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IL7Rα Contributes to Experimental Autoimmune Encephalomyelitis through Altered T Cell Responses and Nonhematopoietic Cell Lineages

Jessica J. Ashbaugh,*+ Roberta Brambilla,* Shaffiat A. Karmally,† Cecilia Cabello,‡ Thomas R. Malek,* and John R. Bethea*+§

A mutation in the IL7Rα locus has been identified as a risk factor for multiple sclerosis (MS), a neurodegenerative autoimmune disease characterized by inflammation, demyelination, and axonal damage. IL7Rα has well documented roles in lymphocyte development and homeostasis, but its involvement in disease is largely understudied. In this study, we use the experimental autoimmune encephalomyelitis (EAE) model of MS to show that a less severe form of the disease results when IL7Rα expression is largely restricted to thymic tissue in IL7RTg IL7Rα−/− mice. Compared with wild-type (WT) mice, IL7RTg IL7Rα−/− mice exhibited reduced paralysis and myelin damage that correlated with dampened effector responses, namely decreased TNF production. Furthermore, treatment of deceased WT mice with neutralizing anti-IL7Rα Ab also resulted in significant improvement of EAE. In addition, chimeric mice were generated by bone marrow transplant to limit expression of IL7Rα to cells of either hematopoietic or nonhematopoietic origin. Mice lacking IL7Rα only on hematopoietic cells develop severe EAE, suggesting that IL7Rα expression in the nonhematopoietic compartment contributes to disease. Moreover, novel IL7Rα expression was identified on astrocytes and oligodendrocytes endogenous to the CNS. Chimeric mice that lack IL7Rα only on nonhematopoietic cells also develop severe EAE, which further supports the role of IL7Rα in T cell effector function. Conversely, mice that lack IL7Rα throughout both compartments are dramatically protected from disease. Taken together, these data indicate that multiple cell types use IL7Rα signaling in the development of EAE, and inhibition of this pathway should be considered as a new therapeutic avenue for MS. The Journal of Immunology, 2013, 190: 4525–4534.

Multiple sclerosis (MS) is a debilitating autoimmune disease of the CNS causing severe neurologic damage in young adults (1–3). MS and its animal model experimental autoimmune encephalomyelitis (EAE) are characterized by extensive inflammation and axonal injury, ultimately leading to severe neurodegeneration (1–5). Although several immune mediators likely contribute to the detrimental microenvironment within the CNS, it is proposed that Th cells are a driving force in the disease, namely through the Th1 and Th17 subsets with their production of inflammatory IFN-γ and IL-17, respectively (6–8). TNF, another effector cytokine, is known to have both pro- and anti-inflammatory effects in EAE and MS (9, 10). These particular cytokines heavily impact pathogenesis; however, the specific etiology behind the autoimmune response is still elusive.

Although environmental factors may influence disease susceptibility (11–16), there is substantial evidence coupling a strong genetic component to MS as well (17–25). Multiple risk alleles have been identified through genome-wide association studies, many of which are related to the immune system (17–23). In particular, single-nucleotide polymorphisms (SNPs) in the gene encoding IL7Rα have emerged through genetic studies of MS patients with differing ethnic backgrounds. The SNP (rs6897932; T244I) most frequently identified in the risk allele is located at an alternative splice site within the SNP background. The SNP with low levels of exon 6 skipping, leading to a modest increase in the soluble isoform of IL7Rα (25, 29). This was supported by quantitative RT-PCR analysis of PBMCs from healthy patients, which presented a decrease in IL7Rα for carriers of the risk allele (25). However, opposing results have been reported in relapsing-remitting MS patients where IL7Rα transcript was increased in PBMCs compared with controls (30). Nevertheless, MS patients also displayed increased soluble IL7Rα in cerebrospinal fluid compared with individuals with other noninflammatory neurologic diseases, suggesting specificity in MS (27). Furthermore, it was recently shown that IL-7–mediated stimulation of IL7Rα promotes Th1 differentiation (31) and is also implicated in the survival and expansion of pathogenic Th17 cells (32) in EAE and MS. Collectively, these findings have shifted the focus of IL7Rα biology from its established roles in lymphocyte development, homeostatic survival, and proliferation (33–37) to its potential contributions in disease settings.

Aside from the immune system, the IL-7/IL7Rα signaling pathway has been proposed to function within the CNS. Indeed, IL7Rα transcripts have been identified in whole mouse brain extracts as...
well as rat cultured subventricular zone progenitors and embryonic neurons (38). Furthermore, IL-7 was found to promote outgrowth and survival of neuronal cultures (38). With respect to astrocytic expression, no transcript was detected in rat primary cultures (38); however, transcript and translated proteins have been reported on human primary astrocytes (39). It has also been reported that astrocytes in human MS brain tissue secrete IL-7 (40). Taken together, these findings highlight the importance of expanding the experimental scope to include non-T cell lineages during the investigation of IL7Rα in EAE.

The main objective of our studies was to determine how IL7Rα contributes to disease development and progression in the EAE model of MS. In this study, we show, both with a genetic (IL7RTgIL7Rα−/− mice) and a pharmacologic approach (anti-IL7Rα Ab administration), that systemic inhibition of IL7Rα signaling greatly reduces the overall severity of EAE. This is accomplished in part by an altered T cell effector response, namely the dramatic loss of TNF-producing T cells. We also show that IL7Rα inhibition in bone marrow (BM)–derived cells alone is not sufficient to induce protection from EAE. This suggests that nonhematopoietic lineages expressing IL7Rα must also partake in disease, for which we have identified astrocytes and oligodendrocytes as plausible candidates. These findings significantly enhance our understanding of IL7Rα function and the impact it has in EAE, underscoring the importance of taking this pathway into consideration when developing new MS therapies.

Materials and Methods

Mice

Experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the University of Miami. All mice used were 2–6 mo of age on the C57BL/6J background. Females were predominantly used; however, a small proportion of males were included to identify sex-linked differences; none was noted. C57BL/6J control mice were obtained from Charles River Laboratories and The Jackson Laboratory. IL7RTgIL7Rα−/− mice were generated as described previously (34, 41). Briefly, mice containing a wild-type (WT) IL7Rα transgene driven by the proximal Ick promoter were crossed to IL7Rα−/− mice (B6.129S7-Ilt7r tm1Imx/J) originally obtained from The Jackson Laboratory.

TSLPR−/− (B6;129-Crlf2tm1Wjl/Mnmc) cryopreserved embryos were obtained from Mutant Mouse Regional Resource Centers, and line resuscitation was carried out by the Transgenic Animal Core Facility at Sylvester University of Miami, Miami, FL), γMT mice (B6.129S2-Igh-6tm1Cgn), Rag1−/− mice (B6.129S7-Rag1 tm1Mom/J), and CD45.1 congenic WT mice (B6.SJL-p7rca/BoA1Tac) were originally obtained from The Jackson Laboratory and Taconic Farms, respectively. IL7Rα−/−GFP mice were a gift from A. Singer (National Cancer Institute, Bethesda, MD). Animals were housed in a virus/Ag-free facility with a 12-h light/dark cycle, controlled temperature and humidity, and provided with water and food ad libitum.

Induction of active EAE and assessment of functional recovery

Age-matched mice were immunized with myelin oligodendrocyte glycoprotein (MOG)35–55 peptide (Biosynthesis, Lewisville, TX), as described previously (42, 43). Briefly, mice were i.p. injected with pertussis toxin (425 ng/mouse) on days 0 and 2. In addition, mice are injected s.c. on days 1 and 7 with 150 µg MOG peptide emulsified in CFA. Clinical signs of EAE are assessed daily using a standard scale of 0–6 as follows: 0, no signs; 1, slight gait; 1.5 moderate to severe loss of tail tone 2, flaccid tail; 2.5 flaccid tail and difficulty in walking 3, complete hind-limb paralysis; 3.5, complete hind-limb paralysis and partial forelimb tone 2, flaccid tail and difficulty in walking 3, complete hind-limb paralysis and total loss of forelimb function 4, moribund; and 6, death. Clinical onset of EAE are assessed daily using a standard scale of 0–6 as follows: 0, no signs; 1, slight gait; 1.5 moderate to severe loss of tail tone 2, flaccid tail; 2.5 flaccid tail and difficulty in walking 3, complete hind-limb paralysis; 3.5, complete hind-limb paralysis and partial forelimb paralysis; 4, total paralysis; 4.5, total paralysis and very poor health (incontinence, etc.); 5, moribund; and 6, death. Clinical onset of EAE is considered when mice first achieve a score of 2. The clinical disease index (CDI) is a measure of disease severity and is calculated as the sum of clinical scores for each mouse over the course of the study.

Histology

Animals were perfused with 0.1 M PBS, followed by 4% paraformaldehyde in PBS, spinal cords (SCs) removed, and postfixed overnight. Tissues were cryopreserved in PBS plus 25% sucrose and cryostat-cut into 15-µm-thick transverse sections. Myelinated white matter was identified in thoracic SC sections with luxol fast blue stain. H&E counterstains further identified inflammatory infiltration and areas of myelin damage. Staining took place at the Histology Core Facility, Miller School of Medicine, University of Miami. Images were obtained with an Olympus BX51 upright microscope.

In vivo neutralization

WT mice were induced with EAE and i.p. injected with anti-IL7Rα (A7R34; BioXcell) or IgG control (Sigma-Aldrich) at 20 µg/g body weight upon disease onset, which was determined once the majority of mice displayed a clinical score of ≥2. A total of 10 injections were administered at 48-h intervals starting from day 19.

Leukocyte isolation from spleen and SC

Individual spleens or SCs were harvested at specified time points. Tissue homogenates were passed through 70-µm cell strainers. RBCs from spleens were lysed with Tris-buffered ammonium chloride (140 mM NH4Cl and 17mM Tris [pH 7.65]). Immune cells from SCs were enriched by negative selection with myelin removal magnetic beads and columns, according to manufacturer’s protocol (Miltenyi Biotec). Cell numbers were assessed by trypan blue staining.

Flow cytometry

Nonsteroidal anti-inflammatory drugs (NSAIDs) were examined for an anti-FcR block (2.4G2) (44) prior to adding the following surface marker Ab conjugates: CD45-FITC (1:300), CD6-PerCP-Cy5.5 (1:80), CD25-PE-Cy7 (1:80), and CD127-biotin (1:100) from eBioscience; CD45.1-biotin (1:200), CD45.2-FITC (1:200), and streptavidin-PE (1:50) from BioLegend; and CD8a-APC-H7 (1:100) from BD Pharmingen. Cells were then fixed overnight with 1% paraformaldehyde in flow cytometry buffer or fixed and permeabilized (Fix/Perm kit; eBioscience) prior to intracellular staining with Foxp3-eFluor450 (1:100) from eBioscience. All incubations were performed at 30 min, 4°C. Cells were analyzed using an Accuri3 or LSRII flow cytometer and FACS Diva software (BD Biosciences).

Ex vivo restimulation

Tissues were harvested and processed as above. Prior to staining, cells were incubated at 107–108 cells/ml with PMA (50 ng/ml; Sigma-Aldrich), ionomycin calcium salt (0.75 µg/ml; Sigma-Aldrich), and 1 µl Golgi Plug protein transport inhibitor (BD Biosciences) for 4 h at 37°C, 5% CO2. Cells were stained, fixed, and permeabilized as above using TNFα-PerCP-eFluor710 (1:100), IFNγ-eFluor450 (1:100), and IL17A-AlexaFluor647 (1:100) intracellular Abs from eBioscience.

Mice chimeras

BM cells were isolated as follows: femur and tibia were collected from both hind limbs of donor mice. BM was flushed out with sterile HBSS, and single-cell suspensions were obtained following repeated cycles of aspiration through a syringe with a 26-gauge needle. T cells were depleted by incubating suspensions with anti-CD4 (RL-172) and anti-CD8 (HO2.2) Abs and fresh Low Tox-M Rabbit Complement (Cedarlane Laboratories) for 45 min at 37°C, 5% CO2. Donor cells were washed with HBSS and then i.v. injected (tail vein, 5 × 106 cells/mouse) into recipient mice that were gamma-irradiated (300–900 rad; GammaCell 40, 251Cs source) 1 d in advance. Recipients were maintained on oral antibiotics for 2 wk post-BM transplant and allowed at least 9 wk recovery before EAE induction.

Immunofluorescence staining

Animals were perfused with 0.1 M PBS, followed by 4% paraformaldehyde in PBS, and SCs were removed and postfixed overnight. Tissues were cryopreserved in PBS plus 25% sucrose and cryostat-cut into 15-µm-thick transverse sections. After blocking for 1 h with 5% normal goat serum in 0.1 M PBS plus 0.4% Triton X-100, sections were incubated overnight at 4°C with primary Abs against glial fibrillary acidic protein (GFAP; mouse, 1:500, BD Biosciences), CCl1 (mouse, 1:500; Calbiochem), and GFAP (rabbit, 1:50; Santa Cruz Biotechnology). Immunoreactivity was visualized either with secondary species-specific fluorescent Abs (Alexa Fluor 594 and 488; Invitrogen). Images were obtained with an Olympus Fluoview 1000 confocal microscope.

Statistical analysis

Statistical analysis of the clinical course of EAE was carried out with the Mann–Whitney U test. For single comparisons, Student t test was applied. p ≤ 0.05 was considered statistically significant.
Results

IL7Rα deficiency yields EAE protection

Although IL7Rα−/− mice would be the obvious choice to directly address the role of IL7Rα in EAE pathophysiology, the use of this genetic model has significant limitations because of the severely impaired T cell development, which is driven by IL7Rα signaling under normal conditions (45, 46). As a result, IL7Rα−/− mice lack the cellular repertoire necessary for normal EAE pathogenesis (47, 48). To circumvent this issue, we turned to the use of IL7RTgIL7Rα−/− mice, which were engineered to express IL7Rα in the thymus of IL7Rα−/− mice. The rescued IL7Rα expression allows for sufficient T cell development while maintaining the IL7Rα-deficient phenotype in extrathymic tissues (Fig. 1A) (34, 41). Analysis of total T cell number in the spleens of naive IL7RTgIL7Rα−/− mice revealed that CD4 T cells were restored to WT levels, and CD8 T cells improved by >50%, all of which were negative for IL7Rα expression by flow cytometry as expected (Fig. 1A). This was in stark contrast to IL7Rα−/− mice where total T cell numbers amounted to <5% of WT (Fig. 1A). Notably, B cell maturation was stunted in both IL7Rα−/− and IL7RTgIL7Rα−/− mice (Fig. 1A) because of the lack of IL7Rα signaling in the BM (49). Although B cells do contribute to EAE/MS pathology (50), they are not required for disease onset or progression in the C57BL/6 background following induction by the MOG35–55 peptide (51) and therefore are not expected to interfere with the EAE paradigm of IL7RTgIL7Rα−/− mice in these studies. To further confirm this, we induced EAE in B cell–deficient μMT mice with the MOG35–55 peptide as previously described (42, 43) and found indeed that they developed EAE similar to WT mice (Fig. 1B).

We proceeded to induced EAE in IL7RTgIL7Rα−/− mice with the MOG35–55 peptide and found that IL7RTgIL7Rα−/− mice developed significantly less severe EAE compared with WT controls. Although onset of disease was similar between groups (Table I), clinical scores were significantly lower in IL7RTgIL7Rα−/− mice and showed steady improvement through the chronic state, whereas WT developed worse EAE. Indeed, the average clinical score of IL7RTgIL7Rα−/− mice corresponded to 1.5 at peak disease (20 dpi) and recovered to a score of 1 at the chronic state (40 dpi), signifying only mild flaccidity of the tail and no hind-limb paralysis (Fig. 2A). On the contrary, WT mice reached clinical scores >2 from peak disease (20 dpi) onward showing varying degrees of hind-limb and forelimb paralysis (Fig. 2A). The difference in EAE severity was also reflected in the significantly reduced CDI and incidence of disease in IL7RTgIL7Rα−/− mice compared with WT (Table I). Histological analysis of myelin with luxol fast blue on thoracic SC cross-sections revealed that IL7RTgIL7Rα−/− mice had fewer and smaller lesions caused by infiltrating leukocytes (Fig. 2B), hence, reduced myelin damage. This paralleled the improved clinical scores of IL7RTgIL7Rα−/− mice compared with WT controls. To further confirm the involvement of IL7Rα in EAE and complement our genetic studies, we administered anti–IL7Rα-neutralizing Ab (A7R34) following induction of EAE. Ab administration was started at peak disease for a more therapeutically relevant treatment paradigm. Mice that received anti–IL7Rα showed significant recovery compared with IgG-treated controls (Fig. 2C), with an EAE profile similar to IL7RTgIL7Rα−/− mice (Fig. 2A). The anti–IL7Rα-mediated recovery correlated with reduced levels of myelin damage in thoracic SC sections at 55 dpi (Fig. 2D). The EAE recovery was not simply because of T cell depletion by Ab-dependent cell-mediated cytotoxicity, because T cells bound to anti–IL7Rα were readily detected during treatment (Supplemental Fig. 1), opening the possibility that instead T cell function may be altered.

The clinical scores and resulting EAE pathology in IL7RTgIL7Rα−/− and anti–IL7Rα-treated WT mice support the hypothesis that IL7Rα signaling does in fact contribute to disease. However, IL7Rα must heterodimerize with either the common γ chain or the thymic stromal lymphopoietin (TSLP) receptor chain to propagate downstream signaling stimulated by IL-7 or TSLP, respectively (52–54). Consequently, signaling mediated by both ligands is defective in IL7RTgIL7Rα−/− and anti–IL7Rα-treated mice. However, functional studies in TSLP-mediated signaling result in the induction of a Th2 response and therefore are not likely to play a role in EAE. To confirm this, we used a TSLPR−/− mouse line whose IL–7/IL7Rα signaling axis remains intact. Under naive conditions, TSLPR−/− mice did not appear to have any deficiencies neither in IL7Rα expression nor in lymphocyte distribution as detected by flow cytometry with a panel of leukocyte markers in the spleen (Supplemental Fig. 2A). We then induced EAE in the TSLPR−/− mice and WT controls using the MOG35–55 peptide as before and found that a deficit in the TSLP-mediated pathway has no effect on the onset or progression of EAE (Sup-

Table I. Clinical parameters of EAE in IL7RTgIL7Rα−/− mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (%)</th>
<th>Day of Onset</th>
<th>CDI</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>93 (14/15)</td>
<td>17.0 ± 0.3</td>
<td>52.8 ± 6.7</td>
</tr>
<tr>
<td>IL7RTgIL7Rα−/−</td>
<td>62 (8/13)*</td>
<td>16.1 ± 0.8</td>
<td>30.5 ± 8.3*</td>
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*Results represent the mean ± SEM.

Supplementary Figure 1. Clinical scores were calculated as the sum of clinical scores for each animal between days 0 and 40.

*p < 0.05 with respect to WT; Student t test.
Anti-IL7R

Fast blue with H&E counterstains. Lower panels with PMA and ionomycin showed that IL7RTg IL7R might be affected during EAE. Ex vivo restimulation of splenocytes investigated how the T cell response from IL7RTg IL7R classes, which are integral to EAE development (31, 32). Therefore, T cell function, particularly with respect to Th1 and Th17 subtypes, has been suggested that IL7Rα deficiency alters T cell activation and cytokine expression.

IL7Rα deficiency alters T cell activation and cytokine expression

Once cognate Ag is recognized by T cells, activation and rapid amplification of the T cell pool occurs to facilitate the immune response (55). Because this response and subsequent CNS infiltration are paramount to EAE pathophysiology, we evaluated T cell numbers and functional state in IL7RTgIL7R−/− and WT mice at the acute phase of EAE (25 dpi). Significantly fewer CD4 and CD8 T cells were found in the spleens of IL7RTgIL7R−/− mice compared with WT (Fig. 3A), whereas T cells infiltrating the SC only showed significant reductions in the CD4 subpopulation (Fig. 3B). Recently, it has been suggested that IL7Rα signaling may also contribute to T cell function, particularly with respect to Th1 and Th17 subclasses, which are integral to EAE development (31, 32). Therefore, we investigated how the T cell response from IL7RTgIL7R−/− mice might be affected during EAE. Ex vivo restimulation of splenocytes with PMA and ionomycin showed that IL7RTgIL7R−/− mice had dramatic changes in both distribution and total number of functional T cell subsets. First, IL7RTgIL7R−/− mice showed a significant reduction (∼50%) in the relative percentage and overall number of splenic T cells producing TNF compared with WT mice (Fig. 3C, 3D, 3F, 3G). Second, the percentage of splenic IFN-γ-producing CD4 T cells increased in IL7RTgIL7R−/− mice, although the actual cell number was similar to WT (Fig. 3C, 3D). In contrast, the percentage of splenic CD8 T cells producing IFN-γ was comparable between WT and IL7RTgIL7R−/− mice, yet total numbers were significantly lower in the IL7RTgIL7R−/− mice (Fig. 3F, 3G). Ex vivo restimulation of SC-infiltrating cells showed significant reductions in both TNF-producing and IFN-γ−producing CD4 T cells in IL7RTgIL7R−/− mice (Fig. 3E); however, no significant differences were detected in CD8 T cells (Fig. 3H). Interestingly, IL-17−producing T cells were minimal to absent during acute disease, both in spleen and SC (Fig. 3I–H).

The overall lack of IL-17−producing T cells at acute disease was unexpected. Nevertheless, Th17 function has been shown to precede that of Th1 in EAE and could occur even prior to the appearance of clinical symptoms (56). To test whether this was the case in our model, we analyzed T cell effector function upon ex vivo stimulation at the predisease time point of 12 dpi (Fig. 4). Similar to acute disease, marked reductions were seen in the overall amount of T cells in IL7RTgIL7R−/− spleens (Fig. 4A). As predicted, IL-17−producing CD4 T cells were higher than in acute disease and were more abundant in WT compared with IL7RTgIL7R−/− mice (Figs. 3C, 3D, 4B, 4C). Interestingly though, splenic TNF and IFN-γ T cell profiles mirrored that of 25 dpi with robust TNF.
production in WT mice that was dramatically reduced in CD4 and CD8 T cells of IL7RTg IL7R<sup>2</sup>/2 mice (Fig. 4B–E). Likewise, IFN-γ–producing CD8 T cells were also significantly diminished in IL7RTg IL7R<sup>2</sup>/2 mice (Fig. 4D, 4E). In the SC, infiltrating immune cells were extremely scarce at 12 dpi, and reliable comparisons could not be made (data not shown). Taken together, IL7R<sup>a</sup> deficiency reduced the classical Th17 phenotype early on in the periphery. This, however, did not persist through acute disease, unlike the effects on TNF or IFN-γ–producing T cells, which were impacted far more and for a longer period of time. Overall, we show that IL7R<sup>a</sup> blockade reduces the T cell response and represents one contributing factor to less severe disease.

EAE pathology is dependent on IL7R<sup>a</sup> expressed on both hematopoietic and nonhematopoietic cells

Although IL7R is most commonly associated with lymphocytes, it has also been identified on dendritic cells, lymphatic microvascular endothelium, and stromal cells (33, 57, 58). In addition, IL7R<sup>a</sup> has been detected in cultured rat hippocampal neurons and mouse subventricular zone progenitors (38) as well as human neuronal and astrocyte cultures (39), warranting the possibility that IL7R<sup>a</sup> may contribute to EAE by signaling directly in the CNS. For that reason, we generated a series of chimeric mice to identify whether IL7R<sup>a</sup> expression on non–BM-derived cells contributes to EAE. Because we showed that IL7RTg IL7R<sup>2</sup>/2 mice have lower T cell–dependent inflammatory responses, we first wanted to determine whether IL7R<sup>a</sup> deficiency on BM-derived cells alone would be sufficient for a reduced EAE phenotype. Therefore, we generated chimeras by transplanting BM cells from IL7RTg IL7R<sup>2</sup>/2 mice or CD45.1 congenic WT mice into lethally irradiated Rag1<sup>2</sup>/2 recipients that retain normal IL7R<sup>a</sup> expression in nonhematopoietic cells but lack lymphocyte competition. Upon reconstitution of the immune compartment (Supplemental Fig. 3), EAE was induced in both chimeras and nonirradiated WT controls. Surprisingly, animals that received IL7RTg IL7R<sup>2</sup>/2 donor BM, hence, lacking IL7R<sup>a</sup> in hematopoietic cells, were not protected from disease. No significant differences
FIGURE 4. IL7Rα deficiency alters T cell responses prior to EAE clinical symptoms. Quantification of total T cell numbers are shown for the spleen of indicated mice at acute EAE (25 dpi) following ex vivo stimulation and flow cytometry analysis. Total CD4 and CD8 T cell populations were analyzed after gating on the CD45 leukocytes (A). Representative dot plots for TNF, IFN, and IL-17 expression within the CD4 (B) and CD8 (D) gates are shown. Cytokine producing CD4 (C) and CD8 (E) T cells were quantified. Results are expressed as the mean ± SEM with n = 13/group from three independent experiments. *p < 0.05, **p < 0.005, ***p < 0.0005; unpaired t test.

were seen in overall clinical scores (Fig. 5A) or incidence of disease between all three groups (Table II). However, chimeric mice receiving the IL7RTgIL7R−/− BM displayed an earlier onset of disease and modest increase in CD45 leukocyte infiltration in all three groups (Fig. 5B). In contrast to the SC, peripheral CD4 and CD8 T cells were drastically reduced in Tg→→ Rag1−/− chimeras (~4-fold) and moderately reduced in WT→→ Rag1−/− chimeras (~2-fold) compared with WT. Reductions in splenic CD4/CD25/Foxp3+ regulatory T cells were only seen in Tg→→ Rag1−/− chimeras (Fig. 5B). Therefore, hindering IL7Rα signaling specifically on BM-derived cells may alter peripheral expansion in the chimeric model but has less effect on leukocyte infiltration of the SC and is not sufficient to protect from disease.

It is important to note, however, that IL7Rα signaling remains functional on all host-derived cell types in these chimeric mice, and therefore, signaling on non–BM-derived lineages is still capable of contributing to EAE progression. For that reason, we wanted to identify whether IL7Rα deficiency on non–BM-derived cells would be sufficient for disease reduction, or whether the IL7Rα deficiency is required on cells from both compartments to achieve protection from EAE. Hence, BM cells from IL7RTgIL7R−/− or CD45.1 congenic WT were transplanted into irradiated IL7R−/− recipients to generate additional chimeric mice. Upon EAE induction, we saw again that chimeric mice receiving WT BM showed no differences in clinical score (Fig. 5C), incidence of disease (Table III), or immune cell infiltration into the SC (Fig. 5D) compared with WT controls through acute disease (24 dpi). However, the chimeric mice generated to have IL7Rα deficiency in both BM and non–BM-derived cell types, hence, resembling the phenotype of IL7RTgIL7R−/− mice, were dramatically protected from EAE (Fig. 5C, Table III) and had little immune infiltration into the SC overall (Fig. 5D). Similarly, peripheral T cell numbers were significantly fewer in Tg→→ IL7R−/− chimeric mice for most T cell subsets (Fig. 5D). Notably, the only peripheral cell type identified as similar between all three groups was CD4/CD25/Foxp3+ regulatory T cells (Fig. 5D), suggesting that differences in effector T cell expansion may be independent of regulatory T cell function. Overall, these data show that blocking IL7Rα in both hematopoietic and nonhematopoietic cell types is required for EAE protection. It is possible then that cells endogenous to the CNS may directly contribute to disease pathogenesis via IL7Rα signaling as well as facilitate proinflammatory function of immunogenic T cells.

IL7Rα expression in the CNS

Although there are multiple potential sources for the nonhematopoietic contributions of IL7Rα signaling in EAE, we chose to focus on the CNS directly because this is the affected target tissue and because the signaling pathway has been implicated in neurons and astrocytes using in vitro model systems (39, 59). Using reporter mice expressing GFP under the IL7Rα promoter and immunofluorescence staining of thoracic SC sections, we show that the IL7Rα-GFP colocalizes with the majority of CC1+ mature oligodendrocytes and a large proportion of GFAP+ astrocytes (Fig. 6). IL7Rα-GFP colocalization was not observed for NeuN+ neurons or CD11b+ microglial cells (data not shown).

Discussion

Our study demonstrates that IL7Rα signaling contributes to EAE through mechanisms involving not only modulation of T cell effector function but also the participation of non–BM-derived cell types that use this pathway to drive disease, in addition to showing novel expression of IL7Rα directly in the CNS. The attenuation of EAE clinical scores and myelin damage seen in our IL7RTgIL7R−/− model, in conjunction with recovery after in vivo neutralization of
IL7Rα, supports the hypothesis that IL7Rα signaling plays a role in EAE pathogenesis. The improved outcome is partially because of select subpopulations of T cells being sensitive to a lack of IL7Rα signaling, diminishing the overall effect of inflammatory cytokine production, particularly TNF. However, it is evident that the scope of IL7Rα expands beyond T cells with respect to EAE pathogenesis, encompassing nonlymphocyte populations, potentially astrocytes and oligodendrocytes local to the CNS. Our findings build upon the genome-wide association studies linking IL7Rα to MS as well as data accumulated in other models of IL7Rα inhibition in EAE, further highlighting the potential of IL7Rα as a target in treatment strategies for MS.

As with most functional studies of signaling pathways, we turned to an IL7Rα-deficient model to further dissect the role of

Table II. Clinical parameters of EAE in Rag1−/− chimera mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (%)</th>
<th>Day of Onset</th>
<th>CFI a,b</th>
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<tr>
<td>WT</td>
<td>74 (14/19)</td>
<td>14.1 ± 0.4</td>
<td>8.7 ± 1.5</td>
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<tr>
<td>WT → Rag1−/−</td>
<td>88 (14/16)</td>
<td>14.9 ± 0.4</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td>IL7RTgIL7R−/− → Rag1−/−</td>
<td>91 (10/11)</td>
<td>11.9 ± 1.6*</td>
<td>15.1 ± 3.1**</td>
</tr>
</tbody>
</table>

*aResults represent the mean ± SEM.
*bDI was calculated as the sum of clinical scores for each animal between days 0 and 18.
*p < 0.05 with respect to WT→Rag1−/−; Student t test; **p < 0.05 with respect to WT; Student t test.
IL7Rα in EAE. However, because of the versatility in downstream effects of IL7Rα signaling, a traditional IL7R−/− line is not suitable for use in the EAE system. It is well known that T cells play a key role in EAE pathogenesis, and IL7R−/− mice are highly deficient in T cell development (3, 45, 46). Walline et al. (60) have shown that IL7R−/− mice are in fact resistant to EAE, associating the effect with reduced IL-17 and IFN-γ production in T cells. Nevertheless, the conclusions were based on the relatively rare production of T cells in IL7R−/− mice (Fig. 1) (45), making it difficult to assess whether the EAE resistance was truly due to altered T cell function or simply the result of insufficient T cell numbers to elicit an immune response in EAE. Therefore, we chose to take advantage of IL7RTgIL7R−/− mice in these studies, which retain ample T cell development while maintaining the IL7Rα-deficient phenotype in nonthymic tissues. Hence, the IL7RTgIL7R−/− mice allow us to more accurately determine how the T cell pool is affected in vivo. Using these unique IL7RTgIL7R−/− mice, we show they too are resistant to EAE, albeit to a lesser extent than IL7R−/− mice, highlighting the importance of T cell number alongside effector function.

Neutralizing Abs have also proven useful as an approach to specifically target signaling pathways and inhibit their function. In this study, we show that a unique anti-IL7Rα mAb, A7R34, yields therapeutic effects by reducing EAE clinical scores along with the associated myelin damage. Recently, it was shown that two additional anti-IL7Rα mAbs, SB/14 or S8G9, were also able to attenuate the clinical scores of MOG-induced EAE by in vivo neutralization (31, 32). The general reduction in EAE phenotype following all three modes of treatment further confirms the involvement of IL7Rα signaling in this model. Moreover, these results also support the assumption that a defective B cell compartment in the IL7RTgIL7R−/− mice is not the cause of their reduced disease, because EAE-induced WT mice treated with anti-IL7Rα have substantial B cell numbers available prior to and during treatment, yet average clinical scores resemble that of IL7RTgIL7R−/− mice.

For many years, IL7Rα signaling was ignored with respect to its contributions in the effector phase of the T cell response, because its expression is downregulated upon activation (61). However, it is becoming more evident that the IL-7 signaling axis can influence select effector subsets prior to receptor downregulation, although to what extent is still unclear. For example, in previous studies neutralizing IL7Rα in vivo with Abs, conclusions differed in that the SB/14 clone alters the Th17 response, whereas clone 28G9 modifies the Th1 response (31, 32). These conflicting results highlight the complexity of IL7Rα signaling, suggesting the possibility that this pathway may differentially affect multiple effector populations. It is also possible that these discordant outcomes could simply be a result of clonal variations in the anti-IL7Rα Abs, which may result in different binding affinities toward the receptor, hence, altering the capacity to neutralize signal. Therefore, we chose to use the IL7RTgIL7R−/− mice to further investigate the effect of IL7Rα inhibition on the entire T cell population and within the various subsets that contribute to the immune response in EAE.

In these studies, we observed that IL7RTgIL7R−/− mice have fewer total T cell numbers in the spleen compared with WT at both preclinical and acute disease time points. Thus, despite having an equivalent CD4 T cell reserve prior to EAE induction, the IL7Rα deficiency dampens the initial T helper response. Likewise, EAE-induced IL7RTgIL7R−/− mice also have fewer CD8 T cells compared with WT; however, the initial differences in naive splenic CD8 T cell number means that the correlation between IL7Rα deficiency and fewer CD8 T cells at 12 and 25 dpi is not necessarily a function of disease. Indeed, studies under homeostatic conditions show that CD8 T cells appear more sensitive to a block in IL7Rα signaling in comparison with CD4 T cells (35, 62), although the reasons behind this general difference have yet to be determined. In fact, one might argue that the lower baseline number of CD8 T cells could in itself contribute to the overall reduction in EAE of IL7RTgIL7R−/− mice. However, it is important to note that this lower number in the spleen did not correlate with reduced CD8 T cell infiltration into the SCs of IL7RTgIL7R−/−

### Table III. Clinical parameters of EAE in IL7R−/− chimera mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Incidence (%)</th>
<th>Day of Onset</th>
<th>CDI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>91 (20/22)</td>
<td>15.6 ± 0.6</td>
<td>25.4 ± 3.5</td>
</tr>
<tr>
<td>WT → IL7R−/−</td>
<td>83 (10/12)</td>
<td>16.0 ± 0.8</td>
<td>27.25 ± 6.0</td>
</tr>
<tr>
<td>IL7RTgIL7R−/−→IL7R−/−</td>
<td>8 (1/13)</td>
<td>23.0</td>
<td>0.4 ± 0.4***</td>
</tr>
</tbody>
</table>

*p values are based on n = 1.

Results represent the mean ± SEM.

CDI is calculated as the sum of clinical scores for each animal between days 0 and 24.

Neurologic severity was scored on a scale of 0 to 9, and clinical severity was scored on a scale of 0 to 4.

No statistics because of n = 1.

*p < 0.0001 with respect to WT, Student t test; **p < 0.0001 with respect to WT, Student t test.

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**FIGURE 6.** IL7Rα expression on astrocytes and mature oligodendrocytes. Representative immunofluorescence staining against IL7Rα-GFP, CC1, and GFAP in thoracic SC sections taken from IL7Rα-GFP reporter mice (top two panels). Anti-rabbit IgG-488 staining of IL7Rα-GFP SC sections, and anti-GFP staining of WT SC sections (bottom two panels) were used as negative controls. Scale bar, 50 µm.
mice. Because there were relatively few significant differences between IL7RTgIL7R−/− and WT mice with respect to the CD8 T cell population in the SC, the negative contributions of IL7Rα signaling are more likely to be of consequence in the CD4 helper subsets.

It is known that Th1 and Th17 cells contribute to the damaging microenvironment in EAE by secreting inflammatory cytokines such as TNF, IFN-γ, and IL-17 that both directly and indirectly lead to destruction of myelin and axonal injury (10, 63–66). Therefore, we went on to examine how T cell function was being affected in EAE-induced IL7RTgIL7R−/− mice. In this study, we show that deficiency in IL7Rα signaling has the most profound effect on TNF-producing CD4 and CD8 T cells. Compared with WT mice, TNF is strikingly less abundant in both peripheral and SC infiltrating cells of IL7RTgIL7R−/− mice, which is in stark contrast to the comparatively small reductions seen in T cell IFN-γ and IL-17.

The profound reduction of TNF-producing T cells is particularly interesting because we have recently shown that the functional outcome of EAE is improved by selectively blocking the secreted form of TNF while permitting transmembrane TNF to signal normally (10). This is because the soluble form of TNF favors TNFR1 binding, which supports chronic inflammation (67), whereas transmembrane TNF preferentially binds TNFR2, promoting anti-inflammatory responses as well as myelin maintenance in the CNS (68, 69). Therefore, the striking drop in TNF-secreting T cells infiltrating into the CNS and in the periphery of IL7RTgIL7R−/− is functionally relevant to the observed EAE protection and is likely affecting the detrimental soluble form of TNF rather than beneficial transmembrane TNF.

In addition to expanding the role of IL7Rα within the T cell pool, our data shed light on other cellular compartments whose IL7Rα function contributes to EAE. Until now, T cells have been the only targets of IL7Rα investigation in this model, and only in the context of systemic IL7Rα inhibition (i.e., genetic manipulation or neutralizing Ab). In our studies, using the BM–chimera model system enabled further dissection of how IL7Rα is functioning in the disease setting. Indeed, we show that IL7Rα cell types other than leukocytes are likely contributing to the pathogenesis of EAE. The severe disease phenotype in IL7RTgIL7R−/− →Rag1−/− chimeric mice, which have functioning IL7Rα on nonhematopoietic cells, denotes that there is at least one additional IL7Rα cell type in the periphery contributing to EAE. However, because WT→IL7R−/− chimeric mice display a classic disease progression, we can also conclude that eliminating IL7Rα signaling within the non–BM-derived compartment alone is insufficient in attenuating EAE. Furthermore, these results support our data showing that normal IL7Rα signaling contributes to the T cell effector response in EAE.

Because only IL7RTgIL7R−/−→IL7R−/− chimeric mice have reduced EAE, it appears that IL7Rα, at the very least, functions through two cellular pathways to collectively drive disease. One speculation is that these cells would also produce downstream effector cytokines, such as TNF, to propagate disease. Compensatory function such as this could explain why EAE still occurs in IL7RTgIL7R−/−→Rag1−/− chimeric mice where T cell function is impaired. It has been shown that microglia and astrocytes are capable of producing TNF during inflammatory responses in the CNS (70, 71), so we hypothesized that IL7Rα may also be expressed on these cells to promote endogenous TNF production during the inflammatory response of EAE. Using IL7Rα–GFAP reporter mice, we positively identified IL7Rα on GFAP+ astrocytes, but not microglia, making astrocytes a strong candidate for further investigation in the IL7Rα-deficient models of EAE. In addition, we identified IL7Rα expression on the cell bodies of CC1+ mature oligodendrocytes. To our knowledge, though, there are no reports of oligodendrocyte TNF production, and therefore, other known functions of IL7Rα, such as cell survival, may play a role in this cell type.

Overall, our data further support the current hypothesis that IL7Rα signaling does contribute to EAE. Although there is some effect on classical IFN-γ-producing Th1- and IL7-mediating Th17 subsets, IL7Rα predominantly drives TNF-producing T cells in the EAE model. In addition, these effector cells appear to work in conjunction with nonhematopoietic cell types, potentially astrocytes, which also use IL7Rα to drive disease. It is now apparent that the IL7Rα signaling pathway functions in many cellular subsets intricately involved in the detrimental aspects of EAE/MS and therefore serves as an optimal target for MS therapies.

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Disclosures
The authors have no financial conflicts of interest.

References
IL7R contributes to EAE through multiple cell types


