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NF-κB Activation Protects Oligodendrocytes against Inflammation

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NF-κB is a key player in inflammatory diseases, including multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE). However, the effects of NF-κB activation on oligodendrocytes in MS and EAE remain unknown. We generated a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes and demonstrated that IκBαΔN expression had no effect on oligodendrocytes under normal conditions (both sexes). Interestingly, we showed that oligodendrocyte-specific expression of IκBαΔN blocked NF-κB activation in oligodendrocytes and resulted in exacerbated oligodendrocyte death and hypomyelination in young, developing mice that express IFN-γ ectopically in the CNS (both sexes). We also showed that NF-κB inactivation in oligodendrocytes aggravated IFN-γ-induced remyelinating oligodendrocyte death and remyelination failure in the cuprizone model (male mice). Moreover, we found that NF-κB inactivation in oligodendrocytes increased the susceptibility of mice to EAE (female mice). These findings imply the cytoprotective effects of NF-κB activation on oligodendrocytes in MS and EAE.

Key words: **EAE; IFN-γ; multiple sclerosis; myelin; NF-κB; oligodendrocyte**

Significance Statement

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS. NF-κB is a major player in inflammatory diseases that acts by regulating inflammation and cell viability. Data indicate that NF-κB activation in inflammatory cells facilitates the development of MS. However, to date, attempts to understand the role of NF-κB activation in oligodendrocytes in MS have been unsuccessful. Herein, we generated a mouse model that allows for inactivation of NF-κB specifically in oligodendrocytes and then used this model to determine the precise role of NF-κB activation in oligodendrocytes in models of MS. The results presented in this study represent the first demonstration that NF-κB activation acts cell autonomously to protect oligodendrocytes against inflammation in animal models of MS.

Introduction

Multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are inflammatory demyelinating diseases of the CNS characterized by inflammation, demyelination, oligodendrocyte death, and axon degeneration (Frohman et al., 2006; Bradl and Lassmann, 2010). It is believed that mature oligodendrocyte death induced by inflammation contributes significantly to the pathogenesis of MS and EAE. A number of studies have shown that protection of mature oligodendrocytes against inflammation decreases the susceptibility of mice to EAE (Hisahara et al., 2003; Mc Guire et al., 2010; Prineas and Parratt, 2012; Lin et al., 2013). Oligodendrocyte regeneration is necessary to repair myelin damage in MS lesions; however, remyelinating oligodendrocytes are prone to apoptosis under inflammatory conditions (Franklin and ffrench-Constant, 2008; Watzlawik et al., 2010; Stone and Lin, 2015). One of the major challenges in MS research is to understand the mechanisms governing the viability of oligodendrocytes and to develop therapeutic strategies that promote oligodendrocyte survival.

The transcription factor NF-κB is a heterodimer or homodimer of the Rel family of proteins, including p65, c-Rel, RelB, p50, and p52. In the quiescent state, NF-κB remains inactive in the cytoplasm through interaction with NF-κB inhibitors (IκBs). Activation of NF-κB involves dissociation from IκBs and trans-

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The authors declare no competing financial interests.

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location into the nucleus, where NF-κB binds to the κB consensus DNA sequence and stimulates transcription of genes that regulate inflammation, cell viability, and cell functions. NF-κB can be activated in a variety of ways, including the IκB kinase 2 (IKK2)-dependent canonical pathway, the noncanonical pathway, and atypical pathways (Hayden and Ghosh, 2012; Mincheva-Tasheva and Soler, 2013). It is known that NF-κB plays a critical role in MS and EAE (Yan and Greer, 2008; Mc Guire et al., 2013). NF-κB is activated in multiple cell types in MS and EAE, including oligodendrocytes, T cells, astrocytes, and microglia/macrophages (Gveric et al., 1998; Bonetti et al., 1999; Lin et al., 2013). A number of studies have shown that NF-κB activation in T cells, microglia/macrophages, or astrocytes promotes the development of MS and EAE by facilitating inflammation (Brambilla et al., 2009; Mc Guire et al., 2013; Yin et al., 2013; Hao et al., 2016).

In vitro studies suggest that NF-κB activation promotes oligodendrocyte survival in response to inflammatory mediators (Nicholas et al., 2001; Hamanoue et al., 2004; Lin et al., 2012). In contrast, one study briefly mentions that IKK2 deletion in oligodendrocytes using the Cre/loxP approach by crossing floxed *IKK2* mice (*IKK2*^{FL} mice) with mice expressing Cre under the myelin oligodendrocyte glycoprotein (MOG) promoter (*MOG/Cre* mice) does not alter the EAE disease course (Raasch et al., 2011). However, the study falls short of excluding the following possibilities: (1) that NF-κB activation is not attenuated in oligodendrocytes of *IKK2*^{FL}*;MOG/Cre* mice during EAE due to insufficient efficiency of Cre-mediated recombination and/or compensation from other NF-κB activating pathways and (2) that inflammation is compromised in *IKK2*^{FL}*;MOG/Cre* mice undergoing EAE due to undesired leaky expression of Cre in inflammatory cells. The minimal alteration of EAE disease course in *IKK2*^{FL}*;MOG/Cre* mice does not rule out the significant impact of NF-κB activation on oligodendrocytes during EAE. Therefore, the effects of NF-κB activation on oligodendrocytes in MS and EAE remain ambiguous.

Our previous studies demonstrated that NF-κB is activated in oligodendrocytes in the CNS of mice undergoing EAE and mice that express IFN-γ, a key proinflammatory cytokine in MS and EAE, ectopically in the CNS (Lin et al., 2012, 2013). Importantly, our previous *in vitro* study showed that expression of IκBαΔN, a super-suppressor of NF-κB, is sufficient to block NF-κB activation in the oligodendroglial cell line Oli-neu in response to IFN-γ and render the cells sensitive to this cytokine (Lin et al., 2012). Therefore, in this study, we sought to determine the role of NF-κB activation in oligodendrocytes in models of MS by exploiting a mouse model that expresses IκBαΔN specifically in oligodendrocytes. Our findings indicate the cytoprotective effects of NF-κB activation on oligodendrocytes in MS and EAE.

Materials and Methods

Mice, cuprizone treatment, and EAE immunization. A summary of mouse lines can be found in Table 1 and a summary of experimental genotypes and crosses can be found in Table 2. *cloxPIκBαΔN* mice, *CNP/Cre* mice, *GFAP/tTA* mice, and *TRE/IFN-γ* mice were on the C57BL/6J background. *cloxPIκBαΔN* mice were crossed with *CNP/Cre* mice to obtain *cloxPIκBαΔN;CNP/Cre* mice and control mice, including *cloxPIκBαΔN* mice, *CNP/Cre* mice, and wild-type mice. *GFAP/tTA* mice were crossed with *CNP/Cre* mice to obtain *GFAP/tTA;CNP/Cre* mice and *TRE/IFN-γ* mice were crossed with *cloxPIκBαΔN* mice to obtain *TRE/IFN-γ;cloxPIκBαΔN* mice. Then, *GFAP/tTA;CNP/Cre* mice were crossed with *TRE/IFN-γ;cloxPIκBαΔN* mice to obtain *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* quadruple mice and *GFAP/tTA;TRE/IFN-γ* double mice. Genotypes were determined by PCR from DNA extracted from tail tips as described previously (Schmidt-Ullrich et al., 2001; Lappe-Siefke et al., 2003; Lin et al., 2004). To repress the expression of the IFN-γ transgene in the astrocytes of *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice and *GFAP/tTA;TRE/IFN-γ* mice, 0.05 mg/ml doxycycline (Dox) was added to the drinking water and provided *ad libitum* from conception (Lin et al., 2004, 2005, 2006).

Table 1. Summary of mouse lines

Mouse line	Description	Citation
cloxPIκBαΔN	Carry a floxed conditional allele of IκBαΔN	Schmidt-Ullrich et al., 2001
CNP/Cre	Express the Cre recombinase under the control of the CNP promoter	Lappe-Siefke et al., 2003
GFAP/tTA	Express the tTA under the control of the GFAP promote	Lin et al., 2004
TRE/IFN-γ	Express IFN-γ under the control of the TRE	Lin et al., 2004

To induce demyelination with cuprizone, 6-week-old male mice were fed a diet of mouse chow containing 0.2% cuprizone (Sigma-Aldrich) for up to 6 weeks. Subsequently, mice were returned to a normal diet for 3 weeks to allow remyelination to occur (Lin et al., 2006, 2014). To induce EAE, 8-week-old female mice were injected subcutaneously in the flank and at the tail base with 200 μg of MOG 35–55 peptide emulsified in complete Freund's adjuvant (BD Biosciences) supplemented with 600 μg of *Mycobacterium tuberculosis* (strain H37Ra; BD Biosciences). Two intraperitoneal injections of 400 ng of pertussis toxin (List Biological Laboratories) were given 24 and 72 h later. Clinical scores (0 = healthy, 1 = flaccid tail, 2 = ataxia and/or paresis of hindlimbs, 3 = paralysis of hindlimbs and/or paresis of forelimbs, 4 = tetraparalysis, 5 = moribund or death) were recorded daily (Lin et al., 2007, 2013, 2014).

All animal procedures were conducted in complete compliance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

PCR and real-time PCR. Deeply anesthetized mice were perfused with ice-cold PBS. Genomic DNA was isolated from the indicated tissues and PCR was performed to determine the removal of the floxed STOP cassette that prevents IκBαΔN translation by Cre recombination in *cloxPIκBαΔN;CNP/Cre* mice as described previously (Schmidt-Ullrich et al., 2001). RNA was isolated from brain using TRIzol reagent (Invitrogen) and treated with DNaseI (Invitrogen) to eliminate genomic DNA. Reverse transcription was performed using the iScript cDNA Synthesis Kit (Bio-Rad). TaqMan real-time PCR was performed with iQ Supermix (Bio-Rad) on the LightCycler 480 System (Roche) as described in our previous studies (Lin et al., 2006, 2007; Lin and Lin, 2010).

Western blot analysis. Brains harvested from mice were rinsed in ice-cold PBS and homogenized using a motorized homogenizer as described previously (Lin et al., 2005, 2008). After incubating on ice for 15 min, the extracts were cleared by centrifugation twice at 14,000 rpm for 30 min. The protein content of each extract was determined by DC Protein Assay (Bio-Rad Laboratories). The extracts (40 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated with a primary antibody against IκBα (1:1000; Santa Cruz Biotechnology, RRID:AB_2235952) or β-actin (1:5000; Sigma-Aldrich, RRID:AB_476694), followed by an HRP-conjugated secondary antibody (1:1000; Vector Laboratories, RRID:AB_2336198). After incubation with ECL Detection Reagents (GE Healthcare Biosciences), the chemiluminescent signal was detected.

Immunohistochemistry (IHC). Anesthetized mice were perfused through the left cardiac ventricle with 4% paraformaldehyde in PBS. The tissues were removed, postfixed with paraformaldehyde, cryopreserved in 30% sucrose, embedded in optimal cutting temperature compound, and frozen on dry ice. Frozen sections were cut using a cryostat at a thickness of 10 μm. For immunohistochemistry, the sections were treated with −20°C acetone, blocked with PBS containing 10% goat serum and 0.1% Triton X-100, and incubated overnight with the primary antibody diluted in blocking solution. Fluorescein (Vector Laboratories, anti-rabbit, RRID:AB_2336197), Cy3 (Millipore, anti-mouse, RRID:AB_11213281, anti-rat, RRID:AB_90854), or enzyme-labeled secondary antibodies (Vector Laboratories, anti-rat, RRID:

Table 2. Summary of mouse crosses

Abbreviated name	Genotype	Cross
IκBαΔN$^+$	*cloxPIκBαΔN*$^+$*;CNP/Cre*$^+$ mice	*cloxPIκBαΔN* mice × *CNP/Cre* mice
IκBαΔN$^-$	*cloxPIκBαΔN*$^+$ mice or *CNP/Cre*$^+$ mice or wild type mice	
IFN-γ$^-$;IκBαΔN$^-$	*GFAP/tTA;TRE/IFN-γ* mice with Dox	*GFAP/tTA;CNP/Cre* mice × *TRE/IFN-γ;cloxPIκBαΔN* mice
IFN-γ$^+$;IκBαΔN$^-$	*GFAP/tTA;TRE/IFN-γ* mice without Dox[a]	
IFN-γ$^-$;IκBαΔN$^+$	*GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice with Dox	
IFN-γ$^+$;IκBαΔN$^+$	*GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice without Dox[a]	

[a]Mice were removed from Dox at E15 for developmental studies and on the first day of cuprizone treatment for cuprizone studies.

AB_2336202; anti-mouse, RRID:AB_2313581) were used for detection. Immunohistochemical detection of CC1 (APC7, 1:50; EMD Biosciences, RRID:AB_2057371), myelin basic protein (MBP,1:1000; Sternberger Monoclonals, RRID:AB_10120129), CD3 (1:50; Santa Cruz Biotechnology, RRID:AB_627010), CD11b (1:50; Millipore, RRID:AB_92930), the active form of p65 (1:50; Millipore, RRID:AB_2178887), glial fibrillary acidic protein (GFAP; 1:200; Agilent Technologies, RRID:AB_10013382), and aspartoacylase (ASPA, 1:1000, kindly provided by Dr. M.A. Aryan Namboodiri at Uniformed Services University of the Health Sciences, Bethesda, MD; Madhavarao et al., 2004) were performed. Fluorescent stained sections were mounted with Vectashield mounting medium with DAPI (Vector Laboratories) and visualized with an Olympus FV1000 confocal microscope.

For cuprizone-treated mice, coronal sections of the fornix region of the corpus callosum corresponding to Sidman sections 241–251 were selected for use and all comparative analyses were restricted to the midline corpus callosum (Sidman et al., 1971). Immunopositive cells were quantified by counting positive cells within the median of the corpus callosum as described previously (Lin et al., 2006, 2014).

Toluidine blue staining and electron microscopy (EM) analysis. Mice were anesthetized and perfused with PBS containing 4% paraformaldehyde and 2.5% glutaraldehyde. The sciatic nerve, spinal cord, cerebellar white matter, and corpus callosum were processed and embedded. For cuprizone-treated mice, brains were sliced into 1 mm sections. The section corresponding to the region of the fornix was trimmed, processed, and oriented so that a cross-section of the corpus callosum was achieved. Thin sections were cut, stained with toluidine blue, and analyzed as described previously (Lin et al., 2013, 2014). Moreover, ultrathin sections were cut, stained with uranyl acetate and lead citrate, and analyzed as described previously (Lin et al., 2013, 2014). We counted the total number of axons, the number of myelinated axons, and calculated the percentage of myelinated axons as described previously (Lin and Lin, 2010; Lin et al., 2014).

T-cell proliferation assay and cytokine assay. Single-spleen cell suspensions were generated from mice with EAE at postimmunization day (PID) 10. Red blood cells were lysed with ACK lysis buffer (Invitrogen), washed, and plated at 5×10^5 splenocytes per well in 96-well microtiter plates. Samples were plated in triplicate. The cells were treated with MOG35-55 peptide (0, 1, 10, or 100 μg/ml) and incubated at 37°C and 5% CO_2. After 48 h, 20 μl of BrdU labeling solution (Millipore) was added to the culture medium for 24 h. Cell proliferation was determined using the Colorimetric BrdU Cell Proliferation kit (Millipore) according to manufacturer's instructions. We quantified the cytokines in the culture supernatants of triplicate wells using the ELISA kits (Thermo Scientific) according to the manufacturer's instructions.

Experimental design and statistical analysis. The sample size for each individual experiment is listed in the corresponding figure legend. For EAE experiments, only female mice were used due to the well known sex differences in EAE. For cuprizone studies, only male mice were used. For developmental studies, both male and female mice were used because no sex differences were observed. EAE clinical score data are presented as mean ± SEM and were compared using a two-way ANOVA with a Sidak's multiple-comparisons test using GraphPad Prism 6 software (RRID:SCR_002798). All other data are expressed as mean ± SD. Comparisons between two groups was evaluated by *t* test using GraphPad Prism 6 software. Multiple comparisons were evaluated statistically with one-way ANOVA with Tukey's posttest using GraphPad Prism 6 software. Phenotypic differences among groups were evaluated with the χ^2 test using GraphPad Prism 6 software. $p < 0.05$ was considered significant.

Results

NF-κB is dispensable for oligodendrocytes under normal conditions

The primary function of oligodendrocytes is to produce the myelin that insulates axons in the CNS. Data indicate that NF-κB has no significant effect on myelin formation and maintenance in the CNS under normal conditions (Blank and Prinz, 2014; Kretz et al., 2014). Mice with deletions in the genes encoding c-Rel, RelB, or p52 do not exhibit myelin abnormalities in the CNS (Hilliard et al., 1999, 2002). Similarly, mice with CNS-restricted deletion of p65, IKK1, IKK2, or IKK3 do not show any myelin defects in the CNS (van Loo et al., 2006; Kretz et al., 2014). Nevertheless, a recent study shows that impaired NF-κB activity is associated with myelin abnormalities in the CNS of patients with Xq28 duplication (Philippe et al., 2013). We generated a mouse model that expresses IκBαΔN, a deletion mutant lacking the N-terminal 36 aa of IκBα that functions as a dominant inhibitor of NF-κB signaling (Krappmann et al., 1996), specifically in oligodendrocytes in the CNS and determined the potential role of NF-κB in these cells under normal conditions.

cloxPIκBαΔN mice (Schmidt-Ullrich et al., 2001) were crossed with *CNP/Cre* knock-in mice (Lappe-Siefke et al., 2003) to obtain *cloxPIκBαΔN;CNP/Cre* mice (IκBαΔN$^+$ mice) and control mice (IκBαΔN$^-$ mice), including *cloxPIκBαΔN* mice, *CNP/Cre* mice, and wild-type mice (Table 1, 2). IκBαΔN$^+$ mice appeared healthy and were indistinguishable from littermate control mice. PCR analysis showed that the floxed STOP cassette that prevents IκBαΔN translation was removed by Cre recombination in the CNS and PNS of IκBαΔN$^+$ mice (Fig. 1*A*). Western blot analysis showed that IκBαΔN was expressed in the CNS of IκBαΔN$^+$ mice (Fig. 1*B*). CC1, a marker for oligodendrocytes, IHC showed that the number of oligodendrocytes in the CNS of 21-d-old IκBαΔN$^+$ mice was comparable to IκBαΔN$^-$ mice (CC, $p = 0.9062$; CE, $p = 0.4333$; SC, $p = 0.7828$; all *t* test; Fig. 1*C*,*D*,*G*). MBP IHC showed that IκBαΔN expression in oligodendrocytes did not alter the degree of myelination in the CNS of 21-d-old IκBαΔN$^+$ mice compared with IκBαΔN$^-$ mice (Fig. 1*E*,*F*). Moreover, CC1 and MBP IHC showed that IκBαΔN expression in oligodendrocytes did not alter oligodendrocyte numbers or the degree of myelination in the CNS of adult mice (CC, $p = 0.5363$; CE, $p = 0.8105$; SC, $p = 0.4502$; all *t* test; Fig. 1*H*–*L*). Therefore, these results demonstrate that NF-κB is dispensable for the viability and function of oligodendrocytes under normal conditions.

Several *in vitro* studies suggest that NF-κB activation is required for Schwann cell differentiation and myelin formation in the PNS (Nickols et al., 2003; Limpert et al., 2013). Nevertheless, a recent report showed that Schwann-cell-restricted deletion of IKK2 has no effect on myelin formation in the PNS *in vivo* (Morton et al., 2013). As described above, the floxed STOP cassette

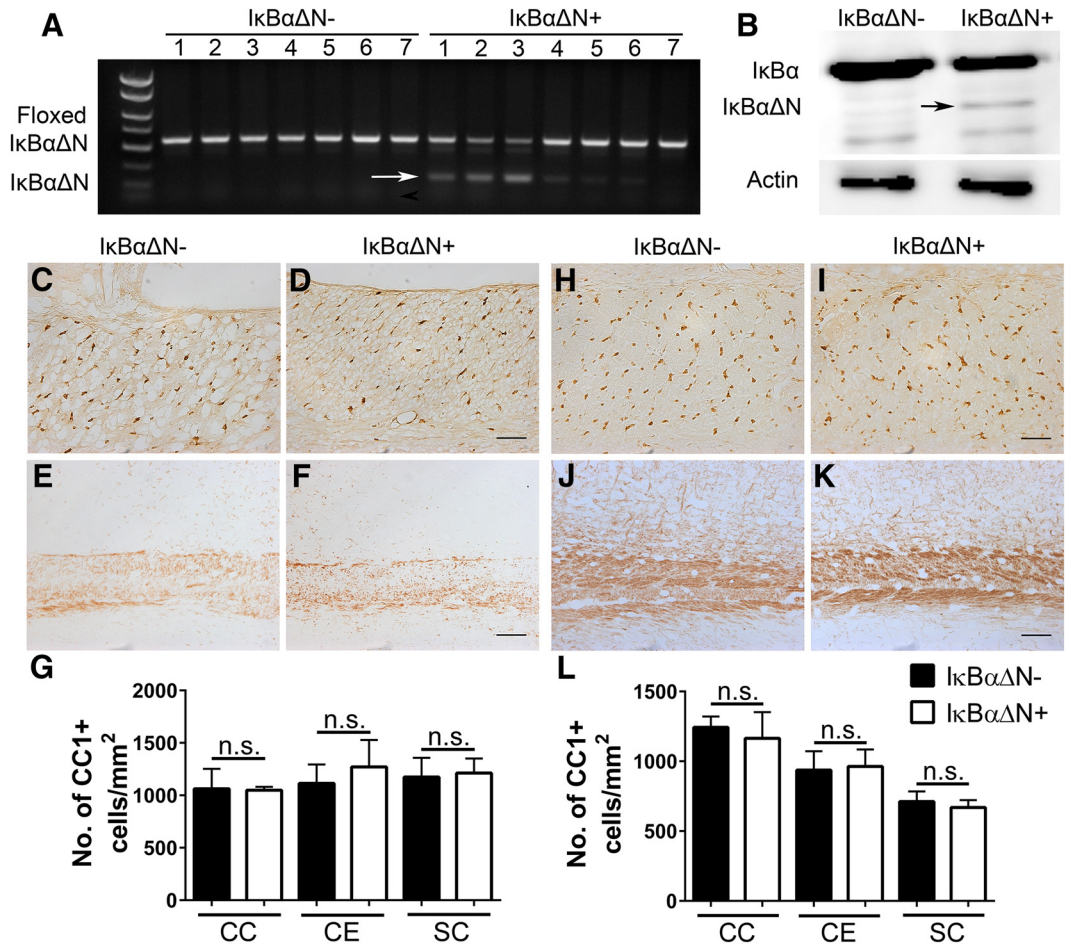

Figure 1. IκBαΔN expression does not affect the myelinating function of oligodendrocytes. ***A***, PCR analysis using genomic DNA showing the floxed IκBαΔN allele (530 base pair) in the brain (1), spinal cord (2), sciatic nerve (3), heart (4), lung (5), liver (6), and kidney (7) of IκBαΔN$^-$ mice and IκBαΔN$^+$ mice; however, IκBαΔN allele (130 bp, arrow) was only detected in the brain (1), spinal cord (2), and sciatic nerve (3) of IκBαΔN$^+$ mice. ***B***, Western blot analysis showing that IκBαΔN$^+$ mice expressed both IκBα and IκBαΔN (arrow) in the brain, whereas control IκBαΔN-mice only expressed IκBα. ***C***, ***D***, ***G***, CC1 IHC showing that oligodendrocyte-specific expression of IκBαΔN did not change oligodendrocyte numbers in the corpus callosum (CC), cerebellum (CE), and spinal cord (SC) of 21-d-old mice. ***E***, ***F***, MBP IHC showing that oligodendrocyte-specific expression of IκBαΔN did not affect myelination in the CC of 21-d-old mice. ***H***, ***I***, ***L***, CC1 IHC showing that oligodendrocyte-specific expression of IκBαΔN did not change oligodendrocyte numbers in the CC, CE, and SC of 56-d-old mice. ***J***, ***K***, MBP IHC showing that oligodendrocyte-specific expression of IκBαΔN did not affect myelination in the CC of 56-d-old mice. Scale bars, 50 μm. $n = 3$ animals. Error bars indicate SD. n.s., Not significant.

that prevents IκBαΔN translation was also removed by Cre recombination in the PNS of IκBαΔN$^+$ mice. Interestingly, toluidine blue staining revealed normal myelination in the developing and adult PNS of IκBαΔN$^+$ mice compared with IκBαΔN$^-$ mice (Fig. 2). These data provide additional evidence that NF-κB is not a major player in Schwann cell differentiation and myelin formation in the PNS under normal conditions.

NF-κB inactivation specifically in myelinating oligodendrocytes exacerbates IFN-γ-induced hypomyelination in the developing CNS

Multiple pathological features of MS lesions are recapitulated in the developing CNS of mice that express IFN-γ ectopically in the CNS, including oligodendrocyte death, myelin loss, T-cell infiltration, microglia/macrophage activation, and upregulation of inflammatory mediators (Corbin et al., 1996; LaFerla et al., 2000; Lin and Lin, 2010; Lin et al., 2014). Using the tetracycline-controllable system, we have generated *GFAP/tTA;TRE/IFN-γ* double-transgenic mice that allow for controllable expression of IFN-γ in the CNS (Lin et al., 2004). We showed that the expression of the transgene IFN-γ is repressed in *GFAP/tTA;TRE/IFN-γ* double-transgenic mice treated with Dox and that IFN-γ becomes detectable in the CNS of these mice after ~14 d of Dox removal (Lin et al., 2005, 2008). Our previous studies showed that the presence of IFN-γ in the CNS of young, developing mice stimulates inflammation, activates NF-κB, and induces myelinating oligodendrocyte death and myelin loss (Lin et al., 2005, 2008; Lin and Lin, 2010; Lin et al., 2012, 2014). Therefore, we determined the effects of NF-κB activation on myelinating oligodendrocytes under inflammatory conditions by using IFN-γ-expressing mice.

GFAP/tTA mice were crossed with *CNP/Cre* mice to obtain *GFAP/tTA;CNP/Cre* mice and *TRE/IFN-γ* mice were crossed with *cloxPIκBαΔN* mice to obtain *TRE/IFN-γ;cloxPIκBαΔN* mice. Then, *GFAP/tTA;CNP/Cre* mice were crossed with *TRE/IFN-γ;cloxPIκBαΔN* mice to obtain *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* quadruple mice and *GFAP/tTA;TRE/IFN-γ* double mice (Table 2). *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice and *GFAP/tTA;TRE/IFN-γ* mice were treated with Dox from conception to repress the transgenic *IFN-γ* expression. One group of *GFAP/tTA;TRE/IFN-γ; cloxPIκBαΔN;CNP/Cre* mice was released from Dox at embryonic day 15 (E15) (IFN-γ$^+$;IκBαΔN$^+$ mice). Another group of *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice never released from Dox served as controls (IFN-γ$^-$; IκBαΔN$^+$ mice). One group of *GFAP/tTA;TRE/IFN-γ* mice was released from Dox at E15 *(IFN-γ$^+$;IκBαΔN$^-$* mice). Another group

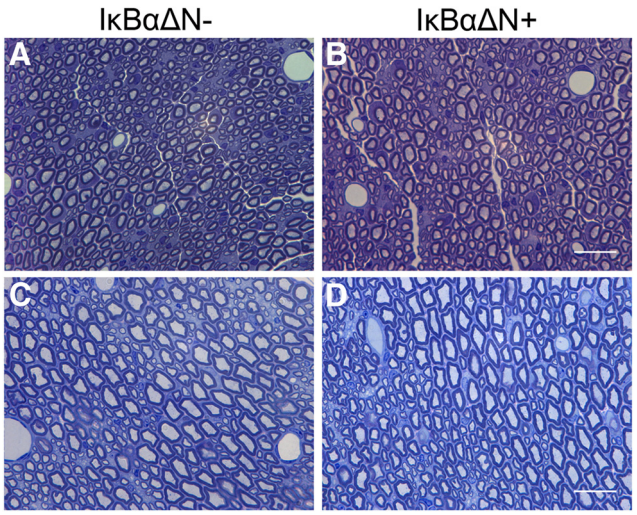

Figure 2. IκBαΔN expression did not affect the myelinating function of Schwann cells. ***A***, ***B***, Toluidine blue staining showing normal myelination in the sciatic nerve of 21-d-old IκBαΔN$^+$ mice compared with IκBαΔN$^-$ mice. ***C***, ***D***, Toluidine blue staining showing normal myelination in the sciatic nerve of 56-d-old IκBαΔN$^+$ mice compared with IκBαΔN$^-$ mice. Scale bars, 10 μm. $n = 4$ animals.

of GFAP/tTA;TRE/IFN-γ mice never released from Dox served as controls *(IFN-γ$^-$;IκBαΔN$^-$* mice*)*. We found that a number of IFN-γ$^+$;IκBαΔN$^-$ mice display tremor and ataxia as described in our previous studies (Lin et al., 2005, 2008, 2014). Importantly, IFN-γ$^+$;IκBαΔN$^+$ mice exhibited a significantly more severe tremoring phenotype compared with IFN-γ$^+$;IκBαΔN$^-$ mice (Table 3).

Double immunostaining for ASPA (a marker for oligodendrocytes) and the active form of p65 showed that immunoreactivity for the active form of p65 was undetectable in the CNS of the control IFN-γ$^-$;IκBαΔN$^-$ mice and IFN-γ$^-$;IκBαΔN$^+$ mice (Fig. 3*A–F*,*M*). A number of cells, including ASPA-positive oligodendrocytes, were positive for the active form of p65 in the CNS of IFN-γ$^+$;IκBαΔN$^-$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest; Fig. 3*G–I*,*M*). Although there were a few cells positive for the active form of p65 in the CNS of IFN-γ$^+$;IκBαΔN$^+$ mice, none of the ASPA-positive oligodendrocytes were positive for the active form of p65 in these mice (Fig. 3*J–M*). Although none of GFAP-positive astrocytes were positive for the active form of p65 in the CNS of IFN-γ$^+$;IκBαΔN$^-$ mice and IFN-γ$^+$;IκBαΔN$^+$ mice (Fig. 3*N*,*O*), the majority of CD11b-positive microglia/macrophages were positive for the active form of p65 in the CNS of these mice (Fig. 3*P*,*Q*). Therefore, these results suggest that oligodendrocyte-specific expression of IκBαΔN blocks NF-κB activation selectively in oligodendrocytes in the CNS of IFN-γ-expressing mice.

As expected, we found that IκBαΔN expression alone had no effect on oligodendrocytes or myelin in the CNS of 18-d-old mice (CC1, $p = 0.4089$; EM, $p = 0.8057$, one-way ANOVA with Tukey's posttest) and that the presence of IFN-γ in the CNS alone noticeably reduced oligodendrocyte numbers ($p < 0.021$, one-way ANOVA with Tukey's posttest) and the degree of myelination in the CNS of 18-d-old mice (Fig. 4*A–C*,*E–G*,*I–K*,*M*,*N*). Interestingly, CC1 IHC showed that the number of oligodendrocytes was further reduced in the CNS of IFN-γ$^+$;IκBαΔN$^+$ mice compared with IFN-γ$^+$;IκBαΔN$^-$ mice ($p < 0.017$, one-way ANOVA with Tukey's posttest; Fig. 4*C*,*D*,*M*). MBP IHC showed noticeably more severe myelin loss in the CNS of IFN-γ$^+$;IκBαΔN$^+$ mice compared with IFN-γ$^+$;IκBαΔN$^-$ mice (Fig. 4*G*,*H*). Moreover, EM analysis showed that there were significantly fewer myelinated axons in the CNS of IFN-γ$^+$;IκBαΔN$^-$ mice compared with IFN-γ$^-$;IκBαΔN$^-$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest), which was further reduced in IFN-γ$^+$;IκBαΔN$^+$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest; Fig. 4*I*,*K*,*L*,*N*). Together, these data suggest that oligodendrocyte-specific expression of IκBαΔN blocks NF-κB activation in oligodendrocytes and results in the increased sensitivity of oligodendrocytes to the detrimental effects of IFN-γ during development.

NF-κB is a master transcription factor that regulates the expression of genes involved in inflammation (Oeckinghaus et al., 2011; Mc Guire et al., 2013; Herrington et al., 2016). We further determined whether oligodendrocyte-specific expression of IκBαΔN influences inflammation in the CNS of IFN-γ-expressing mice. CD11b IHC showed that the presence of IFN-γ in the CNS alone strongly activated microglia/macrophages (Fig. 5*A–C*). Nevertheless, oligodendrocyte-specific expression of IκBαΔN had no significant effect on activation of microglia/macrophages in the CNS of IFN-γ-expressing mice (Fig. 5*C*,*D*). CD3 IHC also revealed comparable T-cell infiltration in the CNS of IFN-γ$^+$;IκBαΔN$^-$ mice and IFN-γ$^+$;IκBαΔN$^+$ mice (Fig. 5*E–H*). Real-time PCR analysis showed comparable levels of IFN-γ in the CNS of IFN-γ$^+$;IκBαΔN$^-$ mice and IFN-γ$^+$;IκBαΔN$^+$ mice (IFN-γ$^-$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^-$ mice, $p < 0.0001$; IFN-γ$^+$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^+$ mice, $p = 0.8957$; one-way ANOVA with Tukey's posttest; Fig. 5*I*). Moreover, the presence of IFN-γ in the CNS alone strongly stimulated the expression of tumor necrosis factor α (TNFα, IFN-γ$^-$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^-$ mice, $p = 0.0008$; one-way ANOVA with Tukey's posttest) and inducible nitric oxide synthase (iNOS, IFN-γ$^-$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^-$ mice, $p = 0.0104$; one-way ANOVA with Tukey's posttest); however, oligodendrocyte-specific expression of IκBαΔN did not affect the expression of TNFα or iNOS significantly in the CNS of IFN-γ-expressing mice (TNFα: IFN-γ$^+$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^+$ mice, $p = 0.9952$; iNOS: IFN-γ$^+$;IκBαΔN$^-$ mice vs IFN-γ$^+$;IκBαΔN$^+$ mice, $p = 0.9987$; one-way ANOVA with Tukey's posttest; Fig. 5*I*). These results suggest that NF-κB inactivation specifically in oligodendrocytes has no effect on inflammation in the CNS of IFN-γ-expressing mice. Collectively, our data indicate that NF-κB activation in myelinating oligodendrocytes cell autonomously protects the cells against inflammation in the developing CNS of IFN-γ-expressing mice.

NF-κB inactivation does not affect the viability or function of oligodendrocytes in the cuprizone model

The cuprizone model is regarded as one of the best mouse models to understand the processes of remyelination in the CNS and is increasingly used to study the mechanisms of remyelination in MS (Matsushima and Morell, 2001; Denic et al., 2011). It is believed that cuprizone acts directly on mature oligodendrocytes, resulting in mature oligodendrocyte apoptosis and subsequent demyelination in the corpus callosum. Oligodendrocytes progenitor cells proliferate and differentiate within the lesion and oligodendrocytes then repopulate the area, resulting in complete remyelination after several weeks of cuprizone removal. A previous study showed that CNS-restricted deletion of IKK2 prevents mature oligodendrocyte apoptosis and demyelination in the cuprizone model; however, oligodendrocyte-restricted deletion of IKK2 had no effect on the demyelination and remyelination processes in this model (Raasch et al., 2011). Herein, we investigated the effects of oligodendrocyte-specific expression of IκBαΔN on

Table 3. IFN-γ^+;IκBαΔN$^+$ mice displayed a more severe tremor than IFN-γ^+;IκBαΔN$^-$ mice

Mouse line	No tremor (%)	Mild tremor (%)	Tremor (%)	Severe tremor (%)
IFN-γ^-;IκBαΔN$^-$ ($n = 40$)	100	0	0	0
IFN-γ^-;IκBαΔN$^+$ ($n = 36$)	100	0	0	0
IFN-γ^+;IκBαΔN$^-$ ($n = 41$)	66	12	15	7
IFN-γ^+;IκBαΔN$^+$ ($n = 20$)	5* ($p < 0.0001$)	5 ($p = 0.5127$)	35* ($p = 0.0228$)	55* ($p < 0.0001$)

Mild tremor indicates tremor only in tail; tremor is tremor in tail and body; severe tremor is whole-body tremor and occasional seizures.

*$p < 0.05$.

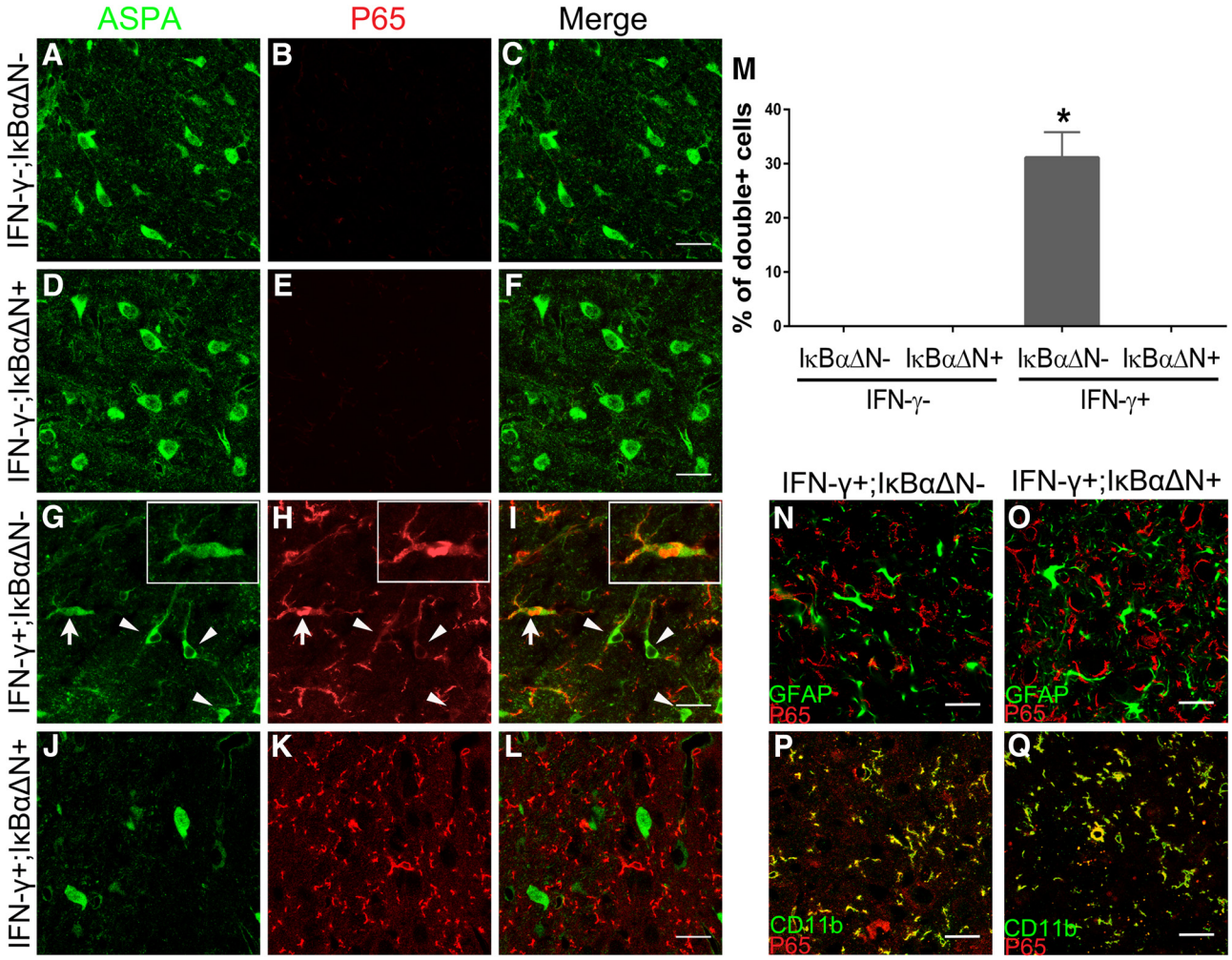

Figure 3. Oligodendrocyte-specific expression of IκBαΔN inhibited IFN-γ-induced NF-κB activation selectively in oligodendrocytes. ***A–F***, ASPA and active p65 double immunostaining showing that active p65 was undetectable in oligodendrocytes in the CNS of 18-d-old IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. ***G–I***, Active p65 became detectable in a number of oligodendrocytes (arrow and arrowheads) of IFN-γ^+;IκBαΔN$^-$ mice. Inset, ASPA and active p65 double-positive cell (arrow). ***J–L***, None of ASPA-positive cells were positive for active p65 in IFN-γ^+;IκBαΔN$^+$ mice. ***M***, Quantitative analysis of oligodendrocytes positive for the active form of p65 in the CNS. ***N***, ***O***, None of GFAP-positive astrocytes were positive for active p65 in either IFN-γ^+;IκBαΔN$^-$ mice or IFN-γ^+;IκBαΔN$^+$ mice. ***P***, ***Q***, The majority of CD11b-positive microglia/macrophages were positive for active p65 in both IFN-γ^+;IκBαΔN$^-$ mice and IFN-γ^+;IκBαΔN$^+$ mice. Scale bars, 20 μm. $n = 4$ animals. Error bars indicate SD. *$p < 0.05$.

oligodendrocyte apoptosis and regeneration in the cuprizone model.

Six-week-old male *cloxPIκBαΔN;CNP/Cre* mice (IκBαΔN$^+$ mice) and control mice (IκBαΔN$^-$ mice), including *cloxPIκBαΔN* mice, *CNP/Cre* mice, and wild-type mice, were treated with 0.2% cuprizone chow for up to 6 weeks. In accordance with previous reports (Matsushima and Morell, 2001; Denic et al., 2011), there was severe oligodendrocyte loss and demyelination in the corpus callosum of IκBαΔN$^-$ mice after 5 weeks of cuprizone treatment compared with untreated mice (Fig. 6*A*,*C*,*E*,*F*,*H*,*J*). We found that oligodendrocyte-specific expression of IκBαΔN did not affect oligodendrocyte loss or demyelination in the corpus callosum after 5 weeks of cuprizone treatment ($p = 0.8814$, *t* test; Fig. 6*A–J*). These results suggest the minimal role of NF-κB in oligodendrocytes during the cuprizone-induced demyelination process.

To determine the effects of NF-κB on remyelinating oligodendrocytes, 6-week-old male IκBαΔN$^+$ and IκBαΔN$^-$ mice were treated with 0.2% cuprizone chow for 6 weeks and then returned to a normal diet for 3 weeks to allow remyelination to occur. CC1 immunostaining showed that the number of remyelinating oligodendrocytes was comparable in the corpus callosum of IκBαΔN$^+$ mice and IκBαΔN$^-$ mice 3 weeks after cuprizone removal ($p = 0.1046$, *t* test; Fig. 6*K*,*L*,*O*). Moreover, MBP immunostaining showed that oligodendrocyte-specific expression

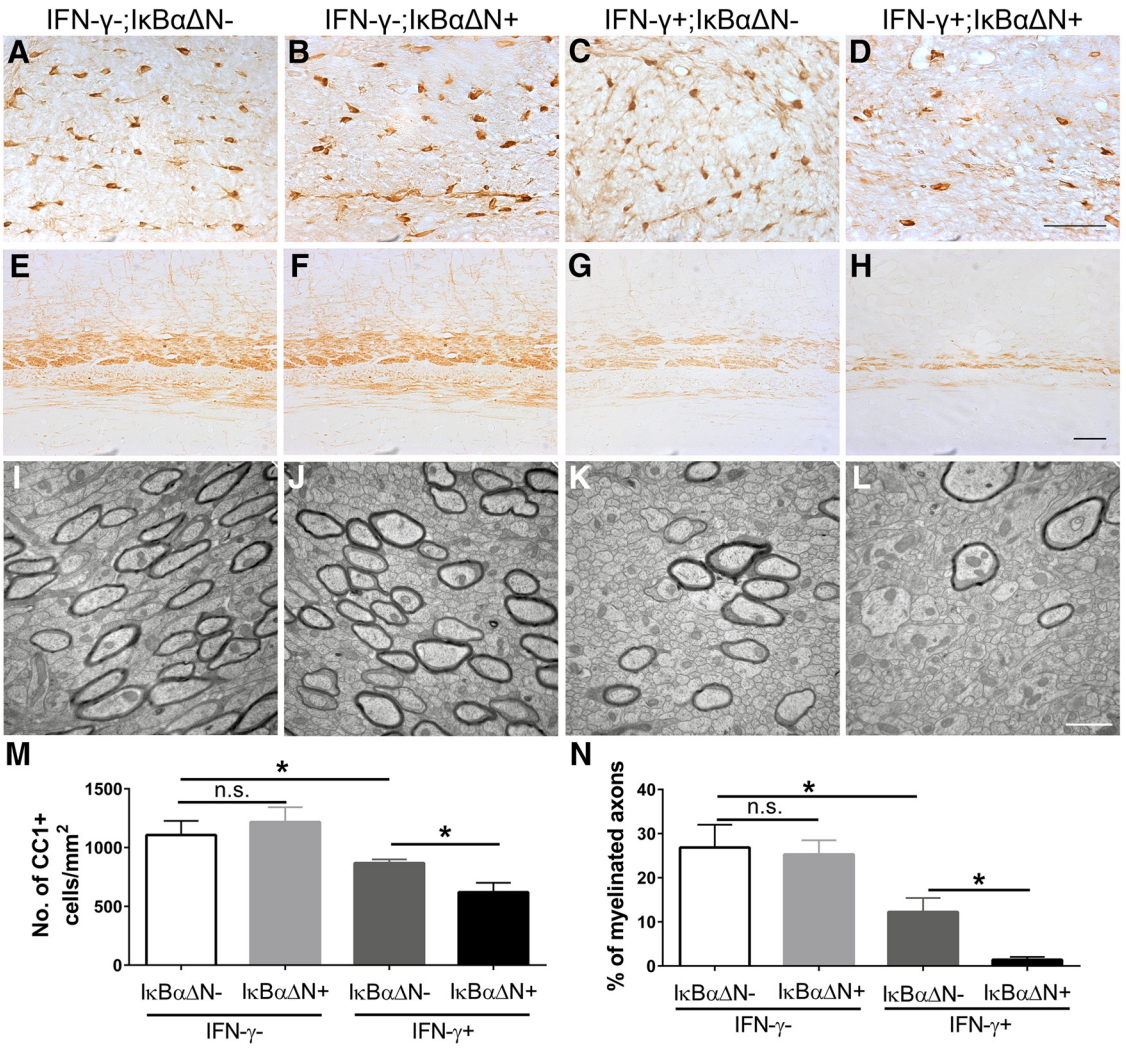

Figure 4. Oligodendrocyte-specific expression of IκBαΔN exacerbated IFN-γ-induced hypomyelination during development. ***A–D, M***, CC1 IHC showing comparable oligodendrocyte numbers in the corpus callosum of 18-d-old IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. Oligodendrocyte numbers were significantly reduced in IFN-γ^+;IκBαΔN$^-$ mice and were further reduced in IFN-γ^+;IκBαΔN$^+$ mice. ***E–H***, MBP IHC showing a comparable degree of myelination in the corpus callosum of 18-d-old IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. The degree of myelination was noticeably reduced in IFN-γ^+;IκBαΔN$^-$ mice and was further reduced in IFN-γ^+;IκBαΔN$^+$ mice. ***I–L***, ***N***, EM analysis showing a comparable percentage of myelinated axons in the corpus callosum of 18-d-old IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. The percentage of myelinated axons was significantly reduced in IFN-γ^+;IκBαΔN$^-$ mice and further reduced in IFN-γ^+;IκBαΔN$^+$ mice. Scale bars: ***A–H***, 50 μm; ***I–L***, 1 μm. $n = 4$ animals. Error bars indicate SD. *$p < 0.05$. n.s., Not significant.

of IκBαΔN did not change the degree of remyelination in the demyelinated lesions significantly 3 weeks after cuprizone removal (Fig. 6*M*,*N*). Therefore, these data suggest the minimal role of NF-κB in remyelinating oligodendrocytes in demyelinated lesions in adult mice.

NF-κB inactivation in remyelinating oligodendrocytes aggravates IFN-γ-induced remyelination failure in the cuprizone model

Our previous studies show that the presence of IFN-γ in the CNS suppresses oligodendrocyte regeneration and remyelination in the cuprizone-induced demyelinated lesions (Lin et al., 2006, 2014). Therefore, we used the cuprizone model to assess the effects of NF-κB activation on remyelinating oligodendrocytes in response to IFN-γ. Six-week-old male *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* quadruple mice and *GFAP/tTA;TRE/IFN-γ* double mice that had been maintained on Dox from conception were treated with 0.2% cuprizone chow for 6 weeks and then returned to a normal diet for 3 weeks. One group of *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice were released from Dox starting on the day of cuprizone treatment (IFN-γ^+;IκBαΔN$^+$ mice). Another group of *GFAP/tTA;TRE/IFN-γ;cloxPIκBαΔN;CNP/Cre* mice were never released from Dox to serve as controls (IFN-γ^-;IκBαΔN$^+$ mice). One group of *GFAP/tTA;TRE/IFN-γ* mice were released from Dox starting on the day of cuprizone treatment (IFN-γ^+;IκBαΔN$^-$ mice). Another group of GFAP/tTA;TRE/IFN-γ mice were never released from Dox to serve as controls (IFN-γ^-;IκBαΔN$^-$ mice).

We found that oligodendrocyte-specific expression of IκBαΔN did not change the number of remyelinating oligodendrocytes or the degree of remyelination significantly in the corpus callosum of the control animals 3 weeks after cuprizone removal (CC1, $p = 0.9552$; EM, $p > 0.9999$; one-way ANOVA with Tukey's posttest; Fig. 7*A*,*B*,*E*,*F*,*I*,*J*,*M*,*N*). Consistent with our previous studies (Lin et al., 2006, 2014), we found that the presence of IFN-γ in the CNS impaired oligodendrocyte regeneration significantly ($p < 0.0037$, one-way ANOVA with Tukey's post-

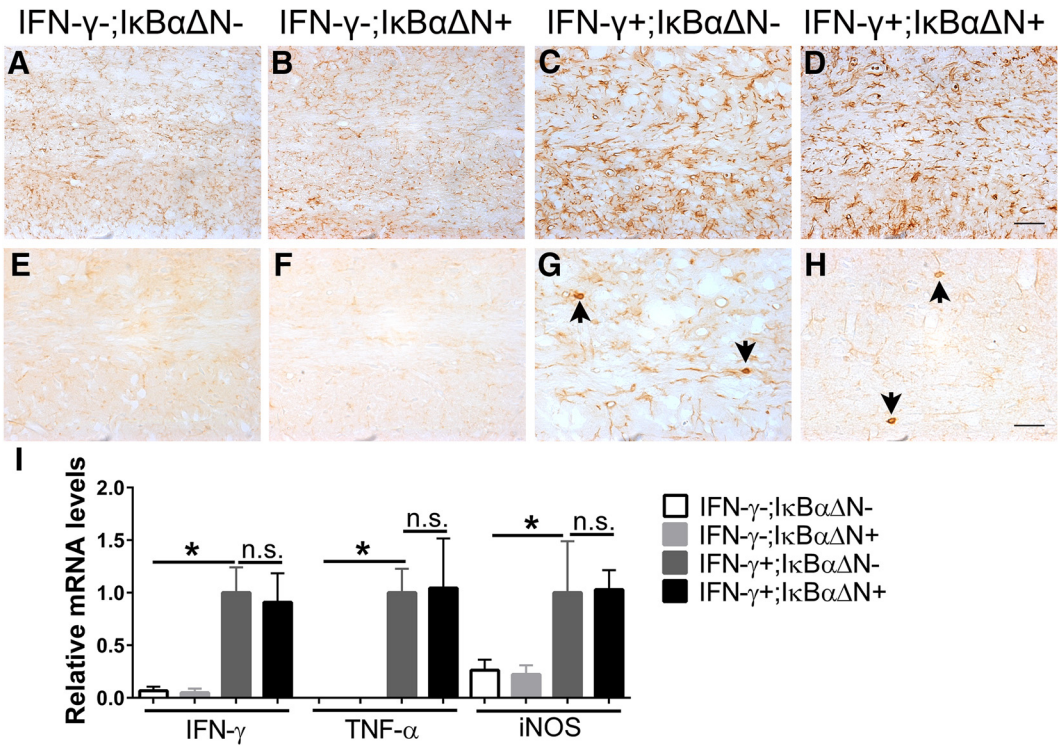

Figure 5. Oligodendrocyte-specific expression of IκBαΔN did not alter inflammation in the CNS of IFN-γ-expressing mice. ***A–D***, CD11b IHC showing strong activation of microglia/macrophages in the corpus callosum of 18-d-old IFN-γ^+;IκBαΔN$^-$ mice compared with IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. However, the degree of microglia/macrophage activation was comparable in IFN-γ^+;IκBαΔN$^-$ mice and IFN-γ^+;IκBαΔN$^+$ mice. ***E–H***, CD3 IHC showing few infiltrating T cells (arrow) in the corpus callosum of 18-d-old IFN-γ^+;IκBαΔN$^-$ mice compared with IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. However, the numbers of infiltrating T cells (arrow) in IFN-γ^+;IκBαΔN$^+$ mice were comparable to those in IFN-γ^+;IκBαΔN$^-$ mice. ***I***, Real-time PCR analysis revealed dramatically increased the levels of IFN-γ, TNF-α, and iNOS in the brains of 18-d-old IFN-γ^+;IκBαΔN$^-$ mice compared with IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice. However, the levels of IFN-γ, TNF-α, and iNOS were comparable in IFN-γ^+;IκBαΔN$^-$ mice and IFN-γ^+;IκBαΔN$^+$ mice. Scale bars, 50 μm. $n = 4$ animals. Error bars indicate SD. *$p < 0.05$. n.s., Not significant.

test) and remyelination in the corpus callosum of IFN-γ^+;IκBαΔN$^-$ mice compared with control IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice (Fig. 7*A–C*,*E–G*,*I–K*,*M*,*N*). Importantly, the number of remyelinating oligodendrocytes in the corpus callosum of IFN-γ^+;IκBαΔN$^+$ mice was further reduced compared with IFN-γ^+;IκBαΔN$^-$ mice ($p < 0.0104$, one-way ANOVA with Tukey's posttest; Fig. 7*C*,*D*,*M*). MBP immunostaining also showed noticeably weaker MBP immunoreactivity in the corpus callosum of IFN-γ^+;IκBαΔN$^+$ mice than IFN-γ^+;IκBαΔN$^-$ mice (Fig. 7*G*,*H*). Moreover, EM analysis confirmed that there were fewer remyelinated axons in the corpus callosum of IFN-γ^+;IκBαΔN$^-$ mice compared with IFN-γ^-;IκBαΔN$^-$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest) and IFN-γ^-;IκBαΔN$^+$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest), which was further reduced in IFN-γ^+;IκBαΔN$^+$ mice ($p < 0.0001$, one-way ANOVA with Tukey's posttest; Fig. 7*I–L*,*N*). Collectively, these data demonstrate that NF-κB inactivation in remyelinating oligodendrocytes increases the sensitivity of the cells to the detrimental effects of IFN-γ in the cuprizone model.

NF-κB inactivation in mature oligodendrocytes increases the susceptibility of mice to EAE

EAE, the primary animal model used in MS research, displays many of the clinical, pathological, and immunological features of MS (Kipp et al., 2017; Lassmann and Bradl, 2017). Our previous study demonstrated NF-κB activation in oligodendrocytes in the MOG-EAE model (Lin et al., 2013). We examined the effects of NF-κB inactivation in mature oligodendrocytes on the development of MOG-EAE. Eight-week-old female *cloxPIκBαΔN;CNP/Cre* mice (IκBαΔN$^+$ mice) and control mice (IκBαΔN$^-$ mice), including *cloxPIκBαΔN* mice, *CNP/Cre* mice, and wild-type mice, were immunized with MOG35-55 peptide to induce EAE. IκBαΔN$^-$ mice developed a typical EAE disease course (Fig. 8*A*,*B*). Interestingly, IκBαΔN$^+$ mice developed very severe EAE: 22 of 27 mice died by PID 21 ($p = 0.0367$ to <0.0001, two-way ANOVA of disease course with a Sidak's multiple-comparisons test, Fig. 8*A*; $p < 0.0001$, *t* test for peak score, Fig. 8*B*). The majority of these mice died suddenly at ~PID 14 (ranging from PID 10–21) before they exhibited classical EAE clinical symptoms such as flaccid tail, hindlimb paresis, or hindlimb paralysis. Surviving IκBαΔN$^+$ mice (5 of 27) displayed EAE clinical symptoms similar to IκBαΔN$^-$ mice (Fig. 8*B*). Because of early, sudden death without obvious clinical symptoms, we were not able to collect CNS tissues to perform histopathologic analyses despite the large number of IκBαΔN$^+$ mice ($n = 27$ animals) used in this experiment.

EAE is initiated by T-cell priming in the mouse peripheral immune system and subsequent autoimmune reactions against oligodendrocytes and myelin in the CNS (Kipp et al., 2017; Lassmann and Bradl, 2017). We investigated whether T-cell priming was altered in IκBαΔN$^+$ mice during EAE. Spleen leukocytes were generated from IκBαΔN$^+$ mice and IκBαΔN$^-$ mice undergoing EAE at PID 10 and their *in vitro* response to a secondary exposure to MOG35–55 peptide was analyzed. Interestingly, *in vitro* recall assays showed that the abilities of T cells generated from IκBαΔN$^+$ mice to proliferate ($p = 0.0996–0.8696$, two-way ANOVA of disease course with a Sidak's multiple-comparisons test) and to produce the cytokines IFN-γ ($p =$

0.3408, *t* test), IL-4 ($p = 0.1058$, *t* test), and IL-17A ($P = 0.0949$, *t* test) were not changed significantly compared with those from IκBαΔN$^-$ mice (Fig. 8*C–F*). A large number of studies demonstrate that impaired NF-κB activation in inflammatory cells suppresses inflammation (Yan and Greer, 2008; Mc Guire et al., 2013; Hao et al., 2016). Therefore, it is unlikely that the exacerbated EAE displayed by IκBαΔN$^+$ mice is due to enhanced inflammation even if there is a leaky expression of IκBαΔN in inflammatory cells in these mice. Conversely, our studies described above showed the detrimental effects of NF-κB inactivation on oligodendrocytes in response to inflammation. Several studies have shown that increased vulnerability of mature oligodendrocytes to inflammation results in increased EAE disease severity (Hisahara et al., 2001; Hussien et al., 2014, 2015). Collectively, these data raise the possibility that NF-κB inactivation in mature oligodendrocytes exacerbates EAE disease severity by rendering oligodendrocytes vulnerable to inflammation.

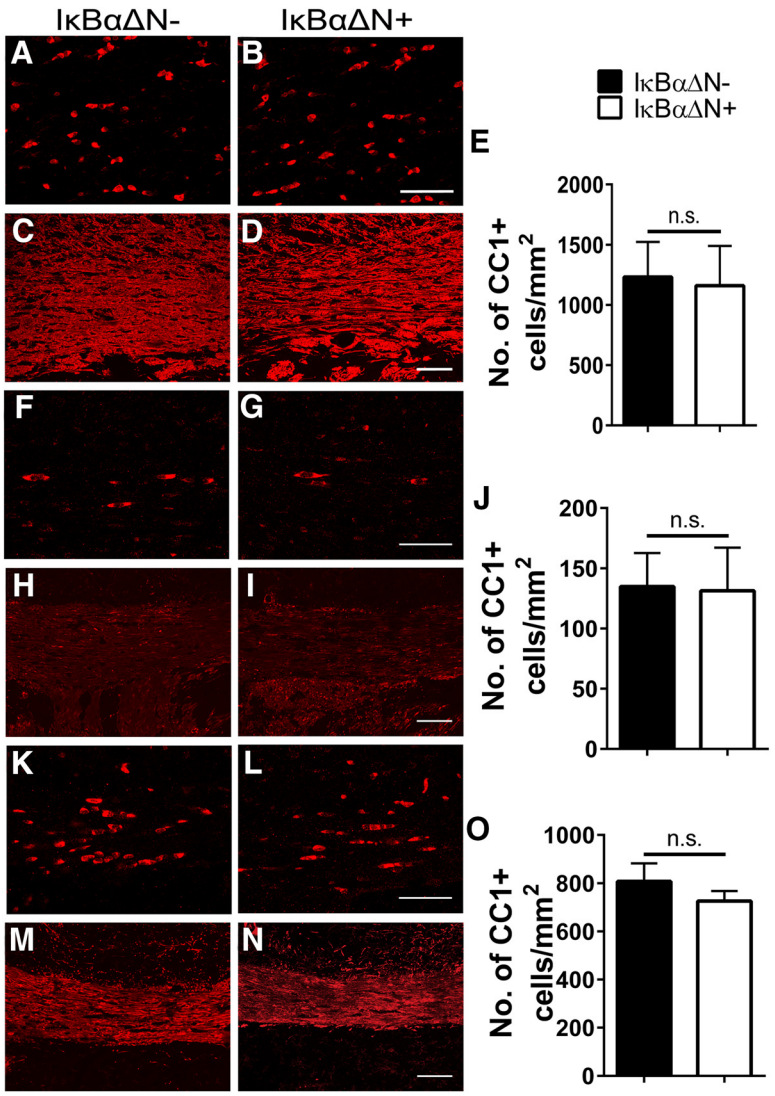

Figure 6. Oligodendrocyte-specific expression of IκBαΔN did not influence demyelination or remyelination in the cuprizone model. ***A***, ***B***, ***E***, CC1 immunostaining showing comparable oligodendrocyte numbers in the corpus callosum of 6-week-old IκBαΔN$^-$ mice and IκBαΔN$^+$ mice. ***C***, ***D***, MBP immunostaining showing comparable, strong myelination in the corpus callosum of 6-week-old IκBαΔN$^-$ mice and IκBαΔN$^+$ mice. ***F***, ***G***, ***J***, CC1 immunostaining showing comparable oligodendrocytes loss in the corpus callosum of IκBαΔN$^-$ mice and IκBαΔN$^+$ mice after 5 weeks of cuprizone treatment. ***H***, ***I***, MBP immunostaining showing comparable demyelination in the corpus callosum of IκBαΔN$^-$ mice and IκBαΔN$^+$ mice after 5 weeks of cuprizone treatment. ***K***, ***L***, ***O***, CC1 immunostaining showing comparable oligodendrocyte regeneration in the corpus callosum of IκBαΔN$^-$ mice and IκBαΔN$^+$ mice 3 weeks after cuprizone removal. ***M***, ***N***, MBP immunostaining showing comparable remyelination in the corpus callosum of IκBαΔN$^-$ mice and IκBαΔN$^+$ mice 3 weeks after cuprizone removal. Scale bars, 50 μm. $n = 4$ animals. Error bars indicate SD. n.s., Not significant.

Discussion

The transcription factor NF-κB plays a critical role in inflammatory diseases by regulating inflammation and cell viability (Oeckinghaus et al., 2011; Mc Guire et al., 2013; Herrington et al., 2016). NF-κB can be activated by diverse stimuli, including various inflammatory mediators, growth factors, and cellular stresses, through a variety of ways, including the IKK2-dependent canonical pathway, the noncanonical pathway, and atypical pathways (Hayden and Ghosh, 2012; Mincheva-Tasheva and Soler, 2013). MS and EAE are full-blown inflammatory diseases in the CNS (Frohman et al., 2006; Lassmann and Bradl, 2017). NF-κB is activated in the CNS cells in these diseases through both the IKK2-dependent pathway and IKK2-independent pathways. It is well documented that NF-κB is one of master regulators of inflammation in MS and EAE (Yan and Greer, 2008; Mc Guire et al., 2013; Hao et al., 2016). Correlative *in vitro* studies point to the cytoprotective effects of NF-κB activation on oligodendrocytes in these diseases (Nicholas et al., 2001; Hamanoue et al., 2004; Lin et al., 2012). However, to date, attempts to understand the role of NF-κB activation in oligodendrocytes in MS and EAE have been unsuccessful.

Oligodendrocyte regeneration and subsequent remyelination is essential and necessary to restore neurological function in MS patients; however, remyelination is insufficient in MS lesions (Franklin and ffrench-Constant, 2008; Kotter et al., 2011). (Re)myelinating oligodendrocytes must produce enormous amounts of membrane proteins and lipids to assemble myelin sheaths. Due to an extremely high rate of metabolism, (re)myelinating oligodendrocytes are highly vulnerable to inflammation (Lin and Popko, 2009; Bradl and Lassmann, 2010). Death of (re)myelinating oligodendrocytes induced by inflammation contributes to remyelination failure in MS (Bradl and Lassmann, 2010; Watzlawik et al., 2010; Stone and Lin, 2015). Therefore, it is important to understand the role of NF-κB activation in (re)myelinating oligodendrocytes in MS lesions. In this study, we generated a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes and demonstrated that oligodendrocyte-specific expression of IκBαΔN had no effect on (re)myelinating oligodendrocytes in the developing CNS and in cuprizone-induced demyelinated lesions. IFN-γ is regarded as a major contributing factor to poor remyelination in MS lesions (Lin et al., 2006, 2014; Stone and Lin, 2015).

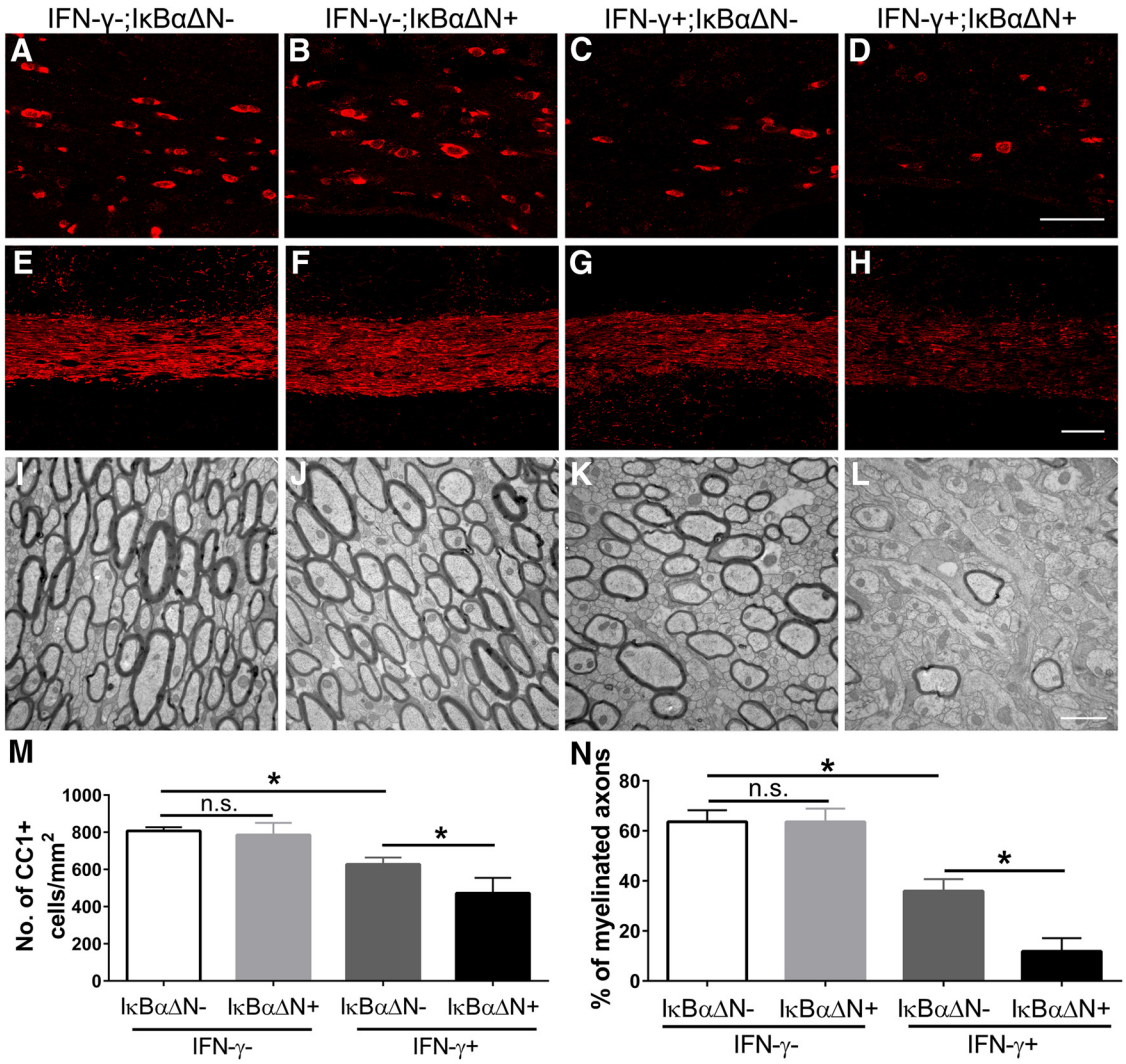

Figure 7. Oligodendrocyte-specific expression of IκBαΔN exacerbated IFN-γ-induced remyelination failure in the cuprizone model. ***A–D***, ***M***, CC1 immunostaining showing comparable oligodendrocyte numbers in the corpus callosum of IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice 3 weeks after cuprizone removal. Oligodendrocyte numbers were significantly reduced in IFN-γ^+;IκBαΔN$^-$ mice and were further reduced in IFN-γ^+;IκBαΔN$^+$ mice. ***E–H***. MBP immunostaining showing a comparable degree of remyelination in the corpus callosum of IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice 3 weeks after cuprizone removal. The degree of remyelination was noticeably reduced in IFN-γ^+;IκBαΔN$^-$ mice and was further reduced in IFN-γ^+;IκBαΔN$^+$ mice. ***I–L***, ***N***, EM analysis showing a comparable percentage of myelinated axons in the corpus callosum of IFN-γ^-;IκBαΔN$^-$ mice and IFN-γ^-;IκBαΔN$^+$ mice 3 weeks after cuprizone removal. The percentage of myelinated axons was significantly reduced in IFN-γ^+;IκBαΔN$^-$ mice and further reduced in IFN-γ^+;IκBαΔN$^+$ mice. Scale bars: ***A–H***, 50 μm; ***I–L***, 1 μm. $n = 4$ animals. Error bars indicate SD. *$p < 0.05$. n.s., Not significant.

Evidence suggests that IFN-γ exerts its detrimental effects on (re)myelinating oligodendrocytes by stimulating inflammation and by acting directly on the cells (Popko and Baerwald, 1999; Lin et al., 2006, 2008; Lin and Lin, 2010; Lin et al., 2014). Interestingly, we showed here that oligodendrocyte-specific expression of IκBαΔN blocked NF-κB activation selectively in oligodendrocytes and exacerbated myelinating oligodendrocyte death and hypomyelination, but did not alter inflammation in the developing CNS of IFN-γ-expressing mice. Moreover, we found that NF-κB inactivation specifically in oligodendrocytes aggravated IFN-γ-induced remyelinating oligodendrocyte death and remyelination failure in the cuprizone model. Collectively, these results suggest that NF-κB activation protects (re)myelinating oligodendrocytes cell autonomously against inflammation in MS lesions.

MS and EAE are thought to be initiated by an autoimmune reaction against oligodendrocytes and myelin (Frohman et al., 2006; Bradl and Lassmann, 2010). Several studies have shown that mature oligodendrocyte apoptosis is the earliest structural change in newly forming demyelinating lesions in MS and EAE (Barnett and Prineas, 2004; Lin et al., 2013). A number of studies have shown that mature oligodendrocytes with an enhanced ability to resist inflammation make mice resistant to EAE (Hisahara et al., 2000; Mc Guire et al., 2010; Lin et al., 2013). In contrast, mature oligodendrocytes with a compromised ability to respond to inflammation render mice susceptible to EAE (Hisahara et al., 2001; Hussien et al., 2014, 2015). We showed here that IκBαΔN$^+$ mice developed extremely severe EAE with very high mortality. Unfortunately, we were not able to collect tissues to assess tissue damage or inflammation in the CNS of these mice due to early, sudden death without noticeable clinical phenotypes. Although a number of studies demonstrate that *CNP/Cre* knock-in mice express Cre specifically in oligodendrocytes and Schwann cells (Lappe-Siefke et al., 2003; Hussien et al., 2014, 2015), the possibility of a leaky expression of IκBαΔN in inflammatory cells in IκBαΔN$^+$ mice cannot be ruled out completely. Nevertheless, we

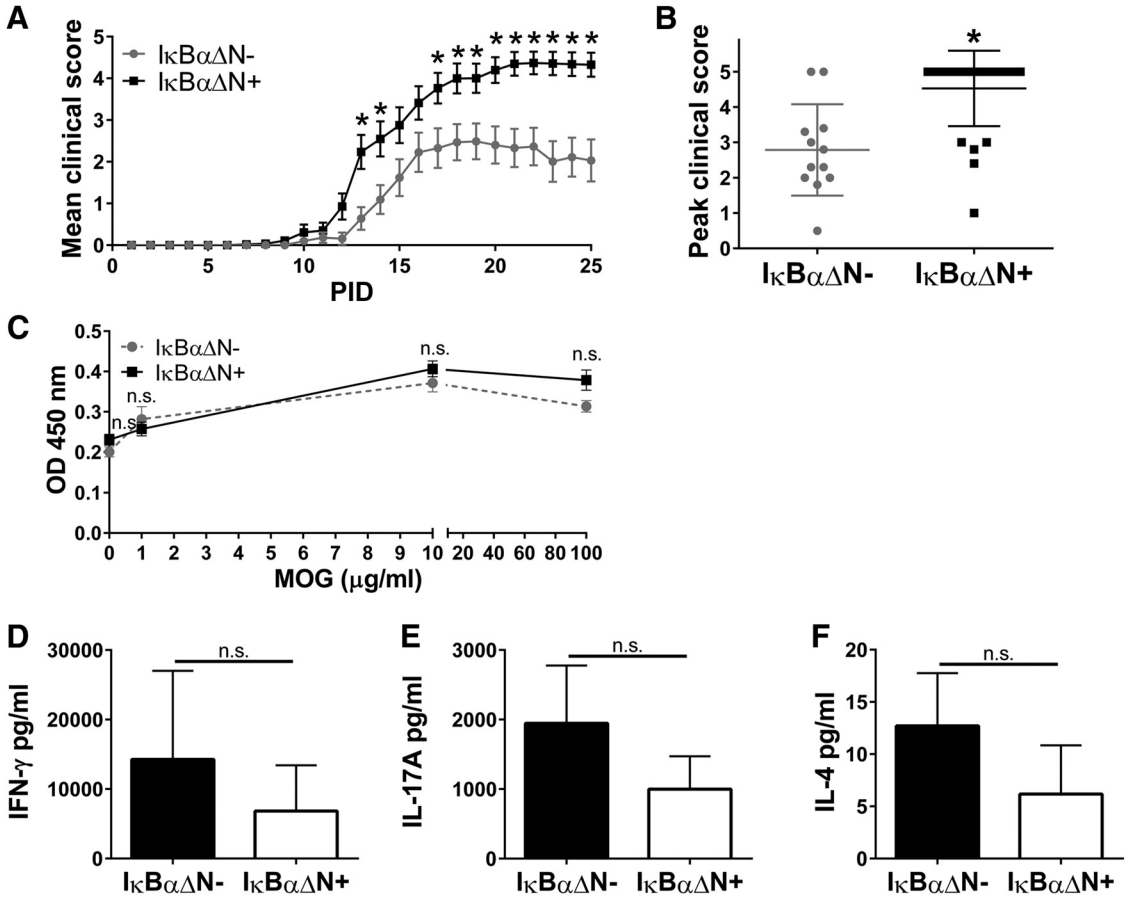

Figure 8. Oligodendrocyte-specific expression of IκBαΔN increased EAE disease severity. ***A***, IκBαΔN$^+$ mice develop much more severe EAE compared with IκBαΔN$^-$ mice. Error bars indicate SEM. IκBαΔN$^-$ mice, $n = 12$ animal; IκBαΔN$^+$ mice, $n = 27$ animal. *$p < 0.05$. ***B***, Peak clinical score for individual mice. IκBαΔN$^-$ mice, $n = 12$ animal; IκBαΔN$^+$ mice, $n = 27$ animal. *$p < 0.05$. ***C***, BrdU cell proliferation assay showing that oligodendrocyte-specific expression of IκBαΔN did not affect T-cell proliferation in response to MOG35–55 peptide. $n = 4$ animals. Error bars indicate SD. n.s., Not significant. ***D–F***, ELISA analysis showing that oligodendrocyte-specific expression of IκBαΔN did not affect significantly the ability of T cells to produce the cytokines IFN-γ, IL-17A, or IL-4 in response to MOG35–55 peptide. $n = 4$ animals. Error bars indicate SD. n.s., Not significant.

showed that T-cell priming was not altered in IκBαΔN$^+$ mice undergoing EAE. Importantly, it is well documented that NF-κB activation in inflammatory cells increases EAE disease severity (Yan and Greer, 2008; McGuire et al., 2013; Hao et al., 2016). Therefore, the possibility that the exacerbated EAE displayed by IκBαΔN$^+$ mice is caused by enhanced inflammation due to an undesired leaky expression of IκBαΔN in inflammatory cells is very remote. Rather, our data suggest that NF-κB activation in mature oligodendrocytes is protective, protecting mice against EAE.

Although NF-κB is increasingly recognized as a prosurvival transcription factor, the precise molecular mechanisms responsible for the cytoprotective effects of NF-κB remain elusive. Several studies have shown that NF-κB activation exerts cytoprotection through the induction of anti-apoptotic genes including cIAPs, cFLIP, Bcl-2, Bcl-x_L, TRAF1, and TRAF2 (Karin and Lin, 2002; Oeckinghaus et al., 2011; Mincheva-Tasheva and Soler, 2013). It is possible that the cytoprotective effects of NF-κB on oligodendrocytes under inflammatory conditions results from the combined upregulation of multiple of these anti-apoptotic genes. Conversely, a recent study showed that upregulation of cFLIP protects cultured primary oligodendrocytes against the cytotoxicity of TNF-α and that downregulation of cFLIP makes oligodendrocytes sensitive to inflammation in the CNS of transgenic mice that ectopically express human IL-1β in astrocytes (Tanner et al., 2015). An alternative, but not mutually exclusive, possibility is that NF-κB activation protects oligodendrocytes against inflammation through induction of cFLIP. Unfortunately, the mouse models used in this study do not allow us to assess the contribution of cFLIP to the cytoprotective effects of NF-κB activation on oligodendrocytes. A mouse model that allows for controllable activation of NF-κB and inactivation of cFLIP specifically in oligodendrocytes would be an ideal model with which to address this important question.

Data indicate that NF-κB is activated in oligodendrocytes in MS and EAE through both the IKK2-dependent pathway and IKK2-independent pathways (Yan and Greer, 2008; Mc Guire et al., 2013). For example, our recent study shows that activation of pancreatic endoplasmic reticulum kinase (PERK) in response to endoplasmic reticulum stress suppresses IκBα translation by phosphorylating translation initiation factor 2α (eIF2α), resulting in NF-κB activation in oligodendrocytes in the CNS of EAE mice (Lin et al., 2013). Moreover, the presence of IFN-γ in the CNS can activate NF-κB in oligodendrocytes through both the IKK2-dependent pathway and IKK2-independent pathways. For example, our previous study demonstrates that IFN-γ can activate NF-κB in oligodendrocytes by suppressing of IκBα translation through the PERK-eIF2α pathway (Lin et al., 2012). We showed here that IκBαΔN expression eliminated NF-κB activation in oligodendrocytes in the CNS of IFN-γ-expressing mice,

suggesting that NF-κB activation in oligodendrocytes under inflammatory conditions via both the IKK2-dependent pathway and IKK2-independent pathways can be blocked effectively by expression of IκBαΔN. In contrast, a previous study shows that oligodendrocyte-specific deletion of IKK2 using *IKK2*^{FL}*;MOG/Cre* mice does not affect the development of EAE (Raasch et al., 2011). The minimal effects of oligodendrocyte-specific deletion of IKK2 in EAE likely reflect the inability of IKK2 deletion alone to impair NF-κB activation in the cells under inflammatory conditions.

In summary, using a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes in the CNS, the results presented in this study demonstrate the dispensable role of NF-κB in oligodendrocytes under physiological conditions and the cytoprotective role of NF-κB activation in oligodendrocytes in animal models of MS. These findings implicate that NF-κB activation protects oligodendrocytes (both mature and remyelinating oligodendrocytes) against inflammation in MS patients, suggesting a therapeutic cytoprotective potential of NF-κB activation in MS. In contrast, a number of studies have shown that NF-κB activation in T cells, microglia/macrophages, or astrocytes enhances inflammation and facilitates the pathogenesis of MS and EAE, implying the therapeutic anti-inflammatory potential of NF-κB inhibition in MS (Brambilla et al., 2009; Mc Guire et al., 2013; Yin et al., 2013; Hao et al., 2016). Therefore, it is unlikely that NF-κB is an ideal therapeutic target for MS due to its paradoxical effects in this disease.

References

Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 55:458–468. CrossRef Medline

Blank T, Prinz M (2014) NF-κB signaling regulates myelination in the CNS. Front Mol Neurosci 7:47. CrossRef Medline

Bonetti B, Stegagno C, Cannella B, Rizzuto N, Moretto G, Raine CS (1999) Activation of NF-kappaB and c-jun transcription factors in multiple sclerosis lesions: implications for oligodendrocyte pathology. Am J Pathol 155:1433–1438. CrossRef Medline

Bradl M, Lassmann H (2010) Oligodendrocytes: biology and pathology. Acta Neuropathol 119:37–53. CrossRef Medline

Brambilla R, Persaud T, Hu X, Karmally S, Shestopalov VI, Dvoriantchikova G, Ivanov D, Nathanson L, Barnum SR, Bethea JR (2009) Transgenic inhibition of astroglial NF-kappa B L improves functional outcome in experimental autoimmune encephalomyelitis by suppressing chronic central nervous system inflammation. J Immunol 182:2628–2640. CrossRef Medline

Corbin JG, Kelly D, Rath EM, Baerwald KD, Suzuki K, Popko B (1996) Targeted CNS expression of interferon-gamma in transgenic mice leads to hypomyelination, reactive gliosis, and abnormal cerebellar development. Mol Cell Neurosci 7:354–370. CrossRef Medline

Denic A, Johnson AJ, Bieber AJ, Warrington AE, Rodriguez M, Pirko I (2011) The relevance of animal models in multiple sclerosis research. Pathophysiology 18:21–29. CrossRef Medline

Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9:839–855. CrossRef Medline

Frohman EM, Racke MK, Raine CS (2006) Multiple sclerosis-the plaque and its pathogenesis. N Engl J Med 354:942–955. CrossRef Medline

Gveric D, Kaltschmidt C, Cuzner ML, Newcombe J (1998) Transcription factor NF-kappaB and inhibitor I kappaBalpha are localized in macrophages in active multiple sclerosis lesions. J Neuropathol Exp Neurol 57:168–178. CrossRef Medline

Hamanoue M, Yoshioka A, Ohashi T, Eto Y, Takamatsu K (2004) NF-kappaB prevents TNF-alpha-induced apoptosis in an oligodendrocyte cell line. Neurochem Res 29:1571–1576. CrossRef Medline

Hao W, Decker Y, Schnöder L, Schottek A, Li D, Menger MD, Fassbender K, Liu Y (2016) Deficiency of IκB kinase β in myeloid cells reduces severity of experimental autoimmune encephalomyelitis. Am J Pathol 186:1245–1257. CrossRef Medline

Hayden MS, Ghosh S (2012) NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes Dev 26:203–234. CrossRef Medline

Herrington FD, Carmody RJ, Goodyear CS (2016) Modulation of NF-κB signaling as a therapeutic target in autoimmunity. J Biomol Screen 21:223–242. CrossRef Medline

Hilliard BA, Mason N, Xu L, Sun J, Lamhamedi-Cherradi SE, Liou HC, Hunter C, Chen YH (2002) Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. J Clin Invest 110:843–850. CrossRef Medline

Hilliard B, Samoilova EB, Liu TS, Rostami A, Chen Y (1999) Experimental autoimmune encephalomyelitis in NF-kappa B-deficient mice: roles of NF-kappa B in the activation and differentiation of autoreactive T cells. J Immunol 163:2937–2943. Medline

Hisahara S, Yuan J, Momoi T, Okano H, Miura M (2001) Caspase-11 mediates oligodendrocyte cell death and pathogenesis of autoimmune-mediated demyelination. J Exp Med 193:111–122. CrossRef Medline

Hisahara S, Araki T, Sugiyama F, Yagami Ki, Suzuki M, Abe K, Yamamura K, Miyazaki J, Momoi T, Saruta T, Bernard CC, Okano H, Miura M (2000) Targeted expression of baculovirus p35 caspase inhibitor in oligodendrocytes protects mice against autoimmune-mediated demyelination. EMBO J 19:341–348. CrossRef Medline

Hisahara S, Okano H, Miura M (2003) Caspase-mediated oligodendrocyte cell death in the pathogenesis of autoimmune demyelination. Neurosci Res 46:387–397. CrossRef Medline

Hussien Y, Cavener DR, Popko B (2014) Genetic inactivation of PERK signaling in mouse oligodendrocytes: normal developmental myelination with increased susceptibility to inflammatory demyelination. Glia 62:680–991. CrossRef Medline

Hussien Y, Podojil JR, Robinson AP, Lee AS, Miller SD, Popko B (2015) ER Chaperone BiP/GRP78 is required for myelinating cell survival and provides protection during experimental autoimmune encephalomyelitis. J Neurosci 35:15921–15933. CrossRef Medline

Karin M, Lin A (2002) NF-kappaB at the crossroads of life and death. Nat Immunol 3:221–227. Medline

Kipp M, Nyamoya S, Hochstrasser T, Amor S (2017) Multiple sclerosis animal models: a clinical and histopathological perspective. Brain Pathol 27:123–137. CrossRef Medline

Kotter MR, Stadelmann C, Hartung HP (2011) Enhancing remyelination in disease–can we wrap it up? Brain 134:1882–1900. CrossRef Medline

Krappmann D, Wulczyn FG, Scheidereit C (1996) Different mechanisms control signal-induced degradation and basal turnover of the NF-kappaB inhibitor IkappaB alpha in vivo. EMBO J 15:6716–6726. Medline

Kretz A, Herrmann KH, Fischer S, Engelmann C, Witte OW, Reichenbach JR, Weih F, Haenold R (2014) Dysfunctional NF-κB and brain myelin formation. Eur J Hum Genet 22:724–725. CrossRef Medline

LaFerla FM, Sugarman MC, Lane TE, Leissring MA (2000) Regional hypomyelination and dysplasia in transgenic mice with astrocyte-directed expression of interferon-gamma. J Mol Neurosci 15:45–59. CrossRef Medline

Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33:366–374. CrossRef Medline

Lassmann H, Bradl M (2017) Multiple sclerosis: experimental models and reality. Acta Neuropathol 133:223–244. CrossRef Medline

Limpert AS, Bai S, Narayan M, Wu J, Yoon SO, Carter BD, Lu QR (2013) NF-κB forms a complex with the chromatin remodeler BRG1 to regulate Schwann cell differentiation. J Neurosci 33:2388–2397. CrossRef Medline

Lin W, Lin Y (2010) Interferon-γ inhibits central nervous system myelination through both STAT1-dependent and STAT1-independent pathways. J Neurosci Res 88:2569–2577. CrossRef Medline

Lin W, Popko B (2009) Endoplasmic reticulum stress in disorders of myelinating cells. Nat Neurosci 12:379–385. CrossRef Medline

Lin W, Kemper A, McCarthy KD, Pytel P, Wang JP, Campbell IL, Utset MF, Popko B (2004) Interferon-gamma induced medulloblastoma in the developing cerebellum. J Neurosci 24:10074–10083. CrossRef Medline

Lin W, Harding HP, Ron D, Popko B (2005) Endoplasmic reticulum stress modulates the response of myelinating oligodendrocytes to the immune cytokine interferon-gamma. J Cell Biol 169:603–612. CrossRef Medline

Lin W, Kemper A, Dupree JL, Harding HP, Ron D, Popko B (2006) Interferon-gamma inhibits central nervous system remyelination

through a process modulated by endoplasmic reticulum stress. Brain 129: 1306–1318. CrossRef Medline

Lin W, Bailey SL, Ho H, Harding HP, Ron D, Miller SD, Popko B (2007) The integrated stress response prevents demyelination by protecting oligodendrocytes against immune-mediated damage. J Clin Invest 117:448–456. CrossRef Medline

Lin W, Kunkler PE, Harding HP, Ron D, Kraig RP, Popko B (2008) Enhanced integrated stress response promotes myelinating oligodendrocyte survival in response to interferon-gamma. Am J Pathol 173:1508–1517. CrossRef Medline

Lin W, Lin Y, Li J, Fenstermaker AG, Way SW, Clayton B, Jamison S, Harding HP, Ron D, Popko B (2013) Oligodendrocyte-specific activation of PERK signaling protects mice against experimental autoimmune encephalomyelitis. J Neurosci 33:5980–5991. CrossRef Medline

Lin Y, Jamison S, Lin W (2012) Interferon-γ activates nuclear factor-κ B in oligodendrocytes through a process mediated by the unfolded protein response. PLoS One 7:e36408. CrossRef Medline

Lin Y, Huang G, Jamison S, Li J, Harding HP, Ron D, Lin W (2014) PERK activation preserves the viability and function of remyelinating oligodendrocytes in immune-mediated demyelinating diseases. Am J Pathol 184: 507–519. CrossRef Medline

Madhavarao CN, Moffett JR, Moore RA, Viola RE, Namboodiri MA, Jacobowitz DM (2004) Immunohistochemical localization of aspartoacylase in the rat central nervous system. J Comp Neurol 472:318–329. CrossRef Medline

Matsushima GK, Morell P (2001) The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol 11:107–116. Medline

Mc Guire C, Volckaert T, Wolke U, Sze M, de Rycke R, Waisman A, Prinz M, Beyaert R, Pasparakis M, van Loo G (2010) Oligodendrocyte-specific FADD deletion protects mice from autoimmune-mediated demyelination. J Immunol 185:7646–7653. CrossRef Medline

Mc Guire C, Prinz M, Beyaert R, van Loo G (2013) Nuclear factor kappa B (NF-κB) in multiple sclerosis pathology. Trends Mol Med 19:604–613. CrossRef Medline

Mincheva-Tasheva S, Soler RM (2013) NF-κB signaling pathways: role in nervous system physiology and pathology. Neuroscientist 19:175–194. CrossRef Medline

Morton PD, Dellarole A, Theus MH, Walters WM, Berge SS, Bethea JR (2013) Activation of NF-κB in Schwann cells is dispensable for myelination in vivo. J Neurosci 33:9932–9936. CrossRef Medline

Nicholas RS, Wing MG, Compston A (2001) Nonactivated microglia promote oligodendrocyte precursor survival and maturation through the transcription factor NF-kappa B. Eur J Neurosci 13:959–967. CrossRef Medline

Nickols JC, Valentine W, Kanwal S, Carter BD (2003) Activation of the transcription factor NF-kappaB in Schwann cells is required for peripheral myelin formation. Nat Neurosci 6:161–167. CrossRef Medline

Oeckinghaus A, Hayden MS, Ghosh S (2011) Crosstalk in NF-κB signaling pathways. Nat Immunol 12:695–708. CrossRef Medline

Philippe O, Rio M, Malan V, Van Esch H, Baujat G, Bahi-Buisson N, Valayannopoulos V, Gesny R, Bonnefont JP, Munnich A, Froyen G, Amiel J, Boddaert N, Colleaux L (2013) NF-κB signalling requirement for brain myelin formation is shown by genotype/MRI phenotype correlations in patients with Xq28 duplications. Eur J Hum Genet 21:195–199. CrossRef Medline

Popko B, Baerwald KD (1999) Oligodendroglial response to the immune cytokine interferon gamma. Neurochem Res 24:331–338. CrossRef Medline

Prineas JW, Parratt JD (2012) Oligodendrocytes and the early multiple sclerosis lesion. Ann Neurol 72:18–31. CrossRef Medline

Raasch J, Zeller N, van Loo G, Merkler D, Mildner A, Erny D, Knobeloch KP, Bethea JR, Waisman A, Knust M, Del Turco D, Deller T, Blank T, Priller J, Brück W, Pasparakis M, Prinz M (2011) IkappaB kinase 2 determines oligodendrocyte loss by non-cell-autonomous activation of NF-kappaB in the central nervous system. Brain 134:1184–1198. CrossRef Medline

Schmidt-Ullrich R, Aebischer T, Hülsken J, Birchmeier W, Klemm U, Scheidereit C (2001) Requirement of NF-kappaB/Rel for the development of hair follicles and other epidermal appendices. Development 128:3843–3853. Medline

Sidman RL, Abervine JB, Pierce ET (1971) Atlas of the mouse brain and spinal cord. Cambridge, MA: Harvard University.

Stone S, Lin W (2015) The unfolded protein response in multiple sclerosis. Front Neurosci 9:264. CrossRef Medline

Tanner DC, Campbell A, O'Banion KM, Noble M, Mayer-Pröschel M (2015) cFLIP is critical for oligodendrocyte protection from inflammation. Cell Death Differ 22:1489–1501. CrossRef Medline

van Loo G, De Lorenzi R, Schmidt H, Huth M, Mildner A, Schmidt-Supprian M, Lassmann H, Prinz MR, Pasparakis M (2006) Inhibition of transcription factor NF-kappaB in the central nervous system ameliorates autoimmune encephalomyelitis in mice. Nat Immunol 7:954–961. CrossRef Medline

Watzlawik J, Warrington AE, Rodriguez M (2010) Importance of oligodendrocyte protection, BBB breakdown and inflammation for remyelination. Expert Rev Neurother 10:441–457. CrossRef Medline

Yan J, Greer JM (2008) NF-kappa B, a potential therapeutic target for the treatment of multiple sclerosis. CNS Neurol Disord Drug Targets 7:536–557. CrossRef Medline

Yin QQ, Liu CX, Wu YL, Wu SF, Wang Y, Zhang X, Hu XJ, Pu JX, Lu Y, Zhou HC, Wang HL, Nie H, Sun HD, Chen GQ (2013) Preventive and therapeutic effects of adenanthin on experimental autoimmune encephalomyelitis by inhibiting NF-κB signaling. J Immunol 191:2115–2125. CrossRef Medline