Liver X receptor agonist regulation of Th17 lymphocyte function in autoimmunity

Jihong Xu, Gail Wagoner, James C. Douglas, and Paul D. Drew

Department of Neurobiology and Developmental Sciences, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

Received October 6, 2008; Revised March 30, 2009; Accepted March 31, 2009. DOI: 10.1189/JLB.1008600

ABSTRACT

CD4^+ Th17 cells are believed to play an important role in the development of a variety of autoimmune diseases including EAE, an animal model of MS. Previously, we and others demonstrated that LXR agonists suppressed the activation of primary glial cells and blocked the development of EAE. The present studies demonstrated that the LXR agonist T0901317 suppressed IL-17A expression from splenocytes derived from Vα2.3/Vβ8.2 TCR transgenic mice and from MOG_{35-55}-immunized C57BL/6 mice. Furthermore, in vitro treatment with IL-23 alone or in combination with MOG_{35-55} induced IL-17A expression from splenocytes derived from MOG_{35-55}-immunized mice, and T0901317 blocked this induction. In vitro treatment with the LXR agonist suppressed IL-23R expression by splenocytes. In addition, in vivo treatment with the LXR agonist suppressed IL-17A and IL-23R mRNA and protein expression in EAE mice. These studies suggest that LXR agonists suppress EAE, at least in part by suppressing IL-23 signaling. Recent studies indicate that the cytokines IL-21 and IL-22 are produced by Th17 cells and modulate immune responses. Our studies demonstrate that the LXR agonist T0901317 suppressed MOG_{35-55}-induced expression of IL-21 and IL-22 mRNA in splenocytes derived from MOG_{35-55}-immunized mice. Finally, we demonstrate that the LXR agonist T0901317 suppressed the development of EAE in an experimental paradigm involving treatment of established EAE. Collectively, these studies suggest that LXR agonists may be effective in the treatment of MS. J. Leukoc. Biol. 86: 000–000; 2006.

Introduction

EAE is an inflammatory disease of the CNS characterized by demyelination and axonal pathology. This disease can be induced in mice following active immunization with a variety of myelin antigens in CFA or through adoptive transfer of CD4^+ Th1 lymphocytes into naïve recipients [1]. EAE is histopathologically and clinically similar to MS and is an established animal model of this human disease [2, 3].

In response to antigen stimulation, naïve CD4^+ lymphocytes become activated, proliferate, and differentiate into phenotypically and functionally distinct effector T cell populations. These effector T cell subsets produce distinct sets of cytokines, which mediate their effector functions. CD4^+ T cells have been classically separated into two subsets, termed Th1 and Th2 cells. Th1 cells have generally been believed to contribute to the development of autoimmune diseases including EAE, and Th2 cells are believed to protect against the development of these diseases [3]. However, several observations led to the suggestion that cells distinct from Th1 cells also contribute to the development of autoimmunity. Recently, studies demonstrated that IL-23, a cytokine related to IL-12, plays an important role in development of EAE [4]. IL-23 was demonstrated to contribute to the development of a distinct, new subset of Th cell, termed Th17 cells, which are now believed to contribute to the development of EAE [5]. Th17 cells are characterized by the production of IL-17A, IL-17F, IL-21, and IL-22 [5–7]. Although IL-23 was first thought to control Th17 cell differentiation, it is now clear that differentiation of this effector cell type is controlled by the cytokines IL-6 and TGF-β, and IL-23 stimulates the maintenance or stability of differentiated Th17 cells [8–10]. More recently, the Th17-derived cytokine IL-21, in combination with TGF-β, has been demonstrated to stimulate the differentiation and expansion of Th17 cells in the absence of IL-6. In addition, IL-21 is believed to act through a positive-feedback loop to stimulate the expansion of Th17 cell populations [7, 11, 12].

A variety of agents has been approved for the treatment of MS. However, these medications do not cure the disease, and better strategies are needed for the treatment of this devastating disease. We have been evaluating the potential of LXR agonists recently for the treatment of MS. LXRs are nuclear receptors that play a critical role in modulating lipid metabolism [13, 14]. In addition to regulating lipid metabolism, LXRs

Abbreviations: DC=dendritic cell, EAE=experimental autoimmune encephalomyelitis, LXR=liver X receptor, m=mouse, MBP=myelin basic protein, MOG=myelin oligodendrocyte glycoprotein, MS=multiple sclerosis, RXR=retinoid X receptor, Treg=T regulatory cell
have been demonstrated more recently to modulate immune responses. Interestingly, LXR agonists suppressed the development of animal models of contact dermatitis and atherosclerosis [15, 16]. This suggested that LXR agonists may be effective in modulating inflammation in the CNS and could be effective in the treatment of MS and other neuroinflammatory disorders. We demonstrated previously that LXR agonists inhibited LPS induction of proinflammatory cytokines and chemokines by primary astrocytes and microglia [17]. Importantly, LXR agonists were also demonstrated to suppress the expression of the Th1 cytokine IFN-γ, and administration of the LXR agonist prior to disease onset blocked the development of EAE [18].

Accumulating evidence suggests that CD4+ Th17 cells are important modulators of EAE and MS [19–21]. The current studies demonstrate that LXR agonists suppress the production of IL-17A from splenocytes activated with appropriate myelin antigens. Our studies demonstrate further that in vivo administration of LXR agonists suppresses IL-17A and IL-23R expression in MOG35–55-immunized mice, suggesting that this agonist blocks the development of EAE, at least in part by inhibiting IL-23 signaling. Finally, our studies demonstrate that LXR agonists block induction of IL-21 and IL-22 mRNA expression in splenocytes derived from MOG35–55-immunized mice. Collectively, these studies suggest that LXR agonists may be effective in the treatment of MS.

**MATERIALS AND METHODS**

**Reagents and animals**
The LXR agonist T0901317 was obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). RPMI 1640, HEPES buffer, sodium pyruvate, and nonessential amino acids were obtained from Cellgro (Herndon, VA, USA). 2-ME was obtained from Invitrogen Corp. (Grand Island, NY, USA). Glutamine and antibiotics were obtained from BioWhittaker (Walkersville, MD, USA). FBS was obtained from Hyclone (Logan, UT, USA). rmIL-23 was obtained from R&D Systems (Minneapolis, MN, USA). C57BL/6 mice were obtained from Harlan (Indianapolis, IN, USA) and bred in-house. Transgenic mice bearing the rearranged V8.2 gene encoding the TCR 

**Induction and clinical evaluation of EAE**
EAE was induced in C57BL/6 wild-type mice by s.c. injection over four sites in the flank with 200 μg/mouse MOG35–55 peptide (C.S. Bio Co., Menlo Park, CA, USA) in an emulsion with IFA supplemented with 5 mg/ml Mycobacterium tuberculosis, strain H37Rv (Difco, Detroit, MI, USA). Pertussis toxin (List Biological Laboratories, Campbell, CA, USA) dissolved in PBS was injected i.p. at 200 μg/mouse at the time of immunization (Day 0) and 48 h later. Mice were scored daily on a scale of 0–6, as described previously [23]: 0, no clinical disease; 1, limping/flaccid tail; 2, moderate hind-limb weakness; 3, severe hind-limb weakness; 4, complete hind-limb paralysis; 5, quadriplegia or paraparesis; 6, death. All mice were 6–10 weeks of age when experiments were performed. The LXR agonist T0901317 was dissolved in DMSO at 50 μg/ml and diluted further with PBS to 25 μg/ml prior to injection. The mice were treated (i.p.) with 25 μg/kg T0901317 or vehicle (1:1 ratio of DMSO plus PBS) daily. Where indicated in the figure legends, mice were deeply anesthetized with halothane and transcardially perfused with PBS, and spinal cords were removed for RNA and protein isolation.

**Cell culture**
Spleens from naïve Vα2.3/Vβ8.2 TCR transgenic mice or from C57BL/6 mice immunized with MOG35–55 11 days earlier were harvested, and single-cell suspensions were obtained by pressing the tissue through a wire mesh screen, as described previously [22]. After RBCs were lysed, and cells were washed, splenocytes were cultured in RPMI-1640 medium containing 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 50 μM 2-ME, 1 mM sodium pyruvate, and 10 mM HEPES solution. Cells were treated with various concentrations of T0901317, relevant myelin peptides, or rmIL-23, as indicated. Cells were harvested, or supernatants were collected at the time-points indicated in the figure legends.

**Cytokine ELISA**
For cytokine analysis, splenocytes were treated as indicated in figure legends. Cytokine levels in tissue culture media were determined by ELISA, as described by the manufacturer (OptEIA Sets, Pharmingen, San Diego, CA, for IFN-γ and R&D Systems for IL-17). ODs were determined using a Spectramax 190 microplate reader at 450 nm, and cytokine concentrations in media were determined from standards containing known concentrations of the proteins.

**RNA isolation and cDNA synthesis**
Total RNA was isolated from splenocytes and spinal cord from MOG-immunized C57BL/6 mice using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA samples were treated with DNaseI (Invitrogen) to remove any traces of contaminating DNA. The RT reactions were carried out using an iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer’s instructions.

**Real-time quantitative RT-PCR assay**
IL-17A, IFN-γ, IL-21, and IL-22 mRNAs were quantified by real-time PCR using an iCycler IQ™ multicolor real-time PCR detection system (Bio-Rad). All primers and TaqMan minor groove binder probes (FAM™ dye-labeled) were designed and synthesized by Applied Biosystems (Foster City, CA, USA). The real-time PCR reactions were performed in a total volume of 25 μL using an iCycler™ kit (Bio-Rad). The levels of IL-17A, IFN-γ, IL-23R, IL-21, and IL-22 mRNAs in splenocytes or spinal cord were calculated after normalizing cycle thresholds against the “housekeeping” gene GAPDH and are presented as the fold change value (2−ΔΔCt: mean threshold) relative to untreated splenocytes or relative to vehicle-treated mice in EAE studies.

**Protein extraction and Western blotting**
Protein extracts were prepared from spinal cord of MOG-immunized C57BL/6 mice or sham-operated mice as described previously [24]. Total proteins were separated electrophoretically on 4–15% Tris-HCl Ready Gels (Bio-Rad). Proteins were transferred electrophoretically to nitrocellulose membranes (NitroBind, MSI, Westborough, MA, USA) and then incubated with rat anti-mIL-17 antibody or rat anti-mIL-23R antibody (R&D Systems) overnight at 4°C. Blots were then incubated with HRP-conjugated goat anti-rat IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. IL-17 and IL-23R protein was detected by ChemiGlow West substrate, as described by the manufacturer (Alpha Innotech, San Leandro, CA, USA), and visualized using a ChemiDoc XRS (Bio-Rad) image analysis system. Actin protein was detected in a similar manner using a rabbit anti-actin polyclonal antibody (Sigma Chemical Co., St. Louis, MO, USA; Catalog #A5060) to verify uniformity in gel loading.

**Flow cytometry**
Splenocytes from C57BL/6 mice immunized with MOG35–55 11 days earlier were isolated by CD90 microbeads (Miltenyi Biotec, Auburn, CA, USA).
Cytes derived from Vβ2.3/Vβ8.2 TCR transgenic mice were activated in vitro with 25 μg/ml MBP in the absence or presence of the indicated concentrations of T0901317 (μM). Supernatants were collected at 48 h, and IFN-γ (A) and IL-17A (B) concentrations were determined by ELISA. Splenocytes from C57BL/6 mice immunized with MOG35–55 11 days earlier were activated in vitro with 25 μg/ml MOG35–55 in the absence or presence of the indicated concentrations of T0901317 (T09; μM). Supernatants were collected at 48 h, and IFN-γ (C) and IL-17A (D) concentrations were determined by ELISA. Values represent the mean ± SEM for a representative experiment run in triplicate. Three independent experiments were conducted. *, P < 0.05, and ***, P < 0.001, versus MBP-treated cultures (A and B) or MOG-treated cultures (C and D).

**Figure 1. LXR agonist T0901317 inhibits cytokine production by splenocytes.** Splenocytes from Vβ2.3/Vβ8.2 TCR transgenic mice, were activated in vitro with 25 μg/ml MBP in the absence or presence of the indicated concentrations of T0901317 (μM). Supernatants were collected at 48 h, and IFN-γ (A) and IL-17A (B) concentrations were determined by ELISA. Splenocytes from C57BL/6 mice immunized with MOG35–55 11 days earlier were activated in vitro with 25 μg/ml MOG35–55 in the absence or presence of the indicated concentrations of T0901317 (T09; μM). Supernatants were collected at 48 h, and IFN-γ (C) and IL-17A (D) concentrations were determined by ELISA. Values represent the mean ± SEM for a representative experiment run in triplicate. Three independent experiments were conducted. *, P < 0.05, and ***, P < 0.001, versus MBP-treated cultures (A and B) or MOG-treated cultures (C and D).

**Statistics**

Data were analyzed by one-way ANOVA, followed by a Bonferroni post-hoc test to determine the significance of difference. Clinical EAE disease scores were evaluated using a two-tailed unpaired Student’s t-test.

**RESULTS**

**Effects of LXR agonist T0901317 on cytokine production by splenocytes derived from Vβ2.3/Vβ8.2 TCR transgenic mice**

Previously, the LXR agonist T0901317 was demonstrated to inhibit antigen (OVA235–359)-stimulated proliferation of CD4+ T cells isolated from the spleens of DO11.10 TCR transgenic mice. Furthermore, these studies indicated that T0901317 inhibited OVA235–359 stimulation of IFN-γ by CD4+ T cells [18]. In the present study, we demonstrate that T0901317 inhibits MBPαβ1–11 stimulation of the Th1 cytokine IFN-γ by splenocytes derived from Vβ2.3/Vβ8.2 TCR transgenic T cells (Fig. 1A). Recently, Th17 cells have been demonstrated to play a critical role in modulating the development of autoimmune disorders including EAE. Interestingly, we demonstrate that T0901317 inhibits the production of IL-17A by splenocytes derived from TCR transgenic mice in a dose-dependent manner (Fig. 1B). This suggests that LXR agonists suppress the development of EAE, at least in part, by inhibiting the activation or differentiation of Th17 cells. As observed in the DO11.10 TCR transgenic system [18], T0901317 also inhibited T cell proliferation in a dose-dependent manner in cells derived from Vβ2.3/Vβ8.2 TCR transgenic mice, and T0901317 did not decrease cell viability at the concentrations evaluated in the present studies (data not shown). This suggests that T0901317 suppression of IL-17A production by splenocytes does not result from effects on cell viability.

**Effects of LXR agonist T0901317 on cytokine production by splenocytes derived from MOG35–55-immunized C57BL/6 mice**

The studies above indicated that the LXR agonist T0901317 suppressed IFN-γ and IL-17A production by a homogeneous population of TCR transgenic T cells. Next, we analyzed the effects of T0901317 on a nonhomogeneous population of splenocytes derived from MOG35–55-immunized C57BL/6 mice. MOG peptide induced IFN-γ (Fig. 1C) and IL-17A (Fig. 1D) production by splenocytes derived from MOG-immunized mice. Furthermore, T0901317 inhibited IFN-γ production significantly at high concentrations and inhibited IL-17A production in a dose-dependent manner. The LXR agonist inhibited T cell proliferation without decreasing cell viability at the concentrations evaluated in these studies (data not shown).

The cytokines IL-6 and TGF-β are critical in the differentiation of Th17 cells. In addition, IL-23 plays a critical role in the maintenance of mature Th17 cells and is a critical modulator of autoimmunity. We evaluated the effects of IL-23 on splenocytes derived from MOG-immunized mice. Our studies demonstrated that in vitro stimulation with IL-23 induced IL-17A production significantly by splenocytes derived from MOG-immunized mice in a dose-dependent manner (Fig. 2A).
synergized with the MOG peptide in inducing the secretion of this cytokine (Fig. 2B). In addition, the LXR agonist inhibited IL-17A production significantly by MOG-primed splenocytes stimulated with IL-23, alone or in combination with MOG, in a dose-dependent manner (Fig. 2, B and C). Similar results were observed in a purified CD4^+ T cell from MOG-immunized C57BL/6 mice (data not shown).

IL-17A is a critical cytokine in driving EAE pathogenesis, and we found that LXR agonists suppress the secretion of this cytokine robustly by activated T cells. We next examined whether T0901317 had an effect on IL-17A expression by CD90^+ lymphocytes, as determined by intracellular cytokine-staining analysis. Our studies indicated that CD90^+ lymphocytes derived from spleen of MOG-immunized mice produced IL-17A following stimulation with PMA plus ionomycin and that T0901317 suppressed the production of this cytokine in a dose-dependent manner (Fig. 3A). Likewise, PMA plus ionomycin induced IFN-\gamma by CD90^+ lymphocytes, and T0901317 suppressed IFN-\gamma production by these cells, although this suppression was weaker than that observed for IL-17A (Fig. 3B).

Effects of LXR agonist on IL-17A, IFN-\gamma, and IL-23R mRNA expression by MOG-primed splenocytes

Real-time RT-PCR analysis was performed to determine whether LXR agonists inhibit mRNA expression of IL-17A and IFN-\gamma, as observed above for protein production. Our data indicated that splenocytes derived from MOG-immunized mice constitutively expressed little IL-17A (Fig. 4A) and IFN-\gamma (Fig. 4B) mRNAs, and MOG peptide potently induced mRNA expression of these two molecules by splenocytes. The LXR agonist T0901317 suppressed MOG induction of IL-17A mRNA expression significantly in a dose-dependent manner and also inhibited IFN-\gamma expression at relatively high concentrations (30 \muM). Thus, T0901317 suppresses IL-17A and IFN-\gamma mRNA expression in a manner similar to that observed for the proteins encoded by these mRNAs (Fig. 1). These data also suggest that this LXR agonist may suppress the production of IL-17A and IFN-\gamma through a mechanism involving repression of the expression of genes encoding IL-17A or IFN-\gamma.
IL-23 effects on the Th17 phenotype are believed to result from this cytokine engaging IL-23R on the surface of these T cells. For these reasons, we investigated the effect of the LXR agonist T0901317 on IL-23R expression. We found that splenocytes derived from MOG-immunized mice constitutively expressed IL-23R mRNA and that the MOG peptide did not induce IL-23R expression significantly (Fig. 4C) on these cells. Treatment with T0901317 (30 μM) inhibited IL-23R mRNA expression significantly by unstimulated splenocytes and MOG-activated splenocytes. These results suggest that LXR agonists inhibit IL-17A production, in part, through down-regulation of IL-23R expression.

**In vivo administration of LXR agonist results in decreased IL-17A, IFN-γ, and IL-23R expression in the spinal cord of EAE mice**

It has been demonstrated previously that the LXR agonist T0901317 suppresses the development of EAE [18]. Here, we wished to determine if T0901317 suppression of EAE was associated with altered expression of IL-17 signaling molecules. Our studies demonstrated that pretreatment with the LXR agonist T0901317 suppressed the development of EAE. In these studies, mice were treated with T0901317 daily, beginning 3 days prior to immunization until 16 days postimmunization, at which time, spinal cords were removed for isolation and analysis of RNA and protein. At the time of sacrifice and removal of spinal cords, vehicle-treated animals had an average clinical score of 2.17, T0901317-treated animals had an average clinical score of 0.6, and IFA-immunized, control animals had a clinical score of 0.0. Our results suggested that T0901317 suppression of EAE was associated with decreased spinal cord expression of IL-17A (Fig. 5A), IFN-γ (Fig. 5B), and IL-23R (Fig. 5C). Treatment of mice with T0901317 also decreased the expression of IL-17A and IL-23R protein in the spinal cord of these animals (Fig. 6). This suggests that the LXR agonist may ameliorate EAE.
development, possibly through down-regulating the production of IL-17A and IL-23R in vivo.

Effects of LXR agonist on Th17 effector cytokine mRNA expression by MOG-primed splenocytes

Recently, Th17 cells have been demonstrated to play an important role in the pathogenesis of EAE. In addition to IL-17A, Th17 cells produce effector cytokines including IL-17F, IL-21, and IL-22. Our current studies demonstrate that splenocytes from mice immunized with MOG35–55 constitutively expressed a low level of IL-21 (Fig. 7A) and IL-22 (Fig. 7B) mRNAs. Furthermore, MOG peptide increased expression of IL-21 and IL-22 mRNAs. Significantly, treatment with T0901317 (30 μM) inhibited IL-21 and IL-22 mRNA expression in these splenocytes. Moreover, IL-23 was shown to stimulate the production of IL-17A mRNA expression (Fig. 7C) and also increased IL-22 mRNA expression (Fig. 7D). T0901317 suppressed the IL-23 induction of IL-17A and IL-22 mRNA expression significantly in a dose-dependent manner. Interestingly, IL-23 did not induce IL-21 mRNA expression significantly in these studies (data not shown). As mentioned previously, T0901317 suppressed IL-23R expression in splenocytes. These studies suggest that T0901317 may suppress splenocyte expression of Th17 cytokines by modulating IL-23R expression. Collectively, these studies suggest that T0901317 modulates the production of effector cytokines produced by Th17 cells and suggest that this LXR agonist could be an important modulator of autoimmunity.

Effects of postimmunization treatment with the LXR agonist on clinical progression of EAE

Previously, administration of LXR agonists prior to immunization was demonstrated to suppress the development of EAE [18]. In the present studies, we demonstrate that administration of the LXR agonist T0901317 around the beginning of clinical signs of EAE was also effective in delaying the onset and decreasing the severity of EAE (Fig. 8). LXR was effective in suppressing EAE, even under conditions where vehicle-treated animals developed severe disease. These studies strongly support that LXR agonists could be effective in the treatment of MS.

DISCUSSION

LXRs are members of the nuclear hormone family of transcription factors. Two LXRs exist, which are characterized by distinct cellular and tissue expression patterns. LXR-α expression is restricted to tissues including adipose and liver and by macrophages, and LXR-β is expressed ubiquitously [25]. The role of LXRs in mediating lipid metabolism is well established [14]. More recently, LXRs have been demonstrated to modulate immune responses. However, the effects of LXRs in altering inflammation in the CNS have not been investigated thoroughly. Previously, we demonstrated that LXR agonists inhibited LPS induction of NO as well as proinflammatory cytokines including IL-6 and IL-1β and the chemokine MCP-1 by primary astrocytes and microglia. LXRs are believed to regulate the expression of LXR-responsive genes as functional heterodimers in association with RXRs. Our studies further demonstrated that LXR and RXR agonists cooperatively suppressed NO production by primary glia, suggesting that LXR agonists act in part through receptor-dependent mechanisms. The transcription factor NF-κB plays a critical role in activating the expression of a variety of proinflammatory molecules. Our studies demonstrated further that LXR agonists blocked LPS induction of IκB degradation and suppressed NF-κB DNA.
binding activity, suggesting an additional mechanism by which LXR agonists may suppress inflammation in the CNS [17]. Importantly, LXR agonists were determined previously to suppress the development of EAE [18]. Although activated glia are believed to contribute to the pathology associated with EAE, this disease is believed to be initiated by autoreactive T cells. The current studies were designed to evaluate the effects of LXR agonists on T cell phenotype and function.

EAE is mediated by myelin-specific CD4\(^+\) Th lymphocytes. These CD4\(^+\) Th cells traditionally have been separated into two classifications [26]. Th1 cells produce IFN-\(\gamma\) and lymphotoxin and facilitate cell-mediated immunity, trigger delayed-type hypersensitivity reactions, and eliminate intracellular pathogens. Th2 cells produce IL-4, IL-5, IL-13, and IL-25 and mediate humoral immune responses, contribute to allergic responses, and control extracellular pathogens [27]. Th1 cells are believed to contribute to the development of autoimmune diseases including MS, and Th2 cells are believed to protect against these diseases. However, several studies suggest that Th1 cells alone are not likely to control autoimmunity. For example, IFN-\(\gamma\) and IFN-\(\gamma\)-deficient mice are more susceptible than wild-type animals to the development of autoimmune disease [28–30]. Furthermore, although IL-12 is critical in the differentiation of Th1 cells, IL-12 deficiency did not suppress development of EAE [31, 32]. Recently, a novel CD4\(^+\) Th cell termed Th17 has been demonstrated to play an important role in modulating autoimmune diseases. Th17 cells are characterized by the expression of IL-17A, IL-17F, IL-21, and IL-22 and are believed to eliminate extracellular pathogens not efficiently cleared by Th1 and Th2 cells. Th17 cell differentiation is principally controlled by the cytokines TGF-\(\beta\) in combination with IL-6 [8–10]. Alternatively, in the absence of IL-6, IL-21 can act together with TGF-\(\beta\) to induce the differentia-
tion of Th17 cells [7, 11, 12]. Interestingly, IL-21 also appears to play a role in the amplification of Th17 cells through a positive-feedback mechanism [7]. Finally, the cytokine IL-23 is now believed to function to increase the stability of the Th17 phenotype [5]. An important role for Th17 cells in modulating autoimmunity is supported by the following observations: First, EAE studies demonstrated that knockout mice for IL-12/23 p40 and IL-23 p19, which heterodimerize to form IL-23, were resistant to development of EAE, demonstrating an important role of IL-23 in this autoimmune disease [4, 31]. Second, Th17 cells are capable of inducing EAE [33]. Third, IL-17 is associated with pathology in autoimmune diseases including EAE, and IL-17 deficiency decreased the severity of EAE [34]. The observation that IL-17 levels are elevated in MS patients further supports a role of Th17 cells in modulating this disease [35].

Our current studies demonstrate that the LXR agonist T0901317 suppresses the production of the Th17-derived cytokine IL-17A following in vitro treatment of splenocytes derived from Vσ2.3/Vβ8.2 TCR transgenic mice as well as from MOG-immunized C57BL/6 mice. T0901317 also suppressed the production of the Th1-derived cytokine IFN-γ by these splenocytes, although less robustly than observed for IL-17A. These studies suggest that LXR agonists may suppress the development of EAE, at least in part through suppression of effector cytokines by Th1 and Th17 cells. The fact that T0901317 inhibits IL-23 stimulation of IL-17A production by splenocytes derived from MOG-immunized mice suggests that LXR agonists may alter IL-23-mediated maintenance and stabilization of Th17 cells. However, it is possible that LXR agonists affect earlier stages of Th17 cell development. As noted previously, IL-6 plays a critical role in Th17 cell differentiation. Importantly, our previous studies demonstrated that T0901317 inhibits IL-6 production by CNS glia, suggesting that LXR agonists may suppress the differentiation and/or restimulation of Th17 cells [17]. We are currently investigating the effects of LXR agonists on peripheral immune cells including DCs to better understand the effects of these agonists on Th17 cell differentiation in the periphery. Interestingly, IL-6 also plays a critical role in controlling the balance between Th17 cells that contribute to pathology in autoimmune diseases and Tregs, which suppress the development of these diseases [7, 8]. These studies demonstrate that in the presence of IL-6, TGF-β stimulates the differentiation of Th17 cells, and in the absence of IL-6, TGF-β favors the production of Tregs. Future studies will determine whether LXR agonists alter the balance between Th17 cells and Tregs.

IL-23 plays a critical role in the maintenance of Th17 cells. This cytokine signals through a heterodimeric receptor consisting of the IL-12Rβ1 chain, which is also common to IL-12R in association with the unique IL-23R subunit. IL-23R is expressed on T cells and NK cells and at lower levels on monocytes and DCs [36]. Inflammatory cytokines including IL-6 increase IL-23R expression on T cells [12]. Our current studies indicate that IL-23R expression is suppressed by in vitro administration of LXR agonists to splenocytes derived from MOG-immunized C57BL/6 mice as well as in vivo in EAE mice. This suggests that LXR agonists may suppress the development of EAE, at least in part, through inhibiting IL-23 signaling.

IL-21 is an effector cytokine produced in abundance by Th17 cells [7, 11, 12]. IL-21 is also produced by Th follicular cells and NK cells [37, 38]. As mentioned previously, IL-21, in combination with TGF-β, is capable of inducing Th17 cell differentiation in the absence of IL-6. Furthermore, IL-6, in combination with TGF-β, is a potent inducer of Th17 cells in wild-type mice, whereas these cytokines produce fewer Th17 cells in IL-21R-deficient mice [7]. These studies demonstrated that IL-21 is important in the amplification of Th17 cells. In addition, IL-21 induces T cell expression of IL-23R, thus facilitating IL-23 stabilization and maintenance of Th17 cells [12]. In addition, administration of IL-21 prior to the induction of EAE increases extravasation of immune cells into the CNS and increases the severity of disease [39]. Like IL-21, IL-22 is an effector cytokine produced by Th17 cells. This cytokine is also produced by NK cells [40]. IL-22 can be pathogenic or alternatively, protect against the development of autoimmune diseases [41]. Interestingly, IL-22-deficient mice were demonstrated to be susceptible to the development of EAE. However, human IL-22 has been demonstrated to damage the blood brain barrier, suggesting that this cytokine may contribute to the development of MS [21]. Our current studies demonstrate that the LXR agonist T0901317 suppresses IL-21 and IL-22 production by splenocytes derived from MOG-immunized mice. This suggests an additional mechanism by which LXR agonists may suppress the development of EAE.

In summary, we have demonstrated that the LXR agonist T0901317 inhibits the production of the Th17 cytokine IL-17A from splenocytes derived from TCR transgenic mice and from MOG-immunized mice. We also demonstrate that in vitro administration of T0901317 suppresses the expression of IL-23R in these splenocytes. Furthermore, T0901317 suppressed the expression of IL-17A and IL-23R in EAE studies. Also, T0901317 suppressed the production of IL-21 and IL-22 mRNA by splenocytes derived from MOG-immunized mice. Finally, T0901317 was effective in the treatment of EAE when administered at the time of development of clinical symptoms. Collectively, these studies suggest that LXR agonists suppress the production of Th17 cells in vitro and in vivo and suggest that these agonists may be effective in the treatment of MS.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (NS 047546), the National Multiple Sclerosis Society, and the Arkansas Biosciences Institute.

REFERENCES


