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Adjuvant Immunotherapy of Experimental Autoimmune Encephalomyelitis: Immature Myeloid Cells Expressing CXCL10 and CXCL16 Attract CXCR3+CXCR6+ and Myelin-Specific T Cells to the Draining Lymph Nodes Rather Than the Central Nervous System

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CFA is a strong adjuvant capable of stimulating cellular immune responses. Paradoxically, adjuvant immunotherapy by prior exposure to CFA or live mycobacteria suppresses the severity of experimental autoimmune encephalomyelitis (EAE) and spontaneous diabetes in rodents. In this study, we investigated immune responses during adjuvant immunotherapy of EAE. Induction of EAE in CFA-pretreated mice resulted in a rapid influx into the draining lymph nodes (dLNs) of large numbers of CD11b+Gr-1+ myeloid cells, consisting of immature cells with ring-shaped nuclei, macrophages, and neutrophils. Concurrently, a population of mycobacteria-specific IFN-γ-producing T cells appeared in the dLNs. Immature myeloid cells in dLNs expressed the chemokines CXCL10 and CXCL16 in an IFN-γ-dependent manner. Subsequently, CD4+ T cells coexpressing the cognate chemokine receptors CXCR3 and CXCR6 and myelin oligodendrocyte glycoprotein (MOG)-specific CD4+ T cells accumulated within the chemokine-expressing dLNs, rather than within the CNS. Migration of CD4+ T cells toward dLN cells was abolished by depleting the CD11b+ cells and was also mediated by the CD11b+ cells alone. In addition to altering the distribution of MOG-specific T cells, adjuvant treatment suppressed development of MOG-specific IL-17. Thus, adjuvant immunotherapy of EAE requires IFN-γ, which suppresses development of the Th17 response, and diverts autoreactive T cells away from the CNS toward immature myeloid cells expressing CXCL10 and CXCL16 in the lymph nodes. The Journal of Immunology, 2012, 188: 2093–2101.

Experimental autoimmune encephalomyelitis (EAE) is an organ-specific model of autoimmunity in the CNS and is a widely studied model of the human autoimmune disease multiple sclerosis (MS) (1). EAE is induced by immunizing mice with myelin Ags emulsified in CFA, with the latter consisting of killed mycobacteria in mineral oil. Mycobacteria express Ags, TLR agonists, and heat shock proteins promoting Th1 responses and delayed-type hypersensitivity against injected self-Ags (2). Because CFA is considered to be a strong adjuvant capable of stimulating cellular immune responses, it is paradoxical that prior exposure to CFA suppresses the severity of EAE (3). Similarly, adjuvant immunotherapy with live mycobacterial infection also ameliorates the severity of EAE (4, 5).

Adjuvant immunotherapy is not limited to experimental autoimmune diseases that are induced with CFA. Notably, live mycobacteria and CFA both suppress spontaneous disease in NOD mice, thus enabling regeneration of pancreatic cells in treated mice (6–11). Adjuvant-mediated suppression of autoimmune disease in rodents can persist as long as a year (12). The success of adjuvant immunotherapy in preclinical models of autoimmune disease has prompted recent clinical trials of adjuvant immunotherapy using live bacillus Calmette-Guérin (BCG) vaccine in humans with autoimmune diabetes (13).

Although both CFA and live mycobacteria suppress EAE and autoimmune diabetes in preclinical models, their mechanisms of immune suppression may not be identical. CFA treatment has been reported to promote T cell dormancy, as demonstrated by adjuvant-treated mice harboring autoreactive T cells capable of transferring autoimmune disease to recipient mice (14). Alternatively, CFA treatment was reported to eliminate a population of TNF-α–susceptible cells, suggesting that CFA treatment resulted in deletion of autoreactive cells rather than dormancy (9). When considering therapies to halt autoimmune destruction, treatments promoting deletion of autoreactive T cells and treatments promoting dormancy of autoreactive T cells may have different outcomes. For this reason, it is important to understand mechanisms of adjuvant immunotherapy by live and killed bacteria.

We have previously demonstrated that an ongoing infection of mice with live mycobacteria suppressed EAE by promoting IFN-γ–producing T cells.
dependent apoptosis of myelin oligodendrocyte glycoprotein (MOG)-specific CD4+ T cells in a bystander fashion (4). In this study, we investigated the mechanism by which killed mycobacteria in CFA attenuates EAE. Our data indicate that CFA does not result in enhanced apoptosis of CNS-infiltrating CD4+ T cells. Instead, we provide evidence for a novel mechanism for adjuvant immunotherapy that is, in part, dependent on IFN-γ. Suppression of EAE by CFA is associated with a rapid simultaneous influx of immature myeloid cells and IFN-γ-producing T cells into the lymph nodes draining the EAE induction site. The myeloid cells in wild-type, but not IFN-γ−/− mice, expressed Th1 cell-attracting chemokines CXCL10 and CXCL16. Subsequently, a large population of CD4+ T cells expressing both CXCR3 and CXCR6 as well as MOG-specific T cells accumulated within the chemokine-expressing lymph nodes, instead of within the CNS. Also, in WT mice, development of the MOG-specific IL-17 response was suppressed by CFA pretreatment; in contrast, adjuvant treatment failed to suppress IL-17 production in IFN-γ−/− mice. IFN-γ−/− mice were not protected from clinical EAE by adjuvant immunotherapy. Thus, adjuvant immunotherapy of EAE requires IFN-γ, which suppresses development of the Th17 response and diverts autoreactive T cells away from the CNS toward immature myeloid cells expressing CXCL10 and CXCL16 in the lymph nodes.

Materials and Methods

Mice

Female mice were used in all experiments. Breeding stocks for IFN-γ−/− C57BL/6 background and C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All investigations involving mice were carried out in accordance with standards and protocols approved by the Institutional Animal Care and Use Committees at the Trudeau Institute, the University of Edinburgh, the Toronto General Research Institute, and Dartmouth Medical School.

Adjuvant treatment

Mice were immunized with CFA on the left flank with 50 μl 1:1 CFA/PBS emulsion. CFA contained 4 mg/ml killed Mycobacterium tuberculosis (H37Ra) and 0.4 mg/ml Mycobacterium butyricum (Difco).

Induction and clinical scoring of EAE

Naïve and adjuvant-treated mice were immunized on the right flank with 100 μg MOG peptide, amino acid residues 35–55 (American Peptide, Sunnyvale, CA) emulsified in CFA (50 μl vol). The dramatic changes in the right inguinal draining lymph node (dLN) in response to EAE induction were evident in adjuvant-treated mice, but not in naïve mice that were induced to develop EAE. At the time of EAE induction and 48 h later, mice were injected i.p. with 200 ng pertussis toxin (Sigma-Aldrich). Mice induced to develop EAE. At the time of EAE induction and 48 h later, the right inguinal draining lymph node (dLN) in response to EAE induction was collected and stained for phenotypic analysis. The lower wells of transwell plates were seeded with 2× 10^5 CD4+ FITC-labeled T cells were added to the upper wells. The cells were cultured for 2 h in a CO2 incubator at 37˚C. After removing top-well inserts, the cells in the lower wells were collected, washed, and stained with a fixable viability marker (eFluor 450; eBioscience, San Diego, CA), then adjusted to 0.4 ml for collection on the flow cytometer. CountBright beads (Invitrogen) were used to calculate the absolute numbers of CD4+FITC− cells in the lower wells according to the manufacturer’s instructions. Using absolute counts and known numbers of cells on the top wells, the percentage of input cells that migrated toward the lower wells was calculated.

ELISPOT assay

The frequency of IFN-γ-producing cells in the CNS and dLNs was determined using an IFN-γ-specific ELISPOT kit (BD Biosciences) according to the manufacturer’s instructions. CNS mononuclear cells (1× 10^6 to 2.5× 10^6 cells/well) and lymph node cells were plated at various densities in an ELISPOT plate coated with rat anti-mouse IFN-γ capture Ab in the presence or absence of 10 μg/ml MOG. In Fig. 2D, 10 μg/ml final concentration of M. tuberculosis (desiccated, sonicated in PBS) was used to stimulate IFN-γ production in the ELISPOT assay. Cells were cultured overnight, lysed, and IFN-γ production was detected using a biotin-conjugated rat anti-mouse IFN-γ detection Ab. Avidin-HRP was then added, and spots were developed with 3-aminio-9-ethylcarbazole. Specific IFN-γ production was calculated by subtracting the background (medium only) from specific Ag-stimulated spots.

Analysis of cytokine production following adjuvant pretreatment

WT and IFN-γ−/− mice were adjuvant pretreated with a s.c. injection of 50 μl CFA on the left flank; control mice were left untreated (n = 6/group). Twenty-one days later mice were immunized with 100 μg MOG in CFA on the right flank. Seven days after MOG immunization, mice were sacrificed and dLN cells and splenocytes (both at 0.5× 10^6/ml) were restimulated with graded doses of MOG. Supernatants were collected after 72 h culture for analysis of cytokine production by ELISA (IL-17/IFN-γ) or Flow-Cytomix cytokine bead array (GM-CSF) according to the manufacturer’s instructions (eBioscience, San Diego, CA).

FACS analysis of cell subsets

Single-cell suspensions in a defined volume were counted on a hemacytometer using trypan blue exclusion of dead cells to determine total live cell numbers. The absolute number of cells was calculated for each sample by applying the percentage of each subset to the total live cells in lymph nodes or CNS. CNS-infiltrating cells and peripheral lymphocytes were prepared for FACS by staining with anti-CD4-PE (L3T4), CD11b-PE (M1/70), Gr-1 (IA8), CD45-allophycocyanin (30-F11), anti-CXCR3 (CXCR3-173), rat anti-mouse CXCR6 (clone 221002), and goat anti-rat PE to detect cell surface expression of CXCR6 (all Abs were from eBiosciences or BD Biosciences). As the unconjugated CXCR6 primary Ab was detected by a PE-conjugated anti-rat secondary Ab, the two-step CXCR6 staining was done, cells were washed, and then other surface Ags were stained with their primary Abs. Cells were gated based on forward and side scatter and propidium iodide or 7-aminoactinomycin D (dead) cells were excluded from analysis.

Chemokine microarray

WT and IFN-γ−/− mice were treated with CFA on the left flank and rested for 3 wk. EAE was then induced by immunizing mice with MOG peptide in CFA, on the right flank, according to the above protocol. Twenty-four hours later the right lymph nodes were isolated and pooled from five mice per group. CD4+CD45+ cells were FACSorted for CXCL10 and CXCL16 expression. The frequency of IFN-γ-producing cells in the CNS and dLNs was de-determined using an IFN-γ-specific ELISPOT kit (BD Biosciences) according to the manufacturer’s instructions. RNase was DNase treated using a DNA-free kit according to the manufacturer’s instructions (Ambion). RNA was reverse transcribed
using SuperScript II reverse transcriptase, random primers, and 2′-deoxy-nucleoside 5′-triphosphates (all Invitrogen). Expression levels in EAE-only samples were compared with those in naive mice to establish any differences resulting from EAE induction. Likewise, expression levels in adjuvant-EAE mice were compared with naive and EAE-only samples. All PCR reactions were done in duplicate, and the mean values of each group were calculated from three to six individual animals.

Results

Adjuvant immunotherapy with CFA attenuated EAE without increasing apoptosis of T cells in the CNS

The adjuvant-treated mice were injected with CFA on the left flank. After resting adjuvant-treated mice for 3 wk, EAE was induced in adjuvant-treated and naive mice by giving a single s.c. injection of MOG peptide emulsified in CFA in the right flank. Induction of EAE in adjuvant-treated mice constituted a secondary challenge with CFA, which was manifested within 24 h as a striking enlargement of the right inguinal lymph nodes draining the second CFA injection site. Lymph node enlargement was only apparent in adjuvant-treated mice and was confined to the lymph nodes draining the site of MOG/CFA immunization. In the experiments that are detailed below we investigated the cellular and molecular changes in the right dLNs of adjuvant-treated mice with EAE compared with EAE-only mice (Fig. 1A).

Treatment of WT mice with CFA 3 wk before inducing EAE significantly attenuated the clinical symptoms of disease (Fig. 1B) compared with non–adjuvant-treated mice with EAE. Previously we had shown that mice infected with live mycobacteria (Mycobacterium bovis, strain BCG) also exhibited attenuated EAE. Importantly, BCG infection increases the apoptosis of activated bystander T cells, indicating that the viability of autoreactive T cells was impaired during BCG infection (4). To investigate whether CFA also increased apoptosis of CNS-infiltrating CD4+ T cells, we measured apoptosis using the annexin V assay. Unlike live BCG infection, CFA pretreatment did not induce high levels of apoptosis in CNS-infiltrating CD4+ T cells on day 17 of disease (Fig. 1C). Thus, although BCG and CFA both attenuated EAE, only live mycobacteria caused high levels of apoptosis of CD4+ T cells in the CNS.

Adjuvant-treated mice responded to EAE induction by rapidly mobilizing immature myeloid cells and mycobacterial Ag-specific IFN-γ+ T cells to the right lymph nodes

In adjuvant-treated mice enlargement of the right lymph nodes at 24 h after EAE induction reflected increased numbers of total cells (8-fold), CD4+ T cells (5-fold), and CD11b+Gr-1+ myeloid cells (20-fold) (Fig. 2A–C). We considered that the re-exposure of adjuvant-treated mice to CFA may have recruited mycobacterial Ag-specific effector T cells to the right dLNs. Indeed, an ELISPOT assay showed that adjuvant-treated mice have a high frequency of mycobacteria-specific IFN-γ+ T cells in the right dLNs within 24 h EAE induction (Fig. 2D). As expected, control mice immunized to develop EAE had no detectable mycobacteria-specific T cells in their right lymph draining nodes at the same time point.

In adjuvant-treated mice with EAE, the increased numbers of mycobacteria-reactive IFN-γ+ cells coincided with accumulation of large numbers of CD11b+Gr-1+ myeloid cells in the right dLNs. Such myeloid cells were present at a low frequencies and numbers in dLNs of naive mice and EAE-only mice at 24 h after immunization (Fig. 2C, 2E). FACS-purified myeloid cells from adjuvant-EAE lymph nodes were examined in cytospin preparations. The cells were heterogeneous, with many having ring-shaped nuclei characteristic of immature myeloid-lineage cells as well as some resembling mature granulocytes and macrophages (Fig. 2F). These results indicate that upon EAE induction in adjuvant-treated mice, there is a rapid and coordinated influx of myeloid cells, CD4+ T cells, and mycobacteria-specific IFN-γ+ cells into the right inguinal lymph nodes draining the site of re-exposure to CFA.

CD11b+Gr-1+ cells in the right lymph nodes of adjuvant-treated WT, but not IFN-γ−/−, mice expressed high levels of IFN-γ-inducible CXCL10 and CXCL16

IFN-γ expressed by T cells in the right dLNs of adjuvant-EAE mice could contribute to adjuvant-mediated protection from EAE. Thus, we tested whether adjuvant could protect IFN-γ−/− mice from EAE. Adjuvant-treated IFN-γ−/− mice initially developed EAE with slower onset (as reflected by significantly reduced clinical severity on day 14) compared with untreated IFN-γ−/− mice. However, by day 21, the disease scores of adjuvant-treated and untreated mice IFN-γ−/− mice with EAE were similar (Fig. 3A). This contrasted with WT mice in which adjuvant treatment provided significant protection from EAE (p = 0.02) throughout the monitoring period (compare Fig. 1B to Fig. 3A). Severe EAE in adjuvant-treated IFN-γ−/− mice demonstrates that IFN-γ contributes to suppression of EAE by adjuvant immunotherapy. The comparative rate of recovery in adjuvant-treated versus untreated IFN-γ−/− mice was not assessed for humane reasons because of the severe EAE in IFN-γ−/− mice.
CD4+ T cells were present at significantly higher numbers in dLNs of adjuvant-treated mice with EAE (Fig. 2B), raising the possibility of their increased migration to or retention within the dLNs. Our preliminary investigations suggested that myeloid cells in adjuvant-treated mice were a source of chemokines. Moreover, although IFN-γ−/− mice were not fully protected by adjuvant treatment, they also accumulated a large population of CD11b+Gr-1− cells in the right dLN upon EAE induction. Such accumulation is consistent with previous work showing that CFA expands a population of immature myeloid cells, which is restrained by IFN-γ (16). Thus, we FACS sorted dLN CD11b+Gr-1− cells from adjuvant-treated WT and IFN-γ−/− mice with EAE and prepared RNA for analysis of chemokine gene expression. Chemokine microarrays showed that myeloid cells from WT adjuvant-treated mice expressed high levels of the chemokines CXCL10 and CXCL16 (Fig. 3B). However, CXCL10 and CXCL16 transcripts were completely absent in myeloid cells from adjuvant-treated IFN-γ−/− mice (Fig. 3B). Both of these chemokines can induce chemotaxis of CD4+ T cells. In a transwell chemotaxis assay, dLN cells from adjuvant-treated mice with EAE, but not dLN cells from EAE-only mice, induced significant migration of CD4+ T cells (Fig. 4A). Moreover, migration of CD4+ T cells toward the adjuvant-treated dLN cells was completely abolished by depletion of the CD11b+ cells. Furthermore, pure populations of CD11b+ cells isolated from adjuvant-treated dLNs induced significant CD4+ T cell migration toward the myeloid cells. These data indicate that the CD11b+ myeloid cells, demonstrated to induce chemotaxis of CD4+ T cells (Fig. 4A–D), constitute the major population of dLN cells in adjuvant-EAE mice that induce chemotaxis of CD4+ T cells.

We also investigated the in vivo kinetics of CD4+ T cell migration into chemokine-expressing dLNs during EAE. In agreement with the in vitro migration assay, we found that CD4+ T cells expressing CXCR3 and CXCR6 accumulated in the right dLNs of adjuvant-treated mice with EAE, but not in dLNs of EAE-only mice (Fig. 4B, 4C).

We also measured the distribution of chemokine receptor-expressing CD4+ T cells between the CNS and dLNs of mice with EAE. Notably, >90% of CNS-infiltrating CD4+ T cells in mice with EAE expressed both CXCR3 and CXCR6 (Fig. 4D). Furthermore, in the inflamed CNS of mice with EAE, CD4+ T cells expressing both CXCR3 and CXCR6 accumulated to significantly greater numbers compared with the CNS of adjuvant-treated mice with EAE (Fig. 4E). The reverse situation was seen in...
In recent years the identification of IL-17–producing Th17 cells and characterization of their importance in driving pathology in EAE (19, 20) have advanced our understanding of the model. The importance of IL-17–producing cells in driving disease and the capacity of IFN-γ to negatively regulate Th17 development (21) provide an explanation for the exacerbated EAE seen in IFN-γ–deficient mice (22, 23). Next we determined whether MOG-driven IL-17 production was altered by adjuvant treatment in WT and IFN-γ−/− mice. IL-17 production was similarly high in adjuvant-treated IFN-γ−/− mice, untreated IFN-γ−/− mice, and WT untreated mice with EAE (Fig. 5A), consistent with their severe EAE clinical scores (Figs. 1B, 3A). However, WT adjuvant-treated mice with EAE had comparatively low IL-17 levels, which was consistent with the mild EAE scores in this group. These data indicate that adjuvant treatment attenuated IL-17 production during EAE in an IFN-γ–dependent manner. This is in line with earlier studies showing that IFN-γ suppresses development of IL-17–producing cells (24).

In addition to IL-17, the importance of GM-CSF in the pathology of EAE has been highlighted in recent studies (25–27), so we determined the impact of adjuvant treatment on MOG-induced GM-CSF production. Adjuvant treatment reduced GM-CSF levels in both WT and IFN-γ−/− mice with EAE (Fig. 5B). These results indicate GM-CSF is suppressed by IFN-γ–independent means. This provides evidence for IFN-γ–independent protective effects, which might account for the delayed onset of EAE in adjuvant-treated IFN-γ−/− mice (Fig. 3A). Additionally, we found no evidence for enhanced production of the regulatory cytokine IL-10 in adjuvant-treated mice with EAE (data not shown), suggesting that adjuvant protection from EAE is due to alterations in the production of proinflammatory cytokines and differences in T cell migration rather than enhanced production of IL-10.

### Discussion

In this study, we investigated the mechanisms of adjuvant immunotherapy of EAE by killed mycobacteria in CFA. We found that adjuvant treatment prior to EAE induction prevented autoreactive MOG-specific T cells from accumulating within the CNS. Instead, adjuvant treatment resulted in accumulation of MOG-specific T cells within the dLN of the EAE immunization site. Adjuvant treatment with CFA did not cause enhanced apoptosis of CNS-infiltrating CD4+ T cells as did live mycobacteria (4). However, we noted that immunization of adjuvant-treated mice to develop EAE led to a coordinated accumulation of large numbers of immature CD11b+Gr-1+ myeloid cells, CD4+ T cells, and...
FIGURE 4. MOG-specific T cells as well as CD4+ T cells expressing both CXCR3 and CXCR6 accumulated within the right dLNs of adjuvant-treated mice with EAE instead of accumulating within the CNS. (A) Results of an in vitro chemotaxis assay. The percentage is shown of input CD4+ T cells that migrated toward the indicated cell populations in the Results of an in vitro chemotaxis assay. The percentage is shown of input CD4+ T cells from dLN of adjuvant-EAE mice on day 1 of EAE. The means and SEM are shown for six mice per point. **p < 0.0001, t test. (B) Total numbers of CD4+CXCR3+ T cells and (C) CD4+CXCR6+ T cells in lymph nodes from adjuvant-EAE and EAE mice on days 3, 7, and 15 after EAE induction. The means and SEM are shown for six mice per group. **p = 0.002 by unpaired t test. (D) Representative dot plot (of n = 6 mice) showing coexpression of CXCR3 and CXCR6 on CNS-infiltrating CD4+ T cells of mice with EAE (day 15). Numbers in quadrants indicate percentage of cells in each subset. Gated on live CD4+ T cells. (E) Absolute numbers of CD4+CXCR3+CXCR6+ T cells in CNS of indicated mice on day 15 of EAE (mean and SEM of six mice per point). **p < 0.0001, t test. (F) Absolute numbers of CD4+CXCR3+CXCR6+ T cells in right dLNs of indicated mice on day 15 of EAE (mean and SEM of six mice per point). **p < 0.0001, t test. (G) Total number of MOG-specific T cells in right (R) and left (L) dLNs depleting of CXCL10 returning to baseline at 24 h after MOG injection. In another report, plasma levels of CXCL10 in MS patients were transiently elevated on day 1, returning to baseline on day 2. A recent study found that among 14 chemokines surveyed by microarray, CXCL10 transcripts and serum levels were strongly correlated with disease activity in MS. In this study, CXCL10 was measured in the cerebrospinal fluid (CSF) of patients with MS and was found to be elevated in patients with active disease. (H) Total number of MOG-specific T cells in right dLNs on day 15 of EAE was determined by an IFN-γ ELISPOT assay (mean and SEM of six mice per point). **p < 0.0001, t test. (I) Total number of MOG-specific T cells in right (R) and left (L) dLNs on day 15 of EAE was determined by an IFN-γ ELISPOT assay (mean and SEM of six mice per point). **p < 0.0001, t test.
Adjuvant treatment suppresses MOG-specific IL-17 production in WT but not IFN-γ−/− mice. WT and IFN-γ−/− mice were adjuvant treated or left untreated as previously described (n = 6/group). Twenty-one days later mice were immunized with MOG/CFA on the right flank. Mice were killed 7 d after MOG/CFA immunization. (A) IL-17-production by splenocytes in response to MOG. (B) GM-CSF production by splenocytes in response to 30 μg/ml MOG.

FIGURE 5. Adjuvant treatment suppresses MOG-specific IL-17 production in WT but not IFN-γ−/− mice. WT and IFN-γ−/− mice were adjuvant treated or left untreated as previously described (n = 6/group). Twenty-one days later mice were immunized with MOG/CFA on the right flank. Mice were killed 7 d after MOG/CFA immunization. (A) IL-17-production by splenocytes in response to MOG. (B) GM-CSF production by splenocytes in response to 30 μg/ml MOG.

The roles of CXCL10 and CXCR3 in CNS inflammation are complex and incompletely understood. CXCL10 protein colocalizing with CXCR3+ cells has been detected within the CNS during MS and other CNS inflammatory diseases (49). However, conflicting data exist on the impact of neutralization or gene deletion of CXCL10 during EAE. Interestingly, the loss of function or neutralization of CXCL10 has been reported to either attenuate or exacerbate EAE in different studies (50–52). Also, the absence of CXCR3 has been reported to either attenuate or exacerbate the severity of EAE (53, 54). Considering these conflicting data, the in vivo neutralization of these molecules to define their protective roles in adjuvant immunotherapy of EAE may not provide unambiguous results. Similarly, as Ab-mediated depletion of Gr−1+ cells in vivo induces resistance to EAE (55), it is not currently possible to isolate the potentially protective role of the CD11b+Gr−1− immature myeloid cells in the dLN of adjuvant-pretreated mice from the pathogenic role of circulating granulocytes.

The above-mentioned studies on CXCL10 and CXCR3 suggest that these chemokines have biological activities in addition to their chemotactic functions, including effects on T cell priming (56). For example, CXCL10 serves as a dominant chemokine in vitro, capable of attracting T cells away from Ag-bearing immunological synapses (57). It has been suggested that elevated expression of CXCL10 within inflamed tissues might enhance the migration of Th1 cells away from dendritic cells presenting Ags in the lymph nodes. It will be of interest for future studies to elucidate the biological effects of myeloid cell-derived CXCL10 on T cell priming within the lymph nodes.

The work in this study adds to a growing body of evidence demonstrating that ongoing Th1 responses are capable of interfering with subsequent Th1 responses in multiple ways. Live infections with virus and mycobacteria promoted IFN-γ–dependent bystander apoptosis of autoreactive T cells (4, 6, 28). Also, exposure to live virus, live mycobacterial infections, and in this study killed mycobacteria all induced IFN-γ–dependent attraction of autoreactive T cells toward sites of ongoing inflammation rather than toward the Ag-bearing target tissues (5, 28, 58). Furthermore, treatment with CFA reversed diabetes in NOD mice and induced persistently increased numbers of CD4+CD25+Foxp3+ regulatory T cells in the pancreatic dLNs of CFA-treated mice (59).

One implication of our work is that mechanisms of interference by ongoing Th1 responses with subsequent Th1 responses should be considered in designing vaccines to promote Th1 responses to infections and tumors. This concept was recently illustrated by abrogation of anti-tumor protection by using CFA as an adjuvant in a tumor vaccine (60). In contrast, a better understanding of multiple mechanisms of immune suppression during Th1 responses may allow us to exploit the immune system to halt cell-mediated
autoimmune destruction. Finally, as suggested by the hygiene hypothesis, ongoing Th1 responses to ubiquitous saprophytic environmental mycobacteria might counteract the subsequent development of autoimmune diseases (61).

Disclosures
The authors have no financial conflicts of interest.

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