IMMUNOPATHOLOGY AND INFECTIOUS DISEASES

Deficiency of I κ B Kinase β in Myeloid Cells Reduces Severity of Experimental Autoimmune Encephalomyelitis

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In experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), peripherally developed myelin-reactive T lymphocytes stimulate myeloid cells (ie, microglia and infiltrated macrophages) to trigger an inflammatory reaction in the central nervous system, resulting in demyelination and neurodegeneration. I κ B kinase β (IKK β) is a kinase that modulates transcription of inflammatory genes. To investigate the pathogenic role of IKK β in MS, we developed strains in which IKK β was conditionally ablated in myeloid cells and established active or passive EAE in these animals. Deficiency of IKK β in myeloid cells ameliorated EAE symptoms and suppressed neuroinflammation, as shown by decreased infiltration of T lymphocytes and macrophages and reduced inflammatory gene transcription in the spinal cord at the peak or end stage of EAE. Myeloid deficiency of IKK β also reduced the transcription of *Rorc* or *Il17* genes in T lymphocytes isolated from lymph nodes, spleen, and spinal cord of EAE mice. Moreover, cultured splenocytes isolated from myeloid IKK β -deficient EAE mice released less IL-17, interferon- γ , and granulocyte-macrophage colony-stimulating factor after treatment with myelin peptide than splenocytes from IKK β wild-type EAE mice. Thus, deficiency of myeloid IKK β attenuates the severity of EAE by inhibiting both the neuroinflammatory activity and the activation of encephalitogenic T lymphocytes. These results suggest IKK β may be a potential target for MS patients, especially when neuroinflammation is the primary problem. (*Am J Pathol* 2016, 186: 1245–1257; <http://dx.doi.org/10.1016/j.ajpath.2016.01.004>)

Multiple sclerosis (MS) is a chronic autoimmune disease characterized by disseminated demyelination, inflammatory infiltrates, and axonal loss in the central nervous system (CNS).^{1,2} The major pathogenic mechanisms of MS have been investigated by using its animal model, experimental autoimmune encephalomyelitis (EAE). In EAE, the disease starts with the peripheral formation of myelin-reactive encephalitogenic T lymphocytes, which are recruited to the CNS, where they initiate neuroinflammation driven by microglia and infiltrated macrophages.^{2,3} Histological examinations in both postmortem MS tissue and in tissue from EAE mice have shown that microglia and infiltrated macrophages, rather than T lymphocytes, are more directly associated with the oligodendrocyte loss and axonal degeneration.^{4–6} Thus, the inflammatory activity of microglia/macrophages determines the severity of

tissue destruction and symptoms of MS. However, the molecular mechanisms regulating the inflammatory activation in these cells are not fully understood.

Studies have shown that microglia and/or macrophages can be deactivated by expression of a suicide gene,⁷ by interference with miR-124,⁸ or by conditional ablation of transforming growth factor- β -activated kinase 1,⁹ resulting in suppression of the neuroinflammatory activation and delay in EAE development. Tumor necrosis factor (TNF)

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receptor—associated factor 3 was also observed to block EAE pathogenesis by inhibiting IL-17—induced NF- κ B and mitogen-activated protein kinase activation.^{10,11}

NF- κ B, a key inflammatory transcription factor, is activated by the I κ B kinase (IKK) complex, consisting of the IKK α and IKK β catalytic subunits and the IKK γ regulatory protein.¹² Deficiency of p50 NF- κ B or IKK β specifically in T cells disturbs the generation of autoantigen-specific T cells, thereby rendering mice resistant to EAE induction.^{13–15} Blockage of NF- κ B activation in astrocytes by ablating genes encoding IKK β or IKK γ , or by overexpressing dominant-negative I κ B α , ameliorates EAE pathology by reducing neuroinflammation.^{16,17} However, the pathogenic role of microglial IKK β —NF- κ B activation in EAE is unclear. Although deficiency of IKK β in microglia has the potential to increase sensitivity of mice to EAE by promoting type 17 helper T-cell (Th17) polarization of CD4⁺ lymphocytes,¹⁸ IKK β deficiency might also inhibit neuroinflammation.

In this study, we investigated the overall pathogenic effects of IKK β in EAE. We induced EAE in mice in which the IKK β expression had been specifically ablated in myeloid cells (including microglia, macrophages, and neutrophils). We compared the symptoms and pathological changes in the IKK β -deficient mice with those of their IKK β -wild-type littermate controls, similarly induced with EAE.

Materials and Methods

Experimental Animals

Ikkb^{fl/fl} mice carrying loxP site—flanked *Ikkb* alleles encoding mouse IKK β were kindly provided by Manolis Pasparakis (University of Cologne, Cologne, Germany).¹⁹ Ikkb^{fl/fl} mice express normal levels of IKK β . LysM-Cre knock-in mice expressing Cre from the endogenous lysozyme 2 gene locus were obtained from The Jackson Laboratory (Bar Harbor, ME; stock number 004781).²⁰ For the EAE experiments, we used mice derived from the cross-breeding between Ikkb^{fl/fl} mice and LysM-Cre knock-in mice with the following genotypes: Ikkb^{fl/fl}Cre^{+/-} (IKK β ablated) and Ikkb^{fl/fl}Cre^{-/-} (normal IKK β expression). All mice from the same litter were recruited without any exclusion so that the EAE phenotype was compared between littermates. To demonstrate the distribution of Cre recombinase-positive cells, LysM-Cre mice were cross-bred to ROSA^{mT/mG} Cre report mice (The Jackson Laboratory; stock number 007676),²¹ which express cell membrane—targeted tomato fluorescent protein before Cre exposure, and cell membrane—targeted green fluorescent protein (GFP) in Cre-expressing cells. All animal experiments were approved by the regional ethical committee in Saarland, Germany.

Establishment of MOG35-55 Peptide-Induced EAE

To establish the active EAE model, 6- to 8-week-old myeloid IKK β -ablated and wild-type mice, as well as the offspring of LysM-Cre mice mated with ROSA^{mT/mG} Cre report mice,

were s.c. immunized with 100 μ g peptide of mouse myelin oligodendrocyte glycoprotein 35-55 (MOG35-55; MEVG-WYRSPFSRVVHLYRNGK; EZBiolab, Carmel, IN) in a complete Freund's adjuvant (BD Biosciences, Heidelberg, Germany) supplemented with 100 μ g *Mycobacterium tuberculosis* (H37Ra; BD Biosciences). This was followed by i.v. injection of pertussis toxin (Enzo Life Sciences GmbH, Lörrach, Germany) 0 and 2 days after MOG35-55 immunization (dpi).

To establish adoptively transferred EAE models, spleen cells were harvested from actively immunized IKK β wild-type mice on 10 dpi. After lysing red blood cells and culturing cells in complete T-lymphocyte culture medium [RPMI 1640 medium with 10% fetal calf serum (PAN-Biotech GmbH, Aidenbach, Germany), 10 mmol/L HEPES, 50 μ mol/L β -mercaptoethanol, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 0.1 mmol/L nonessential amino acids, 100 U/mL penicillin, and 100 μ g/mL streptomycin (all reagents except fetal calf serum were bought from Life Technologies, Darmstadt, Germany)] supplemented with 50 μ g/mL MOG35-55 and 10 ng/mL mouse IL-12 (R&D Systems, Wiesbaden, Germany) in flasks for 24 hours, the swimming cells enriched with encephalitogenic CD4⁺ T lymphocytes were collected, washed, and i.p. injected into IKK β -ablated and wild-type recipient mice (3×10^7 spleen cells per mouse). Two doses of 100 ng per mouse pertussis toxin were i.v. administrated on 0 and 2 days after T-cell transfer.

Assessment of clinical disease activity was performed with the following scale: 0 indicates no disease, 0.5 indicates weak tail or mild hind limb ataxia, 1 indicates limp tail and/or hind limb ataxia, 2 indicates hind limb paresis, 3 indicates hind limb paralysis, 4 indicates hind and fore limb paralysis, and 5 indicates death.

Qualitative Scoring of Demyelination and Inflammatory Cell Infiltration

At the end stage of disease, EAE mice were sacrificed via inhalation of isoflurane (Abbott, Wiesbaden, Germany) and perfused with ice-cold phosphate-buffered saline and 4% paraformaldehyde (Sigma-Aldrich, Taufkirchen, Germany). The lumbar sacral spinal cord was collected and embedded in paraffin. The tissue was cut longitudinally and stained with Luxol—fast blue and cresyl violet (both compounds were bought from Sigma-Aldrich). Qualitative scoring of the degree of demyelination in the spinal cord was performed according to the published protocol.²² Six serial sections per animal with 20- μ m interval in between were examined by a blinded observer (W.H.).

Histological Examination of Inflammatory Infiltrates

The spinal cord was collected as described above and serial transverse sections (2 μ m thick) were mounted onto glass slides. Immunohistochemical staining was performed with the VECTASTAIN Elite ABC kit (Vector Laboratories, Burlingame, CA) and primary antibodies against macrophage/microglia

(rat monoclonal anti-Mac3; clone M3/84; BD Biosciences) and T cells (rabbit polyclonal anti-CD3; catalog number ab5690; Abcam, Cambridge, UK). Sections were developed with diaminobenzidine tetrahydrochloride hydrate (Sigma-Aldrich). Per animal, four equidistant sections with a 100- μ m interval were scored. For quantification of the cell number, Mac3 or CD3-positive cells with hematoxylin-stained nuclei were counted and normalized to the analyzed area (mm²). For T cells, the location of parenchyma and in the perivascular area was distinguished.

Flow Cytometric Analysis of Inflammatory Infiltrates

EAE mice were sacrificed at the peak disease stage, and the whole spinal cords were harvested after phosphate-buffered saline perfusion. The tissue was carefully dissected from the spinal cord to prepare a single-cell suspension, with myelin removed, using the Neural Tissue Dissociation Kit (papain based) and Myelin Removal Beads II (both from Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), according to the manufacturer's protocols. After pelleting cells by centrifugation, we added 80 μ L of blocking buffer containing 25 μ g/mL rat anti-mouse CD16/CD32 antibody (2.4G2; BD Biosciences) and 10% fetal calf serum to prevent non-specific binding. Thirty minutes after blocking at 4°C, cells were incubated with fluorophore-conjugated antibodies against mouse CD4, CD8a, CD11b, Ly-6G, and CD45 (clones GK1.5 for CD4, 53-6.7 for CD8a, 1A8 for Ly-6G, M1/70 for CD11b, 30-F11 for CD45; eBioscience, Frankfurt, Germany). After thorough washing, different inflammatory cell populations were immediately analyzed using flow cytometry (BD FACSCanto II), and absolute numbers of cells were counted using BD Trucount absolute counting tubes (BD Biosciences).

Quantitative Western Blot Analysis Detection of IKK β in CD11b-Positive Myeloid Cells in the Spinal Cord and Blood of EAE Mice

To determine the ablating level of IKK β proteins in microglia and infiltrating macrophages in the spinal cord, the single-cell suspension was prepared from the myeloid IKK β -deficient and wild-type EAE mice, as described above. After blocking with 10% fetal calf serum, rat anti-CD11b monoclonal antibody (clone M1/70; R&D Systems GmbH) at 20 μ g/mL was added to the cells. After 1 hour of incubation at 4°C, cells were washed and positively selected with Dynabeads magnetic beads-conjugated sheep anti-rat IgG (Life Technologies). Similarly, CD11b primary antibody was directly added to the EDTA-containing whole blood, which was collected from EAE mice by intracardial puncture. CD11b-positive cells were selected with magnetic beads. The selected CD11b-positive cells were lysed in 2 $\times$ Laemmli sample buffer and separated using 10% Tris-glycine polyacrylamide gel electrophoresis. After the proteins were transferred onto a nitrocellulose membrane,

quantitative Western blot detection of IKK β was performed with the rabbit monoclonal antibody against IKK β (clone Y466; Abcam) and mouse monoclonal anti- α -tubulin (clone DM1A; Abcam).

Histological Analysis with the Fluorescence Microscope

To demonstrate the efficiency of LysM-Cre-mediated gene recombination in microglia/macrophages and neutrophils, we used paraffin-embedded transverse sections of spinal cord, as described above. Deparaffinized sections were first incubated with the primary antibody, chicken anti-GFP (catalog number ab13970; Abcam), and then with Alexa Fluor 488-conjugated goat anti-chicken IgY (Life Technologies). After thorough washing, rat monoclonal antibody against Mac3 (clone M3/84; BD Biosciences) or neutrophil (clone RM0028-3G23; Novus Biologicals, Abingdon, UK) was added and visualized with Alexa Fluor 546-conjugated goat anti-rat IgG secondary antibody (Life Technologies). Finally, the section was covered with DAPI-contained mounting medium.

To quantify the percentage of GFP-positive cells within Mac3- or neutrophil marker-positive cells, three equidistant sections with a 100- μ m interval per mouse were examined. From each section, five randomly chosen areas containing ≥ 200 of total number of investigated cells were analyzed under fluorescence microscopy (Zeiss AxioImager.Z2 microscope, Göttingen, Germany) with 40 $\times$ objective. With Z-stack scanning, Alexa 546-labelled cells with clear DAPI staining were first imaged. Thereafter, GFP was identified in the green channel by carefully adjusting the Z-levels. Cells with green or red fluorescence alone and with both fluorescence were counted.

To investigate the pathogenic role of IL-1 β in EAE, we incubated the deparaffinized sections of spinal cord with mouse monoclonal antibody against IL-1 β (clone 3A6; New England Biolabs GmbH, Frankfurt am Main, Germany), and then with Alexa Fluor 488-conjugated donkey anti-mouse IgG (Life Technologies). To demonstrate the EAE lesion sites, Mac3 was stained as described above. After thorough washing, the section was mounted with DAPI-contained medium.

Spleen Cell Culture and Enzyme-Linked Immunosorbent Assay Detection of Cytokines after MOG35-55 Stimulation

EAE mice were sacrificed at peak disease (18 dpi) and the spleens were harvested for the preparation of a single-cell suspension. Splenocytes (6×10^5 cells/0.3 mL/well in a 48-well plate) were cultured in triplicate in the complete T-lymphocyte culture medium in the presence (10, 20, and 50 μ g/mL) or absence of the MOG35-55 peptide at 37°C in 5% CO₂ for 72 hours. As a negative control, splenocytes were isolated from C57BL6 mice without MOG35-55 immunization and cultured in the same way. Supernatants were collected and diluted for the measurement of interferon (IFN)- γ , IL-17, IL-10, granulocyte-macrophage colony-stimulating factor

Figure 1 LysM-Cre efficiently excises the floxed *Ikbkb* gene in infiltrated myeloid cells in the experimental autoimmune encephalomyelitis (EAE) lesion site. EAE was induced in the offspring of LysM-Cre mice mated with ROSA^{mT/mG} Cre reporter mice. The reporter protein, green fluorescent protein (GFP), was costained with Mac3 (representing microglia/macrophages; **A**) or with neutrophil marker (**C**). Myeloid cells with LysM-Cre-mediated GFP expression cluster in EAE lesion sites. **B** and **D** show the boxed areas in **A** and **C**, respectively, with a higher magnification. In **B** and **D**, cells marked with **white arrowheads** are GFP and relevant cell marker double positive, cells with **red arrowheads** are the relevant cell marker positive but without GFP expression, and cells with **green arrowheads** are GFP-expressing cells but without immunological staining of the relevant cell marker. **E**: CD11b-positive cells were positively selected from the spinal cord and circulating blood of EAE mice. Quantitative Western blot analysis detected IKKβ proteins and showed that LysM-Cre has efficiently ablated IKKβ proteins in myeloid cells in IKKβ-deficient (kd) EAE mice compared with the IKKβ wild-type (wt) EAE mice. *n* = 7 per group (**E**, spinal cords); *n* = 3 per group (**E**, blood samples). **P* < 0.05, ***P* < 0.01 (*t*-test). IKKβ, IκB kinase β.

(GM-CSF), and IL-1β with commercially available enzyme-linked immunosorbent assay kits (R&D Systems), according to the manufacturer's instructions.

In Vitro T-Cell Proliferation Assay

T lymphocytes were harvested from the draining axillary and inguinal lymph nodes of actively immunized IKKβ-deficient and wild-type mice at 10 dpi, and the cell proliferation was determined with a cell-based dissociation-enhanced lanthanide fluorescence immune assay (DELFI), according to the attached instructions (PerkinElmer, Waltham, MA). T cells were cultured in the complete T-cell culture medium containing MOG35-55 at various concentrations or 25 μg/mL concanavalin A. Cell cultures were pulsed after 72 hours with 5-bromo-2'-deoxyuridine (BrdU). The incorporation of BrdU was assessed 16 hours later through incubating cells with europium ion-conjugated anti-BrdU antibody and reading the fluorescence in the presence of DELFIA Inducer with Synergy Mx Multi-Mode microplate reader (BioTek, Winooski, VT).

Quantitative PCR

To analyze T-cell differentiation, single-cell suspensions were prepared from draining axillary and inguinal lymph nodes and

the spleen of EAE mouse at 10 dpi and from the spinal cord of EAE mouse at 18 dpi. After blocking cells with CD16/CD32 antibodies, T cells were positively selected with Dynabeads magnetic beads-conjugated rat anti-mouse CD4 (clone L3T4; Life Technologies). Total RNA was isolated from T lymphocytes with the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). After reverse transcription, SYBR Green-based real-time PCR (Roche Applied Science, Mannheim, Germany) was used for detecting transcripts of *Foxp3*, *Tbx21* (alias *Tbet*), and *Gata3* genes with the following primers: FoxP3, forward, 5'-GAGTTTCTCAAGCACTGCCA-3' and reverse, 5'-ACTTCTCTCTGGAGGAGGCA-3'; Tbet, forward, 5'-CAACAA-CCCCCTTGCCAAAG-3' and reverse, 5'-TCCCCCAAG-CAGTTGACAGT-3'; and GATA-3, forward, 5'-AGAACCG-GCCCTTATCAA-3' and reverse, 5'-AGTTCGCGCAG-GATGTCC-3'. TaqMan gene expression assays (Life Technologies) were used to detect the transcripts of *Il17a*, *Ifng*, *Rorc*, and *Csf2*. The quantitative PCR was performed with the 7500 Fast Real-Time PCR System (Life Technologies), according to our established protocol.²³

To analyze the neuroinflammation, total RNA was isolated from the spinal cord with Trizol (Life Technologies). Reverse transcription and real-time PCR using TaqMan gene expression assays were performed to detect transcripts of *Tnf*, *Il1b*, *Nos2* (alias iNOS), *Il10*, *Ccl2*, and *Cxcl10*, according to our established protocol.²³

Figure 2 Myeloid cell lineage–restricted ablation of I κ B kinase β (IKK β) ameliorates experimental autoimmune encephalomyelitis (EAE). Myeloid IKK β -ablated (deficient; kd) and wild-type (wt) mice (aged 6 to 8 weeks) were immunized with MOG35–55 in complete Freund's adjuvant. Assessment of clinical disease activity was daily performed until 27 days after immunization. **A:** The result was pooled from four independent experiments (two-way analysis of variance showing the effect of genotype; *t*-test between two groups on individual days). Mice were sacrificed after the clinical monitoring. The spinal cord was isolated, embedded in paraffin, and longitudinally divided into sections. The tissues were then stained with Luxol–fast blue (LFB) and cresyl violet for the analysis of demyelination and inflammatory infiltration. **B:** In the mice with IKK β ablation in myeloid cell lineage, the influx of leukocytes is evident in the pial lining. The parenchymal inflammation is limited, and the myelin sheath is mostly preserved; in IKK β wild-type EAE mice, the extended inflammation has been observed in the parenchyma and the myelin sheath is severely damaged. **C:** There are parenchymal holes at the lesion sites. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 versus kd (*t*-test); †††*P* < 0.001 versus kd between day 9 and day 27 (two-way analysis of variance). *n* = 24 (**A**, kd group); *n* = 18 (**A**, wt group).

Statistical Analysis

Data are presented as means $\pm$ SEM. For multiple comparisons, we used one- or two-way analysis of variance, followed by Bonferroni's, Tukey's honestly significant difference, or Tamhane's T2 post hoc test (the choice of post hoc test depended on the result of Levene's test for homogeneity for determining the equality of variances). We used Spearman's tests to analyze the correlation between spleen cell–released cytokines and the clinical score. Two independent-sample *t* tests were used to compare means for two groups. All statistical analyses were performed with SPSS version 19.0 for Windows (IBM, New York, NY). Statistical significance was set at the level of *P* < 0.05.

Results

LysM-Cre–Mediated IKK β Deletion Is Prominent in the EAE Lesion Site

The rate of LysM-Cre–mediated recombination of floxed gene under physiological conditions is approximately 50% in the microglia of spinal cord and approximately 60% in circulating monocytes.⁹ After our recent observation that LysM-Cre–mediated IKK β ablation significantly inhibits microglial inflammatory activation in an Alzheimer disease mouse model,²³ we tested whether IKK β ablation in myeloid cells also modifies EAE pathogenesis by affecting the neuroinflammatory activation.

We first examined the efficiency of LysM-Cre–mediated gene recombination in EAE lesions. We induced EAE in the offspring of LysM-Cre mice mated with ROSA^{mT/mG} Cre report mice and observed that the report protein GFP-expressing cells clustered in the EAE lesions compared with nonlesion sites (Figure 1, A and C). We further counted GFP-expressing Mac3-positive cells (representing infiltrating macrophages and activated endogenous microglia) and neutrophils in the lesions of EAE mice (Figure 1, A–D). We observed that 87.94% $\pm$ 3.06% Mac-3–positive cells and 84.44% $\pm$ 4.67% neutrophils were GFP positive (*n* = 3 for both counting experiments). In further experiments with quantitative Western blot analysis, we observed that 46.59% $\pm$ 5.23% and 58.22% $\pm$ 11.33% IKK β proteins had been ablated in CD11b-positive cells isolated from the spinal cord and whole blood of EAE mice, respectively (Figure 1E).

IKK β Deficiency in Myeloid Cells Attenuates EAE Symptoms and Demyelination

After confirming that the floxed *Ikkb* gene was efficiently deleted by LysM-Cre in myeloid cells, we investigated the effects of myeloid IKK β on EAE pathology. EAE symptoms were significantly reduced after IKK β ablation in myeloid cells (Figure 2A). The peak disease scores (2.25 $\pm$ 1.12) of IKK β -deficient EAE mice were significantly lower than the scores (3.45 $\pm$ 1.24) of IKK β wild-type control EAE models

Figure 3 Myeloid cell lineage–restricted ablation of IκB kinase β (IKKβ) reduces infiltration of microglia/macrophage and T cell in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice at the end stage of disease. Mice were sacrificed on 35 days after MOG35–55 immunization. The spinal cord was embedded in paraffin and divided transversely into sections. The serial sections were then stained with Luxol–fast blue (LFB; **A** and **D**) and antibodies against Mac3 (**B** and **E**) and CD3 (**C** and **F**). The infiltration of Mac-3– and CD3–positive cells stained in brown is significantly less in IKKβ-ablated EAE mice (IKKβ-deficient; kd; **D**–**F**) than in IKKβ wild-type controls (wt; **A**–**C**). **B** and **C** are enlarged from the boxed area in **A**, and **E** and **F** are enlarged from the boxed area in **D**. CD, cluster of differentiation; Mac-3, lysosomal-associated membrane protein 2.

(*t*-test, $P < 0.001$) (Figure 2A). However, IKKβ deficiency did not affect the onset of EAE symptoms (12.14 ± 2.96 and 11.96 ± 2.08 dpi; *t*-test, $P > 0.05$).

Accordingly, the pathological score²² describing the intensity of inflammatory infiltration and its associated demyelination in the spinal cord at the end stage of EAE disease was significantly higher in IKKβ wild-type mice (6.15 ± 1.01 points) (Figure 2C) than in IKKβ-deficient EAE mice (1.98 ± 1.40 points; *t*-test, $P < 0.001$; $n \geq 7$ per group) (Figure 2B). As negative controls, the spinal cords isolated from both IKKβ-deficient and wild-type mice without EAE induction demonstrated absence of demyelination and inflammatory cell infiltrates (data not shown).

IKKβ Deficiency in Myeloid Cells Reduces Leukocyte Infiltration and Inflammatory Activation in the Spinal Cord of EAE Mice

Because inflammatory activation directly causes demyelination and neurodegeneration in MS,^{4–6} we further investigated the effects of IKKβ on neuroinflammatory activation. At the late stage of EAE (35 dpi), fewer infiltrated microglia/macrophages shown as Mac 3–positive cells were present in the spinal cord of myeloid IKKβ-deficient EAE mice compared with IKKβ wild-type EAE mice (*t*-test, $P = 0.001$) (Figure 3, B and E, and Table 1). IKKβ deficiency in myeloid cells significantly inhibited infiltration of T lymphocytes in the parenchyma (cells at least 30 μm from blood vessels²⁴), as demonstrated by significantly fewer CD3-positive cells in myeloid IKKβ-deficient EAE mice than in IKKβ wild-type controls (*t*-test, $P = 0.016$). Surprisingly, the number of infiltrating T cells in the perivascular area in the spinal cord was not significantly

different between myeloid IKKβ-deficient and wild-type EAE mice (*t*-test, $P = 0.457$) (Figure 3, C and F, and Table 1). Moreover, Luxol–fast blue staining showed that the attenuated demyelination was correlated with decreased infiltration of macrophages/microglia and T cells (Figure 3, A and D, and Table 1).

We used flow cytometry to further examine the inflammatory cell infiltration at the peak disease (18 dpi). The numbers of CD45^{high}CD11b[–], CD45^{high}CD11b⁺, and CD45^{low}CD11b⁺ cells, representing infiltrated T lymphocytes, infiltrated macrophages, and endogenous microglia, respectively, were all correlated to the clinical scores, with the maximal numbers at 3 points of score (Figure 4D). When the clinical score reached 4 points, the numbers of infiltrating inflammatory cells tended to decrease. When comparing IKKβ-deficient with wild-type control EAE mice, we observed that all CD45^{high}CD11b[–], CD45^{high}CD11b⁺, and CD45^{low}CD11b⁺ cells were fewer in the spinal cord of

Table 1 Quantification of Spinal Cord Infiltrates and Demyelination in Histological Analysis

Items	IKKβ wt	IKKβ ablated
Mac3/mm ²	208.7 ± 18.2	79.5 ± 12.7*
CD3/mm ² (parenchyma)	43.3 ± 9.1	10.8 ± 1.4*
CD3/mm ² (perivascular)	48.4 ± 5.9	39.4 ± 10.5
Parenchyma/total (ratio)	0.46 ± 0.03	0.24 ± 0.05*
Demyelination (%)	9.0 ± 0.9	1.6 ± 0.6**

The result is shown in means ± SEM from two independent experiments with total $n = 5$ per group.

* $P < 0.01$, ** $P < 0.001$ versus IKKβ wild-type experimental autoimmune encephalomyelitis mice (*t*-test).

IKKβ, IκB kinase β; wt, wild type.

Figure 4 Myeloid cell lineage–restricted ablation of I κ B kinase β (IKK β) reduces infiltration of myeloid cells and T cells in the spinal cord of experimental autoimmune encephalomyelitis (EAE) mice at the peak of disease. Mice were sacrificed on 18 days after MOG35-55 immunization. The spinal cord from myeloid IKK β -ablated (IKK β -deficient; kd) or wild-type (IKK β wt) EAE mouse was homogenized to prepare a single-cell suspension. **A** and **B**: After staining of CD11b, CD45, CD4, CD8, and Ly-6G, the spinal cord cells were analyzed with flow cytometry (four independent experiments). **C**: Several C57BL/6 mice that were immunized with MOG35-55 but did not develop EAE symptoms were used as negative controls. Few macrophages and T cells were recruited into the spinal cord. **D**: The number of infiltrated cells was correlated with EAE scores when all IKK β kd and wt mice were pooled (one-way analysis of variance, followed with Bonferroni's post hoc test, versus cell number at clinical score of 2). **E–G**: Spinal cords were also collected for the total RNA isolation, which was followed by the real-time PCR to quantify the transcripts of inflammatory genes (*t*-test; from three independent experiments). *n* = 6 (**A**, **B**, **E–G**, kd group); *n* = 9 (**A** and **B**, wt group); *n* = 7 (**E–G**, wt group). **P* < 0.05, ***P* < 0.01. CCL, chemokine ligand; iNOS, inducible nitric oxide synthase; MOG35-55, mouse myelin oligodendrocyte glycoprotein 35-55; TNF, tumor necrosis factor.

myeloid IKK β -deficient EAE mice than in the IKK β wild-type control EAE mice (*t*-test, *P* < 0.05) (Figure 4, A and B, and Table 2). Within the CD45^{high}CD11b⁻ cell population, CD4⁺ but not CD8⁺ cells were significantly decreased by the deficiency of myeloid IKK β (*t*-test, *P* < 0.001) (Figure 4, A and B, and Table 2). Similarly, myeloid IKK β deficiency also decreased CD45^{high}CD11b⁺Ly6G⁺ cells, which represent neutrophils, compared with wild-type EAE mice (*t*-test, *P* = 0.028) (Figure 4, A and B, and Table 2). As negative controls, the mice that were immunized with MOG35-55 but failed to develop EAE symptoms were analyzed for the inflammatory cell infiltration. Few T cells and macrophages had been recruited into the spinal cord (Figure 4C).

Furthermore, myeloid deficiency of IKK β significantly decreased the transcription of *Tnf*, *Il1b*, and *Cxcl10*, but not of *inos* and *ccl-2*, as detected with quantitative PCR (*t*-test, *P* < 0.05) (Figure 4, E–G).

IKK β Deficiency in Myeloid Cells Modulates T-Cell Differentiation and Activation in EAE Mice

In MS/EAE, the intensity of neuroinflammation is determined not only by the inflammatory activity of microglia/macrophages in the CNS, but also by the activation level of peripherally recruited encephalitogenic T lymphocytes.^{2,3} We asked whether the reduced neuroinflammation is because of suppression of T-cell activation by myeloid IKK β deficiency. Using T cells harvested from the spinal cord (Figure 5, A–G) of myeloid IKK β -deficient and wild-type EAE mice at the peak disease (18 dpi) and from the peripheral lymphoid organs, spleen (Figure 5, H–M) and lymph nodes (Figure 5, N–S) at the T-cell priming phase (10 dpi), we observed that the transcriptional level of *Rorc* or *Il17a* in T cells was significantly lower in three locations of myeloid IKK β -deficient than in IKK β wild-type EAE

Table 2 Total Cell Number of Infiltrating Inflammatory Cells in the Spinal Cord

Cell groups	IKK β wt	IKK β ablated
CD45 ^{high} /CD11b ⁻	3.16 $\pm$ 0.31	1.79 $\pm$ 0.37*
CD45 ^{high} /CD11b ⁺	13.07 $\pm$ 1.00	8.93 $\pm$ 1.70*
CD45 ^{low} /CD11b ⁺	4.23 $\pm$ 0.43	1.98 $\pm$ 0.43**
CD45 ^{high} /CD11b ⁻ /CD4 ⁺	2.11 $\pm$ 0.12	0.98 $\pm$ 0.06**
CD45 ^{high} /CD11b ⁻ /CD8 ⁺	0.59 $\pm$ 0.13	0.49 $\pm$ 0.04
CD45 ^{high} /CD11b ⁺ /Ly6G ⁺	1.79 $\pm$ 0.06	1.17 $\pm$ 0.17*

The cell number is shown with means $\pm$ SEM $\times 10^5$ from three independent experiments, with total $n = 6$ in IKK β -ablated mouse group and $n = 9$ in IKK β wild-type mouse group.

* $P < 0.05$, ** $P < 0.01$ versus IKK β wild-type experimental autoimmune encephalomyelitis mice (t -test).

IKK β , I κ B kinase β ; wt, wild type.

mice (t -test, $P < 0.05$) (Figure 5, D, K, and N), whereas myeloid deficiency of IKK β increased the transcription of both *Tbx21* and *Gata3* genes in T cells isolated from spleens (t -test, $P < 0.05$) (Figure 5, J and M). In the T cells isolated from lymph nodes, the transcription of *Tbx21* was also enhanced by deficiency of myeloid IKK β (t -test, $P = 0.045$) (Figure 5P). There were no significant differences in the transcription of other detected genes (eg, *Ifng*, *Foxp3*, and *Gmcsf*) in T lymphocytes between these two groups of EAE mice (Figure 5).

In spleen cells cultured from the EAE models at 18 dpi, secretion of IL-17, IFN- γ , and GM-CSF was strongly triggered by MOG35-55 treatment in a dose-dependent manner (two-way analysis of variance, $P < 0.05$) (Figure 6, A–C). Interestingly, the concentrations of IL-17, IFN- γ , and GM-CSF in the culture medium of spleen cells isolated from myeloid IKK β -deficient mice were significantly lower than those from IKK β wild-type EAE mice (two-way analysis of variance showing effects of genotypes, $P < 0.05$) (Figure 6, A–C). When all results derived from IKK β -deficient and wild-type mice were pooled for an additional analysis, we observed a positive correlation between clinical score and concentrations of IL-17, IFN- γ , and GM-CSF in the medium after stimulation of spleen cells with 50 μ g/mL MOG35-55 (Spearman's correlation tests, $P < 0.05$) (Figure 6, D–F). As negative controls, spleen cells isolated from C57BL/6 mice without MOG35-55 immunization showed no release of IL-17, IFN- γ , and GM-CSF after MOG35-55 stimulation (data not shown). After treatments of MOG35-55, the secretion of IL-10 from cultured spleen cells was slightly, but significantly, increased; similarly, myeloid IKK β -deficient spleen cells released significantly less IL-10 than IKK β wild-type cells (two-way analysis of variance, $P < 0.05$). However, because of low concentrations of IL-10 (< 50 pg/mL) in the culture medium with and without MOG35-55 stimulation, the importance of IL-10 measurements in evaluating effects of myeloid IKK β on T-cell activation and differentiation might be limited.

In the culture of T cells isolated from the draining lymph nodes of myeloid IKK β -deficient and wild-type mice at

10 dpi, restimulation with MOG35-55 antigens, but not with concanavalin A (a non-specific T-cell stimulator), resulted in a significantly greater increase in T-cell proliferation in EAE mice with myeloid IKK β deficiency, compared with IKK β wild-type EAE mice (two-way analysis of variance, $P < 0.05$) (Figure 6G).

IL-1 β Is Not Correlated with the EAE Pathogenesis

Because IL-1 β secretion is enhanced by IKK β deficiency in myeloid cells,^{18,25} we tested whether IL-1 β affects the EAE pathogenesis. In the spinal cord of myeloid IKK β -deficient EAE mice, IL-1 β was positively stained as green dense puncta in areas of the extracellular matrix that were close to a small lesion site, but obviously not colocalized with the lesion (Figure 7A). By contrast, in the control tissue derived from myeloid IKK β wild-type EAE mice with severe cell infiltration, the extracellular matrix close to lesion sites was rarely stained with IL-1 β antibodies in puncta (Figure 7B). There was weak staining of IL-1 β surrounding nuclei, and some of them colocalized with Mac3 staining, which was highly likely the staining of intracellular pro-IL-1 β (Figure 7B). Inside of the lesions, there was nearly no IL-1 β staining (Figure 7C). We also detected IL-1 β in the culture medium of spleen cells derived from EAE mice. Significantly more IL-1 β was released from spleen cells derived from myeloid IKK β -deficient EAE mice than from IKK β wild-type control mice (two-way analysis of variance showing the effects of genotypes, $P < 0.05$) (Figure 7D); however, the IL-1 β level after MOG35-55 stimulation at 50 μ g/mL was not correlated to the clinical score of EAE mice (Spearman's correlation tests, $P = 0.482$) (Figure 7E).

IKK β Deficiency in Myeloid Cells Reduces EAE Symptoms in Passive EAE

As a further test of whether the IKK β -controlled inflammatory activation in microglia/macrophages in the CNS determines the severity of EAE symptoms and demyelination, we established passive EAE by transferring myeloid IKK β -deficient and wild-type recipient mice with activated myelin-reactive T lymphocytes. EAE symptoms were significantly diminished in myeloid IKK β -deficient EAE mice compared with myeloid IKK β wild-type EAE mice ($P < 0.001$) (Figure 8A). The maximal clinical score of disease was also significantly higher for myeloid IKK β wild-type EAE mice than for IKK β -deficient controls (2.727 ± 0.454 versus 1.111 ± 0.200 ; t -test, $P = 0.007$). However, the onset of EAE symptoms in myeloid IKK β -deficient EAE mice (7.222 ± 0.547 days after T-cell transfer) was significantly earlier than the onset in myeloid IKK β wild-type EAE mice (12.455 ± 1.239 days after T-cell transfer; t -test, $P = 0.002$). Comparison of EAE mice with and without normal expression of IKK β showed that deficiency of IKK β in myeloid cells also suppressed transcription of inflammatory cytokines (TNF- α and IL-1 β) and

Figure 5 Myeloid cell lineage–restricted ablation of I κ B kinase (IKK β) differently modulates T-cell differentiation in the spinal cord, spleen, and lymph nodes of experimental autoimmune encephalomyelitis (EAE) mice. Female 6- to 8-week-old myeloid IKK β -ablated (IKK β -deficient; kd) and wild-type (IKK β wt) mice were immunized with MOG35-55 in a complete Freund's adjuvant. T lymphocytes were isolated from the spinal cord at the peak disease [18 days after MOG35-55 immunization (dpi); pooled from three independent experiments; **A–G**], and from the spleen (**H–M**) and draining lymph nodes (**N–S**) at 10 dpi (pooled from two independent experiments). The transcripts of *Il17a*, *Ifng*, *Tbx21*, *Rorc*, *Foxp3*, *Gata3*, and *Gmcsf* genes in T lymphocytes were quantified with quantitative real-time PCR. * $P < 0.05$ (t -test). $n = 9$ per group (**A–G**); $n = 4$ to 6 per group (**H–S**). FoxP3, forkhead box P3; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; RORC, RAR-related orphan receptor gamma.

chemokines (chemokine ligand-2 and CXCL-10) in the spinal cord (t -test, $P < 0.05$) (Figure 8, B and C).

Discussion

In the EAE mouse model, autoimmune reactive T lymphocytes from the periphery start the inflammatory process by activating microglia and the macrophages that have infiltrated the CNS, which results in the demyelination and neurodegeneration characteristic of MS.^{1,2} In our study, we deleted IKK β specifically in myeloid cells (ie, microglia, macrophages, and neutrophils), and observed a strong reduction in the severity of EAE symptoms and demyelination along with attenuated neuroinflammatory activation.

Demyelination and neurodegeneration appear to be closely related with neuroinflammatory activation in the early relapsing phase and progressive stage of MS.²⁶ Inhibition of inflammatory activation in microglia and infiltrated macrophages attenuates the severity of EAE symptoms.^{8–11} NF- κ B is activated in microglia/macrophages in the EAE lesion site.²⁷ Constitutive

activation of NF- κ B in myeloid cells promotes EAE pathogenesis and leads to a more severe clinical course, which is associated with enhanced inflammatory activation.²⁸ In our active and passive EAE models, deletion of IKK β in myeloid cells results in reduced EAE symptoms and neuroinflammation, possibly because of inhibition of NF- κ B activation.²³ Thus, neuroinflammatory activation is a key determinant of the severity of EAE disease. Various inflammatory stimuli, such as cytokines (ie, TNF- α and IL-1 β) and pathogen-associated molecular patterns, activate IKK β , which then induces NF- κ B–dependent transcription of a wide range of immune and inflammatory genes.¹² Moreover, pathogenic helper T cells initiate the inflammatory activation of microglia and macrophages in EAE by releasing GM-CSF.²⁹ Because IKK β is known to bind the GM-CSF receptor,³⁰ IKK β in myeloid cells could be an ideal therapeutic target to inhibit the neuroinflammatory activation in MS.

Neutrophils, as another type of myeloid cells, also infiltrate the EAE lesions. In MS patients, neutrophils have not been appreciated as a driver, perhaps because of their absence in MS lesions; however, this view might need to be updated. Recent studies have shown that the neutrophil/lymphocyte ratio is

Figure 6 Myeloid cell lineage-restricted ablation of I κ B kinase (IKK β) differentially modulates T-cell activation in the spleen and lymph nodes of experimental autoimmune encephalomyelitis (EAE) mice. Myeloid IKK β -ablated (IKK β -deficient; kd) and wild-type (IKK β wt) female mice (aged 6 to 8 weeks) were immunized with MOG35-55 in complete Freund's adjuvant. Spleen cells were isolated at 10 days after MOG35-55 immunization and cultured in the presence of MOG35-55 at different concentrations (0, 10, 20, and 50 μg/mL) for 72 hours (from three independent EAE experiments). **A–C:** Thereafter, the protein levels of IL-17, interferon (IFN)- γ , and granulocyte-macrophage colony-stimulating factor (GM-CSF) in cultured medium were measured with enzyme-linked immunosorbent assay kits (two-way analysis of variance showing the difference between kd and wt groups). **D–F:** All kd and wt EAE mice were pooled for the correlation tests between concentrations of IL-17, IFN- γ , and GM-CSF after treatment of 50 μg/mL MOG35-55 peptides and the clinical scores (Spearman's tests). For the T-cell proliferation assay, T cells were cultured with MOG35-55 or concanavalin A for 72 hours. 5-Bromo-2'-deoxyuridine (BrdU) was then added into the culture medium for 16 hours. **G:** Thereafter, the T-cell proliferation was evaluated by measuring BrdU incorporated into the cellular DNA (two-way analysis of variance showing the difference between kd and wt groups). $n = 6$ to 8 per group (**A–C**); $n = 13$ to 16 per group (**D–F**); $n = 3$ per group (**G**). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. EU, europium.

significantly higher in MS patients than in healthy controls.³¹ Plasma levels of neutrophil-related chemokines, CXCL1 and CXCL5, and neutrophil elastase are correlated with the cumulative magnetic resonance imaging lesion volume.³² In mice, depleting neutrophils by injecting anti-Ly-6G (clone: 1A8) antibody,³³ impairing the neutrophil chemotaxis by deleting CXCR2,³⁴ or inhibiting neutrophil expansion by knocking out granulocyte colony-stimulating factor receptor-encoded gene³² can all prevent development of EAE. As potential mechanisms, neutrophils have been shown to secrete proinflammatory cytokines to promote EAE progress by disrupting the blood-brain barrier and facilitating the maturation of antigen-presenting cells in the CNS.^{33,35} In our IKK β -deficient EAE mice, the efficiency of LysM-Cre-mediated IKK β deletion in neutrophils is nearly 90%. Unfortunately, we cannot yet clarify the specific function of IKK β in neutrophils. It has been reported that deficiency of IKK β increases the secretion of IL-1 β from neutrophils and prevents cell apoptosis.²⁵ However, the general neuroinflammatory activation in our IKK β -deficient EAE mice is still lower than that in IKK β wild-type EAE controls, suggesting that the proinflammatory effect of IKK β deficiency in myeloid IKK β -deficient EAE models appears to be limited.

NF- κ B-related anti-inflammatory therapies have already been considered in the clinic. For example, corticosteroids (methylprednisolone, prednisolone, or dexamethasone), which inhibit NF- κ B activation,^{36,37} have been prescribed for the pharmacological management of acute relapse of MS.^{38,39} The suppressive effect of corticosteroids on TNF- α production in MS patients is even related to their clinical effect.¹⁷

The level of neuroinflammation is not only determined by the inflammatory activity of myeloid cells in the CNS, but also controlled by the encephalitogenic capacity of T cells. The IKK β -NF- κ B signaling pathway plays an important role in the expression of major histocompatibility complex antigen-presenting molecules, costimulatory molecules, and various inflammatory cytokines in dendritic cells, thereby promoting the maturation of antigen-presenting cells and the T-cell activation that follows.^{40–42} However, inhibition of IKK β in myeloid cells has been observed to increase IL-1 β secretion,²⁵ which facilitates Th17 lymphocyte differentiation.¹⁸ Thus, myeloid IKK β might have diverse effects on T-cell activation and differentiation, depending on IL-1 β signaling.

In our EAE mice, deficiency of IKK β in myeloid cells reduces *Rorc* gene transcription in the T lymphocytes

Figure 7 Myeloid I κ B kinase β (IKK β) deficiency facilitated IL-1 β release is not correlated with the experimental autoimmune encephalomyelitis (EAE) pathology. The spinal cords were isolated from myeloid IKK β -deficient (kd) and wild-type (wt) EAE mice at 18 days after MOG35-55 immunization (dpi) and stained with fluorophore-conjugated antibodies against IL-1 β (in green) and Mac3 (in red). In kd mice (**A**), the IL-1 β staining is shown as dense puncta in green fluorescence (arrowheads), which is mainly located in the extracellular matrix; in wt mice (**B**), few IL-1 β puncta can be observed and the IL-1 β staining is shown as a weak green shadow around DAPI-stained nuclei (in blue) and colocalizing with Mac3 (asterisks). In the EAE lesion in wt mice, there are rare IL-1 β staining-positive signals (**C**). Spleen cells isolated from EAE mice at 18 dpi were cultured and stimulated with MOG35-55 at different concentrations for 72 hours. **D**: IL-1 β in the supernatant was measured with enzyme-linked immunosorbent assay (two-way analysis of variance showing the difference between kd and wt groups; from three independent EAE experiments). **E**: Spearman's test was used to test the correlation between IL-1 β concentration after treatment with 50 μ g/mL MOG35-55 and the EAE scores ($n = 14$ including both kd and wt EAE mice). $n = 6$ to 8 per group (**D**); $n = 14$ (**E**, including both kd and wt EAE mice). *** $P < 0.001$.

infiltrated into the spinal cord at the peak disease. In the peripheral lymphoid organs, spleen and lymph nodes, at the T cell—priming stage, the transcription of *Rorc* or *Il17a* gene in T cells was also suppressed. However, myeloid deficiency of IKK β simultaneously enhanced the transcription of *Tbx21* and *Gata3* in the T cells of spleen and increased the transcription of T-bet alone in the T cells of lymph nodes. The *Rorc* gene encodes RAR-related orphan receptor γ , and the *Tbx21* and *Gata3* genes encode T-bet and GATA-3. RAR-related orphan receptor γ and T-bet are two transcription factors driving the differentiation of pro-EAE Th17 and Th1 cells,^{43,44} respectively, whereas GATA-3 polarizes the T-cell differentiation toward anti-EAE Th2 cells.⁴⁵ Thus, myeloid IKK β regulates the T-cell differentiation and exerts different effects at different locations of T cells on the way to the CNS. However, the deficiency of myeloid IKK β appears to constantly inhibit the Th17 cell differentiation.

Moreover, cultured spleen cells derived from myeloid IKK β -deficient EAE mice release less IL-17, IFN- γ , and GM-CSF after the MOG35-55 treatment than spleen cells isolated from IKK β wild-type EAE. The reduced cytokine secretion is in accordance with the attenuated EAE severity. Although we also observed that MOG35-55 stimulation led to higher proliferation activity in T cells isolated from the lymph nodes of myeloid IKK β -deficient EAE mice, and myeloid IKK β -deficient mice who received autoreactive lymphocytes by adoptive transfer displayed symptoms 3 days earlier than IKK β wild-type recipient controls, our study strongly suggests that deficiency of IKK β in myeloid cells has the potential to stop EAE disease progression by suppressing the activation and differentiation of pathogenic T cells.

IL-1 β promotes Th17 lymphocyte differentiation.¹⁸ However, in our myeloid IKK β -deficient EAE mice, the higher secretion of IL-1 β both in the spinal cord and from the cultured spleen cells is not correlated with the severity of EAE

Figure 8 Myeloid cell lineage—restricted ablation of I κ B kinase β (IKK β) ameliorates symptoms and neuroinflammatory activation in the passive experimental autoimmune encephalomyelitis (EAE). Female 6- to 8-week-old myeloid IKK β -ablated (IKK β -deficient; kd) and wild-type (IKK β wt) mice were adoptively transferred with activated encephalitogenic T cells. **A**: The clinical disease activity was daily scored until 25 days after immunization (two-way analysis of variance showing the difference between kd and wt groups; t -test between kd and wt EAE mice on each day; pooled from three independent experiments). **B** and **C**: Thereafter, mice were sacrificed and transcripts of *Tnf*, *Il1b*, *Nos2* (alias iNOS), *Ccl2*, and *Cxcl10* were measured with real-time PCR (t -test; pooled from two independent experiments). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus kd (t -test); ††† $P < 0.001$ versus kd between day 9 and day 27 (two-way analysis of variance). $n = 9$ (**A**, kd mice); $n = 11$ (**A**, wt mice); $n \geq 4$ per group (**B** and **C**). CCL, chemokine ligand; iNOS, inducible nitric oxide synthase; TNF, tumor necrosis factor.

pathogenesis. Thus, the general effects of inhibition of IKK β in myeloid cells on EAE pathogenesis are not significantly changed by the increased IL-1 β secretion.

Deletion of IKK β in neurons promoted neuronal death and exacerbated EAE symptoms in one study,⁴⁶ although ablation of NF- κ B affected neither neuroinflammatory activation nor neuronal death in the EAE model in another study.⁴⁷ Thus, the pathogenic effects of IKK β –NF- κ B might be divergent. The disease status of EAE or MS should be carefully considered before an anti–NF- κ B drug is administered.

In summary, our study has demonstrated that deficiency of IKK β attenuates the severity of EAE by inhibiting the neuroinflammatory activation, and perhaps also suppressing the activation of encephalitogenic T lymphocytes. IKK β could be used as a potential therapeutic target in MS, especially when neuroinflammation is considered to be the primary problem.

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