from autoreactive, activated $CD4^+$ T cells moving from the periphery across the blood brain barrier into the CNS. Most therapeutic agents used in MS (e.g. immunosuppressive and immunomodulatory drugs and cell cycle interruption drugs) are only used for RR-MS. These treatments show some efficiency in lessening the relapse rate in RR-MS and time to progression, but cannot cure MS. Thus, there is a need for new efficient treatments for all types of MS. An increasing number of studies indicate that nuclear factor-κB plays an important role in controlling expression of genes relevant to the pathogenesis of autoimmunity. Genetic factors related to NF-κB may also be determinants of MS susceptibility, as polymorphisms in the molecules involved in regulation of the NF-κB signal transduction pathway differ between RR-MS and progressive MS. Herein, the role of NF-κB in MS will be reviewed and its potential as a new therapeutic target in MS will be considered and compared with existing treatments.

Keywords: Multiple sclerosis, NF-κB, IκB, IKK, autoimmunity, therapy, therapeutic intervention.

1. MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disorder of the central nervous system (CNS) of unknown etiology [1] that affects about 0.2% of the world population [2]. Based on the cells that make up the inflammatory infiltrate in the CNS during MS, and on the study of experimental autoimmune encephalomyelitis (EAE), MS is considered to be an autoimmune disease mediated primarily by $CD4^+$ T cells [3, 4]. The pathological hallmarks of MS are demyelination and axonal damage. Demyelinated lesions can occur anywhere within the brain and spinal cord, leading to disease complexity and heterogeneity of clinical signs and symptoms. The latter can include problems with vision (e.g. optic neuritis, nystagmus), with motor (e.g. paresis, monoparesis, dysarthria) or sensory (e.g. paraesthesia, neuralgia, anaesthesia) function, coordination and balance (e.g. ataxia, intention tremor, vertigo), cognitive function (e.g. depression, dementia) and problems with bladder and bowel functions.

Clinical Course of MS

On the basis of the temporal course of disease, MS can be subdivided into three main clinical subgroups: relapsing–remitting MS (RR-MS), secondary progressive MS (SP-MS) and primary progressive MS (PP-MS) [5] (Table **1**). In RR-MS, which is the initial disease course in more than 80% of patients, exacerbation of neurological symptoms are followed by complete or incomplete remission [6]. RR-MS is twice as prevalent in females as in males. With time, about half of those patients who initially have RR-MS develop SP-MS, in which there is a continuous, irreversible neurological decline unassociated with relapses [6-8].

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Table 1. Differences Between RR-MS and Chronic Progressive MS

	RR-MS	SP-MS	PP-MS
Mean age of onset (years)	30	40	40
Female:male ratio	2-3:1	2-3:1	1:1
Presenting syndrome	Optic nerve (25%) Brainstem (20%) Spinal cord (45% - sensory more common than motor)		Spinal cord (80% - motor much more common than sensory) Brainstem and cerebellum (15%)
Brain lesions on MRI	Moderate	Moderate	Small
Gadolinium-enhancing lesions	Common	Common	Infrequent
Healthy looking white-matter injury	No	Mild	Prominent
Spinal cord atrophy [171]	Limited	Yes	Yes
Typical lesions [172,173]	Focal inflammatory primary demyelinating lesions with relative sparing of axons. Inflammatory infiltrate made up of T cells, B cells, macrophages, activated microglia.	Less active inflammation in focal white matter lesions. Diffuse axonal injury	Less active inflammation in focal white matter lesions. Diffuse axonal injury. Loss of oligodendrocytes.
Antibodies & complement activation [172-174]	Yes	Yes	Yes
Presence of B-cell follicle-like structures in meninges [15]	No	Yes	No
Cortical demyelination [175]	Mild	Mild to prominent	Prominent
Autoimmune reactivity to myelin antigens by blood and CSF cells [19,176,177]	Yes	Yes (but usually less than in RR-MS)	Limited

Ten to 15% of patients with MS have a primary progressive disease course from the onset. Clinically, PP-MS is defined as a disease course without any clinical attacks or remission from onset [6, 9]. The incidence of PP-MS is similar in females and males and PP-MS tends to appear at a later age than does RR-MS. Although at first sight PP-MS and SP-MS are quite different [10-12], these two clinical groups share some features [13-15] and it has been suggested that PP-MS is MS "amputated" from the usual preceding relapsing-remitting phase [16]. In addition to these three major clinical MS subgroups, a small number of patients initially present as PP-MS, but later develop neurologic exacerbations [17]. This pattern of disease is usually called progressive relapsing MS (PR-MS). However, this term has also been used for patients with SP-MS who have clinical exacerbations followed by incomplete remission [6].

Pathogenesis of MS

MS is generally considered to be an autoimmune disease directed against CNS myelin and the myelin-producing cells, the oligodendrocytes; however, as with many other human chronic autoimmune diseases, the primary cause of autoimmunity is unknown. Most studies in people with MS and from the animal model of MS, EAE, which shares some of the clinical and neuropathological features of MS, suggest that Th1 and Th17 $CD4^+$ cells are important in the initiation of episodes of demyelination in the relapsing-remitting phase of MS [3, 18-24]. Several studies have also proposed a role for $CD8^+$ T cells in the subsequent stages of

disease [25, 26]. The activity of these pathogenic T cells is regulated by special regulatory T cells (Treg), which have been reported to be reduced in number and dysfunctional in the peripheral blood of people with MS [27, 28], but increased in number in the CNS [29]. The progressive stage of MS is believed to be secondary to neurodegenerative changes triggered by inflammation. Additionally, there is increased formation of B-cell follicular-like structures in the CNS in progressive MS, strongly suggesting a prominent role for B cells and antibodies in the development of disease progression [15, 30].

A central player in the activation of T cells and other cells thought to be involved in the pathogenesis of MS, including B cells, dendritic cells (DC), macrophages, and CNS resident glia, is the transcription factor NF-κB. NF-κB is induced by a large number of extracellular signals involved in both innate and adaptive immune responses, and is an important mediator in activation of T cells and their expression of pro-inflammatory cytokines, production of antibody by B cells, cytokine production by DC and macrophages, and in the regulation of the susceptibility of these cells to apoptosis (reviewed in [31]). Gene expression profiling has shown similar increases in some genes controlling elements of the NF-κB pathway in both peripheral blood mononuclear cells (PBMC) from MS patients and in EAE models [32]. These properties suggest a role for the NF-κB pathway in MS pathogenesis and, thus, potential as a therapeutic target in MS.

2. NF-κB AND PATHWAYS OF ACTIVATION

NF-κB is a ubiquitous transcription factor that plays an important role in controlling gene expression in inflammation, immunity, cell proliferation and apoptosis [33, 34]. In mammals, NF-κB comprises a family of five protein subunits, p50, p52, RelA (p65), c-Rel, and Rel-B, which share an N-terminal 300 amino acid Rel homology domain that allows them to dimerize, translocate to the nucleus and bind to specific DNA sequences known as κB sites [31, 35] – thereby regulating the expression of a variety of genes [35-37]. Target gene specificity is determined by the specific NF-κB complexes present in different cell types, the κB target site binding specificities of different NF-κB complexes, and the particular protein-protein interactions and post-translational modifications that NF-κB complexes undergo in different contexts [38, 39].

In most resting cells, hetero- or homo- dimers of NF-κB subunits are retained in an inactive form in the cytoplasm through association with any of a family of inhibitory proteins, known collectively as IκB (IκBα, IκBβ, IκBγ, IκBε, BCL3, p100, p105 and IκBL), which mask the DNA binding domains [40]. The IκB proteins all contain an ankyrin repeat of 30-33 amino acids [41, 42]. The specific interaction between NF-κB subunits and IκB is mediated through binding of the ankyrin repeat and the Rel-homology domain of NF-κB. Exposure of cells to a variety of stimuli leads to the rapid phosphorylation, ubiquitination, and ultimately proteolytic degradation of IκB, which frees NF-κB to translocate to the nucleus and activate gene transcription. The phosphorylation of IκB is achieved through the action of the IκB kinase (IKK) complex.

Extracellular stimuli that can induce NF-κB activation include proinflammatory cytokines, bacteria and fungi and their products, viruses and viral products, eukaryotic parasites, physiological stress conditions, physical and oxidative stress, environmental factors, therapeutically used drugs, modified proteins, apoptotic mediators, mitogens, growth factors and hormones, and chemical agents [31, 35, 43, 44] (see http://www.nf-kb.org/inducers/ for a full listing), many of which have been implicated in the development of autoimmune diseases such as MS. Genes regulated by NF-κB include pro-inflammatory cytokines/chemokines and their modulators, inflammatory enzymes such as nitric oxide synthases and the matrix metalloproteinases, cell adhesion molecules, proteins involved in antigen presentation to T cells, acute phase proteins, stress-response genes, growth factors, and regulators of apoptosis [31]. In addition, a range of genes encoding extracellular stimuli of NF-κB activity can also be transcribed by activated NF-κB, resulting in rapid amplification of the NF-κB

pathways and perpetuation of inflammatory responses.

Two major NF-κB activating signal transduction pathways have been described [45], the classical and alternative pathways (Fig. **1**). The classical NF-κB pathway, also called the canonical pathway, is initiated by pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) binding to their specific receptor, leading to the sequential recruitment of various adaptors (for TNF-α signalling these include TNF-receptor-associated death domain protein, receptor-interacting protein and TNF-receptor-associated factor 2) to the cytoplasmic membrane [46]. This is followed by the recruitment (*via* interactions between NEMO (NF-κB essential modulator) and receptor-interacting protein) and activation of the classical IKK complex, which is composed of two catalytic subunits, IKKα and IKKβ, (as homo- or hetero-dimers) and a regulatory subunit known as NEMO or IKKγ. IKK then phosphorylates two N-terminal serines of IκB [47], causing the ubiquitination and degradation of IκBs through the 26S proteasome pathway [48, 49] and allowing NF-κB dimers to enter the nucleus, where they bind to sites in the promoter regions of target DNA elements that have the consensus sequence GGGRNNYYCC (R = purine, N = any base, and Y = pyrimidine) and transiently activate transcription of genes encoding proteins involved in immune or inflammatory responses and cell growth control [50]. The classical pathway usually involves NF-κB heterodimers of p50/p65, complexed to IκBα in unstimulated cells, with IKKβ mediating the phosphorylation-induced proteolysis of IκBα [51]. The NF-κB/IκB complex can shuttle between the cytoplasm and the nucleus [52], but because the export rate from nucleus to cytoplasm is higher than import from cytoplasm to nucleus, the complex is primarily cytoplasmic. The NF-κB/IκB complex in the nucleus cannot initialize gene transcription, as the DNA-binding domain is blocked by IκB. Classical pathway activation usually occurs within minutes of the initial stimulus.

In contrast, the alternative NF-κB pathway, also called the non-canonical pathway, is NEMO-independent and is initiated by cytokines such as lymphotoxin β [53], B-cell activating factor [54] or the CD40 ligand [55] and viruses such as Epstein Barr virus [56]. Activation of the alternative pathway is a much slower process than activation of the classical pathway, taking several hours. This pathway is thought to operate primarily in the immune system, playing a role in B-cell, T-cell and secondary lymphoid organ development [57]. Interaction of the stimuli with cell surface receptors leads to the activation of the NF-κB inducible kinase, which then phosphorylates IKKα to trigger the processing of p100, *via* interaction with the S9 subunit of the 19S proteasome, to mature p52. The p52 subunit can then dimerize with RelB, p65 or c-Rel. Once generated, p52/RelB dimers are free to move to the nucleus, whereas p52/p65 or p52/c-Rel dimers are first captured by IκBs and then activated through the classical pathway [57]. Recently, it has been found that IKKα accelerates both the turnover of p65 and its removal from pro-inflammatory gene promoters [58], suggesting that the alternative pathway plays a major role in resolution of the early inflammatory process and to the onset of tolerance to self [59, 60]. Thus, cross-regulation between the classical and alternative signaling pathways appears to be crucial in promoting an optimally protective response that is balanced between inflammation and tolerance [58].

Atypical NF-κB signaling pathways that are independent of IKK, but that still require the proteasome, have also been described [61-64]. In these atypical pathways, phosphorylation of IκBα residues other than those N-terminal serine residues phosphorylated by IKK occurs. For example, UV radiation induces IκBα degradation *via* the proteasome, but the targeted serine residues are located within a C-terminal cluster that is recognized by the p38-activated casein kinase 2. Oxidative stress leads to NF-κB activation *via* phosphorylation of the N-terminal tyrosine at residue 42, and the Syk protein tyrosine kinase is required for H_2O_2-mediated NF-κB activation.

In addition, constitutive activation of NF-κB pathways has been described in many situations, such as during embryonic development, the development of B cells, macrophages and cancer cells, in some cells in the CNS, and in some inflamma-

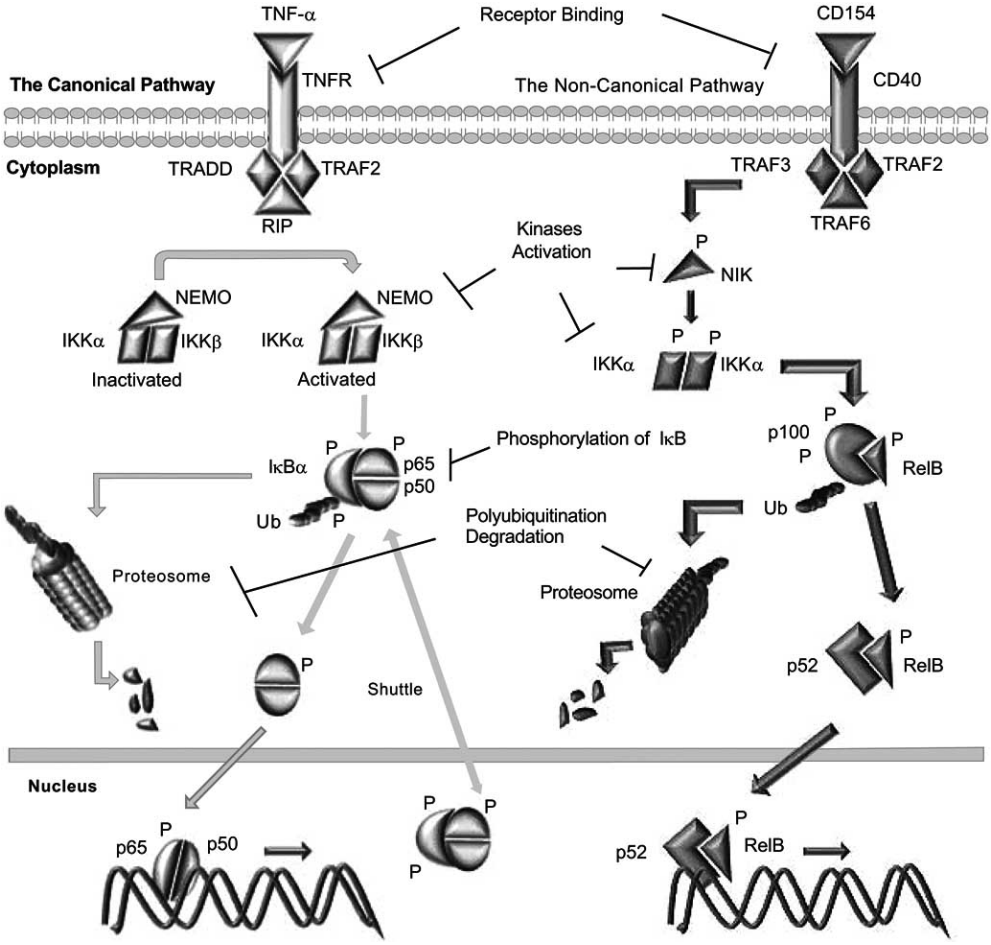

Fig. (1). NF-κB signal transduction pathways and potential target sites for inhibition of the pathways for treatment of MS. The classical pathway (on the left) is triggered by pro-inflammatory cytokines (e.g. TNF-α). Upon binding of the pro-inflammatory cytokine to the receptor, various adaptor proteins (TRAFs, RIP) are recruited to the membrane. Interactions between NEMO and RIP lead to recruitment and activation of the IKK complex. Activated IKK induces phosphorylation, ubiquitination and degradation of IκB-α, releasing the NFκB dimer to enter the nucleus, where it can bind with κB sites in the gene promoter region and initiate gene transcription. In the alternative pathway (on the right), TRAF is recruited following receptor engagement. This leads to activation of NIK, phosphorylation of IKKα, and phosphorylation, ubiquitination and cleavage of the C-terminal part of NF-κB p100 to generate p52. Dimers of p52 and RelB can then translocate to the nucleus to initiate gene transcription. Inhibition of NF-κB activation can be achieved at different stage in the pathways, as indicated by ⊥ symbols.

tory and autoimmune diseases [65, 66]. IκBβ appears to be a key element associated with constitutive activation of NF-κB in many different types of cells. IκBβ binds the same NF-κB dimers as does IκBα; however, IκBβ is only degraded after more sustained stimulation [65]. Degradation of IκBβ requires the phosphorylation of Ser19 and Ser23, whereas phosphorylation of Ser32 and Ser36 is required for degradation of IκBα. IκBβ also differs from IκBα in that it has six ankyrin repeats where IκBα has five. The specific number of ankyrin repeats in IκB has been proposed to play an important role in determining NF-κB subunit binding [66]. In addition, the gene encoding IκBα, but not IκBβ, contains a κB binding site, which allows it to be rapidly restored through NF-κB initiated transcription and in turn bind to NF-κB to quickly stop further activation. In contrast, IκBβ and IκBε are synthesized constitutively and re-establish NF-κB inhibition with a relatively slow time-course.

3. ROLE OF NF-κB IN CELLS TYPES INVOLVED IN MS

NF-κB plays unique and distinct functions in different types of cells. The fine regulation of NF-

κB activation depends on the type of cell, the stimulus applied to it, the adaptors recruited by the stimulus, the residues of IKK and IκBs that are phosphorylated or otherwise post-translationally modified, and the composition of the NF-κB dimers. Much remains to be understood regarding the exact mechanisms of NF-κB action in many cell types, and how modulation of NF-κB activation as a therapeutic modality might affect the body as a whole. Some of the mechanisms of NF-κB activation for cells involved in the development of MS are discussed below.

Lymphocytes

The NF-κB pathway fulfills diverse roles in the development, maturation, and homeostasis of lymphocytes, and has been extensively studied and recently reviewed [34, 67, 68]. Both classical and alternative NF-κB pathways play crucial roles in development of immature B and T cells and in the maintenance of mature lymphocytes. Both pathways are also required for the development of secondary lymphoid organs such as the spleen and lymph nodes [68]. Activation of NF-κB after recognition of antigen by the T or B cell receptors is critical for regulation of genes involved in pivotal mechanisms such as proliferation of activated lymphocytes, IL2 production, differentiation of Th cells to Th1, Th2 or Th17 phenotypes, antibody class switching, and survival. There is evidence that persistent lymphocyte NF-κB activation occurs in lesions of many chronic inflammatory diseases, including MS [43], which contributes to autocrine/par-acrine loops of inflammatory cytokines and growth factors capable of maintaining activation of non-immune cells within the lesions.

Much of what is known of the role of NF-κB in immune cells has come from studies using conditional knockout of components of the NF-κB pathway. B cells that lack IKKβ survive poorly [69] and do not make strong antibody responses upon antigen stimulation [70]. Additionally, studies of B cells from patients with X-linked anhidrotic ectodermal dysplasia with hyper-IgM syndrome, who have deficient expression of NEMO, have shown that the classical NF-κB pathway regulates V(D)J recombination in B cells, which is essential to produce an Ig repertoire with a large range of Ag specificities. The generation of memory B cells and somatic hypermutation were also markedly deficient in these cells, confirming a role for NF-κB in B cell maturation [71].

Deletion of IKKβ in T cells has shown that IKKβ is not absolutely required for survival of naive peripheral T cells but plays a major role in the generation of Treg, natural killer T (NKT) cells and memory T cells [72, 73]. This is of interest for MS, where Treg are decreased in their number and function [27]. In contrast, T cell-specific deletion of NEMO or replacement of endogenous IKKβ with a kinase-dead mutant is incompatible with mature T cell generation and/or persistence [72]. In general, pro-inflammatory T cell responses appear to be mediated through the classical NF-κB pathway. Much attention in MS and other autoimmune diseases recently has focused on the role of the pro-inflammatory IL-17-secreting Th17 cells. IL-17 has been shown to be a potent activator of the classical NF-κB pathway [74].

There is also much interest in the role of activation of the alternative NF-κB pathway in the generation of Treg. Several receptor pairs involved in T cell signaling are also responsible for regulating T cell activation through a process of "reverse signaling", whereby there is immediate feedback to the antigen-presenting cells upon their initiation of the forward signal in the T cell. Depending on the type of antigen-presenting cell and the particular receptor pair, this can result in activation of an immunoregulatory pathway of tryptophan catabolism, which is initiated by the enzyme indoleamine 2,3-dioxygenase (IDO), resulting in the expression of anti-inflammatory cytokines such as IL-10 by the antigen-presenting cell and generation of Treg [75]. Non-canonical NF-κB activation is necessary for the induction of IDO in response to reverse signaling, possibly due to the presence of a binding site for p52/p65 dimers in the promoter of the gene encoding IDO [76-78].

Dendritic Cells

Dendritic cells (DC) play an essential role in both induction of immune responses and the development of autoimmunity. Ligation of CD40 on DC induces early production of inflammatory media-

tors *via* canonical NF-κB signaling, as well as late expression of the anti-inflammatory enzyme IDO *via* the alternative pathway of NF-κB signaling [60]. In addition, alternative pathway NF-κB signaling down-regulates pro-inflammatory cytokine production in DCs and selective activation of this pathway results in non-inflammatory DCs that suppress T-cell activation and promote the development of Treg [60].

Macrophages

Macrophages are important inflammatory cells in the CNS during MS, and represent the most numerous cell type present in the active MS lesion. The NF-κB classical pathway can be robustly activated within macrophages by many stimuli, and contributes to the induction of genes responsible for the antigen processing and presentation abilities of the macrophages [79]. Conditional knockout of IKKβ in macrophages leads to dramatic decreases in the levels of IL6, IL12 and the chemokine CXCL1 [80, 81]. IKKα, on the other hand, negatively controls classical NF-κB signaling in macrophages and promotes the resolution of inflammation [58], suggesting that the alternative pathway may operate in a similar manner in macrophages as in DC.

CNS Cells

In the CNS, NF-κB subunits display a development and spatial regulation in their expression (review in [82, 83]). NF-κB appears to play a complex role in integrating signaling between and within neurons and glial cells (microglia and astrocytes) and there is relatively high constitutive activity of NF-κB in brain tissue compared with other tissues [84], particularly in areas of high metabolic activity, e.g. hippocampus and cortex [85]. This is thought to be due, at least in part, to the activation of the NF-κB pathway by stimulation through glutamate receptors. Traumatic or degenerative damage to the CNS induces numerous signaling events that stimulate NF-κB activity in all cells. In neurons undergoing excitotoxic- or age-related degeneration, NF-κB activation has been proposed to have a protective, anti-apoptotic role [84], and inactivation of the gene encoding IκBα results in increased susceptibility of neurons to oxidative, excitotoxic and metabolic insults. In contrast, focal or global ischaemia activates p50/p65 dimers and promotes cell death [86].

NF-κB activation controls microglial migration to sites of injury [87], where their effects can be either neuroprotective or destructive, depending on the stimulus, duration and threshold levels of the microglia. In normal appearing white matter, p65, but not c-Rel, p50 or IκB, has been found in the nuclei of microglia [88]. Activation of the NF-κB activity in microglial cells and astrocytes, such as occurs in MS, usually results in the production of proinflammatory cytokines and potentially neurotoxic mediators, such as reactive oxygen species and excitotoxins [82]. This is not unexpected, as microglia and astrocytes share many behaviors with macrophages.

Of particular relevance to MS is the finding that NF-κB, induced in oligodendrocytes by factors that are produced by non-activated microglia and which signal through a PDGFα receptor signaling pathway, has a pro-survival role and promotes maturation of oligodendrocyte progenitors [89]. There is conflicting evidence regarding the role of NF-κB activation in mature oligodendrocytes, with some studies showing that translocation of p50, p65 and c-Rel containing dimers mediates apoptotic cell death of mature oligodendrocytes [90], but others finding that NF-κB activation can prevent cell death. In MS the latter may be the case, since there is a paucity of oligodendrocyte apoptosis in active lesions [91].

4. NF-κB IN MS AND EAE

Several studies over the last 10 years have investigated the localization of NF-κB in MS brain tissue. In active MS plaques, p50, p65, c-Rel and IκB were all found in the nuclei of infiltrating macrophages, and p65 was also present in the nuclei of hypertrophic astrocytes [88, 91]. Activated p65 was also increased in the nuclei of some oligodendrocytes in active plaques, but not in oligodendrocytes in normal-appearing white matter, and no IκB could be detected in the nuclei of oligodendrocytes in any area [91]. Perivascular infiltrating lymphocytes in active plaques were reported to have c-Rel, but not p50 or p65, in the nuclei [88]. In addition, cDNA microarray analysis

Table 2. Current Therapeutic Agents Used in MS

Drug	Mode of Action	Effects
Corticosteroids	Immunosuppression	Decrease duration and severity of relapses in some patients. No long term impact [1,178]
Cyclophosphamide, azathioprine, cyclosporine, methotrexate, cladribine)	Immunosuppression/cell cycle interruption	Modest effects with associated toxicity
Beta interferon (Betaseron/Rebif/Avonex)	Immunomodulation Decreased blood-brain barrier permeability? [148] Induction of Treg? [179]	Reduce relapse rate in RR-MS. Modest effect in delaying disability. No effect in SP-MS or PP-MS. (Reviewed in [180])
Copaxone (Glatiramer acetate)	Immunomodulation (?altered peptide ligand)	Some effect on relapse rate and modest effect in delaying disability. No effect in SP-MS or PP-MS [181-184]
Mitoxantrone (Novantrone)	Cell cycle interruption	Short term effects in RR-MS and SP-MS, but cardiotoxicity limits use. Risk of secondary acute myelogenous leukemia [185-187]
Natulizumab (Tysabri – anti-VLA-4 antibody)	Blocks movement of T cells and other leukocytes (apart from neutrophils) into CNS	Good efficacy in RR-MS in reducing relapse rate. Not tested in SP-MS or PP-MS. Risk of progressive multifocal leukoencephalopathy in patients who carry JC virus [188-190]
Intravenous immunoglobulin	Immunomodulation	Limited effects in RR-MS. No effects in SP-MS [191,192]

of brain tissue showed that genes related to NF-κB are often upregulated in tissue from patients with MS compared to controls [92-99]; there is, however, a fair amount of variability in the individual reports, probably due to differences in the disease state and treatment history of the patients, the particular microarray platform used, and whether or not the tissue tested was from an area of active inflammation and demyelination. An excellent review of these microarray studies has recently been published [32] and thus they will not be considered here.

Other studies in humans have focused on the activation of NF-κB in PBMC from MS patients and healthy controls. These results have been somewhat conflicting, probably reflecting the small numbers of patients tested, differences in their clinical state at the time of testing, and the different NF-κB subunits assessed. Flores and colleagues [100] tested NF-κB activation in PBMC from 11 healthy controls and 10 untreated RR-MS patients in remission and did not find any differences between the groups. In contrast, Eggert and colleagues [101] found elevated levels of DNA-binding p50, but not p65, in a group consisting of 5 patients with RR-MS, 10 with SP-MS and 5 with PP-MS, compared to 24 healthy controls. Two studies have recently used microarray technology to investigate differences in gene expression in PBMC of healthy individuals and people with MS [102, 103]. One of these compared levels of gene expression in a group of 22 RR-MS patients undergoing a relapse with 20 RR-MS patients who were in remission [102]. They found 1,578 gene transcripts that significantly differentiated acute relapse from remission; these transcripts were enriched in genes relating to apoptotic-related pathways, including over-expression of p65 in PBMC from acute relapse. The other study [103] compared samples from 21 healthy controls and 24 patients with MS (mostly of the RR-MS type), and did not find significant upregulation of genes encoding NF-κB subunits, although there was increased expression of the TNF receptor in MS patients compared to controls. It was not specified whether MS patients in this later study were in relapse or remission.

A large number of studies have addressed the role of NF-κB activation in EAE [104-131]. Extrapolating findings from the EAE model to MS requires some caution, because data from differential gene expression studies suggest that, whereas EAE is an immunological disease that targets a healthy brain /spinal cord with very little intrinsic alteration of brain function, the pathogenetic processes in MS brain involve a target tissue that is itself functionally altered [32]. In addition, since EAE is usually induced by injection of myelin proteins or peptides in complete Freund's adjuvant, which is a potent activator of the canonical pathway of NF-κB, the results may be skewed somewhat in favor of this pathway. Generally, it has been found that p65 and p50, but not c-Rel, RelB or p52, are the prototypic inducible NF-κB subunits in the CNS during EAE [113,118,124]. This is supported by findings from van Loo and colleagues [132] who showed that ablation of NEMO or IKKβ, but not IKKα, in non-microglial CNS cells resulted in ablation of EAE, suggesting that canonical NF-κB activation in the CNS has a mainly pathogenic effect. In the periphery, c-Rel is critical for development of encephalitogenic T cells [114]. Many of these EAE studies have shown that drugs which decrease NF-κB activation can lead to protection against EAE [104, 107, 110, 116, 120-123, 126, 128, 129, 131-135]. This will be discussed further below.

5. NF-κB POLYMORPHISMS AND MS

The MHC class II region has been linked with susceptibility to MS, with carriage of HLA.

DRB1*1501 promoting an approximately 3- to 4- fold relative risk in most populations [136, 137]. The MHC region contains about 160 genes [138], including those encoding TNF and IκBL. As noted earlier, TNF-α is the prototypic cytokine for induction of the classical NF-κB pathway. TNF-α levels are increased in blood and cerebrospinal fluid from MS patients and in demyelinated plaques in the CNS. TNF-α also regulates myelin basic protein gene transcription through NF-κB in a human oligodendroglioma cell line [139]. However, TNF polymorphisms have not been found to associate with susceptibility to MS [140, 141].

IκBL is related to other IκB family members, although it only contains two complete and one partial ankyrin repeats, whereas other IκB proteins contain 6-7 [142]; however, the ankyrin repeats in IκBL are sufficient for interaction with members of the NFκB family. The IκBL protein is most similar to IκBα. The gene encoding IκBL (*IKBL*) is located in the central MHC region [143] and contains several structural polymorphisms. A polymorphism at position 738 in exon 4, which results in an amino acid change from cysteine to arginine in the IκBL, has been linked with predisposition to MS and is additionally associated with HLA DRB1*1501 [140, 144, 145]. It is thought that this polymorphism would affect phosphorylation of the IκBL, causing it to dissociate from NF-κB. Although significantly over-represented in RR-MS [145], thus far there have been no studies to test whether NF-κB activation is altered in RR-MS patients who carry this *IKBL* polymorphism. Polymorphisms in *IKBL* also influence the risk for development of systemic lupus erythematosus and Sjögren's syndrome [146]. Since systemic lupus erythematosus and Sjögren's syndrome are primarily antibody-mediated disorders, this raises the possibility that *IKBL* may play a role in regulating B cell responses.

Another polymorphism implicated in PP-MS is an 8 base insertion in the promoter region of *NFKBIA*, the gene encoding IκBα, that is located on chromosome 14q13 [145]. *NFKBIA* is highly polymorphic, but the difference in frequency between healthy controls and MS patients was a significant decrease in the frequency of the promoter region insertion in PP-MS compared to controls. The 8 base insertion in *NFKBIA* occurs in a region containing a binding site for heat shock protein, a molecule involved in immune regulation and innate immunity. The significance of the association of this polymorphism with PP-MS, but not RR-MS or SP-MS remains to be elucidated, but suggests possible differences in regulation of innate immunity in the different types of MS.

6. CURRENT THERAPEUTIC AGENTS IN MS AND THEIR EFFECTS ON THE NF-κB PATHWAY

Current therapeutic agents for MS mainly target autoimmune and inflammatory aspects of the disease and thus are designed using strategies of immunosuppression, immunomodulation, and cell cycle interruption. Commonly used agents and their mode(s) of action are summarized in Table **2**. Most of these pharmacological agents are only used for treatment of RR-MS, and while some have shown efficacy in lessening the relapse rate in RR-MS and time to progression, they cannot cure MS and there are few treatments available for PP-MS and SP-MS. Some of these agents are now thought to act by mechanisms that regulate the NF-κB pathway.

Beta interferon (IFN-β) is one of the most commonly used drugs in the treatment of MS. It has been proposed that IFN-β works by shifting the immune response in MS from a pro-inflammatory Th1 to anti-inflammatory Th2 type, and that inhibition of NF-κB activation is involved in this process [120, 147]. However, other studies suggest that IFN-β acts in a more complex way, actually increasing the pro-inflammatory responses in PBMC from people with MS [148], pointing to activation of NF-κB pathways. Treatment of human primary microglia cell cultures with IFN-β induces NF-κB inhibitor degradation and NF-κB activation in these cells, resulting in increased levels of production of chemokines such as RANTES and MIP-1β [149]. Activation of NF-κB induced by IFN-β is modulated by a protein, p202a, which belongs to the interferon-inducible p200 family and which inhibits sequence-specific binding to DNA [150]. Interestingly, there appear to be differences in response to IFN-β in females and males; it has been speculated that this might be due to the effects of the hormone estradiol on basal levels of NF-κB activation [151].

Glucocorticoids such as methylprednisolone, which is used for treatment of acute exacerbations in MS, act by binding to a cytosolic glucocorticoid receptor, which is subsequently activated and translocated to the nucleus. Once in the nucleus, the glucocorticoid receptor either binds to DNA and switches on the expression of anti-inflammatory genes or acts indirectly to repress the activity of a number of distinct signaling pathways such as NF-κB [152]. In one study, pulse therapy with methylprednisolone in MS patients decreased the level of activated p65 subunits in PBMC, leading to reduced levels of transcriptionally active pro-inflammatory NF-κB [101]. Some of the other immunosuppressive drugs that are less-frequently used in the treatment of MS, such as azathioprine, also suppress NF-κB indirectly. In human $CD4^+$ T cells, azathioprine inhibits binding of a protein known as Rac1 to GTP. This suppresses activation of NF-κB and other genes by Rac1, and leads to induction of T cell apoptosis [153].

The mechanism of action of glatiramer acetate is not certain, and several different proposals have been put forward, including that it acts in a similar manner to altered peptide ligands to modulate the T cell response by acting as a partial agonist. However, in one study, treatment of a human astrocyte cell line with glatiramer acetate led to decreased activation of NF-κB and reduced production of the chemokine RANTES [154], suggesting that it may exert indirect effects on the NF-κB pathway.

7. EXPERIMENTAL STUDIES ON NF-κB BLOCKERS FOR TREATMENT OF MS

Targeting NF-κB is an attractive therapeutic option for RR-MS, where inflammatory CNS infiltration correlates closely to relapses of the disease. Several strategies will need to be considered for inhibiting NF-κB activation in MS, in order to control the level of suppression. Generally, blocking early steps in the activation pathway will lead to a more non-specific type of suppression. Sites to target could include cell-surface receptors upstream of the NF-κB pathway, signal transduction that activates IKK, IκB degradation, NF-κB translocation, and interfering with the binding of NF-κB to DNA. Hundreds of molecules active at one or more of these sites have been identified [48]. A selection of molecules active at different steps in the NF-κB pathway (see Fig. **1**) and that have been investigated in EAE models are described below.

Most EAE studies show that activation of NF-κB occurs *via* the canonical pathway, and strategies that block binding of pro-inflammatory cytokines to their receptors, such as anti-TNFα antibodies and soluble TNF receptors, have been successfully trialed in EAE [155, 156]. While showing promise in EAE, anti-TNFα antibody trials in MS patients had the opposite effect and actually appeared to worsen the disease [157, 158].

Recently, much attention has been given to the use of the curcumin, a major constituent of the spice turmeric, in the treatment of a wide variety of diseases, including cancer, infection, and autoimmune disease. Curcumin appears to act by inhibiting activation of NIK, thereby blocking the non-canonical NF-κB pathway [159]. Treatment with curcumin every other day reportedly reduces the incidence of disease and mean clinical scores of mice that have been immunized to induce EAE [160].

Further down the NF-κB pathway, several studies have investigated molecules that inhibit activation of the IKK complex for their effects in EAE. Peptides of the NF-κB essential modifier-binding domain of IKKβ or IKKα have been used to specifically decrease NF-κB activation, without affecting basal NF-κB activity, and were strongly protective against the induction of EAE in mice [107]. In another study, the IKKβ inhibitory compound PS-1145 reduced neuroantigen-specific proliferation and cytokine production *in vitro* and diminished clinical signs of EAE *in vivo* [161].

Utilizing agents that prevent the degradation of IκB, Moreno and colleagues [122] found that methylthioadenosine prevented acute EAE and reversed chronic EAE by preventing IκBα degradation. In another study, Aktas and colleagues [104] used a component of green tea that blocks the catalytic activities of the 20S/26S proteasome complex, leading to the accumulation of IκBα and inhibition of NF-κB activation, to protect from relapses of EAE. Moreover, this molecule protected against injury induced by N-methyl-D-aspartate or TRAIL in living brain tissue and directly blocked the formation of neurotoxic reactive oxygen species in neurons. Vanderlugt and colleagues [162] showed that administration of the proteasome inhibitor PS-519 during the remission phase of EAE, following acute clinical disease, significantly reduced the incidence of clinical relapses, CNS histopathology, and T cell responses to both the initiating and relapse-associated neuroantigen epitopes. These authors found that continuous administration of PS-519 was required to prevent disease relapse: drug withdrawal led to recovery of T cell function and onset of disease relapses within 10-14 days.

Thymoquinone (black cumin/Roman coriander) has been reported to inhibit the activation of NF-κB specifically by suppressing the direct binding of nuclear p65 to DNA [163]. Thymoquinone, administered after the appearance of clinical signs in a rat model of EAE, prevented perivascular cuffing and infiltration of mononuclear cells into the brain and spinal cord and ameliorated clinical signs of disease [121].

Estrogens represent another potential treatment of MS that is thought to rely on inhibition of NF-κB activation. It is well known that many MS patients have a reduced frequency of relapses during pregnancy [164-167], due at least in part to altered levels of sex hormones such as estriol, estradiol and progesterone. Studies looking at the effects of administration of estriol point to inhibition of NF-κB as regulating T cell transmigration into the CNS and cytokine production [168]. The effects of estrogens during pregnancy may be mediated *via* induction of IDO in dendritic cells [169]. Given that IDO is thought to be a major regulator of the NF-κB pathway, it is likely that estrogens would have down-regulatory effects on immune cells, although their effects on cells of the CNS remain to be determined.

One caveat in studies on the effects of NF-κB inhibitors in EAE is that none of them have addressed possible changes in measures such as oligodendrocyte or neuronal cell apoptosis. These parameters need to be assessed in order to determine if positive effects of the inhibitors outweigh potentially damaging effects.

8. TARGETING NF-κB SIGNALING PATHWAY AS A THERAPEUTIC STRATEGY IN THE TREATMENT OF RELAPSING-REMITTING AND PROGRESSIVE MS

The therapeutic efficacy of conventional MS drugs is limited by the deleterious effects of global immunosuppression. In this context NF-κB inhibitors may represent an alternate therapeutic approach for MS; however, given the broad involvement of NF-κB in regulating cellular processes, prolonged systemic inhibition of NF-κB might have unwanted side-effects. Even so, there may well be a place for modulation of NF-κB activity at different phases or stages of MS. For RR-MS, short-term treatment with inhibitors that block the classical NF-κB pathway at times of disease activity are predicted to dampen the inflammatory response, particularly if used in combination with glucocorticoids or other molecules that induce the generation of Treg. During RR-MS, induction of NF-κB activity in neurons and oligodendrocytes may have a beneficial effect in protecting against apoptosis of these cells, and thus strategies that target NF-κB inhibitors to immune-related cells might have distinct advantages. This could potentially be done either by gene delivery of NF-κB inhibitors, or by using inhibitors that are not CNS penetrant. Considerable effort is currently underway to develop potent and selective NF-κB pathway inhibitors. Some of the potential effects of inhibiting the NF-κB pathway in different cells involved in MS are summarized in Table **3**.

As noted earlier, there are currently few therapies available for treatment of progressive MS. It is unlikely that treatments that rely solely on immunosuppression or immunomodulation of T cell responses will have a strong impact on this phase of disease, as not only does there appear to be an inability to switch off the inflammatory response, but axonal and neuronal degeneration appear to play an at least equivalent role in MS progression. Strategies for progressive MS may therefore need to target several components of the NF-κB pathway in different ways in different types of cells. There is chronic activation of macrophages, microglia, and other inflammatory cells in the CNS, which could potentially be related to persistent activation of NF-κB through IκBβ, as opposed to transient activation through IκBα [65]. Thus, inhibiting phosphorylation of IκBβ in macrophages may provide one arm of the treatment. It addition, novel IκB's, such as IκBL, may be related to the development of certain types of MS [145]. While little is currently known of IκBL, it is likely that the phosphorylation of this molecule will differ slightly from other IκB's, thus allowing one to target specific kinases in order to inhibit this molecule. Differences in the promoter region of the gene encoding IκBα in PP-MS patients, compared to other types of MS, suggest that the innate immune response may be aberrantly regulated in PP-MS. Therapeutic strategies targeting IκBα may therefore be beneficial in PP-MS. In progressive MS, it may also be efficacious to actually activate, rather than inhibit, the NF-κB pathway in some circumstances. For example, chronic inflammation might be cleared through the action of Treg, which could be induced through stimulation of the alternative pathway in antigen presenting cells. Finally, targeted activation of NF-κB pathways in neurons and oligodendrocyte precursors may protect those cells from degeneration. Conceivably, this could be achieved by administering trophic factors that activate NF-κB signaling, or through induction of a mild metabolic stress response, such as dietary restriction [82, 170].

We suggest that the main considerations for the type of NF-κB therapy to be used in particular patients will relate to the features of that individual's MS, and that this will not be a "one type fits all" therapeutic option.

9. CONCLUSIONS

The NF-κB pathway plays critical roles not only in the normal functioning of both the immune system and the nervous system, but also in development of and protection against disease in these systems. Modulation of NF-κB as a means of controlling MS will require further knowledge regarding the specific molecular interactions leading to activation of pro-inflammatory responses during the course of different types and phases of MS.

Table 3. Putative Effects of Inhibition of NF-κB in Different Types of Cells Involved in MS

Cell Type	Benefit	Harm
T lymphocyte	⇓ activation ⇓ cytokine expression ⇑ apoptosis susceptibility	Generalized immunosuppression
B lymphocyte	⇓ antibody production	Generalized immunosuppression
Monocytes/ macrophages	⇓ cytokine expression	⇓ development of monocytes to macrophages
Microglia	⇓ accumulation in lesion ⇓ cytokine expression	? ⇓ protective effects
Astrocytes	⇓ cytokine expression ⇓ accumulation in lesion and scar formation	
Neurons	? ⇓ cell death	⇑ susceptibility to oxidative, excitotoxic and metabolic insults ⇑ apoptosis susceptibility ⇑ MHC class I expression
Oligodendrocytes	? ⇓ cell death	⇑ apoptosis susceptibility

Studies in the EAE model, while informative, will need to be supplemented with additional information obtained from tissues of patients with MS. In particular, it will be important to determine the pathways leading to NF-κB activation in the CNS during attacks of disease and recovery phases, to identify patterns of activation correlating with the clinical course and features of disease. It will also be essential to develop a more thorough understanding of the role of NF-κB in nervous system health and disease: the highly differentiated cell types and subtypes in the CNS and their interdependency on one another create a particularly challenging landscape for investigators in determining such parameters as the subunits activated in different cell types, the target sequence preferences of the subunits, and the outcomes that they induce in individual cells.

ABBREVIATIONS

BCL = B-cell leukaemia

CNS = Central nervous system

CXCL1 = Chemokine (C-X-C motif) ligand 1

DC = Dendritic cells

EAE = Experimental autoimmune encephalomyelitis

GTP = Guanosine triphosphate

H2O2 = Hydrogen peroxide

HLA = Human Leukocyte Antigen

IDO = indoleamine 2,3-dioxygenase

IFN-β = Beta interferon

IκB = Inhibitor of NF-κB

IκBL = Inhibitor of κB-like

IKK = Iκ kinase complex

IKKα = Alfa subunit of the IKK complex

IKKβ = Beta subunit of the IKK complex

IL-17 = Interleukin-17

MHC = Major histocompatibility complex

MIP-1β = Macrophage inflammatory protein-1β

MS = Multiple sclerosis

NEMO = NF-κB Essential Modulator

NF-κB = Nuclear factor-κB

NIK = NF-κB Inducing Kinase

NKT cell = Natural killer T cell

PBMC = Peripheral Blood Mononuclear Cell

PP-MS = Primary progressive MS

RANTES = Regulated upon Activation, Normal T-cell Expressed and Secreted

RR-MS = Relapsing–remitting MS

SP-MS = Secondary progressive MS

Syk = Spleen tyrosine kinase

Th1 = T helper 1

Th17 = T helper 17

Th2 = T helper 2

TNF-α = Tumor necrosis factor-α

Treg = Regulatory T cells

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