Pharmacological interventions targeting nuclear factor-kappa B signaling in multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory neurodegenerative disease of the central nervous system (CNS). Pathological characteristics of the disease include activation of CNS-intrinsic immune cells, such as microglia and astrocytes, and loss of neuronal connections, myelin and blood-brain barrier (BBB) integrity as well as peripheral immune cell infiltration into the brain. MS has long been considered a predominantly immunological disease, which has led to the development of essentially only immune-directed medications. Within this traditional "outside-in" MS hypothesis, a dysregulation of the peripheral immune system causes immune cell infiltration into the CNS, leading to autoreactivity against myelin sheath components and secondary BBB dysfunction. However, recent findings indicate that overactivation of microglia and astrocytes represents an important first step in MS pathology, as appears to be the case for other neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Within this new hypothesis of CNS-intrinsic neuroinflammation in MS – also known as the "inside-out" model (Titus et al., 2020), the transcription factor nuclear factor-kappa B (NFκB) plays a central role in the brain. In CNS cells, various triggers, such as bacterial and viral infections, oxidative stress and other cellular stressors like protein misfolding and DNA damage, lead to NFκB activation in CNS-immune cells and subsequent production of pro-inflammatory cytokines and adhesion molecules, activation of the inflammasome complex, apoptosis and cell cycle arrest. The production of pro-inflammatory molecules causes a microenvironment which provokes CNS-cell degeneration, and is detrimental for (re)myelination by oligodendrocytes and neuronal regeneration. Neuroinflammatory cascades in the CNS also prevent microglia and astrocytes from exerting their regenerative effects on oligodendrocytes and neurons. In addition, microglia and astrocytes reinforce each other's negative effects via cytokine-mediated feedback mechanisms, which create a negative loop that further affects the environment for CNS-cell regeneration. Targeting the NFκB pathway may be especially attractive for the treatment of MS as this transcription factor is also involved in regulating inflammatory processes within both the innate and the adaptive peripheral immune systems.

CNS-intrinsic effects of U.S. Food and Drug Administration (FDA)-approved MS drugs via the modulation of NFκB signaling: In addition to their effects on peripheral immune cells, most of the commonly prescribed FDA-approved MS drugs (Fingolimod, Dimethyl Fumarate, Glatiramer Acetate (GA), Teriflunomide, Interferon-β (IFNβ), Laquinimod, Ocrelizumab, Alemtuzumab and Natalizumab) modulate the neuroinflammatory NFκB pathway in microglia and astrocytes as well (De Kleijn and Martens, 2020). Furthermore, the MS drug-induced amelioration of the glial and neuronal damage observed in various MS animal models - as well as in animal models of other neurodegenerative diseases – appears to be attributable to a direct effect of these drugs on microglia and astrocytes. This new perspective paves the way for the development of novel MS drugs that, through the dampening of overactive CNS immune cells via the modulation of NFκB signalling, stimulate CNS-cell regeneration. Therefore, drugs currently under exploration for the treatment of non-immunological neurodegenerative diseases or cerebrovascular disorders and that target suppression of CNS-intrinsic inflammation via NFκB may be investigated in connection with their repurposing for the treatment of MS. Furthermore, a combination of drugs, i.e., combinatorial as opposed to monotherapy, may be considered. Interestingly, single-nucleotide polymorphisms (SNPs) that lead to increased cellular expression of NFκB or modulation of its signalling cascades have been found to be associated with MS. In addition, a remarkable number of MS-associated genes contain NFκB-binding sites, which again highlights the importance of NFκB in MS.

Overlap in CNS pathology between MS and other neurodegenerative diseases: The CNS pathologies of MS and other neurodegenerative diseases display important similarities, such as microgliosis and astrogliosis – an increase in the number of pro-inflammatory microglia and astrocytes, respectively –, and increased CNS levels of pro-inflammatory cytokines. In frontal brain tissue of patients suffering from AD, PD and MS, microglia-specific mRNA expression was elevated and these profiles were remarkably correlated among the three diseases. The astrocyte-specific mRNA expression profiles in brain tissues of MS and PD, but not AD, patients were also highly interrelated. This reflects the reactive (inflammation-associated) microglial and astrocytic phenotypes which have been described in the CNS of AD and PD as well as MS patients. Although AD is primarily thought to be a grey matter neuronal disease, metabolic oligodendrocytic alterations and myelin sheath damage are also present, resembling the demyelination observed in MS lesions. In addition to microglial and astroglial inflammatory phenotypes, and neuronal and oligodendrocyte degeneration, MS, AD and PD are all characterized by the presence of reactive oxygen species and mitochondrial injury as well.

Effects of NFκB-targeting compounds in MS: In view of the above-mentioned overlap in CNS pathology, the compounds that are currently proposed for the treatment of non-immunological neurodegenerative diseases, and that reduce the NFκB-induced pro-inflammatory status of microglia and astrocytes represent attractive candidates for repurposing in MS. Strategies to target the NFκB signaling cascade include the modulation of 1) membrane-bound receptors that activate NFκB, 2) intermediate activators in the NFκB signaling cascade, such as inhibitory κB (IκB) kinases (IKKs), 3) proteasomal degradation (via phosphorylation) of NFκB inhibitor alpha (IκBα), 4) NFκB nuclear translocation and 5) target DNA-binding of NFκB factors (**Figure 1**). Examples of such NFκB-targeting compounds are polyphenols (such as resveratrol, quercetin and catechin), α-tocopherol, curcumin (analogues), nonsteroidal anti-inflammatory drugs (NSAIDs such as aspirin), berberine, minocycline, antioxidants, rapamycin and corticosteroids.

A limited number of preclinical studies have described the effects of pharmacological NFκB modulators on the most commonly used animal model for MS, experimental autoimmune

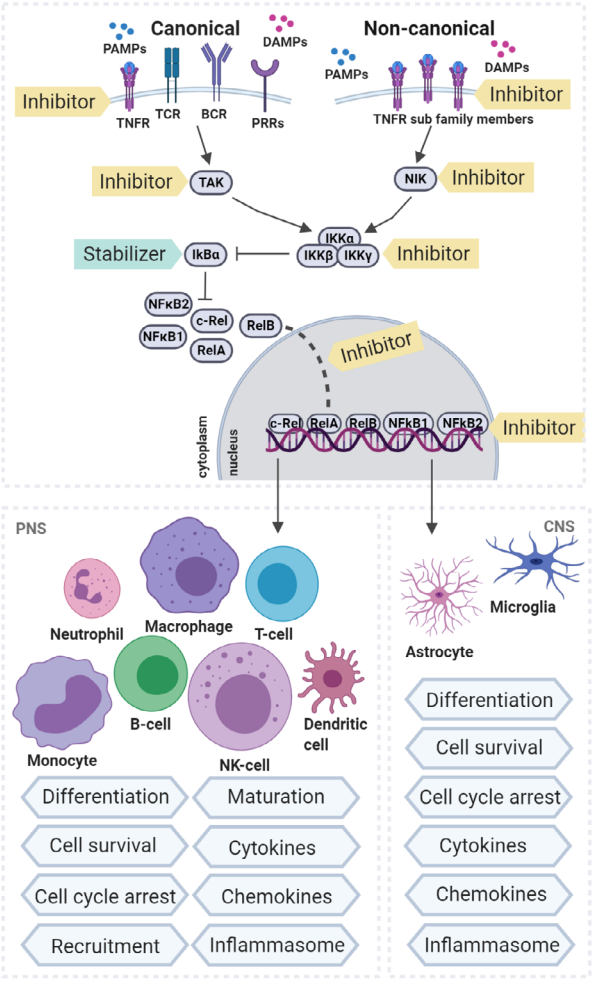

Figure 1 | **Molecular effects of pharmacological modulators on the canonical and non-canonical NFκB signalling pathways in various cell populations of the peripheral and central nervous systems.** Figure 1 was created with BioRender (www.Biorender.com). BCR: B-cell receptor; CNS: central nervous system; c-Rel: proto-oncogene C-Rel; DAMP: damage-associated molecular patterns; IKK: IκB kinase; IκBα: NFκB inhibitor alpha; NFκB: nuclear factor-kappa B; NFκB1: NFκB subunit 1; NFκB2: NFκB Subunit 2; NIK: NFκB inducing kinase; NK-cell: natural killer cell; PAMP: pathogen-associated molecular patterns; PNS: peripheral nervous system; PRR: pattern recognition receptors; RelA: avian v-rel reticuloendotheliosis viral oncogene homolog A/NFκB p65 subunit; RelB: avian v-rel reticuloendotheliosis viral oncogene homolog B; TAK: TGF-β-activating kinase; TCR: T-cell receptor; TNFR: tumor necrosis factor receptor.

encephalitis (EAE). Both the NFκB inhibitor pyrrolidine dithiocarbamate (Pahan and Schmid, 2000) and IKKα/β inhibitors (Greve et al., 2007) ameliorated EAE symptoms and improved peripheral measures of inflammation. Furthermore, peptides that bind to the NFκB essential modifier (NEMO/IκBγ) binding domains of IκBα and IκBβ inhibited formation of the active NFκB complex without changing the baseline levels of NFκB itself and caused anti-inflammatory effects on both the peripheral nervous system (PNS) and CNS in mouse models of EAE (Dasgupta et al., 2004).

Compounds directed towards the NFκB complex have also been studied in clinical MS trials (recently reviewed in Ramadass et al., 2020). Ibudilast (MN-166), a nonspecific phosphodiesterase inhibitor that is also an inhibitor of cell membrane receptors in the NFκB pathway, has reached phase-2 clinical trials in progressive and relapsing-remitting MS (RRMS) patients. Following treatment with Ibudilast, patients showed significant attenuation of both retinal thinning and brain atrophy. Furthermore, even phase-3 trials have been initiated in RRMS patients for Evobrutinib and Sanofi's SAR-442168, which are small molecules that inhibit NFκB through the Bruton's tyrosine kinase signalling pathway. Yet, a phase-1 clinical trial with the IKKα/β inhibitor MLN-0415 in MS patients has unfortunately been discontinued due to side effects. The antibiotic minocycline inhibits NFκB p65 phosphorylation and nuclear translocation, but combinatorial therapy of minocycline and IFNβ1a did not lead to a significant beneficial effect in RRMS patients (Sørensen et al., 2016). However, a recent trial within the RECYCLINE study indicates that minocycline combined with GA may have beneficial effects on magnetic resonance imaging endpoints in RRMS patients (Metz et al., 2009).

Some of the NFκB-targeting compounds are of natural origin. Curcumin, a natural spice obtained from the roots of Curcuma longa, resulted in an amelioration of EAE-induced demyelination, via a decrease in T-cell and Th1-cell proliferation through c-Jun N-terminal Kinase/Statins/NFκB inhibition (Natarajan and Bright, 2002) or Th17 cell-mediated inflammation (Xie et al., 2009). Furthermore, the major constituent of green tea, epigallocatechin-3-gallate, suppressed the clinical symptoms and the NFκB-mediated brain and peripheral inflammation in proteolipid protein-1-induced EAE, either alone (Aktas et al., 2004) or in combination with the MS drug GA (Herges et al., 2011). Another plant-extracted polyphenol, resveratrol, also reversed clinical EAE symptoms through the induction of peripheral T-cell apoptosis by reducing NFκB signalling via the aryl hydrocarbon receptor (Singh et al., 2007). The flavonoid luteolin reduced transmigration of peripheral immune cells over the BBB in rat models of EAE (Hendriks et al., 2004). Finally, vitamin A showed a positive antioxidative effect on the T-cell balance in IFNβ1a-treated MS patients (Saboor-Yaraghi et al., 2015). Unfortunately, the effects of these natural NFκB modulators have been studied almost exclusively on NFκB-signalling in PNS inflammation and not on NFκB-signalling in CNS cell types, a caveat in the field especially in the light of the CNS-intrinsic hypothesis of MS.

The ubiquitin-proteasome system is crucial for both activation of NFκB-signalling and termination of its transcriptional response via proteasomal degradation of IκBα, and may therefore represent another new drug target for the treatment of MS. Interestingly, genetic variations segregating in multiple independent MS families have recently been identified in the gene encoding the ubiquitin-proteasome system-associated E3 ubiquitin ligase RNF213. Furthermore, compounds directed towards additional major signalling regulators and down-stream targets of NFκB, such as c-Jun N-terminal Kinase, statins, mitogen-activated protein kinase/extracellular signal-regulated kinases and the mammalian target of rapamycin complex, may be considered as MS therapeutics. For instance, the mammalian target of rapamycin complex modulator rapamycin reduced the phosphorylation of NFκB and IKKβ in lipopolysaccharide-stressed rats (Mengke et al., 2016). Yet, modulation of these

molecules may have many nonspecific side effects due to their broad expression patterns and functions in various cell types.

Conclusions and future perspectives: Currently, MS is primarily considered an immunological disease triggered by CNS infiltration of peripheral immune cells rather than a microglia-/astrocyte- and neurodegeneration-induced autoimmune disease. Nevertheless, many FDA-approved MS drugs have recently been shown to have a marked effect on the NFκB pathway in CNS cells, and their specific molecular effects on microglia and astrocytes should therefore be elucidated in more detail. In addition, searches for novel MS drugs should not only focus on modifying immune cell populations in the brain or repairing the BBB, but also on improving the brain microenvironment for effective CNS-cell regeneration via modulation of NFκB signalling. An important question that remains to be explored in this connection is how to specifically target NFκB, since this transcription factor has dual and even opposite roles under certain circumstances, such as its pro- as well as anti-apoptotic role under stress conditions. Given that NFκB not only acts as a mediator of inflammatory processes in peripheral immune cells but also plays a clear role in microglia and astrocytes, the effects of NFκB-directed drugs in MS should be considered at both peripheral and central levels. Finally, the translation of the CNS effects of NFκB-targeting drugs from animal models to humans is not straightforward because the pathological mechanisms causing NFκB activation in MS animals do not necessarily correspond with the processes inducing NFκB signalling in MS patients. Furthermore, none of the current animal models used to study MS fully recapitulates MS pathology, with cuprizone inducing mainly demyelination and CNS inflammation, and EAE being a model linked to the "outside-in" hypothesis. Thus, exploring the effects of NFκB-targeting compounds on MS patient-derived three-dimensional stem cell systems may be an appealing additional option. In any case, the new perspective of NFκB being a central drug target in MS is in line with the recently proposed CNS-intrinsic model of MS pathogenesis and may lead to an expansion of the current repertoire of drugs to treat this complex immune-mediated neurodegenerative disease.

Figure 1 was created with BioRender (www.Biorender.com).

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