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Inhibition of ERK MAPK Suppresses IL-23- and IL-1-Driven IL-17 Production and Attenuates Autoimmune Disease

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IL-17-producing CD4+ T (Th17) cells are pathogenic in many autoimmune diseases. The induction and expansion of Th17 cells is directed by cytokines, including IL-23 and IL-1β, produced by innate immune cells through activation of pathogen recognition receptors. The NF-κB and IFN regulatory factor families of transcriptional factors mediate IL-12 production; however, distinct signaling pathways appear to be required for IL-23 production. In this study, we show that inhibition of ERK MAPK suppressed IL-23 and IL-1β production by dendritic cells stimulated with TLR or dectin-1 agonists but did not affect IL-12p70 production. Furthermore, an ERK inhibitor suppressed the ability of Ag-pulsed TLR-activated dendritic cells to induce Ag-specific Th17 cells in vivo, but interestingly also inhibited the induction of Th1 cells. Treatment with an ERK inhibitor attenuated experimental autoimmune encephalomyelitis (EAE), when administered either at the induction phase of acute EAE or during remission in the relapsing-remitting EAE model. This was associated with significant suppression of autoimmune-specific Th17 and Th1 responses. The suppressive effect of the ERK inhibitor on attenuation of EAE was reversed by administration of IL-12 and IL-23. Our findings suggest that ERK MAPK plays a critical and hitherto undescribed role in activating innate production of IL-23 and IL-1β, which promote pathogenic T cell responses, and therefore represents an important target for therapeutic intervention against autoimmune diseases. The Journal of Immunology, 2009, 183: 1715–1723.

nterleukin-17-producing T cells (Th17 cells) and IFN-γ-secreting Th1 cells are discrete populations of inflammatory T cells that have distinct and possibly complementary roles in the pathogenesis of autoimmune diseases and in protection against infection (1–4). The differentiation of Th1 cells is mediated by IL-12, whereby the IL-12 family member, IL-23, especially in synergy with IL-1, plays an essential role in the induction or expansion of murine and human Th17 cells (1, 5–7). IL-6 or IL-21 with TGF-β can also promote the differentiation of Th17 cells from naive CD4+ T cells (8, 9). IL-1R and IL-23-defective mice are resistant to the development of experimental autoimmune encephalomyelitis (EAE) (5, 10). Furthermore, anti-IL-23 therapy reduced IL-17 concentrations in serum and in the CNS and prevented disease relapse in relapsing-remitting EAE (11). Consequently, IL-23 and IL-17 are now considered to be important new targets for treating autoimmune diseases in humans (12). In contrast, the roles of IL-12 and IFN-γ are less clear, with evidence for suppression of Th17 development by IFN-γ (2, 3) and exacerbated autoimmunity in IL-12- or IFN-γ-defective mice (10, 13), but also reports that Th1 cells do have pathogenic roles in certain autoimmune diseases (14–16).

IL-12 and IL-23 are produced by cells of the innate immune system, such as dendritic cells (DC), following binding of pathogen-associated molecular patterns or endogenous danger signals to TLR, NOD-like receptor, or other pathogen recognition receptors, such as dectin-1 (6, 7). However, the induction of IL-12 and IL-23 are differentially regulated by TLR and NOD-like receptor-activated DC (7). NF-κB, IFN regulatory factor (IRF)1, and IRF8 signaling pathways play important roles in the expression of IL-12p35 and IL-12p40 and in driving Th1 responses (17–20). In contrast, it has been demonstrated that IL-6 and IL-23 production in DC by agonists of dectin-1, and consequent development of Th17 cells, is mediated by signaling via Syk and CARD9 (21). However, the signaling molecules involved in promoting the IL-23 production are still unclear. A study on the role of IRF1 demonstrated that this transcriptional factor was essential for IL-12-driven Th1 responses but was not required for IL-23-dependent IL-17 production (20).

In this study, we examined the role of ERK MAPK in TLR-induced IL-23 and IL-1β production by DC and the subsequent expansion of Th17 cells. We found that inhibition of MEK1/2, the kinase upstream of ERK, suppressed IL-23 and IL-1β production by DC stimulated with the TLR4 agonist, LPS, but also with the dectin-1 agonist, curdlan, and with heat-killed Mycobacteria tuberculosis (MTB). In contrast, IL-12p70 production was not affected by inhibition of ERK activation. Adoptive transfer experiments with Ag-pulsed and TLR agonist-activated DC revealed that ERK activation played a major role in their ability to promote Th17 responses in vivo. Furthermore, inhibition of ERK suppressed autoantigen-specific Th17 and Th1 responses and attenuated EAE, when administered at the induction of acute EAE or therapeutically in a relapsing-remitting model. These findings provide new evidence that IL-12 and IL-23 production use distinct

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3 Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; IRF, IFN regulatory factor; DC, dendritic cell; EAU, experimental autoimmune uveitis; KLH, keyhole limpet hemocyanin; MOG, myelin oligodendrocyte glycoprotein; MTB, Mycobacteria tuberculosis; PLP, proteolipid protein; PT, pertussis toxin.

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signaling pathways in DC and demonstrate that the ERK signaling pathway may be an important target for new therapeutic interventions against T cell-mediated autoimmune diseases.

Materials and Methods

Mice

C57BL/6 and SJL/J mice were purchased from Harlan U.K. All mice were maintained according to European Union regulations, and experiments were performed under license from the Department of Health and Children and with approval from the Trinity College Dublin Bioresources Ethics Committee.

Dendritic cells

Bone marrow-derived immature DC were prepared by culturing bone marrow cells from C57BL/6 mice in medium containing 10% FCS supplemented with 20 ng/ml GM-CSF. On day 3, fresh medium containing 20 ng/ml GM-CSF was added. On day 6, semiadherent cells were removed using 0.02% EDTA (Sigma-Aldrich). Cells were recultured in medium supplemented with 20 ng/ml GM-CSF. On day 8, fresh medium containing 20 ng/ml GM-CSF was added. On day 10, loosely adherent cells were collected, washed, and used for assays. DC were cultured with LPS (100 ng/ml; Alexis), heat-killed MTB (2–50 μg/ml; Chondrex), or curdlan (100 μg/ml; Wako) with or without the MEK1/2 inhibitors U0126 (Calbiochem) or PD98059 (Calbiochem) or the Calbiochem MEK1/2 inhibitor (1–10 μM). Supernatants were recovered after 24 h, and IL-1β (R&D Systems), IL-23-β (eBioscience), IL-12βp70 (R&D Systems), and IL-12βp40 (BD Pharmingen) concentrations in the supernatants were determined by ELISA.

Western blotting

Murine bone marrow-derived DC were incubated with medium or LPS (100 ng/ml) alone or in the presence of increasing concentrations of the MEK1/2 inhibitor U0126. Cell lysates were prepared and resolved on 12% SDS-PAGE gels and transferred to nitrocellulose membrane. Blots were incubated with anti-phospho-ERK or anti-ERK (Santa Cruz Biotechnology) and with horseradish peroxidase-conjugated secondary Abs and developed using ECL.

Real time RT-PCR

RNA was harvested from the DC using the TRIzol (Invitrogen)-chloroform method, followed by reverse transcription into cDNA using a Quantitect Reverse Transcription kit (Qiagen). Real-time RT-PCR for the detection of IL-12βp35, IL-23p19, and IL-1β mRNA was performed using predesigned TaqMan gene expression assays (Applied Biosystems). 18s ribosomal RNA was used as an endogenous control. Samples were assayed on an Applied Biosystems 7500 Fast Real Time PCR machine.

DC transfer

DC were stimulated for 24 h with medium only, keyhole limpet hemocyanin (KLH; 20 μg/ml), or KLH and LPS (100 ng/ml) with or without U0126 (1 μM). Cells were washed and transferred (5 × 10^5) s.c. to naive mice. After 7 days, lymph node cells (1 × 10^6/ml) were restimulated in vitro with KLH (2–50 μg/ml) or medium only. Supernatants were recovered after 72 h, and IL-17, IFN-γ, IL-10 and IL-13 concentrations were quantified by ELISA.

Induction and assessment of EAE and treatment with a MEK1/2 inhibitor

C57BL/6 mice were immunized s.c. with 150 μg of myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (GenScript) emulsified in CFA supplemented with 4 mg/ml heat-killed MTB (Chondrex). Alternatively, SJL/J mice were immunized s.c. with 150 μg of proteolipid protein (PLP) peptide 139–151 (GenScript) emulsified in CFA, supplemented with 4 mg/ml heat-killed MTB. All mice were injected i.p. with 500 ng of pertussis toxin (PT; Kaketsuken) on days 0 and 2. In the MOG-induced EAE model, the MEK1/2 inhibitor U0126 (50 μg/mouse) was administered s.c. to C57BL/6 mice with the MOG and CFA on day 0 and again on days 1 and 2 or on days 5, 7, 9, 11, 13, 15 and 17. In the PLP-induced relapsing remitting EAE model, the MEK1/2 inhibitor U0126 (50 μg/mouse) was administered s.c. to SJL/J mice every 2 days from day 18 postimmunization. EAE was also induced by adoptive transfer of MOG-specific T cells. Lymph node and spleen cells from C57BL/6 mice with EAE were cultured in vitro for 3 days with MOG, MOG and IL-1β plus IL-23, or MOG and IL-1β plus IL-23 in the presence of a neutralizing anti-IFN-γ Ab or with MOG and IL-12. Cells were washed and transferred i.v. to naive C57BL/6 mice (5 × 10^6/mouse). In certain experiments, mice were treated with U0126 (50 μg/mouse) by s.c. administration at the time of T cell transfer and again 1 and 2 days later. Alternatively, DC were stimulated with MOG (50 μg/ml) and heat-killed MTB (10 μg/ml), with or without U0126 (5 μM). Cells were washed and transferred s.c. to naive C57BL/6 mice (1 × 10^6 cells/mouse). Disease severity was recorded as follows: grade 0, normal; grade 1, limp tail; grade 2, wobbly gait; grade 3, hind limb weakness; grade 4, hind limb paralysis; and grade 5, tetraparalysis/death.

Ag-specific proliferation and IL-17 and IFN-γ production

Lymph node cells (1 × 10^6 cells/ml) were stimulated with MOG or PLP peptides (1–50 μg/ml). After 3 days of culture, IL-17 (R&D Systems) and IFN-γ (BD Pharmingen) concentrations in the supernatants were determined by ELISA. Proliferation was determined on day 4 by [3H]thymidine incorporation as described previously (5).

Intracellular cytokine staining

Spleen or lymph node cells (2 × 10^6 cells/ml) were stimulated for 4 h with PMA (10 ng/ml) and ionomycin (1 μg/ml with brefeldin A (5 μg/ml) for the last 2 h. Cells were washed and blocked with Fcy block (1 μg/ml; BD Pharmingen) before extracellular staining for surface CD3 or CD4 (BD Pharmingen). Cells were then fixed and permeabilized (Fix and Perm cell permeabilization kit; Caltag Laboratories) and stained for intracellular IL-17 or IFN-γ. Flow cytometric analysis was performed using a Cyan ADP Flow Cytometer (DakoCytomation).

Statistical analysis

Data were compared by unpaired t test or by one- or two-way ANOVA. Where significant differences were found, the Tukey-Kramer multiple comparisons test was used to identify differences between individual groups.

Results

ERK MAPK is required for IL-23, but not IL-12, production by DC

Previous studies had suggested that distinct signaling pathways were required for production of IL-12 and IL-23 and the development of Th1 and Th17 cells (18). In this study, we examined the role of ERK MAPK in IL-12 and IL-23 production. Because we have shown that IL-1 is required for the induction or expansion of Th17 cells, we also determined the role of ERK in IL-1 production. We first demonstrated that DC expressed endogenous phosphorylated ERK, which was enhanced following stimulation with LPS and suppressed by culture with the MEK1/2 inhibitor U0126 (Fig. 1A). We next examined the effect of inhibiting ERK on IL-12, IL-23, and IL-1β production and found that U0126 inhibited IL-23 and IL-1β, but not IL-12βp70, production by LPS-activated DC (Fig. 1B). Furthermore, LPS-induced IL-23p19 and IL-1β, but not IL-12βp35, mRNA expression in DC was suppressed by the ERK inhibitor (Fig. 1C).

The suppressive effect of the MEK1/2 inhibitor on IL-23 and IL-1β production was not confined to TLR4 activation, as U0126 also suppressed IL-23 and IL-1β production by DC in response to the dectin-1 agonist curdlan (Fig. 2). Furthermore, suppression of LPS- or curdlan-induced IL-23 and IL-1β was observed with three distinct inhibitors of MEK1/2, U0126, PD98059, and a commercially available (Calbiochem) MEK1/2 inhibitor over a range of inhibitor concentrations (Fig. 2).

Because MTB includes a range of pathogen-associated molecular patterns and is used as the adjuvant to induce many experimental autoimmune diseases in mice, including EAE, we examined the effect of inhibiting ERK signaling on cytokine production by DC stimulated with heat-killed MTB. Incubation of DC with U0126 suppressed IL-1β and IL-23 production but had little effect on IL-12βp40 production in response to heat-killed MTB (Fig. 3). These findings demonstrate that distinct
signaling pathways in DC mediate IL-23 and bioactive IL-12 production.

**Inhibition of ERK in DC suppresses their ability to induce Th17 responses in vivo**

Because IL-1 and IL-23 production by innate immune cells promotes IL-17 production by T cells, we examined the effect of inhibiting ERK on the ability of TLR-activated DC to drive Th17 responses. We also examined the effect on Th1 and Th2 responses.

DC were pulsed in vitro with the Ag, KLH, alone or with LPS, in the presence or absence of U0126 and transferred s.c. to naive mice. Assessment of KLH-specific cytokine production by lymph node cells ex vivo 7 days after transfer revealed that Ag-specific IL-17 and IFN-γ production was significantly suppressed in mice injected with DC that had been pulsed with Ag and LPS in the presence of the MEK1/2 inhibitor (Fig. 4A). In contrast, IL-10 and IL-13 production, induced by transfer of DC pulsed with KLH, was not suppressed by U0126 (Fig. 4B). To provide further evidence that the inhibitory effect on Th17 responses did not result from a nonspecific effect on DC function or viability, we also examined the effect of the ERK inhibitor on LPS-induced DC maturation and viability. Incubation of DC with LPS for 24 h significantly enhanced MHC class II and CD40 expression.Coincubation with the ERK inhibitor did not affect class II or CD40 expression or reduce cell viability, as determined by propidium iodide staining (Fig. 4C). Our findings suggest that ERK activation in DC plays a major role in IL-23 and IL-1β production and in promoting the induction of Th17 and Th1 cells in vivo.

**Treatment with an ERK inhibitor suppresses autoantigen-specific Th17 and Th1 cells in vivo and attenuates EAE**

It has been reported that IL-23, IL-1, and Th17 cells have a pathogenic role in many experimental autoimmune diseases, including...
EAE (1, 5, 10), collagen-induced arthritis (22), and experimental autoimmune uveitis (EAU) (14). Furthermore, inhibition of ERK can attenuate arthritis in mice, although the mechanism of suppression was not clear and its effect on Th17 responses was not examined (23). In this study, we examined if blocking ERK activation might attenuate the symptoms of EAE by suppressing the induction or expansion of autoantigen-specific T cells. We found that the incidence and severity of EAE were significantly reduced in mice treated with the ERK inhibitor, although this was only significant at the higher concentration of MOG used in mice treated with the ERK inhibitor, although this was only significant at the higher concentration of MOG used in mice treated with the ERK inhibitor (Fig. 5D), suggesting that the ERK inhibitor suppressed the induction or expansion of Th1 and Th17 cells in vivo (Fig. 5D). The percentage of CD4+ T cells secreting IL-17 was consistently lower than that observed for CD3+ T cells. This can be accounted for by a population of IL-17-secreting γδ T cells, which are also suppressed by the ERK inhibitor (data not shown). We have found that IL-1 and IL-23, as well as promoting IL-17 production by memory Th17 cells, also promote IL-17 by γδ T cells. Furthermore, γδ T cells and CD4+ T cells both contribute to IL-17 production in mice with EAE. (C. E. Sutton et al., manuscript in preparation).

Administration of the ERK inhibitor on days 5–18 after the induction of EAE had a less significant effect on clinical scores (Fig. 5E), suggesting that once autoantigen-specific T cells are induced, inhibition of IL-1 and IL-23 production was not as effective at modulating the course of disease in this model.

We next determined the efficacy of ERK inhibition in PLP-induced relapsing-remitting EAE in SJL/J mice, which more closely resembles the course of disease in multiple sclerosis patients. Administration of U0126 after the acute phase of disease resulted in significant attenuation of clinical symptoms compared with mice treated with vehicle alone (Fig. 6A). This
was associated with significant suppression of PLP-specific IL-17 production (Fig. 6B). These findings demonstrate that inhibition of ERK attenuates the development of acute EAE when administered during the induction phase and also prevents relapse in the relapsing-remitting model of EAE. Furthermore, the attenuation is associated with significant suppression of autoantigen-specific IL-17 production.

**MOG-specific T cells expanded with Ag in the presence of IL-1 and IL-23 induce EAE following adoptive transfer in vivo**

We have previously shown that IL-1 and IL-23 promote IL-17 production by CD3+ T cells from naïve mice (5). The findings of the present study demonstrate that ERK plays an important role in the production of IL-23 and IL-1β but not IL-12. Surprisingly, inhibition of ERK suppressed both IL-17 and IFN-γ production in vivo. Therefore, we examined the influence of IL-1β and IL-23 on activation of MOG-specific Th1 and Th17 cells in vitro. Mice were immunized with MOG and CFA and sacrificed at day 10. Lymph node cells were restimulated in vitro with MOG alone or in combination with IL-23, IL-1β, or IL-1 plus IL-23. The results showed that IL-23 significantly enhanced MOG-induced IL-17 production and that this was further augmented by addition of IL-1β (Fig. 7A). In contrast, IFN-γ production was enhanced by culture with MOG in the presence of IL-1β, but this was not further augmented by addition of IL-23 (Fig. 7A).

We next examined the ability of MOG-specific T cells activated in vitro with MOG, IL-1β, and IL-23 to transfer EAE to naive mice. Lymph node and spleen cells from mice with EAE cultured in vitro with MOG, IL-1, and IL-23 were restimulated in vitro with MOG alone or in combination with IL-1, IL-1β, or IL-1 plus IL-23. The results showed that IL-23 significantly enhanced MOG-induced IL-17 production and that this was further augmented by addition of IL-1β (Fig. 7A). In contrast, IFN-γ production was enhanced by culture with MOG in the presence of IL-1β, but this was not further augmented by addition of IL-23 (Fig. 7A).
EAE was induced in SJL mice by immunization with PLP in CFA followed by PT. Mice were treated with PBS (control) or U0126 (50 μg/mouse) every 2 days from day 18. Arrows indicate treatment days. Data are means ± SEM for triplicate stimulations of lymph node cells from individual mice (n = 9/group) and are representative of two experiments.
suggest that in our system, a major effect of the ERK inhibitor is to suppress MTB-induced IL-1 and IL-23 production by DC, which promote induction of IL-17-producing T cells, but that the ERK inhibitor may also suppress endogenous IL-1 and IL-23 production in vivo, required for expansion or survival of Th17 cells.

The attenuating effect of the ERK inhibitor on Th17 and Th1 responses and EAE is mediated via suppression of IL-1 and IL-23 production

To confirm that the suppressive effect of the ERK inhibitor on Th17 responses and on the clinical course of EAE was mediated by inhibition of IL-23 and IL-1β, we added back these cytokines to see if we could reverse the suppression. EAE was induced in C57BL/6 mice by immunization with MOG and CFA, and the ERK inhibitor was administered on days 0, 1, and 2 with or without IL-23 or IL-1β and IL-23. Assessment of IL-17 production in the supernatants of MOG-stimulated lymph node cells by ELISA demonstrated that treatment with the ERK inhibitor during immunization with MOG and CFA completely suppressed the induction of IL-17-producing T cells (Fig. 8A). This was partially reversed by coadministration of IL-23 and completely reversed by coadministration of IL-1β and IL-23. MOG-specific IL-17 production was also enhanced but not significantly when mice were immunized with MOG and CFA in the presence of IL-1β and IL-23, without the ERK inhibitor (Fig. 8A). Intracellular cytokine staining on lymph node cells from mice immunized with MOG and CFA revealed that the frequency of IL-17 producing by CD3+ T cells was reduced by administration of the ERK inhibitor, and this was reversed by coadministration of IL-23 (Fig. 8B). Assessment of IL-17 production by CD4+ T cells confirmed that treatment with U0126 suppressed the development of Th17 cells, which was reversed by administration of IL-23 in vivo (Fig. 8B).

We also examined the effect of IL-1β and IL-23 on the suppressive effect of the ERK inhibitor on the course of disease in the EAE model. Consistent with our earlier data, treatment with the ERK inhibitor significantly reduced the symptoms of EAE (Fig. 8C). The attenuating effect of the ERK inhibitor on the development of EAE was reversed by coadministration of IL-1β and IL-23 (Fig. 8C). Treatment with IL-1β and IL-23 in the absence of the ERK inhibitor did not alter the course of disease. These findings suggest that the ERK inhibitor attenuates the clinical signs of EAE by suppressing IL-1β and IL-23 production.

Discussion

It has previously been reported that MyD88-mediated activation of NF-kB, IRF1, and IRF8 are required for IL-12p35 and IL-12p40 gene expression and driving Th1 responses (17–20). However, these studies failed to find a role for these transcriptional factors in IL-23-driven Th17 responses and suggested that alternative signaling molecules may be involved (18, 20). Our study demonstrates that ERK MAPK is an essential component of an alternative pathway required for IL-23-dependent IL-17 production.

The findings demonstrate that the TLR- and dectin-1-mediated activation of IL-23 and IL-1β production by DC is mediated through activation of ERK and that this plays a critical role in promoting Th17 responses in vivo. Although IL-6 and TGF-β have a clearly established role in the differentiation of naïve T cells into Th17 cells (8, 9, 24) and IL-21 is involved in an autocrine amplification loop (25–27), the present study supports previous reports that IL-23 and IL-1 play a major role in the induction and expansion of Th17 cells. Studies in EAE and EAU models have demonstrated that IL-23p19−/− mice produce less IL-17 and are resistant to induction of autoimmunity (10, 14). Furthermore, treatment with anti-IL-23 can suppress IL-17 production and attenuate clinical signs of autoimmunity in EAE and EAU models (11, 14). In the EAE model, adoptive transfer of T cells from wild-type into IL-23p19−/− mice or IL-1R1−/− mice demonstrated that IL-23 and IL-1 play a critical role in the development of Th17 cells (5, 28).

This study has shown that inhibition of MEK1/2 suppresses the clinical signs of disease in two EAE models by suppressing autoantigen-specific T cell responses. It has previously been reported that ERK1/2−/− mice have increased susceptibility to EAE (29). Although these findings appear to be at variance with our study, they are not directly comparable, because the
MEK1/2 inhibitors used in our study block ERK1 and ERK2, whereas the knockout mice are only defective in ERK1. Furthermore, the ERK inhibitor was only administered during or after the induction of EAE, whereas the knockout mice lack ERK1 through the course of their development. Interestingly, we previously reported that inhibition of ERK enhanced IL-17 production by T cells in vitro (5), whereas the present study shows that inhibition of ERK suppresses IL-23 and IL-1β production by DC. Furthermore, in vitro treatment of DC with an ERK inhibitor before pulsing with Ag and a TLR agonist suppressed the ability of the DC to promote the induction of Ag-specific IL-17 production in vivo. Moreover, addition of an ERK inhibitor during in vitro pulsing of DC with MOG and MTB suppressed their ability to induce clinical signs of EAE in naive mice. Treatment with the ERK inhibitor also suppressed, although not significantly, the induction of EAE by transfer of MOG-specific Th1 and Th17 cells but not to as great an extent. These findings suggest that the dominant effect of the ERK inhibitor in vivo is suppression rather than enhancement of Th17 responses, and this reflects its ability to inhibit innate IL-1 and IL-23, which promote the development and expansion of Th17 cells. We have recently demonstrated that IL-1 and IL-23, as well as activating memory Th17 cells, also promote IL-17 production by γδ T cells and that both populations are a source of IL-17 in mice with EAE (C. E. Sutton et al., manuscript in preparation). In this study, we found that attenuation of EAE by treatment with the ERK inhibitor was associated with suppression of IL-17 production by γδ T cell as well as CD4⁺ T cells. Therefore, the effect of the ERK inhibitor suppresses innate cytokines that stimulate unconventional as well as conventional T cells.

Our data clearly demonstrate that treatment with an ERK inhibitor reduces the severity of autoimmune disease in two EAE models, and this is consistent with its protective efficacy against collagen-induced arthritis (23). The ERK inhibitor prevented relapse when administered during remission in the relapsing-remitting model, but interestingly only had a significant protective effect in the acute MOG-induced model when administered during induction of EAE but not after development of disease. This correlated with suppression of the induction of IL-17-producing T cells. It has previously been shown that anti-IL-23 therapy reduced IL-17 concentrations in serum and in the CNS and prevented disease relapse in relapsing-remitting EAE (11). Furthermore, treatment with anti-IL-23 reduced IL-17 production and attenuated EAU, when administered immediately before and after induction of disease but not at the effector stage of the disease (14).

Surprisingly, inhibition of ERK suppressed IFN-γ as well as IL-17 production. This effect was independent of IL-12, as the ERK inhibitors did not suppress TLR-induced IL-12 production by DC but inhibited IL-23 and IL-1β production. Culture of MOG-specific T cells with Ag in the presence of rIL-23 and IL-1β-enhanced IFN-γ and IL-17 production. Furthermore, adoptive transfer of a mixed population of MOG-specific Th1 and Th17 cells cultured in vitro in the presence of IL-1- and IL-23-induced EAE. In contrast, transfer of polarized Th1 cells, expanded by culture with MOG and IL-12, or Th17 cells, expanded by culture with IL-1β and IL-23 in the presence of neutralizing IFN-γ, induced only transient symptoms of EAE, which were significantly milder than those induced by the combination of Th1 and Th17 cells. These findings are consistent with a pathogenic role for Th1 as well as Th17 cells in EAE (15, 16) and uveitis (14) and suggest that a combination of the two populations may be optimum for induction of EAE, with each having different functions in the disease process. Indeed, it has been demonstrated IL-23-driven T cells promote neutrophil infiltration into white matter lesions, whereas macrophages were more prominent in the inflammatory CNS infiltrate driven by Th1 cells (15). Our findings suggest that blocking IL-1 and IL-23 by targeting the ERK signaling pathway in innate immune cells is an effective means of suppressing the induction or expansion of Th1-, Th17-, and IL-17-secreting γδ T cells and may be a promising approach in the design of new immunotherapeutic drugs against autoimmune and chronic inflammatory diseases.

Disclosures

K.M. is a co-founder and shareholder in opsona therapeutics a start-up company involved in the development of anti-inflammatory drugs.

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