Regulation of Interleukin (IL)-12 Receptor \( \beta_2 \) Subunit Expression by Endogenous IL-12: A Critical Step in the Differentiation of Pathogenic Autoreactive T Cells

By John T. Chang, Ethan M. Shevach, and Benjamin M. Segal

From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892

Summary

The interleukin (IL)-12 receptor (R) \( \beta_2 \) subunit is the critical molecule involved in maintaining IL-12 responsiveness and controlling T helper cell type 1 lineage commitment. We demonstrate that IL-12 and interferon (IFN)-\( \gamma \) play separate, but complementary, roles in regulating IL-12R \( \beta_2 \) expression on antigen-specific CD4\(^+\) T cells. These results are consistent with our previous observation that IL-12 can promote autoimmune disease through IFN-\( \gamma \)-independent as well as -dependent pathways. Therefore, we compared the induction of IL-12 by, and the expression of the IL-12R \( \beta_2 \) subunit on, myelin basic protein (MBP)-specific T cells from experimental allergic encephalomyelitis (EAE)-susceptible SJL (H-2\( ^s \)) mice and from EAE-resistant B10.S mice (H-2\( ^s \)). B10.S mice had an antigen-specific defect in their capacity to upregulate the IL-12R \( \beta_2 \) subunit. Defective expression was not secondary to the production of suppressive cytokines, but to a failure of B10.S MBP-specific T cells to upregulate CD40 ligand expression and to induce the production of IL-12. IL-12R \( \beta_2 \) expression as well as encephalitogenicity of these cells could be restored by the addition of IL-12. These results suggest that the development of immunotherapies that target the IL-12R \( \beta_2 \) subunit may be useful for the treatment of autoimmune diseases.

Key words: autoimmunity • experimental allergic encephalomyelitis • T helper cell type 1 lymphocytes • Interferon \( \gamma \) • CD40 ligand

Animal models of organ-specific autoimmune diseases are uniformly mediated by autoreactive Th1 lymphocytes, whether they develop spontaneously or are induced by immunization with autoantigen in CFA. For example, the production of IFN-\( \gamma \) and/or TNF-\( \alpha \) has implicated in the pathogenesis of experimental allergic encephalomyelitis (EAE),\(^1\) myasthenia gravis, collagen-induced arthritis (CIA), insulin-dependent diabetes mellitus (IDDM) in nonobese diabetic mice, and the spectrum of organ-specific autoimmune disease that follows neonatal thymectomy (1–8). Th1 cells have also been implicated in the pathogenesis of organ-specific diseases in humans, including multiple sclerosis, IDDM, inflammatory arthritis, and autoimmune thyroiditis (9–12). Although successful induction of autoimmune diseases in mice genetically deficient in IFN-\( \gamma \), TNF-\( \alpha \), or lymphotxin \( \alpha \) have cast doubt on the involvement of a single proinflammatory Th1 cytokine in disease pathogenesis (13–16), and have even demonstrated protective effects of some of these proinflammatory mediators, studies in a number of disease models demonstrate a critical role for IL-12 in the generation of Th1 effectors (17). IFN-\( \gamma ^{-/-} \) mice exhibit enhanced susceptibility to EAE as well as to CIA, whereas IL-12 \( ^{-/-} \) mice are completely resistant to induction of either disease (18–20). Furthermore, neutralization of IL-12 in IFN-\( \gamma ^{-/-} \) mice protected them from both EAE and CIA. In SJL mice, administration of a polyclonal antiserum to IL-12 was protective during either the induction or effector phase of adoptively transferred EAE (20, 21). Similarly, neutralization of IL-12 in mice with established colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) led to rapid and complete recovery (22); furthermore, anti-IL-12, but not anti-IFN-\( \gamma \), was recently found to be therapeutic in another model of autoimmune colitis that arises in IL-10 \( ^{-/-} \) mice (23). Interference with the biological activity of IL-12 may therefore be an appropriate target for the treatment of autoimmune disease in humans.

Collectively, these studies suggest that the successful differentiation and/or function of autoimmune effector cells must be dependent on their ability to respond to quantities of IL-12 that are available in the microenvironment during critical time points. Studies from several groups have dem-

---

\(^{1}\)Abbreviations used in this paper: CD40L, CD40 ligand; CIA, collagen-induced arthritis; EAE, experimental allergic encephalomyelitis; MBP, myelin basic protein; NP, nucleoprotein; PLP, proteolipid protein.
demonstrated that the expression of the IL-12 receptor plays a critical role in determining the Th1/Th2 balance during the course of an immune response. The IL-12Rβ2 subunit is not expressed on resting T cells, but is induced at low levels after engagement of the TCR by antigen. Maintenance of a level of IL-12Rβ2 subunit expression sufficient to allow signaling by IL-12 and therefore Th1 differentiation is critically dependent on the relative amounts of IFN-γ and IL-4 in the microenvironment during T cell priming (24–26). IL-4 inhibits IL-12Rβ2 subunit expression, but this inhibition can be overcome by IFN-γ, even in cells that have begun to differentiate along the Th2 pathway. Although the IL-12Rβ2 subunit can be induced in the presence of exogenous IL-12 in the absence of IFN-γ and IL-4, the role of endogenous IL-12 itself in direct induction of IL-12Rβ2 expression has not yet been examined. The direct induction of IL-12Rβ2 expression may be particularly relevant to the development of autoimmune effector cells in IFN-γ−−/− mice.

In this report, we initially used cytokine-deficient mice and neutralizing anti-cytokine mAbs to critically examine the relative roles of IL-12 and IFN-γ in the induction and maintenance of the IL-12Rβ2 subunit on CD4+ T cells that have been primed against a conventional foreign antigen. We demonstrate that in wild-type mice IL-12 and IFN-γ play separate as well as complementary roles in regulating IL-12Rβ2 expression. However, sustained expression of IL-12Rβ2 can be induced in IFN-γ−−/− mice by an IL-12-dependent pathway. These findings raised the possibility that the regulation of IL-12Rβ2 expression by IL-12 itself may play a role in the generation of pathogenic autoreactive T cells. Therefore, we extended these studies and compared the induction of the IL-12Rβ2 subunit on MBP-specific T cells derived from a strain of mice (SJL, H-2b) that is highly susceptible to EAE with T cells from a strain (B10.S, H-2d) that is resistant to EAE. We demonstrate that B10.S mice have an antigen-specific defect in their capacity to upregulate the IL-12Rβ2 subunit. This defective expression of the IL-12Rβ2 subunit is not secondary to the production of suppressive cytokines or to a decreased functional avidity of the TCR, but to a failure of the B10.S MBP-specific T cells to upregulate CD40 ligand (CD40L) expression, which is an important stimulus for the endogenous production of IL-12 by APCs. Because IL-12Rβ2 expression as well as the encephalitogenicity of these cells can be restored by the addition of exogenous IL-12 or IL-12–inducing agents (27, 28), we classify the B10.S MBP-specific cells as a novel population of autoreactive preautoimmune T (Tpre-A) cells that have emerged from the thymus with a defect in their capacity to differentiate into mature Th1 effector cells. However, these autoreactive cells retain the potential to differentiate into pathogenic T cells upon exposure to IL-12 induced by environmental or infectious agents.

Materials and Methods

Mice. SJL/J and C57BL/6 mice were obtained from the National Cancer Institute. Breeding pairs of C57BL/6 IL-12−−/− (N6) and C57BL/6 IFN-γ−−/− were originally provided by J. Magram (Hoffman-La Roche, Nutley, NJ) and D. Dalton and T. Stewart (Genentech Inc., South San Francisco, CA), respectively. B10.S mice were obtained from both MCLaughlin Research Institute and Taconic Farms. All mice were housed under specific pathogen-free conditions. They were exclusively female and between 2 and 4 mo of age when experiments were started.

Peptides. Peptides corresponding to residues 260–283 of Influenza A nucleoprotein (NP, 260–283, AR SALILR GSV AH KSCL PACVY GP), residues 87–106 of myelin basic protein (MBP, 87–106, VVHF FKN IVP TR TPPPSQ GK), and residues 139–151 of proteolipid protein (PLP, 139–151, HSLG K LWHPDKF) were synthesized and purified by HPLC by the Laboratory of Molecular Structure, Peptide Synthesis Laboratory (NIAID, National Institutes of Health [NIH]). Chicken OVA was purchased from Sigma Chemical Co.

Immunization. Mice were immunized subcutaneously at four sites over the flanks with an emulsion consisting of equal volumes of CFA (DIFCO Labs.) and antigen dissolved in PBS. Doses of antigen used were as follows: MBP, 100 μg; N, P, OVA, 5 μg; OVA, 100 μg; PLP, 100 μg.

Disease Induction. For disease induction by adoptive transfer, donor mice were immunized with 100 μg of MBP or PLP in CFA (1:1); 10–14 d later, draining LN cells were cultured for 96 h with MBP or PLP. Recovered cells (6×106) were injected intraperitoneally into naive syngeneic recipients that were examined daily for signs of EAE and rated on a five-point scale as previously described (20).

Cell Cultures. 10–14 d after immunization, draining LN cells (axillary and inguinal) were removed and processed as previously described (20). In brief, single cell suspensions of spleen or LN tissue were prepared by passage through wire mesh and red blood cells lysed with ACK buffer (NIM Media Uinit). Cells (4×106/ml) were cultured in RPMI 1640 containing 10% FCS and standard supplements (25) for 24–72 h in the presence of MBP, N, 50 μg/ml, N, P, 5 μg/ml, PLP, 100 μg/ml, or OVA (100 μg/ml). To generate short-term lines, draining LN cells were cultured in MBP or OVA for 4 d, washed extensively, and rested for 7 d in complete media. For maintenance of lines, T cells (106/ml) were restimulated with antigen in the presence of irradiated, syngeneic splenocytes (4×106/ml) every 10 d. For IL-12 assays, T cells (106/ml) were restimulated with or without antigen in the presence of syngeneic peritoneal exudate macrophages (5×106/ml). To generate peritoneal exudate macrophages, B10.S or SJL mice were injected with 3 ml fluid thioglycollate media (NIM, Media Uinit). 3 d later, macrophages were removed from the peritoneum of treated mice, washed, and used in experiments. In some experiments, cells were purified using T cell enrichment columns (R&D Systems), T and/or NK cell depletion was performed by treating cells with anti-Thy1.2 (clone H O13.4) culture supernatants or anti-NK1.1 antibody (clone PK136) followed by treatment for 45 min at 37°C with rabbit complement (Cedarlane Labs.).

Proliferation Assays. LN cells (5×106/0.2 ml) were cultured with various concentrations of antigen or with media alone for 4 d in 96-well round-bottomed plates (Costar Corp.). Cells were pulsed for the final 16 h of culture with 1 μCi of [3H]TdR (Amersham) and counted as previously described (20).

Antibodies and Cytokines. Where specified, cytokines or neutralizing antibodies were added to the primary cultures as follows: IL-12, 20 ng/ml (gift of S. Wolfr, Genetics Institute, Cambridge, MA); recombinant murine IFN-γ, 30 ng/ml (PharMingen); recombinant murine IL-10, 10 ng/ml (PharMingen); mouse IFN-γ, 10 μg/ml (clone XMG 1.2); rat anti–mouse IL-12, 10 μg/ml.
Northern blot analysis. Total RNA was isolated from LN cell cultures using RNAzol RNA isolation solvent (Tel-Test). Samples (10 μg of total RNA per lane) were run on a 1.2% agarose gel containing MOPS buffer and formaldehyde, and blotted onto a Hybond-N nylon membrane (Amersham). Membranes were baked for 2 h at 80°C, then probed for murine IL-12R β2 subunit, murine IL-12R β1 subunit, or β-actin. The following primer sets (Bio-Synthesis) were used to generate oligonucleotide cDNA probes: IL-12R β2 forward CTTG CAC CCA CTC ACA TTA AC; IL-12R β2 reverse CAG TGG GCT TTTG CCC TGT GGC; IL-12R β1 forward GAG GAG GCG GCT CTC CTC AG; IL-12R β1 reverse ACA TTA CTC CTC TCT CTC CAG GG. The β-actin primer set was purchased from Clontech. PCR was performed for 40 cycles with the following parameters: 94°C, 30 s; 58°C, 30 s; 72°C, 1 min. cDNA probes were run on a 1.2% agarose gel, and purified using the Wizard PCR DNA purification system (Promega). PCR fragments (50 ng) were labeled with [32P]dCTP using an oligolabeling kit (Pharmacia). Blots were prehybridized for 1 h at 42°C, followed by overnight hybridization with labeled probe at 42°C. Blots were then washed for 30 min in 2× SSC, 0.1% SDS buffer (room temperature) followed by 30 min in 0.1× SSC, 0.1% SDS buffer (55°C for IL-12R β2 and IL-12R β1; 65°C for β-actin).

PCR...
to induce any detectable IL-12p40 production (Fig. 2 A). In contrast, SJL MBP- and NP-reactive T cell lines induced comparable amounts of IL-12p40 production from macrophages (Fig. 2 B).

Because the interaction of CD40L on T cells and CD40 on APCs is a major stimulus for IL-12 production, these results raised the possibility that the failure of activated B10.S MBP-reactive T cells to express sufficient levels of CD40L upon activation is responsible for their failure to prime APCs to produce IL-12. We used a competitive PCR mimic to quantitate the relative levels of CD40L mRNA expressed upon antigenic activation by B10.S MBP- and NP-reactive LN cells. MBP-reactive LN cells failed to upregulate CD40L mRNA, whereas NP-reactive LN cells upregulated CD40L mRNA eightfold over unstimulated background levels (Fig. 3). Thus, the relative deficiency in CD40L expression by B10.S MBP-reactive LN cells may account for their failure to induce IL-12 production by APCs.

MBP-specific T cells from EAE-resistant B10.S Mice Fail to Upreregulate IL-12Rβ2 Subunit mRNA. To test directly whether the failure of the B10.S MBP-specific T cells to induce IL-12 leads to a deficiency in the expression of adequate levels of IL-12Rβ2 subunit, draining LN cells from SJL and B10.S mice that had been primed 12 d previously with a combination of MBP and NP in CFA were cultured in vitro for 72 h with either MBP or NP and evaluated for IL-12Rβ2 expression. LN cells from SJL mice upregulated IL-12Rβ2 mRNA strongly in response to stimulation with either MBP or NP (Fig. 4 A, left). In contrast, LN cells from B10.S mice upregulated IL-12Rβ2 subunit mRNA only in response to stimulation with NP, but not MBP (Fig. 4 A, right). Since NK cells and dendritic cells have been reported to express IL-12R (26, 31), T cells were purified from LN cells from immunized B10.S mice and depleted of NK cells. These T cells were combined with T cell-depleted, NK cell-depleted splenocytes from naive B10.S mice and stimulated for 72 h, then evaluated for IL-12Rβ2 subunit expression. Results similar to those shown in Fig. 4 A were obtained, thus excluding dendritic cells or NK cells as the source of IL-12R observed (Fig. 4 B). In contrast to the IL-12Rβ2 subunit, which was induced only upon antigen stimulation, IL-12Rβ1 subunit was constitutively expressed with no consistent patterns of antigen-induced upregulation (Fig. 4 A), in agreement with previously published reports (25, 26).

IL-12Rβ2 Subunit mRNA Expression Correlates with Susceptibility to EAE. We have previously demonstrated that the addition of pharmacological concentrations of IL-12 to cultures of B10.S MBP-reactive LN cells restored their ability to produce IFN-γ and converted them into encephalitogenic effectors (28). Indeed, the addition of exogenous IL-12 restored the ability of the B10.S MBP-reactive LN cells to upregulate the expression of IL-12Rβ2 subunit mRNA in an antigen-specific, dose-dependent manner (Fig. 5 A). Although the addition of IFN-γ failed to restore the ability of these cells to produce IFN-γ upon secondary

![Image](https://example.com/image1.png)
stimulation (28), modest induction of IL-12Rβ2 subunit expression was seen (data not shown). The addition of neutralizing antibodies to IL-12 or IFN-γ to cultures of NP-reactive B10.S LN cells (Fig. 5B) or MBP-reactive SJL LN cells (Fig. 5C) inhibited the ability of these cells to upregulate IL-12Rβ2 subunit mRNA in response to antigenic stimulation. Thus, the complementary roles of endogenous IL-12 and IFN-γ for optimal expression of IL-12Rβ2 subunit seen in the response of C57BL/6 mice to OVA can be extended to a second foreign antigen (NP) and an autoantigen (MBP).

Role of IL-10 in the Expression of IL-12Rβ2 Subunit mRNA. One possible explanation for the failure of B10.S MBP-specific T cells to induce IL-12 production and to upregulate IL-12Rβ2 expression is that a major component of the response to MBP in this strain is mediated by Th2 cells. However, we have previously failed to demonstrate production of IL-4 or IL-10 by B10.S MBP-specific T cells and treatment of B10.S mice with anti-IL-4 in vivo or anti-IL-10 in vitro did not result in the induction of IFN-γ production (28). Furthermore, addition of anti-IL-4 in vitro failed to restore the ability of these cells to express IL-12Rβ2 subunit (data not shown). However, our recent demonstration that IL-10 production by antigen nonspecific CD4+ T cells regulates the proinflammatory effects of IL-12 in vivo prompted us to more carefully examine the role of IL-10 in the priming of B10.S mice to MBP in vivo (20). B10.S mice were immunized with MBP/NP and simultaneously treated with neutralizing anti-IL-10 mAbs. Neutralization of IL-10 in vivo and in vitro did not restore the ability of MBP-reactive LN cells to express IL-12Rβ2 subunit (Fig. 6A) or to produce IFN-γ (data not shown). However, it should be emphasized that IL-10 had a potent downregulatory effect on the expression of IL-12Rβ2 in vitro as the addition of exogenous IL-10 to cultures of either B10.S NP-reactive or SJL MBP-primed LN cells strongly inhibited upregulation of the expression of the IL-12Rβ2 subunit (Fig. 6, B and C).

The Failure of B10.S MBP-specific T cells to Fully Differentiate into Th1 Effector Cells Does Not Appear to Be Secondary to a Decreased Functional avidity of the TCR. One of the problems with the comparison of immune responses between B10.S and SJL mice at the population level even with a well-defined peptide antigen is that these two strains differ by many background genes including the TCR gene complex (32). Thus, as a result of distinct positive/negative selection events in the thymus, the B10.S MBP-specific T cell repertoire may be quantitatively smaller or exhibit an overall lower affinity for the MBP87-106 peptide I-A^b complex compared with similarly restricted SJL T cells. The average lower affinity of the B10.S MBP-specific TCRs might result in impaired induction of CD40L and a cascade of events leading to defective upregulation of the IL-12Rβ2 subunit. Targoni and Lehmann have shown that immunization with low doses of peptide would generate recall responses only from T cells with a high affinity TCR for their MHC–peptide ligand (33). We immunized B10.S and SJL mice with different amounts (50, 100, or 400 μg) of MBP87-106 in CFA and measured T cell proliferation in response to a broad range of peptide concentrations in vitro.
The Defect in IL-12R β2 Subunit Expression by B10.S M BP-specific T Cells Does Not Extend to Other Myelin Autoantigens. Several studies have suggested that B10.S mice have a global defect in their responses to myelin autoantigens as the incidence and/or severity of EAE in B10.S mice immunized with PLP139-151 or with whole spinal cord homogenate was significantly less than that seen in similarly immunized SJL mice. To examine whether a failure of IL-12R β2 subunit expression was also seen in response to PLP, we immunized B10.S mice with a combination of MBP and PLP in CFA. PLP- but not MBP-reactive LN cells upregulated IL-12R β2 subunit strongly in response to antigenic stimulation (Fig. 8 A). Furthermore, PLP- but not MBP-reactive LN cells produced large quantities of IFN-γ in response to antigenic stimulation in secondary cultures (Fig. 8 B). To further address the question of whether the encephalitogenicity of primed LN cells correlated with their ability to express the IL-12R β2 subunit, we immunized B10.S mice with PLP139-151 or MBP87-106 in CFA. 10 d later, we stimulated draining LN cells in vitro with PLP or MBP for 4 d, then transferred the cells into naive B10.S recipients. Transfer of PLP-reactive LN cells resulted in a 30% incidence of EAE, whereas transfer of MBP-reactive LN cells failed to induce EAE in any mice (Fig. 8 C). Thus, the capacity of LN cells to transfer EAE was found to correlate with their ability to express IL-12R β2 subunit upon activation.

Discussion

Although CD4+ Th1 lymphocytes have been implicated as the effector cells in both animal models and human organ-specific autoimmune diseases, the failure to identify a single effector cytokine that is responsible for pathogenicity has suggested that cytokine-targeted therapeutic approaches to autoimmune disease should be directed against the primary cytokine responsible for the differentiation of Th1 cells, IL-12. Because the IL-12R β2 subunit has been identified as the critical molecule involved in maintaining IL-12 responsiveness and in controlling Th1 lineage commitment, we have focused our studies on critically examining the requirements for induction of the IL-12R β2 subunit on CD4+ T cells responding to autoantigens as well as conventional foreign antigens. We have used cytokine-deficient mice to establish a critical role for IL-12 itself, independent of IFN-γ, in the induction of IL-12R β2 expression. Although IFN-γ was capable of upregulating IL-12R β2 on T cells derived from IL-12−/− mice, the level of expression was always less than that seen on T cells from wild-type and IFN-γ−/− mice. As IL-12−/− mice have been found to have markedly deficient Th1 responses and to be resistant to Th1-mediated autoimmune diseases (34), the significance of this IL-12-independent, IFN-γ-dependent pathway of IL-12R β2 upregulation remains to be determined. More importantly, our studies with neutralizing antibodies in vitro have clearly shown that both IL-12 and IFN-γ play critical roles in the upregulation of IL-12R β2 on restimulation of antigen-primed T cells in vitro from wild-type mice.

We have previously defined a critical role for IL-12 in the cytokine cascade needed for activation of encephalitogenic MBP-specific T cells from EAE-resistant B10.S mice.
IL-10 to the in vitro culture exerted a powerful downregulation of Th1 differentiation of encephalitogenic effectors is supported by the suppression of EAE induction by the administration of blocking CD40L mAbs (35, 36). Our previous studies have shown that the primary defect in the immune response of the B10.S mouse to MBP is defective expression of CD40L on the MBP-specific T cell population with subsequent failure to generate IL-12 production from APCs. This in turn leads to defective expression of sufficient levels of IL-12Rβ2 needed for Th1 differentiation. The importance of CD40/CD40L interactions in the differentiation of encephalitogenic effectors is supported by the suppression of EAE induction by the administration of blocking CD40L mAbs (35, 36). Our previous studies have ruled out excessive production of IL-4 as the mechanism responsible for defective MBP-specific Th1 differentiation in the B10.S mouse (28). Neutralization of IL-10 in vivo and in vitro also did not restore IL-12Rβ2 expression by the MBP-specific T cells. However, addition of exogenous IL-10 to the in vitro culture exerted a powerful downregulatory influence on expression of IL-12Rβ2 subunit by antigen-specific T cells primed in vivo under Th1 conditions. Presumably, IL-10 acted directly on the APCs in the cultures to inhibit IL-12 production and thereby mimicked the effects of the addition of anti–IL-12 (37). However, a direct effect of IL-10 on the responding T cells remains a possibility. In any case, it appears that once IL-12Rβ2 subunit expression is induced by IL-12 in vivo, IL-10 can act to limit the level of functional IL-12Rβ2 ultimately expressed.

Indeed, this step may be the most important therapeutic effect of IL-10 in the treatment of autoimmune disease.

The antigen-specific defect we have observed in the CD40L/IL-12/IL-12Rβ2 subunit pathway in MBP-specific B10.S T cells should be contrasted with the more global defects of the B10.S strain in the response to myelin-derived autoantigens reported by others (38, 39). It is likely that this antigen-specific defect is superimposed over and above a more global defect. First, although the addition of exogenous IL-12 to cultures of B10.S MBP-reactive T cells restored their capacity to transfer EAE, the resultant disease was monophasic and less severe than the relapsing-remitting course manifested by SJL recipients of syngeneic MBP-primed T cells (28). Second, although we have demonstrated that B10.S PLP-reactive T cells can induce disease upon adoptive transfer, it should be noted that disease incidence and severity were much less than those observed in SJL mice. Furthermore, production of IFN-γ was only observed during secondary but not primary stimulation in vitro. The differences between our results and those of Encinas et al., who reported complete resistance of B10.S mice to PLP-induced EAE, may be secondary to the different disease induction protocols used (active induction versus passive transfer) or differences in the mouse colonies consequent to genetic drift (38). Thus, B10.S mice appear to have inherited a set of traits that confer a certain degree of protection against autoimmune phenomena in general, distinct from the antigen-specific defect in the IL-12 pathway. This set of traits is presumably mediated by the products of non–H-2 background genes and may also be responsible for the monophasic (as opposed to relapsing-remitting) course of EAE exhibited by B10.PL mice as well as for the resistance of C57BL/6 mice to autoimmune orchitis. In this context, it is interesting to note that one of the genetic loci found to be important in susceptibility to EAE (eae7) colocalizes to the same region of chromosome 11 as Orch3, a susceptibility locus in autoimmune orchitis (39). Hence, in the case of the B10.S response to MBP, we believe that absolute resistance arises as a result of the antigen-specific defect superimposed on a more global pattern of resistance to the manifestations of autoimmune disease.

What then is the mechanism responsible for the generation of this antigen-specific defect in the capacity of a population of autoreactive T cells to differentiate into pathogenic Th1 effector cells? The simplest explanation is that it is secondary to the size of the T cell repertoire specific for MBP. We believe that this is unlikely as the magnitude of the proliferative responses and of IL-2/IL-3 production by B10.S mice to MBP does not differ significantly from that of SJL mice (28). However, this question will only be able to be addressed quantitatively when specific peptide–MHC binding T cells are measured in this model. It is also possible that the affinity of the interaction of the anti-MBP TCRs from B10.S mice with the MBP peptide–I-A2.
References

1. Xu, H., L.V. Rizzo, P.B. Silver, and R.R. Caspi. 1997. Uveitogenicity is associated with a Th1-like lymphokine profile: cytokine-dependent modulation of early and committed effector T cells in experimental autoimmune uveitis. Cell. Immunol. 178:69–78.

2. Germann, T., J. Szeliga, H. Hess, S. Storkel, F.J. Podlaski,
14. Ferber, I.A., S. Brocke, C. Taylor-Edwards, W. Ridgway, C. Frei, K., H.P. Eugster, M. Bopst, C.S. Constantinescu, E. Navikas, V., and H. Link. 1996. Review: cytokines and the induction of experimental autoimmune encephalomyelitis. Immunol. 108:1092–1098.

15. Constantinescu, C.S., M. Wysocka, B. Hilliard, E.S. Ventura, K.W., D.J. Shuster, K.M. Gillooly, R.R. Warrier, S.E. Connaughton, L.B. Hali, L.H. Arb, M.K. Gately, and J. Magram. 1996. Reduced incidence and severity of collagen-induced arthritis in interleukin-12-deficient mice. Eur. J. Immunol. 26:2933–2938.

19. M.athys, P., K. Vermeire, T. Mitera, H. Heremans, S. Huang, and A. Billiau. 1998. Anti-IL-12 antibody prevents the development and progression of collagen-induced arthritis in IFN-gamma receptor-deficient mice. Eur. J. Immunol. 28:2143–2151.

20. Segal, B.M., S.K. Dwyer, and E.M. Shevach. 1998. An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J. Exp. Med. 187:537–546.

21. Leonard, J.P., K.E. Wolburger, and R. Goldman. 1995. Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J. Exp. Med. 181:381–386.

25. Szabo, S.J., A.S. Dighe, U. Gubler, and K.M. Murphy. 1996. Reduced incidence and severity of collagen-induced arthritis. Proc. Natl. Acad. Sci. USA 93:1099–1108.

27. Grohmann, U., M.L. Belladonna, R. Bianchi, C. Orabona, J. Agee, P. Marrack. 1989. Analysis of CD4+ T cells with Th1-like cytokine profile predominate in retroorbital lymphocytic infiltrates of Graves' ophthalmopathy. J. Clin. Endocrinol. Metab. 77:1120–1124.

28. Szabo, S.J., A.S. Dighe, U. Gubler, and K.M. Murphy. 1997. Regulation of the interleukin (IL)-12R beta 2 subunit expression in vivo. J. Immunol. 158:495–521.

29. Segal, B.M., D.M. Klinman, and E.M. Shevach. 1996. CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. Proc. Natl. Acad. Sci. USA 93:2499–2504.
36. Samoilova, E.B., J.L. Horton, H. Zhang, and Y. Chen. 1997. CD40L blockade prevents autoimmune encephalomyelitis and hampers TH1 but not TH2 pathway of T cell differentiation. J. Mol. Med. 75:603-608.

37. D'Andrea, A., M. Aste-Amezaga, N.M. Valiante, X. Ma, M. Kubin, and G. Trinchieri. 1993. Interleukin 10 (IL-10) inhibits human lymphocyte interferon-γ production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. J. Exp. Med. 178:1041-1048.

38. Encinas, J.A., M.B. Lees, R.A. Sobel, C. Symonowicz, J.M. Greer, C.L. Shovlin, H.L. Weiner, C.E. Seidman, J.G. Seidman, and V.K. Kuchroo. 1996. Genetic analysis of susceptibility to experimental autoimmune encephalomyelitis in a cross between SJL/J and B10.S mice. J. Immunol. 157:2186-2192.

39. Butterfield, R.J., J.D. Sudweeks, E.P. Blankenhorn, R. Korngold, J.C. Marini, J.A. Todd, R.J. Roper, and C. Teuscher. 1998. New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J. Immunol. 161:1860-1867.

40. Harrington, C.J., A. Paez, T. Hunkapiller, V. Mannikko, T. Brabb, M. Ahearn, C. Beeson, and J. Goverman. 1998. Differential tolerance is induced in T cells recognizing distinct epitopes of myelin basic protein. Immunity. 8:571-580.

41. Thornton, A.M., and E.M. Shevach. 1998. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J. Exp. Med. 188:287-296.

42. Martin, R., R. Voskuhl, M. Flerlage, D.E. McFarlin, and H.F. McFarland. 1993. Myelin basic protein-specific T-cell responses in identical twins discordant or concordant for multiple sclerosis. Ann. Neurol. 34:524-535.

43. Pelfrey, C.M., L.R. Tranquill, A.B. Vogt, and H.F. McFarland. 1996. T cell response to two immunodominant proteolipid protein (PLP) peptides in multiple sclerosis patients and healthy controls. Mult. Scler. 1:270-278.

44. Pette, M., K. Fujita, B. Kitze, J.N. Whitaker, E. Albert, L. Kappos, and H. Wekerle. 1990. Myelin basic protein-specific T lymphocyte lines from MS patients and healthy individuals. Neurology. 40:1770-1776.

45. Jingwu, Z., R. Medaer, G.A. Hashim, Y. Chin, E. van den Berg-Loonen, and J.C. Raus. 1992. Myelin basic protein-specific T lymphocytes in multiple sclerosis and controls: precursor frequency, fine specificity, and cytotoxicity. Ann. Neurol. 32:330-338.

46. Voskuhl, R.R., R. Martin, and H.F. McFarland. 1993. A functional basis for the association of HLA class II genes and susceptibility to multiple sclerosis: cellular immune responses to myelin basic protein in a multiplex family. J. Neuroimmunol. 42:199-207.