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Midkine inhibits inducible regulatory T cell differentiation by suppressing the development of tolerogenic dendritic cells

Yoshifumi Sonobe,* Hua Li,* Shijie Jin,* Satoshi Kishida,† Kenji Kadomatsu,† Hideyuki Takeuchi,* Tetsuya Mizuno,* and Akio Suzumura*

Midkine (MK), a heparin-binding growth factor, reportedly contributes to inflammatory diseases, including Crohn’s disease and rheumatoid arthritis. We previously showed that MK aggravates experimental autoimmune encephalomyelitis (EAE) by decreasing regulatory CD4+CD25+Foxp3+ T cells (Tregs), a population that regulates the development of autoimmune responses, although the precise mechanism remains uncertain. In this article, we show that MK produced in inflammatory conditions suppresses the development of tolerogenic dendritic cells (DCregs), which drive the development of inducible Treg. MK suppressed DCreg-mediated expansion of the CD4+CD25+Foxp3+ Treg population. DCregs expressed significantly higher levels of CD45RB and produced significantly less IL-12 compared with conventional dendritic cells. However, MK downregulated CD45RB expression and induced IL-12 production by reducing phosphorylated STAT3 levels via src homology region 2 domain-containing phosphatase-2 in DCreg. Inhibiting MK activity with anti-MK RNA aptamers, which bind to the targeted protein to suppress the function of the protein, increased the numbers of CD11clowCD45RB+ dendritic cells and Tregs in the draining lymph nodes and suppressed the severity of EAE, an animal model of multiple sclerosis. Our results also demonstrated that MK was produced by inflammatory cells, in particular, CD4+ T cells under inflammatory conditions. Taken together, these results suggest that MK aggravates EAE by suppressing DCreg development, thereby impairing the Treg population. Thus, MK is a promising therapeutic target for various autoimmune diseases. The Journal of Immunology, 2012, 188: 2602–2611.

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Abbreviations used in this article: CD, Crohn’s disease; DC, dendritic cell; DCcon, conventional dendritic cell; DCreg, regulatory T cell-inducing tolerogenic dendritic cell; dLN, draining lymph node; EAE, experimental autoimmune encephalomyelitis; iTreg, inducible regulatory T cell; KO, knockout; MK, midkine; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PD-L1, programmed cell death ligand 1; pSTAT, phosphorylated STAT; RA, rheumatoid arthritis; rm, recombinant murine; SHP, src homology region 2 domain-containing phosphatase; Treg, regulatory T cell; WT, wild-type.

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results suggest that MK produced by CD4+ T cells aggravates EAE by suppressing DCreg development and subsequent iTreg differentiation. Thus, expanding the iTreg population by inhibiting MK activity and DCreg proliferation may provide a useful strategy for treating autoimmune diseases, including MS.

Materials and Methods

Animals

Female C57BL/6 (H2b) and BALB/c (H2d) mice were obtained from Japan SLC. MK-knockout (KO) mice (C57BL/6) were generated as described previously (19). Mice were bred in the animal facility of the Research Institute of Environmental Medicine at Nagoya University. The protocols for animal experiments were approved by the Animal Experiment Committee of Nagoya University.

Generation of DCs

DCs were generated as previously described (20). In brief, immature conventional DCs (DCcons) were generated by incubating bone marrow cells in complete DMEM containing 10% FBS, 2 mM l-glutamine, and 20 ng/ml GM-CSF (R&D Systems) for 6 d, with or without 100 ng/ml recombinant MK. Immature DCregs were generated with 20 ng/ml GM-CSF, 20 ng/ml recombinant murine (rm)IL-10, and 20 ng/ml recombinant human TGF-β1, with or without 100 ng/ml recombinant MK. Immature DCcons or DCregs were then stimulated to become mature DCs with 100 ng/ml LPS for 24 h.

Flow cytometry

Cells were resuspended in FACS buffer (PBS supplemented with 2% FBS and 0.01% sodium azide). Nonspecific staining was blocked using rat anti-FcR Abs (BD Biosciences) for 30 min at 4˚C, after which the cells were stained with FITC–anti-CD11c (HL-3), PE–anti-CD45RB (16A), and PE–anti-CD86 (GL-1) Abs from BD Biosciences, as well as PE–anti-MHC class I (AF6-88.5), PE–anti-MHC class II (M5/114.15.2), PE–anti–CD80 (16-10A1), and PE–anti–CD86 (GL-1) Abs from BD Biosciences, as well as PE–anti-programmed cell death ligand 1 (PD-L1; MIH5) Abs from eBioscience. To detect Tregs, cells were incubated with PerCP–anti-CD4 Abs, cells were stained with FITC–anti-IFN-γ (XMG1.2; BD Biosciences) and PE–anti-IL-17 (TC11-18H10; BD Biosciences) Abs. The samples were examined with a Cytomics FC500 system (Beckman Coulter) and analyzed with CXP Software Ver. 2.0 (Beckman Coulter).

Suppression assay

CD4+CD25+ T cells were isolated from spleens of BALB/c mice using a MACS system (Miltenyi Biotec). CD4+CD25+ T cells (H2b) were cocultured for 4 d with DCcons, MK-treated DCcons, DCregs, or MK-treated DCregs from C57BL/6 mice (H2b) at 1:1 ratios in the presence or absence of rIL-12p70, anti–IL-12p40 Abs (C17.8; BD Biosciences), or SHP-2 inhibitor (NSC87877; Calbiochem). The percentage of CD4+CD25+Foxp3+ Tregs was determined by flow cytometry. The levels of IFN-γ in the culture supernatant were measured by ELISA.

Adaptive transfer of DCs

Immature DCcons or DCregs treated or not with MK were pulsed for 24 h with 20 μg/ml myelin oligodendrocyte glycoprotein (MOG)35–55 and 100 ng/ml LPS and were transferred i.v. into wild-type (WT) mice (5×105 cells/mouse). One day later, EAE was induced as described below.

EAE

MOG-induced EAE was induced as described previously (19). In brief, mice were injected s.c. with 0.2 ml emulsion containing 200 μg MOG35–55 in PBS combined with an equal volume of CFA containing 300 μg heat-killed Mycobacterium tuberculosis H37Ra. Mice were injected i.p. with pertussis toxin on the day of immunization and 2 d after immunization (200 ng/mouse). Mice were scored daily according to the following

![FIGURE 1. MK suppresses DCreg development. (A and B) Allogeneic CD4+CD25+ T cells from BALB/c mice were cocultured with each subset of DCs from C57BL/6 mice for 4 d. Percentages of CD4+CD25+Foxp3+ Tregs were assessed by flow cytometry (n = 6). *p < 0.05, **p < 0.001. (C) Treg function was evaluated using proliferation assays in which CD4+CD25- T cells and MACS-sorted CD4+ T cells expanded by DCcons, MK-treated DCcons, DCregs, or MK-treated DCregs in MLR were cultured in the presence of 5 μg/ml plate-bound anti-CD3 (145-2C11; BD Biosciences) and 2 μg/ml soluble anti-CD28 (37.51; BD Biosciences) Abs for 3 d at the indicated ratio. BrdU was added for the final 16 h, and proliferation was evaluated using a BrdU cell-proliferation assay kit (Calbiochem). Results obtained with CD4+CD25- T cell were set to 1 (n = 3).](http://www.jimmunol.org/content/jimmunol/2603/1/F1.jpg)
scale: 0, normal; 1, limp tail or mild hind limb weakness; 2, moderate hind limb weakness or mild ataxia; 3, moderate to severe hind limb weakness; 4, severe hind limb weakness, mild forelimb weakness, or moderate ataxia; 5, paraplegia with moderate forelimb weakness; and 6, paraplegia with severe forelimb weakness, severe ataxia, or moribundity.

**RNA aptamers**

Anti-MK RNA aptamers were produced by Ribomic, essentially as described previously (21). In brief, RNA aptamers were raised against MK using systematic evolution of ligands by exponential enrichment (SELEX). The functionally optimized 49-mer RNA molecule was modified with fluorine and O-methyl at the 2$^\text{'}$ position of each ribose and with a cholesterol moiety and inverted dT tags at the 5$^\text{'}$ and 3$^\text{'}$ ends, respectively. Anti-MK RNA aptamers were administered to EAE mice at a dose of 15 mg/kg every other day from the day of immunization.

**Histological analysis**

EAE mice were intracardially perfused with PBS at 28 d after immunization; spinal cords were fixed with 4% paraformaldehyde and then embedded in paraffin. Ten-micrometer-thick sections were stained with H&E, as described previously (19). Stained sections were observed under a microscope. Inflammatory foci were identified as perivascular clusters containing $\geq$20 mononuclear cells.

**Isolation of CNS mononuclear cells**

CNS mononuclear cells were prepared from the brains and spinal cords of EAE mice intracardially perfused with PBS from the left ventricle 28 d after immunization (22). The tissues were then passed through a nylon mesh (NB 60; NBC). The CNS-infiltrating mononuclear cells were enriched using a 30% Percoll gradient.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** MK induces IL-12 production in DCregs. Each subset of DCs was expanded, as described in Materials and Methods. After treatment with LPS (100 ng/ml) for 24 h, levels of surface molecules and cytokines were assessed. (A and B) Expression of surface molecules in each DC subset was assessed using flow cytometry. Representative data are shown in (A), whereas average results are shown in (B) ($n = 3$). (C) The levels of IL-12, IL-6, TGF-β, IL-10, and IL-27 in culture supernatant containing each DC subset were analyzed using ELISAs. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. 

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Cytokine ELISA
ELISA kits specific for mouse IL-12p70, IL-6, IL-10, and IFN-γ were obtained from BD Biosciences. An ELISA kit specific for mouse TGF-β was purchased from R&D Systems, and kits specific for IL-23 and IL-27 were obtained from eBioscience.

Western blotting
Cells were lysed in TNE buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, and 0.1% SDS) with a protease inhibitor mixture (Roche) and a phosphatase inhibitor mixture (Sigma). Thirty micrograms of protein from total cell lysates was examined on Western blots, as previously described (23), using anti–SHP-2 (polyclonal; Cell Signaling), anti-pSTAT3 (D3A7; Cell Signaling), anti-STAT3 (84; BD Biosciences), anti-MK (H-65; Santa Cruz Biotechnology), and anti–α-tubulin (DM1A; Sigma) Abs.

Quantitative RT-PCR
RNA was extracted with an RNeasy Mini kit (Qiagen) and reverse transcribed with oligo(dT)12–18 (Invitrogen), according to the manufacturer’s protocol. CD4+ T cells were stimulated with rmIL-12 (10 ng/ml; R&D Systems), rmIFN-γ (5 ng/ml; R&D Systems), and anti–IL-4 Abs (11B.11; 10 μg/ml; BD Biosciences) for Th1 cells; mouse IL-4 (2 ng/ml; R&D Systems) and anti–IFN-γ Abs (XMG1.2; 10 μg/ml; BD Biosciences) for Th2 cells; human TGF-β1 (5 ng/ml), IL-6 (30 ng/ml), and anti–IFN-γ Abs for Th17 cells; human TGF-β1 (5 ng/ml) for iTregs; and IL-4 (2 ng/ml) and human TGF-β1 (5 ng/ml) for Th9 cells.

Statistical analysis
Differences between the mean values were analyzed using the two-tailed Student t test, with Welch’s correction. The p values < 0.05 were considered significant.

Results
MK suppresses Treg differentiation by inhibiting DCreg development
We analyzed the effects of MK on DCreg development in the presence of TGF-β and IL-10. Allogeneic responses of BALB/c-derived CD4+CD25− T cells with C57BL/6-derived DCregs induced CD4+CD25+Foxp3+ Treg differentiation (Fig. 1A, 1B). However, MK suppressed DCreg-mediated differentiation of CD4+CD25+Foxp3+ Tregs (Fig. 1A, 1B). The levels of MK were negligible in the culture supernatant (Supplemental Fig. 1). To examine the suppressor activity of these in vitro-induced CD4+ T cells, CD4+CD25+effector T cells were stimulated with anti-CD3 and anti-CD28 Abs in the presence of CD4+ T cells expanded by each subset of DCs. CD4+ T cells that developed in response to DCregs suppressed anti-CD3 Ab- and anti-CD28 Ab-induced proliferation of the DC4+CD25− effector T cells (Fig. 1C). However, MK-stimulated DCregs did not suppress proliferation of effector T cells (Fig. 1C). Taken together, these results suggest that MK suppresses expansion of the DCreg population.

MK suppresses DCreg-mediated iTreg differentiation by inducing IL-12 production
We next analyzed molecules related to Ag presentation, including MHCs, B7 family molecules, and cytokines, in each DC subset using flow cytometry and ELISA. B7-1 (CD80) and B7-2 (CD86) expression levels in DCregs were significantly lower than
in DCcons (Fig. 2A, 2B). Expression levels of MHC class I and II, B7-1 (CD80), B7-2 (CD86), and B7-H1 (PD-L1) were similar with and without MK treatment (Fig. 2A, 2B), showing that MK did not affect the expression of molecules related to Ag presentation. However, DCregs expressed significantly higher levels of CD45RB, and addition of MK decreased the expression (Fig. 2A, 2B). In addition, levels of the inhibitory cytokines IL-10 and TGF-β, as well as the type 1 regulatory T cell-inducing cytokine IL-27, in the culture supernatant were similar for the various DC subsets (Fig. 2C). In contrast, DCregs produced significantly less IL-6 and IL-12 compared with DCcons. IL-23 concentrations were less than the detection threshold in the culture supernatant (data not shown). Notably, MK significantly increased IL-12 production in DCregs, whereas IL-6 and IL-23 levels were not affected (Fig. 2C). These results suggest that MK-stimulated DCregs do not cause naive CD4+ T cells to differentiate into iTregs because of IL-12 production in the DCregs. To address this hypothesis, we examined the effects of IL-12 produced by MK-treated DCregs on iTreg differentiation using rIL-12– and anti–IL-12–neutralizing Abs. rIL-12 downregulated DCreg-mediated iTreg differentiation during the allogeneic responses (Fig. 3). Furthermore, MK-mediated suppression of iTreg differentiation by DCregs was ameliorated by anti–IL-12–neutralizing Abs (Fig. 3). Although the addition of anti–IL-12–neutralizing Abs upregulated the percentage of CD4+ CD25+ Foxp3+ Tregs in MLR using DCcons, it was not significantly different compared with isotype control. However, MK-treated DCregs did not induce the differentiation of IFN-γ-producing Th1 cells (Fig. 4). Together, these results suggest that MK suppressed DCreg-induced iTreg differentiation by inducing IL-12 production.

MK suppresses iTreg differentiation by upregulating SHP-2 expression and inducing IL-12 production

Because we previously showed that MK induced SHP-2 expression in CD4+ T cells (19), we next assessed SHP-2 expression in bone marrow cells by Western blotting. MK tripled the expression of SHP-2 in bone marrow cells (Fig. 5A). Moreover, IL-10–induced STAT3α phosphorylation was inhibited by MK, an effect that was reversed by the SHP-2 inhibitor NSC87877 (Fig. 5B). This SHP-2 inhibitor also inhibited IL-12 production by MK-stimulated DCregs (Fig. 5C), as well as CD4+CD25+Foxp3+ Treg differentiation from CD4+CD25− T cells induced by MK-treated DCregs in allogeneic responses (Fig. 5D, 5E). Taken together, these results suggest that MK suppresses expansion of the DCreg population by downregulating pSTAT3 levels via SHP-2.

MK-treated DCregs do not suppress autoimmune inflammation in vivo

To determine whether MK-induced suppression of DCreg development impairs CD4+CD25+Foxp3+ Treg differentiation in vivo, C57BL/6 mice were injected with one of four DC subsets (DCcons, DCregs, MK-stimulated DCcons, and MK-stimulated DCregs), and EAE was induced. Injecting DCregs significantly suppressed the development of EAE, whereas the disease was significantly more severe in mice injected with MK-stimulated DCregs compared with mice injected with untreated DCregs.

**FIGURE 5.** MK induces IL-12 production in DCregs by upregulating SHP-2 levels. (A) Bone marrow cells were cultured with MK for 6 d. Levels of SHP-2 in whole-cell lysates were analyzed by Western blotting. (B) Levels of pSTAT3 in cells cultured for 6 d with the indicated cytokines and SHP-2 inhibitor (SHP2I). (C) Levels of IL-12 in supernatant containing DCcons, DCregs, or MK-treated DCregs in the presence or absence of SHP2I were analyzed by ELISA. (D and E) Percentages of CD4-gated CD25+Foxp3+ cells in allogeneic responses induced by DCcons, DCregs, or MK-treated DCregs, in the presence or absence of SHP2I, were assessed by flow cytometry. *p < 0.05.
(Fig. 6A). Injection of DCregs reduced the infiltration of cells in the spinal cord at 28 d after immunization, whereas inflammatory foci were increased in meninges of mice that received MK-stimulated DCregs compared with mice injected with untreated DCregs (Fig. 6B). The percentage and number of CD4\(^+\)CD25\(^+\) Foxp3\(^+\) Tregs among the cells obtained from draining lymph nodes (dLNs) of EAE mice injected with DCregs were significantly higher than in any of the other mice with EAE (Fig. 6C). Similar changes were not observed in the CNS, however (Fig. 6C, Supplemental Fig. 2). Accordingly, the percentage and number of IL-17- and IFN-\(\gamma\)-producing CD4\(^+\) T cells obtained from the CNS of EAE mice injected with DCregs were significantly lower than data obtained from mice with EAE injected with MK-treated DCregs, whereas the groups showed similar IL-17- and IFN-\(\gamma\)-producing CD4\(^+\) T cell populations from the dLNs (Supplemental Fig. 3A, 3B). These results suggest that MK inhibits the ability of DCregs to induce Treg differentiation in vivo.

**MK suppresses expansion of the DCreg population in vivo**

We then examined the number of CD11clowCD45RB\(^+\) DCregs in MK-KO mice and EAE mice treated with anti-MK RNA aptamers, which bind to MK to suppress the function of the protein (19). Anti-MK RNA aptamers reduced the clinical EAE scores (Fig. 7A) and infiltration of cells in the spinal cord 28 d after immunization (Fig. 7B). The percentages and numbers of CD11clow CD45RB\(^+\) DCregs and CD4\(^+\)CD25\(^+\)Foxp3\(^+\) Tregs in dLNs of mice treated with anti-MK RNA aptamers were higher than in PBS-treated mice (Fig. 7C, 7D). Reduced levels of IL-12p40 mRNA expression, but not IL-12p35 or IL-23p19 mRNA expression, were also noted in dLN DCs (Fig. 7E). In contrast, significantly fewer IFN-\(\gamma\)- and IL-17-producing CD4\(^+\) T cells had infiltrated into the CNS in mice treated with anti-MK RNA aptamers compared with PBS-treated mice (Fig. 7F).

MK-KO mice showed significantly milder EAE symptoms compared with WT mice (Supplemental Fig. 4A). The percentages of CD11clowCD45RB\(^+\) DCregs and CD4\(^+\)CD25\(^+\) Foxp3\(^+\) Tregs in dLNs of MK-KO mice were higher than in WT mice (Supplemental Fig. 4B, 4C). In contrast, fewer IFN-\(\gamma\)- or IL-17-producing CD4\(^+\) T cells infiltrated into the CNS of MK-KO mice compared with WT mice (Supplemental Fig. 4D). Taken together, these results support MK as a suppressor of DCreg expansion in mice with EAE.

**MK is produced by CD4\(^+\) cells in vitro and in vivo**

We next attempted to identify the cellular source of MK. Expression levels of MK mRNA and MK protein were significantly elevated in the dLNs of EAE mice compared with naive mice (Fig. 8A, 8B). MK mRNA expression was induced in CD11b\(^+\), CD4\(^+\), and CD8\(^+\) cells from the dLNs of EAE mice (Fig. 8A). MK protein expression was also induced in CD4\(^+\) T cells from the dLNs of EAE mice (Fig. 8C). Because CD4\(^+\) T cells play a critical role in the development of EAE, we focused on MK expression in CD4\(^+\) T cells in vitro. MK mRNA and MK protein expression were significantly upregulated in CD4\(^+\) T cells stimulated with anti-CD3 and anti-CD28 Abs or Con A (Fig. 8D, 8E). In addition, CD4\(^+\)CD62L\(^+\) naive T cells showed markedly increased MK mRNA levels in response to anti-CD3 and anti-CD28 Abs (Fig. 8F). Interestingly, Th1 cells, which were induced in the presence

**FIGURE 6.** MK inhibits the suppressive functions of DCregs in EAE. (A) Clinical EAE scores in mice that underwent adoptive transfer of each DC subset (n = 6). (B) H&E staining of lumbar spinal cords from EAE mice that underwent adoptive transfer of each DC subset. Arrows show inflammatory foci. Boxed area: original magnification ×20. Scale bars, 200 \(\mu\)m. (C) Number of CD4\(^+\)CD25\(^+\)Foxp3\(^+\) cells in the CNS and dLN harvested 28 d after immunization. Cells were counted by multiplying the percentage of CD4\(^+\)CD25\(^+\)Foxp3\(^+\) cells by the absolute number of cells obtained from the CNS or dLN. \(*p < 0.05, **p < 0.001.\)
of IL-12 and IFN-γ, expressed significantly higher levels of MK mRNA compared with Th0 cells (Fig. 8G). These results suggest that MK produced by CD4+ T cells plays an important role in the symptoms of EAE by suppressing DCreg expansion.

Discussion

Our previous report showed that inhibiting MK induced Treg expansion and suppressed autoimmune responses. In this study, we showed that MK suppressed Treg development by inhibiting expansion of the DCreg population. Stimulating bone marrow cells with TGF-β and IL-10 resulted in the development of DCregs, which induced CD4+CD25+Foxp3+ Treg differentiation. However, it is still unclear how DCregs induce iTreg differentiation. We also showed that DCregs expressed higher levels of PD-L1 and lower levels of IL-12. PD-L1 reportedly induces iTreg development and maintains this cellular population (24). Thus, PD-L1 may contribute to iTreg differentiation from naive T cells. Furthermore, DCons induce Th1 cell differentiation owing to higher levels of CD80 and CD86 expression and IL-12 production. CD28-mediated signaling is known to induce T cell activation to promote Th1 cell differentiation in the presence of IL-12. Although our data indicated that CD80, CD86, and PD-L1 were highly
expressed in DCs, high doses of soluble CD28 were reported to overcome PD-L1–induced negative regulation of CD4+ T cells (25). Overall, higher expression levels of PD-L1 and lower expression levels of CD80 and CD86 may be important for DC-mediated iTreg-induction processes.

STAT3 activation is important for the development of tolerogenic DCs or suppression of DCs that lead to aggressive immune responses (26–28). Injecting small interfering RNA specific for STAT3 in myeloid cells led to immune activation and antitumor activity (29). In particular, IL-10 reduces IL-12 production in DCs by inhibiting NF-κB recruitment to the IL-12p40 promoter in a STAT3-dependent manner (30). Consistent with this, we showed that MK downregulated the levels of pSTAT3 and induced IL-12 production in DCregs. Thus, decreasing pSTAT3 levels in DCs exacerbated inflammatory processes by inducing IL-12 production. We also showed that MK suppressed STAT3 activation in DCs by inducing SHP-2 expression. In fact, mice lacking SHP-2 signaling show prolonged STAT3 phosphorylation (31). In addition, SHP-2 is required for discoidin domain receptor-induced suppression of STAT3 phosphorylation (32). Interestingly, low-density lipoprotein receptor-related protein, which is thought to be the receptor for MK, was shown to associate with SHP-2 in a human follicular thyroid carcinoma cell line (33). These results suggest that SHP-2 signaling plays an important role in MK-induced suppression of STAT3 phosphorylation in DCs. MK-induced SHP-2 signaling also inhibits IL-10–induced suppression of IL-12 production, which is a STAT3-mediated response. Therefore, inhibiting MK may be a useful strategy to treat autoimmune diseases, because such an approach would upregulate p-STAT3 levels and regulate immune responses in myeloid cells.

Recently, Th17 cells, a CD4+ T cell subset, have been implicated in EAE development (34–36). Moreover, studies using neutralizing Abs (37) and KO mice (38–40) showed that the Th1 cytokine IFN-γ plays an important role in suppressing EAE. In contrast, MOG-reactive Th1 cells are reported to induce EAE (41, 42). These findings suggest that factors other than IFN-γ mediate the effects of Th1 cells in EAE. In the current study, we showed that MK is produced by CD4+ cells during EAE development. In addition, in vitro studies revealed that MK expression was strongly induced in Th1 cells compared with naive Th cells. Thus, MK may be one of the factors that contributes to Th1 cell-mediated exacerbation of EAE. Of note, IFN-γ KO mice show accelerated collagen-induced arthritis, a model of RA (43), whereas anti–IL-12 Abs prevent this disease in IFN-γ KO mice (44), suggesting that Th1 cells also have a prominent role in RA. In addition, Th1 cells contribute to CD (45). Because MK has been detected in the synovial fluid of patients with RA (18) and is more strongly expressed in CD (16), MK may play a role in these Th1 cell-induced autoimmune diseases by suppressing DCreg development and subsequently expanding the Treg population.

In this study and in a previous article, we demonstrated the efficacy of anti-MK RNA aptamers in mice with EAE, suggesting that a similar approach may be useful for MS. Aptamers are single-stranded oligonucleotides that bind to proteins with high affinity and affect function of the target (46). Toxicologic studies have shown that aptamers do not produce the same adverse effects observed with antisense oligonucleotides (47–49), such as immune stimulation, complement activation, and anticoagulation. In this article, we showed that administration of anti-MK RNA aptamers enhanced DCreg development in dLNs during EAE. Cytokine production and Ag presentation by DCs are critical for differentiation of Th cells to initiate autoimmune processes. Thus, expansion of the DCreg population in response to anti-MK RNA aptamers regulates Treg development at early stages. Anti-MK
therapy may be a particularly useful strategy for treating MS and other autoimmune diseases.

Disclosures

No financial conflicts of interest.

References


