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ORIGINAL ARTICLE

JAK inhibition ameliorated experimental autoimmune encephalomyelitis by blocking GM-CSF-driven inflammatory signature of monocytes

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Abstract Monocytes are key effectors in autoimmunity-related diseases in the central nervous system (CNS) due to the critical roles of these cells in the production of proinflammatory cytokines, differentiation of T-helper (Th) cells, and antigen presentation. The JAK–STAT signaling is crucial for initiating monocytes induced immune responses by relaying cytokines signaling. However, the role of this pathway in modulating the communication between monocytes and Th cells in the pathogenesis of multiple sclerosis (MS) is unclear. Here, we show that the JAK1/2/3 and STAT1/3/5/6 subtypes involved in the demyelination mediated by the differentiation of pathological Th1 and Th17 and the CNS-infiltrating inflammatory monocytes in experimental autoimmune encephalomyelitis (EAE), a model for MS. JAK inhibition prevented the CNS-infiltrating CCR2-dependent Ly6C^{hi} monocytes and monocyte-derived dendritic cells in EAE mice. In parallel, the proportion of GM-CSF^{+}CD4^{+} T cells and GM-CSF secretion were decreased in pathological Th17 cells by JAK inhibition, which in turns converted CNS-invading monocytes into antigen-presenting cells to mediate tissue damage. Together, our data highlight the ther-

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apeutic potential of JAK inhibition in treating EAE by blocking the GM-CSF-driven inflammatory signature of monocytes.

1. Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory autoimmune disease characterized by progressive demyelinating lesions and axonal damage, ultimately causing neurodegeneration in the central nervous system (CNS)[1]. MS lesions harbor various immune cells, among which auto-reactive helper T (Th) cells and monocytes are crucial for their pathogenic potentials and for the activation of inflammatory phagocytes[2,3]. The abnormal production of cytokines during an autoimmune response underlies the pathogenic effects of these inflammatory cells[4]. However, how cytokines cause the demyelinating and destructive effects of Th cells and myeloid cells in MS is unclear. Therefore, to develop effective therapeutics against MS, it is crucial to explore the coordinated control of cytokines among the executive cells associated with tissue damage.

The cytokine-driven Janus kinase (JAK)−signal transducer and activator of transcription (STAT) signaling pathway are involved in innate and adaptive immunity and contributed to inflammatory and immune responses, which are essential for the immunopathology of autoimmune diseases[5,6]. The JAK family of non-receptor protein-associated tyrosine kinases, including JAK1, JAK2, JAK3, and tyrosine kinase 2, relay signals downstream of type I and type II cytokine receptors through STATs[7]. More than 50 cytokines, including interleukins (ILs), interferons, and, colony-stimulating factors, rely on the JAK−STAT signaling pathway to modulate cell proliferation, differentiation, and survival in addition to immune responses[8,9]. The relationships between JAKs and various diseases have been revealed *via* studies on functional mutations and polymorphisms of JAKs in cell culture, mice, or humans[10,11]. Therefore, inhibition of JAK activity is key to suppressing abnormal inflammatory and immune responses. With the approval of the JAK inhibitor tofacitinib by the U.S. Food and Drug Administration (FDA) for the treatment of rheumatoid arthritis in 2012, the JAK family has been validated as a new and effective therapeutic target in inflammatory and autoimmune diseases[12]. To date, 10 JAK inhibitors have been approved to treat various autoimmune diseases driven by innate or adaptive immune mechanisms, but no JAK inhibitor has been approved for the treatment of MS yet[13,14]. Accordingly, the identification of efficient JAK inhibitors is not only helpful for the treatment of MS but also for understanding the JAK−STAT-related mechanisms underlying the pathogenesis of MS.

It is widely accepted that $CD4^+$ T cells are important cellular players in the pathogenesis of MS, which can be modeled in animals *via* experimental autoimmune encephalomyelitis (EAE)[3,15]. Among $CD4^+$ cells, differentiation and functions of Th1 and Th17 cells are categorized according to the specific cytokines they produce, including interleukin 17 (IL-17), granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon-gamma (IFN-γ), which are mediators of the JAK−STAT signaling pathway[16,17]. Myeloid cells, such as monocytes and monocyte-derived dendritic cells (moDCs), are major effectors of the tissue destruction of the CNS in EAE[18]. Especially, circulating $Ly6C^{hi}$ monocytes contribute to the demyelination damage in EAE by infiltrating the CNS[19]. Therefore, the role of the cytokine-driven JAK−STAT signaling in the immune responses of monocytes and T cells in the CNS remains unclear. Whether inhibition of the abnormal activation of the JAK−STAT signaling to suppress the monocytes activating pathological Th cells can improve EAE should be explored.

Herein, this study explored whether the JAK−STAT pathway by a JAK inhibitor can suppress EAE progression. The JAK inhibitor Z526 significantly alleviated EAE by inhibiting Th1 and Th17 differentiation and resulting in a reduction in GM-CSF secretion. Consequently, we further demonstrate that decreased GM-CSF derived from Th17 cells fails to activate $Ly6C^{hi}CCR2^+$ monocytes and monocyte-derived dendritic cells (moDCs) and reduces inflammatory myeloid cells infiltrating the CNS. These results suggest that the JAK2−STAT5 pathway relays the GM-CSF signaling in CNS-infiltrated monocytes, which consequently become antigen-presenting cells. Collectively, these results show the therapeutic potential of JAK inhibition and provide interesting therapeutic avenues for MS patients.

2. Materials and methods

2.1. Experimental animals

Female C57BL/6 mice (20−22 g, aged 8−12 weeks) were purchased from the Beijing HuaFuKang Bioscience Co., Ltd. (Beijing, China). Animals (5 per cage) were housed at room temperature (22 ± 2 °C) in specific pathogen-free conditions under a 12-h light/12-h dark cycle, with food and water provided *ad libitum*. All the animal experimental procedures used in this study were approved by the Animal Care and Use Committee of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (approval no. 00005958). The animal study also accorded with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.2. EAE induction and treatment

To induce EAE, 8−12 weeks-old female mice were immunized with 300 μg MOG_{35-55} emulsified in CFA (made with 4 mg/mL heat-killed Mtb H37Ra in incomplete Freund's adjuvant). The immunization was performed *via* subcutaneous injection into two different sites on each hind flank, with 50 μL emulsion used per injection, resulting in four injections and 200 μL emulsions in total per mouse. On Day 0 and Day 2 post-immunization, 200 ng pertussis toxins were intravenously administered. Clinical signs of EAE and weight change were daily assessed according to the following scores: 0, no clinical sign of disease; 1, limp tail; 2, paraparesis (weakness, incomplete paralysis of one or two hind

limbs); 3, paraplegia (complete paralysis of two hind limbs); 4, paraplegia with forelimb weakness or paralysis; 5, moribund state or death[20,21]. For treatment, Z526 (3, 10, and 30 mg/kg) or vehicle (CMC-Na) were intragastrically administered daily from Day 10 postimmunization for 10 days.

For adoptive transfer EAE, purified $CD4^+$ T cells from EAE mice (immunized for 10 days with 300 μg MOG_{35-55} emulsified in CFA) were cultured in the presence or absence of Z526 (10 μmol/L) under MOG_{35-55} challenge and Th17-polarizing conditions. The final processed cells were adoptively transferred to recipients. The recipient mice also intravenously received 200 ng pertussis toxins on Day 0 and Day 2 post-immunization. Mice were daily observed for the clinical signs of EAE as described above.

2.3. Isolation of mouse $CD4^+$ naïve T cells and Th cells differentiation

Mouse $CD4^+$ naïve T cells were isolated from female C57BL/6 mice lymph nodes by magnetic negative selection using the MojoSort $CD4^+$ Naïve T Cell Isolation Kit (480040, BioLegend), and then cultured in RPMI-1640 containing 10% Fetal Bovine Serum (FBS), 1 μg/mL anti-CD3 (100312, BioLegend) and 5 μg/mL anti-CD28 (102121, BioLegend) in the presence or absence of Z526 or/and cytokines that drive differentiation into Th1 or Th17 subsets. For Th1 cell differentiation, anti-IL-4 (10 μg/mL) and IL-12 (10 ng/mL) were added into a culture. For Th17 differentiation, anti-IL-4 (10 μg/mL), anti-IFN-γ (10 μg/mL), TGF-β (10 ng/mL), IL-6 (20 ng/mL), IL-23 (20 ng/mL), and IL-1β (20 ng/mL) were added to polarize naïve $CD4^+$ T cells. Z526 was added at five different concentrations, ranging from 10 mmol/L to 0.1 mmol/L (3-fold dilutions) at the initiation of $CD4^+$ naïve T cells polarization to monitor the effect on T cells differentiation. After 5 days, T cells were harvested and restimulated with phorbol 12-myristate 13-acetate (20 ng/mL) and ionomycin (1 μg/mL) for 4 h, then blocked with brefeldin A (420601, BioLegend) and monensin solution (420701, BioLegend) for 2 h. After surface staining with anti-CD4 antibody (1:100, 100510, BioLegend), T cells were permeabilized with the Foxp3/Transcription Factor Staining Buffer Set (00-5523-00, eBioscience) and stained with anti-IFN-γ (1:100, 505810, Biolegend), anti-IL-17A (1:100, 506903, Biolegend) and anti-GM-CSF (1:100, 505404, Biolegend) antibody to determine T cells differentiation by flow cytometry. Flow cytometry analysis was performed with BD verse and data were analyzed with FlowJo V10 software. In addition, cell supernatant was collected to determine cytokines IFN-γ and IL-17A with the ELISA method according to the manufacturer's instruction.

2.4. BMDC culture and siRNA transfection

Bone marrow cells were collected from the femur and tibia of mice and placed into phosphate-buffered saline. The cells were resuspended in the BMDC culture medium (RPMI-1640 with 10% FBS, 50 μmol/L 2-mercaptoethanol, penicillin/streptomycin, 20 ng/mL recombinant mice GM-CSF, and 10 ng/mL recombinant mice IL-4) and then cultured for 5 days to obtain BMDCs for further analyses. For siRNA transfection, siRNA targeting STAT5 and negative control siRNA were purchased from GenePharma. The following sequences were used: STAT5 sense 5′-CCCACUUCAGAAACAU-GUCACUGAA-3′; negative control sense 5′-UUCUCCGAACGUGUCACGUTT-3′. BMDCs were transfected with the STAT5 siRNA or negative-control siRNA for 48 h by using RNAi Max (Thermo Fisher Scientific) according to the manufacturer's instructions.

2.5. Histopathological analysis

For histopathological analysis, the spinal cord was harvested from the EAE and Z526-treated groups, embedded in paraffin, and sectioned at 5-μm thickness. Subsequently, the sections were stained with hematoxylin/eosin (HE) or Luxol fast blue (LFB), or subjected to immunofluorescence analysis for myelin basic protein (MBP), and then assessed for immune-cell infiltration and demyelination *via* standard procedures. CNS infiltrated T cells and DCs were immunofluorescence stained with anti-CD4 and anti-CD11c antibodies.

2.6. Statistical analysis

Data are presented as mean ± standard error of mean (SEM) of three or more independent experiments unless stated otherwise. All the statistical analyses were performed using the GraphPad Prism 8 software (GraphPad Software Inc., La Jolla, CA, USA). The clinical scores of the groups were compared using a two-factor analyzed of variance (ANOVA) followed by Tukey's *post hoc* test. The other experiments were analysis using one-way ANOVA followed by an appropriate *post hoc* test. $P < 0.05$ is considered statistical significance.

3. Results

3.1. JAK inhibition ameliorates the inflammatory demyelination in EAE mice

Immune cells infiltrating the white matter of the spinal cord are the main effector cells exerting the myelin attack in EAE, with $CD4^+$ T cells playing a pathogenic role in this process, especially the cytokine-activated JAK−STAT signaling-driven Th1 and Th17 cells. To investigate the precise regulatory role of the JAK−STAT signaling in the pathogenesis of MS, a mouse model of MS was established by inducing EAE in mice *via* injection of the MOG_{35-55} peptide. Peripheral blood mononuclear cells, lymphocytes, and CNS-infiltrated mononuclear cells from EAE mice at the peak of disease onset were collected to analyze the expression profile of JAK/STAT isoforms. The results showed that hyperphosphorylation of JAK1/2/3 and its effector molecules STAT1/3/5/6 were involved in the pathological functional alterations of immune cells in EAE mice (Fig. 1A). Inhibition of abnormal activation of the JAK−STAT signaling pathway in immune cells may alleviate the symptoms of EAE, so our study further investigated the therapeutic effect of the previously discovered JAK inhibitor Z526 for the treatment of EAE. Z526 is a novel JAK inhibitor discovered by us with potent inhibition on phosphorylation of the JAK family (Supporting Information Fig. S1A−S1E). Mice with EAE received oral administration of 3, 10, or 30 mg/kg Z526, starting on Day 10 after the MOG_{35-55} immunization (Fig. 1B). These Z526-treated mice displayed lower morbidity and symptom severity than the vehicle-treated controls (Fig. 1C). Additionally, the incidence rate of EAE in the Z526-treated mice was markedly lower, consistent with the lower clinical scores, than those in the controls (Fig. 1D). To

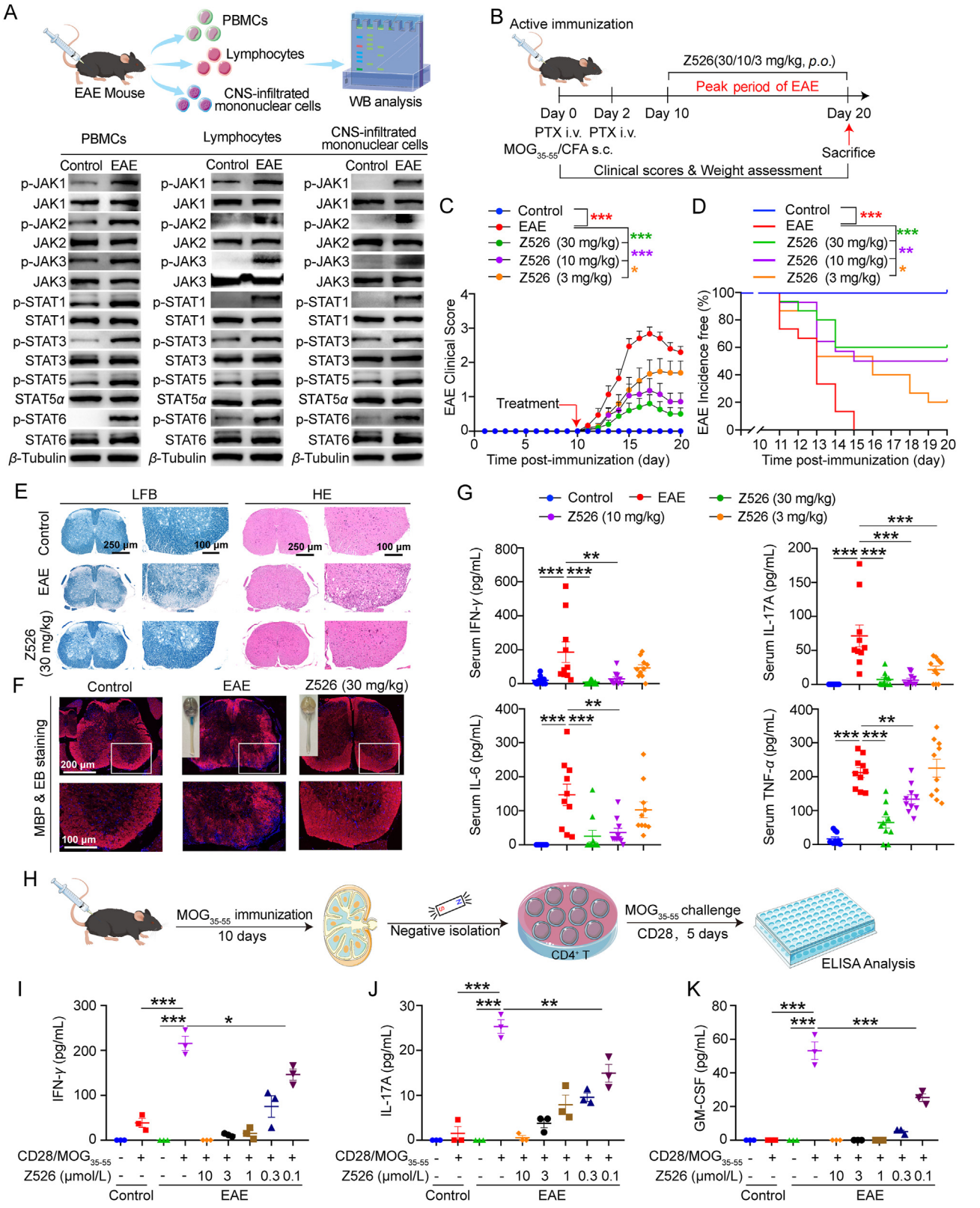

Figure 1 JAK inhibition is resistant to EAE pathogenesis and inflammation. (A) Experimental scheme of the therapeutic effect of Z526 for the analysis of EAE; C57BL/6 mice were immunized on Day 0 and were treated with different doses of Z526 (3, 10, and 30 mg/kg) from Day 10 for 10 days. Clinical score (B) and incidence free rate (C) were assessed every day in control, EAE mice, and Z526-treated EAE mice ($n = 15$). Spinal cord sections were stained by LFB and HE (D) and MBP&EB (E). (F) Inflammatory cytokines IFN-γ, IL-17A, IL-6 and TNF-α in serum were measured by ELISA method ($n = 10$). (G) Antigen-specific cytokines were determined from purified $CD4^+$ T cells of MOG_{35-55} immunized C57BC/6 mice after re-stimulated with MOG_{35-55} *in vitro* by ELISA method. (H) Experimental scheme of effect of Z526 on the

determine whether the therapeutic effects of the JAK inhibitor on the neurological manifestation of EAE are related to the demyelination of the spinal cord white matter, the lumbar spinal cord of the Z526-treated mice was analyzed *via* LFB staining, HE staining, and MBP immunostaining. We found that the JAK inhibition significantly attenuated the inflammatory demyelination, caused by massive immune-cell infiltration, in the white matter of the spinal cord (Fig. 1E). In addition, immunofluorescence staining of the lumbar spinal cord for MBP also confirmed the role of the JAK inhibitor Z526 in stabilizing the structure and function of the CNS myelin sheath (Fig. 1F). In addition, our study found that Z526, a pan-JAK inhibitor, was superior to the selective JAK1/2 inhibitor baricitinib and the selective JAK3 inhibitor Z583[22] in the treatment of EAE mice (Supporting Information Fig. S2), suggesting that there may be a joint involvement of JAK1/2/3 isoforms in the autoimmune response to central demyelination in EAE mice.

Increased blood−brain barrier permeability causes peripheral exposure of central myelin protein components, which triggers adaptive autoimmunity and allows peripheral immune cells to enter the CNS, thereby leading to inflammatory demyelination. The Evans Blue staining revealed that the JAK inhibitor Z526 protects the integrity of the blood−brain barrier (Fig. 1F). As expected, serological tests showed high levels of IFN-γ (Th1 marker) and IL-17A (Th17 marker) in the peripheral blood of EAE mice and typical levels of inflammatory cytokines IL-6 and TNF-α (Fig. 1G). Z526 significantly reduced the secretion of the aforementioned inflammatory cytokines and alleviated the clinical signs of EAE. To further evaluate the effect of JAK inhibitors on MOG_{35-55} immunization, an *ex vivo* assay was performed. Specifically, the effect of JAK inhibition on the secretion of antigen-specific cytokines by $CD4^+$ T cells was assessed. Briefly, female C57BL/6 mice were immunized by MOG_{35-55} peptide for 10 days. Subsequently, $CD4^+$ T cells were harvested from lymph nodes and then co-treated with MOG_{35-55} peptide and an anti-CD28 in the presence or absence of the JAK inhibitor Z526 for 5 days (Fig. 1H), Z526 significantly inhibited the secretion of the antigen-specific cytokines IFN-γ (Fig. 1I), IL-17A (Fig. 1J), and GM-CSF (Fig. 1K). Together, these data show that the JAK inhibitor Z526 alleviated the neurological severity and disease progression of EAE by reducing the secretion of inflammatory cytokines, suggesting that JAK is a viable drug target against MS.

In order to further estimate the druggability and clinical value of Z526, we carried out a preliminary assessment of its safety and metabolism. The acute toxicity of Z526 was evaluated *via* oral administration of a signal dose of 2 g/kg Z526 in mice ($n = 10$; five males and five females) and the hematological parameters also were detected on Day 14. During the surveillance period, no acute toxic effects were observed and the LD_{50} of Z526 was found to be > 2 g/kg by oral administration (Fig. S2A and S2C). Preliminary pharmacokinetic (PK) analysis showed that an oral dose of 30 mg/kg Z526 exhibited a reasonable PK profile, with a $t_{1/2}$ of 11.54 h (Fig. S2B). Furthermore, *in vitro* ADME analysis revealed that Z526 has excellent metabolic stabilities against human liver microsome, plasma, and whole blood (Supporting Information Tables S1−S3).

3.2. JAK inhibition prevents the pathogenic differentiation of T cells during EAE

Activated $CD4^+$ T cells are the main drivers of EAE pathogenesis. The Z526-induced reduction in the IFN-γ, IL-17A, and GM-CSF levels in serum and lymph nodes suggested that JAK inhibition prevents the differentiation of Th1 and Th17 cells. Thus, we further analyzed the pathogenic $CD4^+$ Th cells in the peripheral lymph nodes and CNS of EAE mice *via* flow cytometry. Briefly, grinding of lymph nodes into single cell suspensions for cell surface and intracellular staining after restimulation and secretion blocking, JAK inhibition significantly inhibited Th1 and Th17 differentiation characterized by a reduction in the proportion of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}17A^+$, and $CD4^+GM\text{-}CSF^+$ cell populations (Fig. 2A and B). We then analyzed the pathogenic Th phenotypes in the brain and spinal cord by using the 70%/30% percoll method (Fig. 2C). The JAK inhibitor Z526 significantly reduced the infiltration of $CD4^+$ T cells and the pathological Th1 and Th17 differentiation in the CNS by suppressing IFN-γ, IL-17A, and GM-CSF secretion (Fig. 2D and E).

JAK inhibition suppressed the differentiation of peripheral and central Th cells in EAE mice, as evidenced by reduced production of cytokines, including IFN-γ, IL-17A, and especially GM-CSF. To further validate the effect of JAK inhibition on the differentiation of Th cells, we cultured terminally differentiated Th cell populations and evaluated the effect of Z526 on their differentiation and cytokine secretion. Briefly, purified $CD4^+$ T cells from C57BL/6 mice were differentiated into Th cells (Th0, Th1, and Th17) *via* previously described methods (Fig. 3A). The JAK inhibitor Z526 significantly suppressed the proliferation of $CD4^+$ T cells (Fig. S2A−S2F) and inhibited the differentiation of naïve $CD4^+$ T cells into Th1 cells in a concentration-dependent manner, characterized by decreased IFN-γ production (Fig. 3B) with an IC_{50} value of 1.37 μmol/L (Fig. 3C). In addition, Z526 also suppressed the differentiation of naïve $CD4^+$ T cells into Th17 cells under Th17-polarizing conditions by abolishing IL-17A (Fig. 3D) and GM-CSF (Fig. 3F) production, with the IC_{50} value of 2.20 μmol/L (Fig. 3E) and 2.77 μmol/L (Fig. 3G), respectively. Compared with terminally differentiated Th1 cells, Th17 cells are more pathogenic in EAE. Thus, we assessed whether the JAK−STAT signaling is involved in the differentiation of Th17 cells and analyzed the mechanism whereby Z526 inhibited the secretion of IL-17A and GM-CSF. Upon stimulation by an array of cytokines (TGF-β1, IL-2, IL-6, IL-23, and IL-1β), Th17 cells undergo phosphorylation of the transcription factors STAT3 and STAT5, among which the phosphorylation of STAT5 has been shown to be crucial for the formation of pathogenic Th17 cells and GM-CSF expression[23−25]. The JAK inhibitor Z526 significantly inhibited the phosphorylation of STAT3, especially STAT5 (Fig. 3H−J) in pathogenic Th cells. In addition, Z526 reduced the mRNA level of *Il-17a* (Fig. 3K) and *Gm-csf* (Fig. 3L) in these cells. Together, these results show that JAK−STAT inhibition suppresses Th1 and Th17 differentiation, as evidenced by the reduced levels of IFN-γ, IL-17A, and GM-CSF upon Z526, treatment and this suppression attenuates the encephalitogenic and tissue-damaging effects of Th cells.

secretion of antigen-specific cytokines by $CD4^+$ T cells from MOG_{35-55} immunized mice. The production of IFN-γ (I), IL-17A (J) and GM-CSF (K) by $CD4^+$ T cells were measured by ELISA; and the IC_{50} values of Z526 (0.1, 0.3, 1, 3, and 10 μmol/L) for inhibiting MOG_{35-55}-specific cytokines secretion were calculated. All data are from three independent experiments. Data are presented as mean ± SEM, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

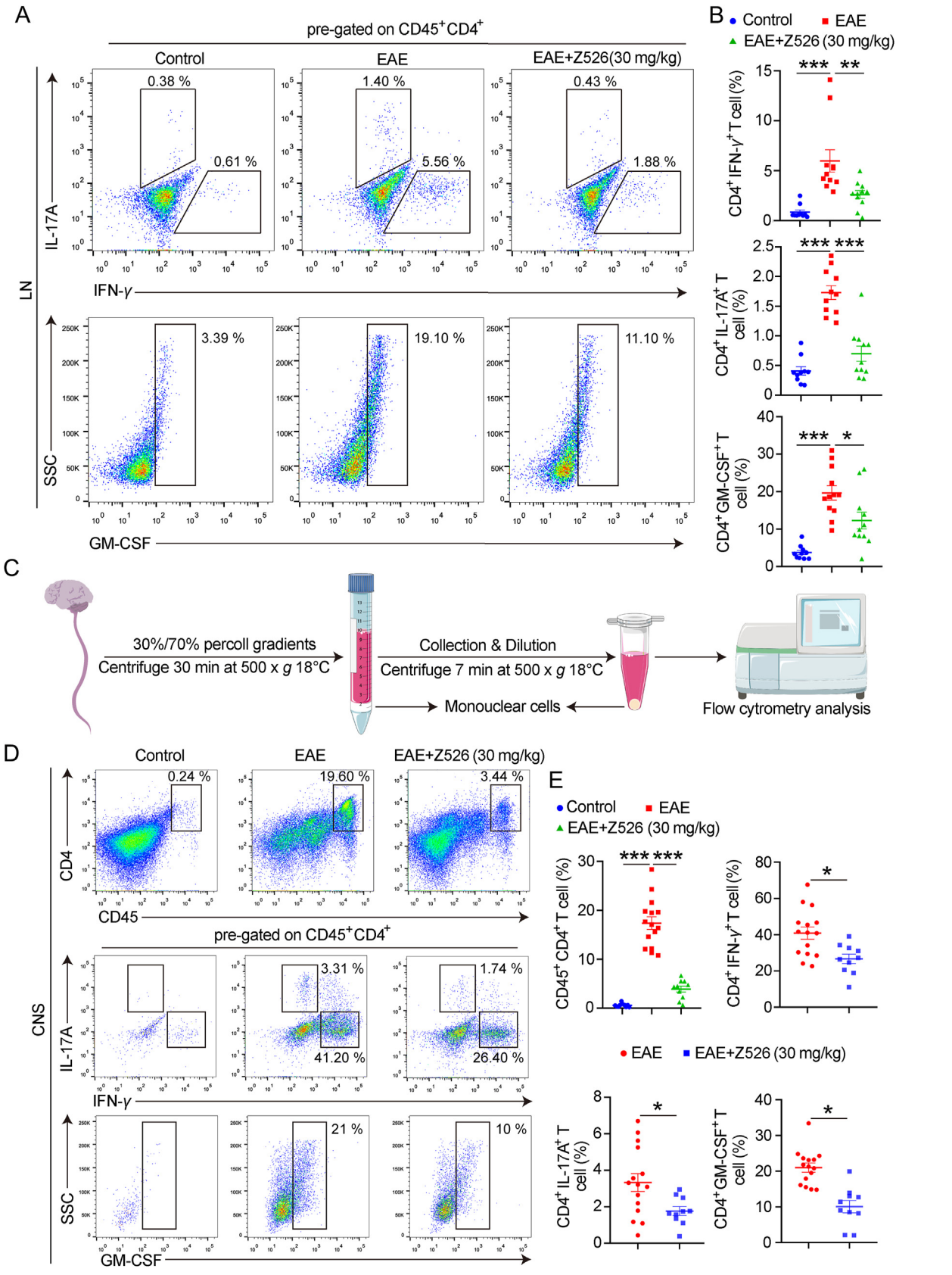

Figure 2 JAK−STAT inhibition suppressed Th1 and Th17 cells differentiation *in vivo*. (A, B) Subpopulations of pathogenic Th1 and Th17 cells differentiated in peripheral lymph nodes were analyzed by intracellular staining IFN-γ, IL-17A and GM-CSF. (C) Mononuclear cells in brain and spinal cord were collected with 30%/70% percoll gradients. (D, E) Subpopulations of pathogenic Th1 and Th17 cells differentiated in CNS were analyzed by intracellular staining IFN-γ, IL-17A and GM-CSF. All data are from three independent experiments. Data are presented as mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

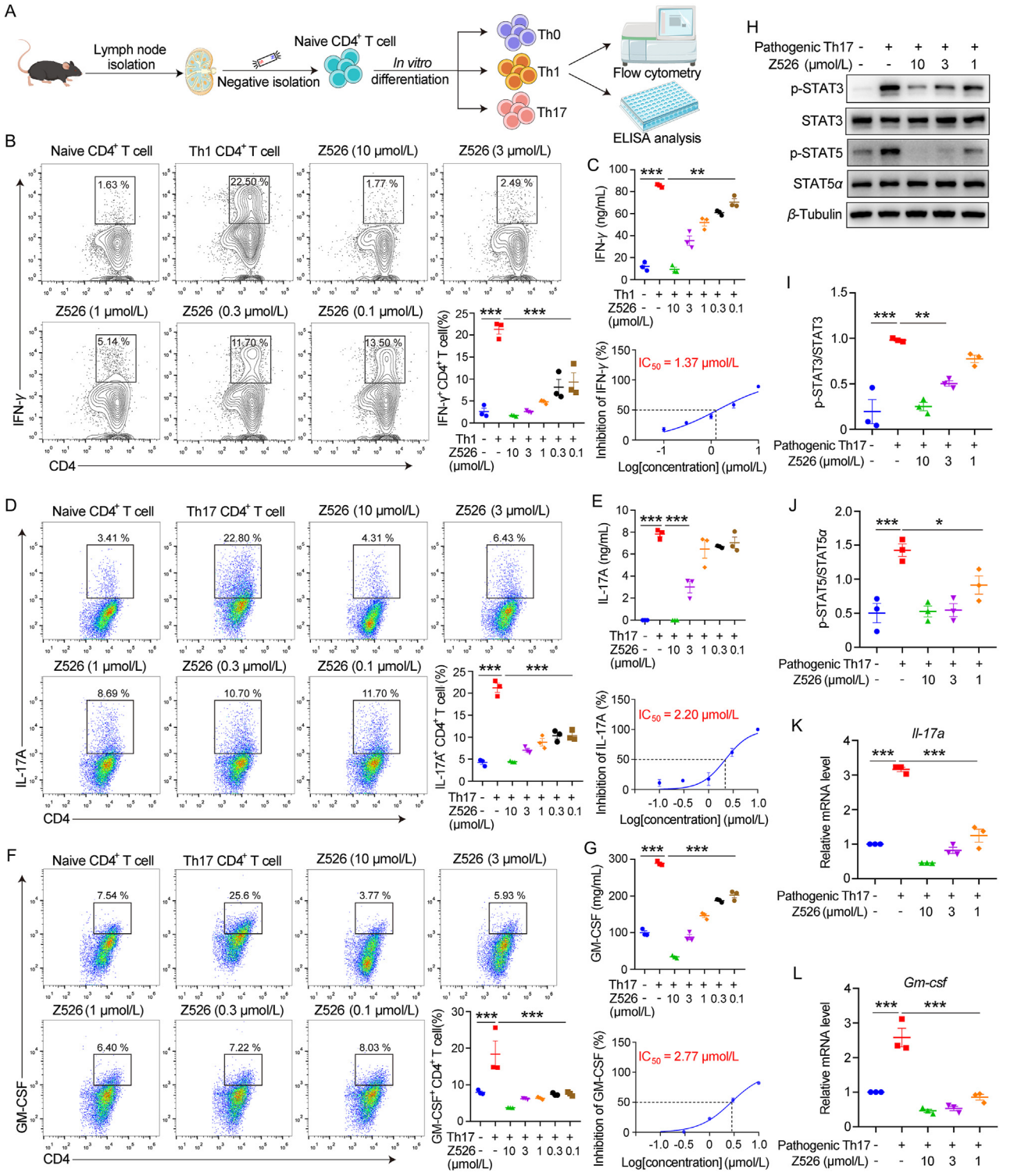

Figure 3 JAK−STAT inhibition suppressed Th1 and Th17 cells differentiation *in vitro*. Naïve CD4$^+$ T cells from lymph nodes of C57BL/6 mice were isolated to perform the proliferation and differentiation assays. (A) Schematic for testing the role of Z526 in mouse Th cell *in vitro* differentiation. CD4$^+$ T cells were stimulated with anti-CD3/anti-CD28 and cytokines in the presence or absence or absence of Z526 (0.1, 0.3, 1, 3, and 10 μmol/L) for 5 days; the expression of IFN-γ (B), IL-17A (D) and GM-CSF (F) were analyzed by flow cytometry. The production of IFN-γ (C), IL-17A (E) and GM-CSF (G) by Th1 and Th17 cells in the culture supernatant was measured by ELISA; the IC_{50} values of Z526 (0.1, 0.3, 1, 3, and 10 μmol/L) for inhibiting T cell differentiation were calculated. (H−J) JAK−STAT signaling pathway affected by Z526 was determined in pathogenic Th17 cells by Western blot. The mRNA expression of IL-17A (K) and GM-CSF (L) in Th17 cells were determined by real-time qPCR. All data are from three independent experiments. Data are presented as mean ± SEM; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.3. *JAK inhibition prevents CNS-infiltration of CCR2-dependent Ly6C^{hi} monocytes and moDCs during EAE*

It is widely accepted that CD4$^+$ Th cells, especially Th1 and Th17 phenotypes, are the prime mediators of EAE or MS. However, CNS-infiltrating myeloid rather than lymphoid cells are responsible for much of the tissue damage and neurological decline. Especially, CCR2-dependent inflammatory Ly6C^{hi} monocytes and their progeny, inflammatory moDCs, have been proposed to be the crucial effector in the development of EAE[26]. Thus, we explored whether the alleviation of EAE by JAK inhibition is due to decreased CNS infiltration of inflammatory monocytes and their progeny. Briefly, mononuclear cells were isolated from the peripheral blood and CNS of Z526-treated mice and then labeled with an array of antibodies to identify diverse cell populations. We observed that inflammatory myeloid cells (CD45$^+$CD11b$^+$) (Fig. 4A and F), including neutrophils (CD45$^+$CD11b$^+$Ly6G$^+$) (Fig. 4B and G), monocytes (CD45$^+$CD11b$^+$Ly6C$^+$) (Fig. 4C and H), and especially CCR2-dependent monocytes (CCR2$^+$CD45$^+$CD11b$^+$Ly6C$^+$) (Fig. 4D and I) and their progeny of inflammatory moDCs (CD45$^+$CD11b$^+$

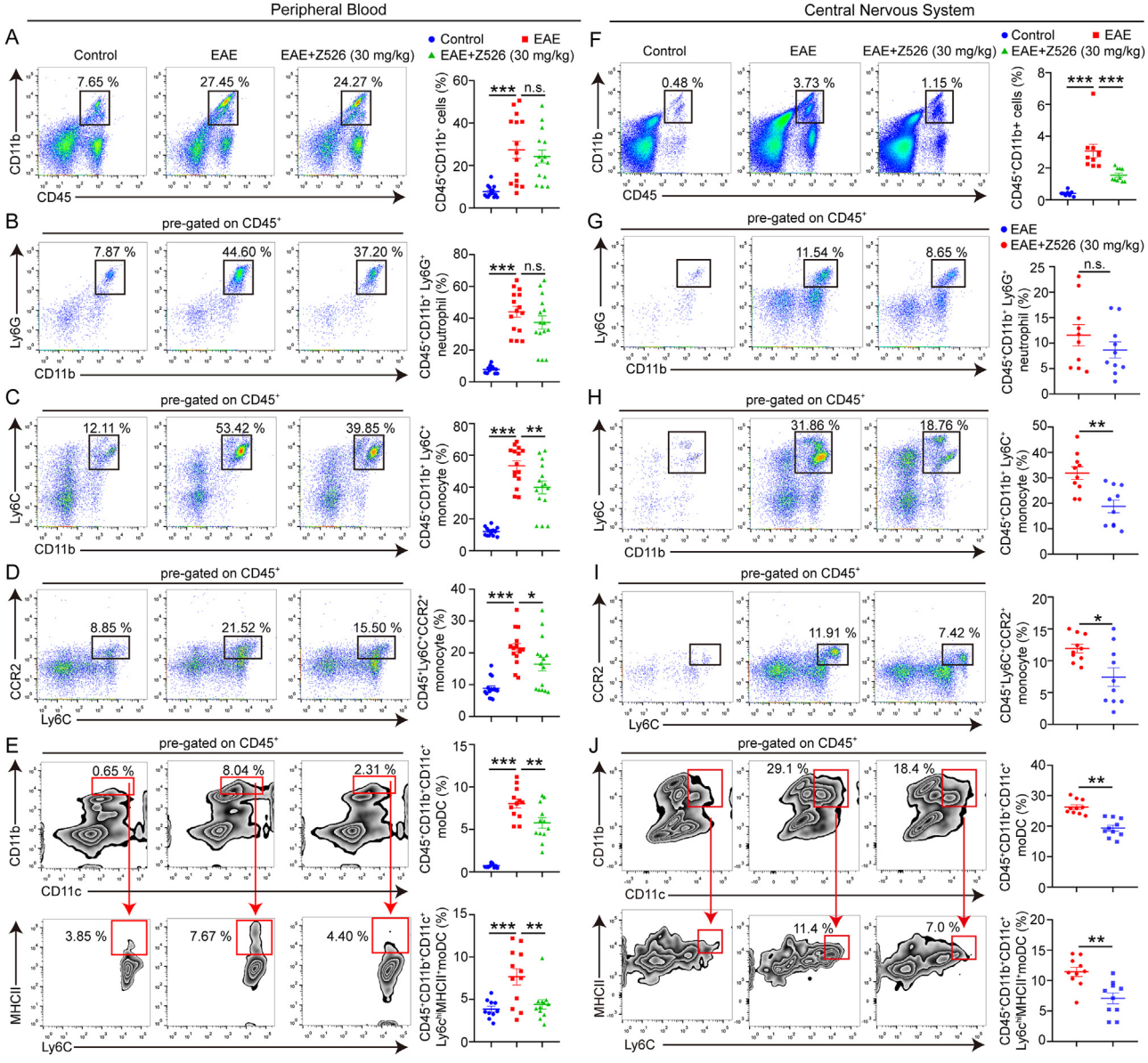

Figure 4 JAK inhibition attenuates CCR2-dependent Ly6C^{hi} monocytes and moDCs recruitment to the CNS. Mononuclear cells from peripheral blood in EAE mice and Z526-treated EAE mice were stained with an array of different flow antibodies to identify inflammatory myeloid cells (CD45$^+$CD11b$^+$) (A), including neutrophil (CD45$^+$CD11b$^+$Ly6G$^+$) (B), monocyte (CD45$^+$CD11b$^+$Ly6C$^+$) (C) especially CCR2-dependent monocyte (CCR2$^+$CD45$^+$CD11b$^+$Ly6C$^+$) (D), and moDCs (CD45$^+$CD11b$^+$CD11c$^+$Ly6C^{hi}MHCII$^+$) (E) (n = 15 for A–C, n = 12 for D and E). Mononuclear cells from the CNS were also stained with flow antibodies to identify inflammatory myeloid cells (CD45$^+$CD11b$^+$) (F), including neutrophil (CD45$^+$CD11b$^+$Ly6G$^+$) (G), monocyte (CD45$^+$CD11b$^+$Ly6C$^+$) (H) especially CCR2-dependent monocyte (CCR2$^+$CD45$^+$CD11b$^+$Ly6C$^+$) (I), and moDCs (CD45$^+$CD11b$^+$CD11c$^+$Ly6C^{hi}MHCII$^+$) (J) (n = 10). Data are presented as mean ± SEM. n.s., not significant. *P < 0.05, **P < 0.01, ***P < 0.001.

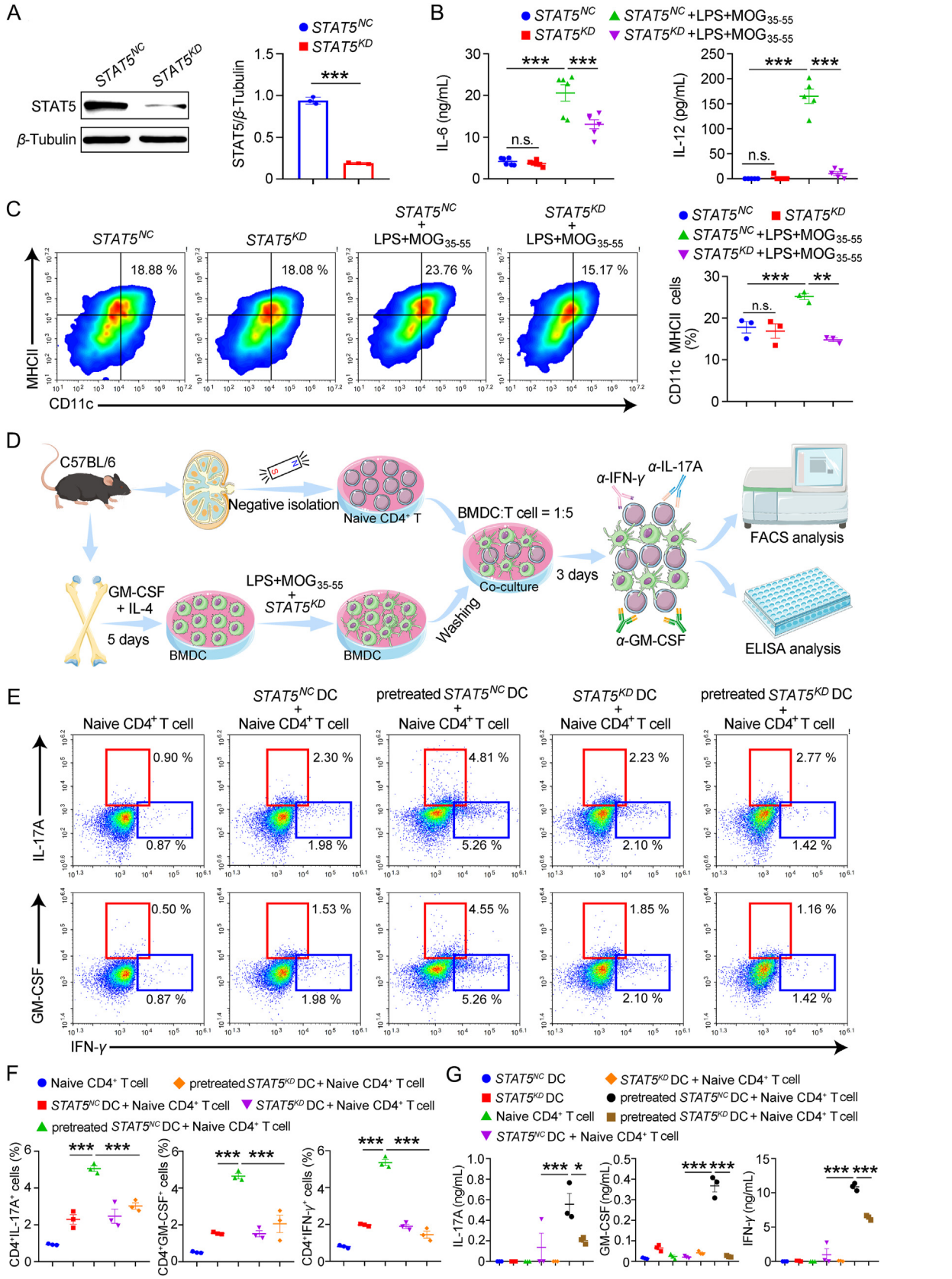

Figure 5 STAT5 is responsible for the antigen-presenting ability of moDCs. (A) STAT5 was knocked down in BMDC and verified by Western blot. (B) BMDC-derived polarizing factors IL-6 and IL-12 were determined by ELISA. (C) Percentage of $CD86^{+}CD11c^{+}$ and $MHCII^{+}CD11c^{+}$ was analyzed by flow cytometry. (D) Schematic for STAT5 knockdown BMDCS co-cultured with naïve $CD4^{+}$ T cells. (E, F) The expression of IFN-γ, IL-17A and GM-CSF in the co-culture system was analyzed by flow cytometry. (G) The production of IL-17A, GM-CSF and IFN-γ by

$CD11c^+Ly6C^{hi}MHCII^+$) (Fig. 4E and J) were highly abundant in the peripheral blood and CNS in mice with EAE. JAK inhibition significantly attenuated the proliferation and invasion of CCR2-dependent $Ly6C^{hi}$ monocytes (Fig. 4I) and moDCs (Fig. 4J) rather than neutrophils (Fig. 4G) in the CNS. Together, these results show that JAK inhibition alleviates EAE not only by suppressing pathogenic Th1 and Th17 phenotypes but also by targeting the myeloid-effector compartment comprising CCR2-dependent $Ly6C^{hi}$ monocytes and moDCs. Collectively, we demonstrated that the JAK-STAT pathway mediates the central homing of myeloid cells, especially inflammatory $Ly6C^{hi}$ monocytes, and moDCs.

3.4. The JAK2−STAT5 signaling is responsible for the antigen-presenting ability of moDCs

Inflammatory monocytes and their progeny moDCs are the main drives of EAE pathogenesis. Of note, the communication between pathogenic Th cells with monocytes is linked by GM-CSF, and monocytes are the primary targets of GM-CSF derived from pathogenic Th cells. As a proinflammatory growth factor, GM-CSF signaling is transmitted by employing the JAK2 subtype and its downstream effector STAT5, which is a transcription factor crucial for the formation of pathogenic Th cells and the antigen-presenting ability of moDCs. Phosphorylation of STAT5 downregulates GM-CSF in pathogenic Th cells. To determine the role of the JAK−STAT signaling in the formation of inflammatory monocytes, BMDCs were cultured to analyze the role of STAT5 in the antigen-presenting ability of myeloid cells. Briefly, monocytes isolated from the bone marrow of C57BL/6 mice were cultured in the presence of GM-CSF and IL-4, which maintain the immature state of these cells, and subjected to STAT5 knock-down. The knock-down efficiency in these BMDCs was >90% (Fig. 5A). When stimulated with lipopolysaccharide (LPS) and MOG_{35-55}, BMDCs displayed obvious protrusions, which were indicative of maturation characterized by secretion of APC-derived polarizing factors and expression of co-stimulatory molecules. The STAT5 knockdown in BMDCs significantly inhibited the secretion of IL-6 and IL-12 (Fig. 5B), which promoted Th17 and Th1 differentiation, respectively. In addition, the expression of the co-stimulatory molecule MHCII was also significantly reduced (Fig. 5C), suggesting that the downregulation of STAT5 in BMDCs suppresses the ability of these cells to present antigens to T cells. Therefore, BMDCs in which STAT5 was knocked down were cultured with naïve $CD4^+$ T cells at a ratio of 1:5, and the differentiation ability of T cells was assessed after 3 days (Fig. 5D). BMDCs can deliver signals to T cells upon MOG_{35-55} antigen stimulation, then naïve Th cells can be polarized to Th1 and Th17 subsets by BMDC-derived cytokines, namely IFN-γ, IL-17A, and GM-CSF. BMDCs with knocked down STAT5 cannot effectively communicate with T cells due to the diminished antigen-presenting ability, resulting in decreased numbers of IFN-γ^+CD4^+, IL-$17A^+CD4^+$, and especially GM-CSF^+CD4^+ T cells (Fig. 5E and F) and reduced secretion of the cytokine IFN-γ, IL-17A and GM-CSF (Fig. 5G). Together, these results show that GM-CSF-activated moDCs exhibit potent antigen presentation upon MOG_{35-55} stimulation and thereby promote the differentiation of Th1 and Th17 cells to participate in the destructive demyelination process, in which STAT5 plays a decisive role.

3.5. The JAK inhibition suppresses the maturation of moDCs

Given that GM-CSF-dependent pathogenesis of represent executors moDCs is characterized by central recruitment and antigen presentation through STAT5, the mechanism of inhibiting JAK against EAE may be related to inhibiting phosphorylation of STAT5 to weaken pathogenicity caused by excessive antigen presentation of moDCs. To evaluate the effect of JAK inhibition on moDCs maturation, monocytes isolated from the bone marrow of C57BL/6 were cultured in the presence of GM-CSF and IL-4 to obtain BMDCs. After 24 h of LPS and MOG_{35-55} stimulation, DCs gradually underwent apoptosis due to over-activation. JAK inhibition significantly suppressed the apoptosis state of DCs (Fig. 6A), which excluded abnormal DCs function caused by apoptosis. In addition, the JAK inhibitor Z526 concentration-dependently decreased the levels of IL-1β (Fig. 6B), IL-6 (Fig. 6C), and IL-12 (Fig. 6D), which are required for Th17 and Th1 differentiation in the culture supernatant of DCs, with the IC_{50} values of 2.91, 3.46, and 0.16 μmol/L, respectively. Mature DCs directly mediate the activation of T cells *via* the co-stimulatory molecules CD86, CD80, and MHCII. Our results demonstrated that after being stimulated with LPS or antigen MOG_{35-55}, Z526-treated $CD11c^+$ DCs expressed significantly lower levels of CD86 and MHCII than those in the untreated mDCs, and the effect depended on the Z526 concentration used (Fig. 6E). Together, these data suggest that the improvement of EAE by the JAK inhibitor is due to the inhibition of moDC maturation.

3.6. Suppression of the JAK2−STAT5 signaling reduces the antigen presentation of moDCs

Given the important role of STAT5 phosphorylation in the GM-CSF expression and antigen-presenting ability of DCs, we analyzed the role of JAK−STAT signaling in mature DCs. Our results revealed that the JAK inhibitor Z526 not only significantly inhibited the phosphorylation of the JAK2 and STAT5 after LPS and MOG_{35-55} co-stimulation (Fig. 7A−E) but also inhibited the GM-CSF-stimulated phosphorylation of JAK2 and STAT5 (Fig. 7F−H) more significant than other cytokines (Supporting Information Fig. S3A−S3F). Our data also showed that NLRP3 was highly abundant in the demyelinated regions of the spinal cord white matter in EAE mice (Fig. 7I). Activation of the NLRP3 inflammasome causes secretion of IL-1β, which is not only an active participant in pathogenic Th17 differentiation to induce GM-CSF secretion but also promotes CNS-invasion of $CCR2^{hi}$ monocytes through the GM-CSF signaling[27]. JAK inhibition significantly suppressed the NLRP3 activation while alleviating the inflammatory demyelination in EAE mice (Fig. 7I). This inhibition would greatly improve the pathological attack caused by IL-1β and the pathogenicity of the Th17 phenotype. Furthermore, in DCs activated by LPS and MOG_{35-55}, we also reconfirmed that Z526 could suppress the antigen-presenting ability by inhibiting the JAK2−STAT5 signaling, thereby suppressing the subsequent activation of the NLRP3 inflammasome and IL-1β secretion (Fig. 7A). In addition, we co-cultured Z526-treated DCs and naïve $CD4^+$ T cells and evaluated the differentiation ability of Th cells to confirm the role of JAK2−STAT5 in the antigen-presentation ability of DCs (Fig. 7J). Our results demonstrate that Z526-treated DCs could not present the antigen MOG_{35-55} to

$CD4^+$ T cells in the supernatant was measured by ELISA. All data are from three independent experiments. Data are presented as mean ± SEM. n.s., not significant. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

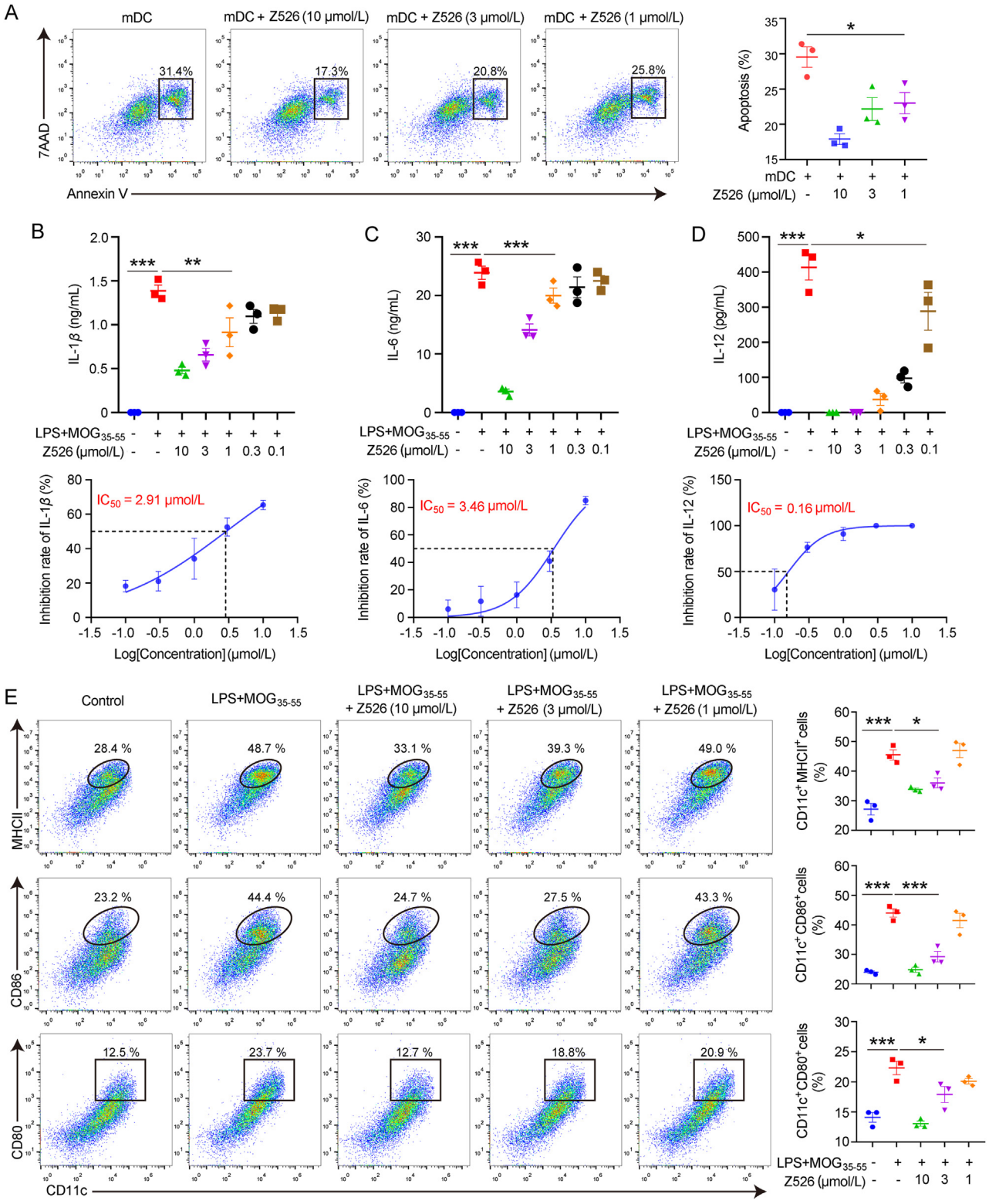

Figure 6 JAK inhibitor Z526 suppressed moDCs maturation and antigen presentation. (A) Annexin V and 7AAD were stained on DCs to evaluate the effect of JAK inhibitor Z526 on apoptosis by flow cytometry. IL-1β (B), IL-6 (C) and IL-12 (D) in mature DCs were determined by ELISA. (E) CD11c, CD86 and MHCII were labeled to determine the effect of Z526 in DCs maturation and antigen presentation. All data are from three independent experiments. Data are presented as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

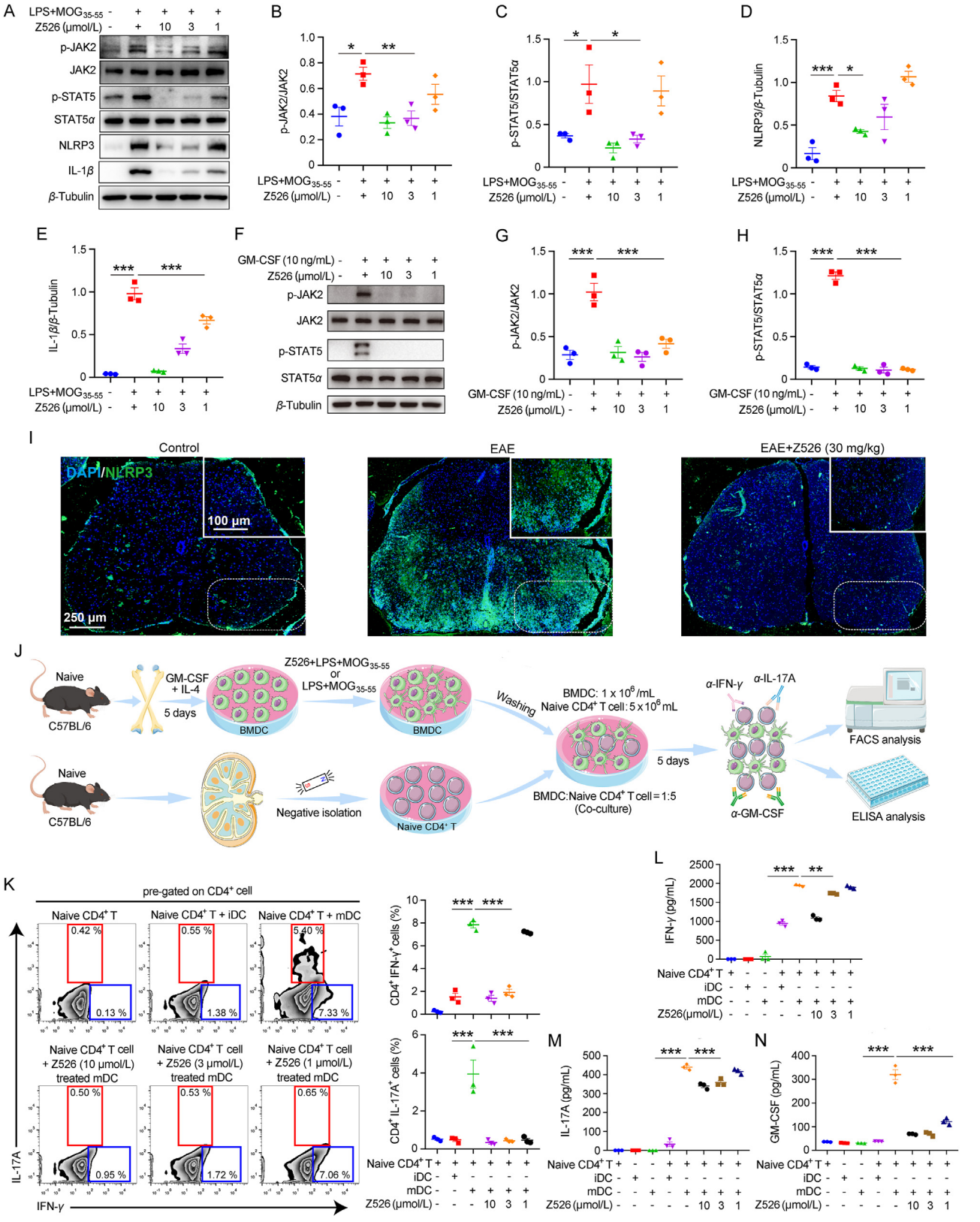

Figure 7 Failed antigen presentation of moDCs is related to decrease JAK2−STAT5 signaling. Z526 inhibited the expression of p-JAK2, p-STAT5, NLRP3 and IL-1β after stimulated by LPS and antigen MOG_{35-55} (A−E) and GM-CSF (F−H) in DCs from C57BL/6. (I) Spinal cord sections of EAE and Z526-treated EAE mice were stained by NLRP3. (J) Schematic for Z526-treated BMDCS co-cultured with naïve $CD4^+$ T cells. (K) The expression of IFN-γ and IL-17A in the co-culture system was analyzed by flow cytometry. The production of IFN-γ (L), IL-17A (M) and GM-CSF (N) by $CD4^+$ T cells in the supernatant was measured by ELISA. All data are from three independent experiments. Data are presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

$CD4^+$ Th cells and therefore exhibited failed Th1 and Th17 differentiation, as evidenced by reduced numbers of IFN-γ^+CD4^+, IL-$17A^+CD4^+$, and especially GM-CSF^+CD4^+ T cells (Fig. 7K), in addition to reduced levels of IFN-γ (Fig. 7L), IL-17A (Fig. 7M), and GM-CSF (Fig. 7N) in the supernatant of the co-cultivation system. Taken together, these results indicate that inhibition of the JAK2−STAT5 signaling suppressed the antigen-presenting ability of moDCs. As a result, these cells cannot present the antigen MOG_{35-55} to Th cells effectively, and the differentiation and cytokine secretion of the pathological Th cells are suppressed.

3.7. JAK inhibition prevents GM-CSF-induced CNS-invading inflammatory monocytes and moDCs in Th17-induced adoptive transfer EAE mice

We demonstrated that GM-CSF produced by Th17 cells not only coordinates the recruitment of monocytes and moDCs to the CNS, but also licenses them the ability to present antigens, and the JAK2−STAT5 signaling plays an indispensable role in this process. In addition, the JAK inhibitor Z526 had a significant effect on the differentiation of pathological Th17 cells by inhibiting the activation of JAK−STAT signaling pathways induced by various cytokines. To further elaborate the relationship among monocytes, moDCs, and Th17 cells during the alleviation of EAE by Z526, we constructed a mouse model of EAE induced *via* adoptive Th17 cell transfer. Briefly, C57BL/6 mice were immunized with the MOG_{35-55} peptide for 10 days. Then, their lymph nodes were isolated and lymphocytes were cultured with the MOG_{35-55} peptide under Th17 differentiation conditions in the presence or absence of Z526. Then, the treated cells were intravenously injected into recipients (Fig. 8A). Notably, the mice subjected to the Th17-induced adoptive transfer began to show signs of paralysis on Day 8, and those subjected to the Z526-treated-Th17-induced adoptive transfer showed delayed onset of EAE and significantly reduced clinical scores (Fig. 8B). We also found that after Z526 treatment, the peripheral serum of mice with EAE showed significantly reduced levels of the typical Th17 cytokines GM-CSF and IL-17A (Fig. 8C). In addition, the inflammatory demyelination in the white-matter region of the spinal cord due to infiltration of immune cells, such as $CD4^+$ T cells, was also ameliorated (Fig. 8D). As we described previously, without the effect of the GM-CSF secreted by Th17 cells, myeloid cells, especially $Ly6C^{hi}$ monocytes, and moDCs, lost their ability of CNS infiltration and inflammatory attack. Thus, Z526 treatment significantly reduced the infiltration of $CD45^+$ myeloid cells (Fig. 8E), especially $Ly6C^{hi}$ monocytes ($CD45^+CD11b^+Ly6C^{hi}$) (Fig. 8F) and their progeny inflammatory moDCs ($CD45^+CD11b^+$ $CD11c^+Ly6C^{hi}MHCII^+$) (Fig. 8G), compared with the infiltration levels in EAE induced *via* adoptive transfer of Th17-cells. Together, our results indicate that Z526 suppressed Th17 differentiation and GM-CSF production by inhibiting the JAK2−STAT5 signaling, thereby decreasing the JAK2−STAT5-mediated antigen-presenting capacity of moDCs and CNS recruitment of monocytes and moDCs and ultimately alleviating the pathological demyelination in EAE.

4. Discussion

MS is the prototypical inflammatory disease with immune damage in the CNS. Differentiated autoreactive $CD4^+$ T cells cause CNS inflammation by priming a phenotypic transformation of myeloid cells *via* the secretion of GM-CSF. Importantly, as a pro-inflammatory growth factor, GM-CSF binds to type I cytokine receptors to activate the JAK2−STAT5 pathway, consequently exerting its effects on the generation, survival, and activation of myeloid cells[28]. It is secreted by pathogenic T cells, and numerous studies have shown its importance in driving autoimmunity. GM-CSF initiates the infiltration of inflammatory monocytes and progeny into the CNS during EAE. However, the mechanism whereby the JAK−STAT signaling participates in MS pathogenesis and the therapeutic feasibility of JAK inhibitors remain unknown. Our study hints that the isoforms of JAK1/2/3 and downstream molecule STAT1/3/5/6 were involved in the pathological functional alterations of immune cells in EAE mice. In addition, the therapeutic effect of the pan-JAK inhibitor Z526 for EAE is superior to selective JAK1/2 inhibitor baricitinib and selective JAK3 inhibitor Z583. It suggests that multiple JAK subtypes may be involved in the autoimmune response of central demyelination in the EAE mice. Among them, the JAK2−STAT5 pathway is especially contributed to the development of EAE by relaying the GM-CSF signaling in CNS-infiltrating myeloid cells to exert inflammatory effects and that JAK inhibition markedly attenuates inflammatory signature of myeloid cells.

Myelin-reactive T cells, such as Th1 and Th17 lymphocytes, are generally considered the major drivers of pathogenesis in both MS and EAE[29]. In contrast, the JAK−STAT signaling pathway plays an important role in the autoimmune response to MS by participating in the differentiation of $CD4^+$ Th cells, such as the differentiation of Th1 by JAK1/2/3−STAT4 and Th17 by JAK1/2/3−STAT3 axis. Activated Th1 and Th17 cells migrate into the CNS and produce the pathogenic cytokines IL-17, IFN-γ, and GM-CSF to communicate with other immune cells, thereby causing inflammatory autoimmune demyelinating disease[30,31]. Various cytokines, including IL-6, IL-9, IL-17, IL-22, IL-23, and GM-CSF, are involved in the activation, differentiation, and migration of autoreactive T cells in MS and EAE pathogenesis[32]. The JAK−STAT signaling is an evolutionarily conserved pathway activated by a large number of cytokines, growth factors, and interferons to regulate cell growth and differentiation[33]. JAK is a key molecule in the transmission of cytokine signals to the cell nucleus for transcriptional induction and is involved in the cytokine-induced activation of T cells during MS pathogenesis. There is limited information about the mechanism whereby the JAK−STAT pathway mediates T-cell activation and about the therapeutic effect of JAK inhibition on EAE. We demonstrated that JAK inhibition by Z526 significantly attenuated the development of EAE. We observed that this JAK inhibitor significantly suppressed Th1 and Th17 differentiation in lymph nodes and reduced the number of CNS-infiltrated $CD4^+$ T cells in EAE mice. These observations supported the notion that inhibition of the formation of pathological Th cells contributed to the alleviation of neuroinflammatory demyelination[34]. Therefore, JAK may serve as a valuable target for the treatment of MS through inhibition of Th cell differentiation. As a cytokine-dependent signaling pathway, the JAK−STAT signaling plays an essential role in the signaling of numerous cytokines that have been implicated in the differentiation of Th phenotypes. Differentiated Th cells are major drivers of both MS and EAE pathogeneses and secrete typical pro-inflammatory cytokines, such as IFN-γ, IL-17A, and GM-CSF, which together participate in the proliferation and differentiation of Th cells. We demonstrated that JAK inhibition not only significantly attenuates CD3/CD28-stimulated secretion of pro-inflammatory cytokines and cell

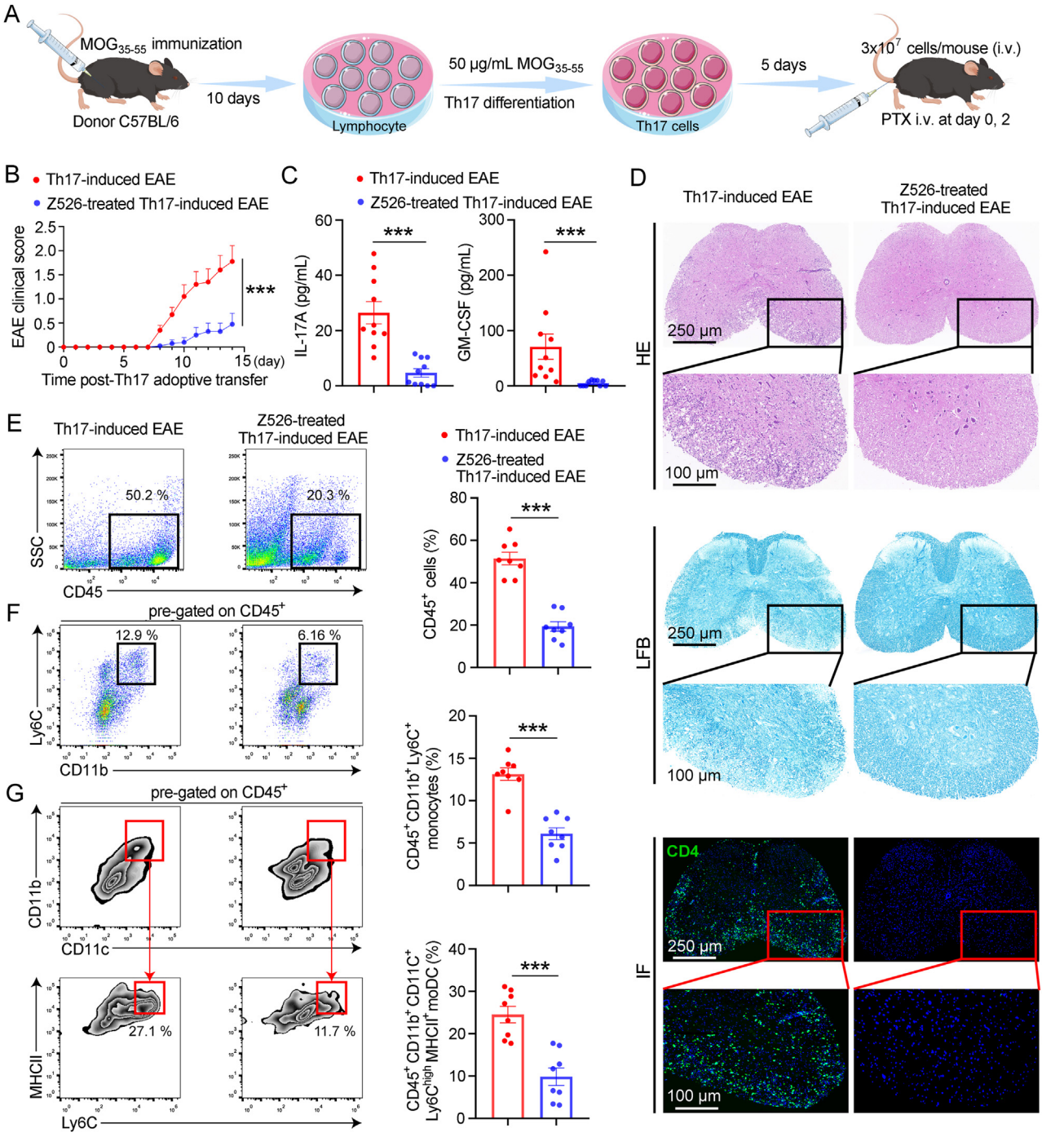

Figure 8 Effect of JAK inhibition on Th17-induced adoptive transfer EAE. (A) Schematic for evaluating the effect of Z526 on Th17-induced adoptive transfer. Briefly, C57BL/6 mice were immunized with MOG_{35-55} peptide (300 μg) for 10 days, lymph node cells were isolated and then MOG_{35-55}-specific T cells were cultured with MOG_{35-55} peptide (25 μg/mL) under Th17 differentiation conditions in the presence or absence of Z526 (10 μmol/L) for 5 days. T cells (30×10^6) were intravenously injected into C57BL/6 mice ($n = 10$). (B) Classical EAE clinical score were recorded every day after injection. (C) Cytokines IL-17A and GM-CSF in Th17 cells and Z526-treated Th17 cells induced adoptive transfer EAE serum were determined by ELISA method. (D) Spinal cord sections were stained by LFB, HE, MBP and CD4. Mononuclear cells from CNS in Th17 cells and Z526-treated Th17 cells induced adoptive transfer EAE were stained with an array of different flow antibodies to identify inflammatory myeloid cells ($CD45^+$) (E), including $Ly6C^{hi}$ monocytes ($CD45^+CD11b^+Ly6C^{hi}$) (F) and moDCs ($CD45^+CD11b^+CD11c^+Ly6C^{hi}MHCII^+$) (G) ($n = 8$ per group). Data are presented as mean ± SEM. ***$P < 0.001$.

proliferation in $CD4^+$ T cells but also inhibits Th1 and Th17 differentiation. The suppressive effect of inhibiting the JAK−STAT signaling on Th-cell differentiation underlies the observed therapeutic effect.

Although pathogenic Th cells play a key role in driving EAE, Th cells are not the major executors of the resulting tissue destruction in the CNS. There is no direct evidence that CNS-infiltrated T lymphocytes themselves can kill myelin-producing

oligodendrocytes or transect axons alive[35]. Activated and inflammatory myeloid cells, such as monocytes and progeny moDCs, are highly abundant in the inflamed CNS tissue in MS and are the major executors priming the demyelinating damage[35]. Pathological T cells and the cytokines they secrete may direct myeloid cells to invade the CNS, where myeloid cells constitute the effector cells that cause demyelination. Our study demonstrated the abnormal abundance of myeloid cells in three major pathological sites in mice with EAE—peripheral blood, lymph nodes, and the CNS—and the cell types affected by JAK inhibition. Our data showed that monocytes, especially inflammatory CCR2-dependent Ly6C^{hi} monocytes, and progeny moDCs were highly abundant in mice with EAE. JAK inhibition by Z526 significantly reduced the proportion of monocytes and moDCs in the peripheral blood. Studies have also established a clear role for the chemokine MCP-1 and its cell-surface receptor CCR2 in trafficking monocytes to the CNS. During the acute progressive phase of EAE, activated pathogenic T cells must secondarily recruit leukocytes to the CNS to perpetuate the inflammation and cause tissue damage, and this process requires the CCR2 signaling[36]. Our data demonstrated that upon JAK inhibition by Z526, the CCR2 expression on the surface of monocytes is reduced, which presumably explains the significant reduction in the numbers of CNS-infiltrating monocytes and inflammatory moDC. However, how effecter Th cells communicate with myeloid cells remains elusive.

Activated autoreactive CD4$^+$ T cells recruit myeloid cells into the CNS and license them with an inflammatory phenotype, thereby playing a central role in EAE development, progression, and remission. Of note, activated Th cells communicate with monocytes and moDCs *via* GM-CSF. GM-CSF is the only known cytokine that is produced by pathogenic Th cells and required for the progression of EAE. The ability to respond to GM-CSF with CNS tissue damage implicates monocytes and moDCs as the primary targets for the GM-CSF derived from pathogenic Th cells[37,38]. Given the crucial role of GM-CSF in the communication between auto-reactive Th cells and myeloid cells, it is considered a key cytokine underlying the demyelinating injury in the CNS during EAE. JAK−STAT pathway is an evolutionarily conserved signaling employed by more than 50 cytokines[22]. As a pro-inflammatory growth factor, GM-CSF relays on JAK2 and a combination of STAT5 licensing myeloid cells for tissue damage. We demonstrated that JAK inhibition suppressed the secretion of GM-CSF by Th cells, consequently reducing the tissue damage caused by myeloid cells *in vivo* and *in vitro*. Our data suggested that the JAK2−STAT5 pathway regulates the production of GM-CSF by pathogenic Th cells and GM-CSF signaling to monocytes and moDCs. In addition to driving the inflammatory signature of CCR2$^+$ Ly6C^{hi} monocytes/moDCs and licensing CNS infiltration,[39] another important role of GM-CSF is to confer moDCs with a pathological phenotype of cytokine secretion and possible antigen presentation[40]. Therefore, to investigate whether GM-CSF-activation of the JAK−STAT signaling can account for the altered antigen-presenting ability of moDCs, we profiled the inhibitory type of JAK−STAT in BMDCs. We found that JAK suppression significantly prevented the phosphorylation of STAT5, which is downstream of JAK2, induced by GM-CSF. Our results further detailed that BMDCs failed to respond to the antigen MOG_{35-55} after knocking down *STAT5*, which directly affected the maturation of BMDCs by inhibiting the expression of the costimulatory molecules CD86 and MHCII while reducing the production of APC-derived Th-polarizing factors IL-6 and IL-12.

STAT5 is a transcription factor crucial for the formation of pathogenic cells, and STAT5 phosphorylation is vital for the expression of GM-CSF[41]. To further clarify the regulatory role of JAK2−STAT5 in EAE, naïve CD4$^+$ T cells were co-cultured with *STAT5*-knocked-down BMDCs to confirm that these two cell types communicate with each other in EAE. The data revealed that T-cell differentiation to pathological Th1 and Th17 failed due to suppressed antigen presentation and cytokine secretion of BDMCs. Thus, STAT5 is responsible for the antigen-presenting ability of BMDCs. In our experiment, JAK inhibition exhibited an outstanding limiting effect on the antigen presentation and cytokine secretion of BMDCs, which in turn failed to induce the proliferation of pathogenic Th cells producing GM-CSF. The lack of antigen presentation by BDMCs due to JAK2−STAT5 inhibition significantly impaired the differentiation of CD4$^+$ T cells to the Th1 and Th17 phenotypes. These results imply that the efficacy of JAK inhibition on EAE was implicated in the antigen presentation of myeloid cells and differentiation of T lymphocytes. Circulating inflammatory Ly6C^{hi} monocytes contribute to EAE encephalitogenic pathology by infiltrating the inflamed CNS. Ly6C^{hi} monocytes also can acquire antigen-presenting function and the DC signature to exert proinflammatory tasks during the effector phase of EAE[42,43]. These cells have been directly linked to the demyelination progress. Our data showed that JAK inhibitor-treated Th17 cell adoptive transfer-induced EAE mice had less GM-CSF production and markedly reduced demyelinating lesions and inflammatory cell infiltration. Therefore, the numbers of CNS-invaded myeloid cells, especially Ly6C^{hi} monocytes, and moDCs, were significantly reduced due to the suppressed recruitment of the pathological Th17 cells, secreting GM-CSF. Consequently, it is suggested that inhibition of the JAK−STAT signaling can provide a therapeutic environment in EAE by reducing the GM-CSF level and thereby suppressing the tissue-destructive capabilities of monocyte-derived inflammatory-cell infiltrates.

5. Conclusions

By using EAE mice, this study explored the involvement of the JAK−STAT pathway in the progression of EAE and whether JAK can be a viable therapeutic target. GM-CSF plays a crucial role in demyelinating damage in MS *via* driving CNS-invading inflammatory monocytes and progeny moDCs and licensing antigen-presenting signature. The JAK2−STAT5 pathway is required for the intracellular transmission of the GM-CSF signaling. Here, our study showed that JAK inhibition significantly ameliorates inflammatory demyelination in mice with EAE. We demonstrated that inhibition of the JAK−STAT signaling suppresses the secretion of GM-CSF derived from pathogenic Th17 cells and prevents the CNS-infiltration of inflammatory monocytes and moDCs and their antigen presentation. These findings suggest that blocking the JAK−STAT signaling can be a successful therapeutic strategy in MS, and JAK is a viable target to this end.

Acknowledgments

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Author contributions

Shuai Shao, Lan Sun, and Tiantai Zhang designed the study. Shuai Shao, Chenjian Chen, Gaona Shi, Yu Zhou, Yazi Wei and Lei Wu performed the experiments, analyzed the data, and prepared the figures and tables. Shuai Shao and Chenjuan Chen contributed to the animal studies. Shuai Shao, Lan Sun, and Tiantai Zhang wrote the paper. Lan Sun and Tiantai Zhang performed supervision, project administration, and funding acquisition. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2023.07.026.

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