Antibodies Against IL-12 Prevent Superantigen-Induced and Spontaneous Relapses of Experimental Autoimmune Encephalomyelitis

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Immunization of (PL/J × SJL/J)F₁ mice with myelin basic protein (MBP) induces relapsing experimental autoimmune encephalomyelitis (EAE). Relapses occur 7 to 10 days after recovery from the initial paralysis. Staphylococcal enterotoxins (SE) A or B, administered after recovery from the initial paralysis, induce immediate relapses. IL-12 is involved in the induction of EAE. Here, we show that SEA and SEB induce IL-12 in splenocytes from (PL/J × SJL/J)F₁ mice in vitro and increase the level of IL-12 in the sera of mice treated with these superantigens. IL-12 administration mimics SE in inducing spontaneous relapses and in enhancing the severity and frequency of spontaneous relapses. IL-12 neutralization blocks SE-induced and subsequent relapses of EAE, and, when administered after recovery from the initial attack, prevents spontaneous relapse. This is the first report of prevention of relapses of EAE with anti-IL-12 Ab, an approach which may prove useful in the prevention of exacerbations in multiple sclerosis. The Journal of Immunology, 1998, 161: 5097–5104.

Experimental autoimmune encephalomyelitis (EAE), an animal model for the human disease multiple sclerosis (MS), is a T cell-mediated central nervous system (CNS) autoimmune disease. The autoreactive T cells, directed against neuroantigens including myelin basic protein (MBP), are of the Th1 type (producing IFN-γ and TNF and promoting cell-mediated immunity). These cells preferentially use the Vβ8 TCR in Lewis rats and H2b mice (1). The superantigen (SAG) staphylococcal enterotoxin (SE) B (SEB) is a potent T cell activator stimulating a large proportion of Vβ8 T cells and has been postulated to trigger autoimmunity by stimulating autoreactive T cells (2). SEB induces relapsing paralysis in PL/J (3, 4) and (PL/J × SJL/J)F₁ mice (3) that have recovered from the first acute EAE attack. This property of SEB has been attributed to its ability to activate Vβ8 T cells and has given rise to analogies with the frequent precipitation of MS relapses by infectious events (5).

(PL/J × SJL/J)F₁ mice, when immunized with whole MBP, also have a high incidence of spontaneous relapses (6). These typically occur between days 23 and 32 after immunization, (7–10 days after recovery from the initial EAE episode) (6) (C.S.C. and A.R., unpublished observations). Thus, spontaneous relapses in (PL/J × SJL/J)F₁ mice are distinguishable from the SEB-induced relapses (3), which occur within 1 to 3 days after SEB administration. Interestingly, staphylococcal enterotoxin A (SEA), a SAG that does not activate Vβ8 T cells, can induce similar relapses (3, 4), suggesting that the mechanisms of SAG-induced relapses are not strictly Vβ8-dependent.

Staphylococcal SAG bind the MHC class II molecule of the APC outside of the binding groove and, subsequently, as a binary complex bind to the Vβ region of the TCR (2). This process induces cytokines both in the T cell and in the APC (7). SAG binding to the MHC class II molecule on the APC induces signaling (8), cytokine gene transcription (9) and secretion (10), and nitrite production (11). Staphylococcal SAG preferentially induce Th1 cytokines (12–14). SAG binding is enhanced by IFN-γ, suggesting that cooperation of T cells and MHC class II-positive APC is needed for optimal SAG-induced responses (15).

An important cytokine produced by activated APC is IL-12. This heterodimeric cytokine, consisting of the p35 and p40 subunits, induces IFN-γ production by T and NK cells (16, 17) and is pivotal in the ability of a neutralizing anti-IL-12 Ab to prevent both actively induced EAE (C.S.C. and A.R., unpublished observations) and adoptively transferred EAE (20), and by the increased encephalitogenicity of neuroantigen-reactive T cells stimulated with IL-12 (20, 21). In addition, exogenous IL-12 induces relapses in an otherwise typically monophasic form of EAE in Lewis rats (22). Thus, it is of interest to determine whether endogenous IL-12 plays a role in EAE relapse, characterized in our mouse model by both spontaneous and SAG-induced relapses. Moreover, because SAG, including staphylococcal SAG, can induce IL-12 production by APC in vitro (23–25), it is interesting to determine this ability in vivo (particularly because SAG may have different effects in vitro and in vivo) and to investigate whether this induction plays a role in the relapsing paralysis of EAE.

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4 Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; CNS, central nervous system; MBP, myelin basic protein; SAG, superantigen; SE, staphylococcal enterotoxin; SEA, staphylococcal enterotoxin B; SEB, staphylococcal enterotoxin A.
Staphylococcus aureus is a potent inducer of IL-12 (26). Moreover, recent evidence indicates that the ability of several bacteria-derived substances, including staphylococcal products, to overcome resistance to EAE is mediated through IL-12 induction (27). The animals were sex and age matched within each experiment. Previous studies in our laboratory and others’ (6) showed no differences between male and female mice of this strain in terms of EAE susceptibility and frequency of relapses.

Materials and Methods

Mice

Male or female (PL/J × SJL/J)F1 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The animals were sex and age matched with IFN-γ. Recombinant murine IL-12 was a generous gift of Dr. Maurice Gately (Hoffmann-La Roche, Nutley, NJ). Murine rIFN-γ was purchased from PharMingen (San Diego, CA). Monoclonal anti-mouse IL-12 Abs C17.8 (rat IgG2a), C15.1 (rat IgG1), and C15.6 (rat IgG1) were previously described (30). FIA and Mycobacterium tuberculosis H37 Ra were purchased from Difco (Detroit, MI). A 1:1 mixture resulted in CFA with 5 mg/ml M. tuberculosis. Bordetella pertussis toxin was obtained from List Biological Laboratories, (Campbell, CA). Rat IgG was purchased from Sigma.

Induction of EAE

Mice, 10 wk old, under anesthesia were immunized on day 0 in the hind footpads and at three sites on each side of the back with a total of 300 μl of: 1) (v/v) mixture of MBP (2.67 mg/ml in PBS) and CFA containing 5 mg/ml M. tuberculosis H37Ra. On days 0 and 2, 400 ng B. pertussis toxin were given i.p.

Clinical scoring

Mice were weighed daily and observed for clinical signs of disease for more than 40 days postimmunization. A clinical scoring system with a scale of 0 to 5, with 0.5 points for intermediate signs, was used: 0, normal; 1, flaccid tail, abnormal gait; 2, hind leg weakness or severe ataxia; 3, minimal hind leg movement; 4, hind leg and forelimb paralysis; 5, moribund due to EAE, with impaired breathing and little or no spontaneous movement. Mild disease had to be observed for 2 days or more and be confirmed by two independent, blinded observers to be considered positive. The score increment was defined as the maximal increase, during a relapse, in the clinical score from the preepilepsy score.

Induction of relapses and treatment after recovery from first EAE episode

Mice that had recovered from the acute disease (day 18 or 19 postimmunization) were injected i.p. with a single dose of 50 μg SEB or 25 μg SEA, both in PBS, or with 100 mg murine rIL-12 in PBS with 1% mouse serum. On the day of the above treatment and on the subsequent 2 days, some of the mice received 1 mg/day of either anti-IL-12 mAb CB.17.8 or control rat IgG. The mice were observed daily for development of relapsing disease. Relapses occurred 1 to 3 days after SE administration. Relapses with onset occurring 3 to 5 days following the treatment were attributed to the intrinsically relapsing nature of EAE in these mice and coincided with relapses in mice that had been immunized on the same day but received no treatment following recovery from the initial episode. However, we cannot rule out the possibility that relapses occurring during this period may also be related to the effect of the SAG in the SAG-treated animals.

Measurement of cytokines in serum and splenocyte supernatants

Mice were given the above doses of SEA or SEB in 100 μl PBS via i.p. injection. Control mice were given 100 μl PBS. Blood was harvested in heparinized tubes by retro-orbital bleeding at 6 h and 24 h, centrifuged, sera removed and diluted at concentrations of 1:10 for IL-12 p40 and IL-12 p70 measurement.

For in vitro cytokine studies, spleens of mice were homogenized to single-cell suspensions by passage through a stainless steel mesh. RBC were removed by hypotonic lysis in NH4Cl-containing buffer. IL-12 p40 and IL-12 p70 assays were performed in total spleen cell populations stimulated either with medium alone (RPMI 1640 containing 5% FBS with antibiotics) or with the same medium containing SEA or SEB, 2.5 μg/ml. IL-12 p40 was assayed in supernatants of spleen cells of mice using recombinant murine IL-12 as standard, following a two-site RIA as described (30). Briefly, samples were placed in 96-well plates (Dynatech Laboratories, Chantilly, VA) coated with 5 μg/ml of C17.15 anti-mouse IL-12 mAb and incubated overnight at 4°C. Plates were washed, and 125I-labeled C15.6 was added. Bound radioactivity after 6 h incubation at 4°C was measured in a microplate scintillation counter (Topcount, Packard, Meriden, CT). Samples were assayed in triplicate. IL-12 p70 was measured in a biologic assay based on IL-12-dependent induction of IFN-γ in murine splenocytes, as described (30). The sensitivity of RIA detecting IL-12 p40 is 30 pg/ml, and the sensitivity of biologic assay detecting IL-12 p70 is 3 pg/ml. IFN-γ was assayed in supernatants of spleen cells of mice using recombinant murine IFN-γ as standard according to a two-site RIA as described (30).

Histopathology

Animals under anesthesia were perfused through the heart with 10% phosphate-buffered formalin. Brains and spinal cords were fixed in formalin, dehydrated through graded alcohols, and embedded in paraffin. Five-micrometer sections of brain and spinal cord were cut serially and mounted on poly-L-lysine-coated glass slides. Five spinal cord sections and five brain sections obtained at similar levels from each mouse were stained. For assessment of demyelination, sections were stained with hematoyxlin-eosin. For evaluation of demyelination, sections were stained with Luxol fast blue and counterstained with cresyl violet. The extent of inflammation and demyelination was scored on a scale of 0 to 3, based on the fraction of tissue section quadrants containing lesions out of 20, as previously described (31): 0, absent; 1, mild (1–7 quadrants); 2, moderate (8–13 quadrants); 3, severe (14–20 quadrants); and 0.5 points for intermediate degrees of histologic severity.

Statistical analysis

The two-tailed Student’s t test was used to assess the significance of differences in clinical scores between groups. Incidences of EAE relapses were compared using the χ² test.

Results

SE induce IL-12 in vitro

To determine whether SE induce IL-12 production by splenocytes (most likely APCs) in vitro, we harvested spleen cells from naive (PL/J × SJL/J)F1 mice and measured the production of IL-12 p40 in cell culture supernatants 6 and 24 h after stimulation with SE in vitro, with or without IFN-γ (100 U/ml) pretreatment. Both SEA and SEB induced IL-12 p40 in (PL/J × SJL/J)F1 splenocytes without IFN-γ pretreatment (data not shown), confirming results of studies using murine peritoneal macrophages (24). This induction was consistently higher when splenocytes were pretreated with IFN-γ (100 U/ml) for 16 to 18 h before stimulation. Figure 1A shows the results of a typical experiment, in which induction of IL-12 p40 by SEA and SEB is demonstrated in murine splenocytes after pretreatment with IFN-γ. Without IFN-γ pretreatment, the corresponding IL-12 p70 measured by biologic assay was either just above the detection limit (≤2 pg/ml) or absent. In contrast, IFN-γ pretreatment resulted in consistently detectable amounts of IL-12 p70 after SE stimulation. Figure 1B shows an example of IL-12 p70 induction by SEA and SEB after IFN-γ pretreatment measured in the same supernatants as in Figure 1A. Stimulation with IFN-γ alone did not induce significant levels of IL-12 p40 or IL-12 p70 (data not shown). The slight increase in IL-12 p40 after 24 h in culture (as seen in Fig. 1A) is also noted with splenocytes
that were allowed to adhere for 24 h without IFN-γ or other stimuli; this increase is never associated with detectable IL-12 p70.

To determine whether IL-12 induction by SE correlates with induction of IFN-γ, we measured IFN-γ in supernatants of SE-stimulated spleen cells. Large amounts of IFN-γ, up to 40 ng/ml, were detected after both SEA and SEB stimulation, the peak of IFN-γ corresponding to that of IL-12 p40 and IL-12 p70 at 24 h after stimulation (data not shown).

**SE induce IL-12 in vivo**

The in vitro and in vivo effects of SAG can be different, particularly with respect to T cell activation vs anergy. In contrast, SE are known to induce cytokines, including TNF, both in vivo and in vitro (32). However, although neutralization of TNF alone significantly delayed SEB-induced relapses, it did not completely prevent them (3). IL-12 is involved in EAE induction (20) and synergizes with TNF (33, 34). Therefore, we investigated whether in vivo SE administration increases IL-12. We measured serum IL-12 p40 in mice 6 and 24 h after the i.p. administration of 25 µg/mouse SEA or 50 µg/mouse SEB. We demonstrated that this treatment induces increased levels of p40 IL-12 in the sera of these mice (Fig. 2). The induction kinetics is similar to that shown in murine endotoxic shock (30). We also measured the levels of IL-12 p70 heterodimer in these sera and observed consistently detectable levels only at the 6-h time point (data not shown).

**SE-induced relapses are prevented by anti-IL-12 Ab**

Previous studies have shown that anti-IL-12 Abs prevent acute monophasic EAE, while IL-12 increases its severity (20). We examined whether the neutralizing anti-IL-12 mAb C17.8 could inhibit or prevent SEB- or SEA-induced EAE relapses. After recovering from the initial acute EAE episode (day 18 or 19), mice received SEA 25 µg/mouse or SEB 50 µg/mouse i.p. On the day of SE treatment and on the following 2 days, a group of mice received additional treatment with anti-IL-12 mAb (1 mg/mouse i.p. per treatment), while the control group received control rat IgG (same dose). As shown in Table I and Figure 3, the incidence and severity (measured as mean increment in clinical score and mean maximal score) of SAG-induced EAE relapses was significantly reduced (p < 0.001 for both SEA and SEB) by treatment with anti-IL-12 Ab, while treatment with control rat IgG was ineffective.

**SE enhance severity and frequency of EAE relapses**

The above results confirm the ability of SE to induce clinical relapses in (PL/J × SJL/J)F1 mice. In addition, this strain of mice

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**Table I. Effect of anti-IL-12 on SE-induced EAE relapse**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SEB</th>
<th>SEB + αIL-12</th>
<th>SEB + rat IgG</th>
<th>SEA</th>
<th>SEA + αIL-12</th>
<th>SEA + rat IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of relapses (%)</td>
<td>11/12 (91.6)</td>
<td>0/12 (0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7/7 (100)</td>
<td>6/8 (75)</td>
<td>0/8 (0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>Mean score increment (SD)</td>
<td>1.27 (0.44)</td>
<td>0 (0)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 (0.26)</td>
<td>0.83 (0.12)</td>
<td>0.06 (0.1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 (0.1)</td>
</tr>
<tr>
<td>Mean maximal score (SD)</td>
<td>1.54 (0.43)</td>
<td>0.23 (0.14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.31 (0.26)</td>
<td>0.92 (0.71)</td>
<td>0.12 (0.18)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 (0.44)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mice were immunized with MBP + CFA. After recovering from the initial acute EAE episode (day 18 or 19), mice received SEB (25 µg) or SEA (50 µg). On the day of SE treatment and on the following 2 days, one group of mice received anti-IL-12, while a control group received rat IgG.

<sup>b</sup> Significant difference with control rat IgG treatment (p < 0.001).
EAE episode. We examined whether SE administration influences
neither SE nor IL-12 treatment after the recovery from their initial
typical occurrence 7 to 10 days after recovery from the initial
incidence of spontaneous relapses of 75% (range: 50–100%), with
munization protocol. Our unpublished results have shown a mean
ing between 20% (3) and 100% (6), depending in part on the im-
has a tendency toward spontaneous relapses, with incidence vary-
ing between 20% (3) and 100% (6), depending in part on the im-
munization protocol. Our unpublished results have shown a mean
incidence of spontaneous relapses of 75% (range: 50–100%), with
typical occurrence 7 to 10 days after recovery from the initial
paralysis. In this paper, we use the term “spontaneous relapses” to
denote relapses that occur in immunized mice that have received
neither SE nor IL-12 treatment after the recovery from their initial
EAE episode. We examined whether SE administration influences
the occurrence or severity of spontaneous relapses. We found that
animals treated with SE after recovery from the initial EAE event,
in addition to experiencing an immediate SE-induced relapse, had
an increased incidence and severity of later relapses (p < 0.0001)
(Table II and Fig. 3). Anti-IL-12 Ab treatment at the time of SE
administration significantly decreased the incidence and severity
of relapses, while administration of the control Ab rat IgG at the
time of SE administration had no effect.

Histopathologic evaluation of the brain and spinal cord was per-
formed on selected mice from the SE + control Ab- and SE +
anti-IL-12-treated groups. Mice were sacrificed at day 33 postim-
umunization. SE + control Ab-treated mice exhibited mild perivascular
and meningeal inflammation in the brain and severe inflam-
mation involving the meninges, the perivascular spaces, and
parenchyma, and accompanied by demyelinating changes in the
spinal cords (Fig. 4, A and B). In contrast, histopathology in SE +
anti-IL-12-treated mice revealed no inflammatory or demyelina-
ting changes (Fig. 4C), with the exception of minimal meningeal
infiltration in a single case out of three in SEB + anti-IL-12-
treated mice (Table III). The differences between SE + control Ab
and SE + anti-IL-12 were statistically significant (p < 0.05).

Severity and frequency of EAE relapses are enhanced by IL-12
and suppressed by anti-IL-12 Ab

We postulated that anti-IL-12 treatment may suppress relapses in
mice not given SE treatment. We also wanted to determine
whether systemic administration of murine rIL-12 can mimic SE
effects by inducing EAE relapses and/or affecting the severity of
relapses. After recovery from the initial bout of EAE, five mice
received anti-IL-12 mAb C17.8 (1 mg/mouse/day i.p. for three
consecutive days) while four control mice received the same quan-
tities of rat IgG. None of the anti-IL-12-treated mice developed
relapses, whereas three of four (75%) control Ab-treated mice had
relapses. The mean severity of the relapse (±SD) in the rat IgG-
treated group was 1.125 (±1.031); the mean increment was 1.125
(±0.25). The difference was statistically significant (p = 0.04
compared with the rat IgG-treated group) (Fig. 5). The protective
effect of anti-IL-Ab was longer lasting than the ~15 days of per-
 sistence of these Abs in the circulation. None of the five animals
followed ≥50 days developed a relapse, in contrast to the average
of 60% of untreated mice that developed at least a third attack.

After recovery from the initial EAE episode (day 18 or 19), 10
mice received 100 ng i.p. of murine rIL-12. Six mice (60%) de-
veloped a relapse on the day following IL-12 administration
(mean ± SD score increment, 1.08 ± 0.44). No relapses were
noted immediately following the cytokine treatment in the remain-
ing four mice. However, all mice receiving IL-12 treatment had a
significantly more severe relapse. These relapses occurred at the
same time (day 24–30) as the spontaneous relapses in mice not
receiving treatment after recovery from the initial paralysis. How-
ever, the incidence (10/10 or 100%) was higher than that of spontan-
eous relapses in the animals without this treatment (6/9 or

### Table II. Effect of anti-IL-12 on SE-enhanced spontaneous EAE relapses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>None</th>
<th>SEB</th>
<th>SEB + oIL-12</th>
<th>SEB + rat IgG</th>
<th>SEA</th>
<th>SEA + oIL-12</th>
<th>SEA + rat IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of relapses (%)</td>
<td>6/9 (66.7)</td>
<td>12/12 (100)</td>
<td>2/12 (16.7)</td>
<td>7/7 (100)</td>
<td>7/8 (87.5)</td>
<td>0/8 (0)</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>Mean score increment (SD)</td>
<td>1.08 (0.2)</td>
<td>3.57 (1.2)</td>
<td>0.18 (0.38)</td>
<td>2.5 (1.4)</td>
<td>2.31 (1.7)</td>
<td>0.08 (0.2)</td>
<td>2.6 (1.65)</td>
</tr>
<tr>
<td>Mean maximal score (SD)</td>
<td>1.11 (1.54)</td>
<td>3.64 (1.4)</td>
<td>0.2 (0.4)</td>
<td>2.93 (1.6)</td>
<td>2.43 (1.7)</td>
<td>0.25 (0.24)</td>
<td>2.6 (0.24)</td>
</tr>
</tbody>
</table>

* Mice were immunized with MBP + CFA. After recovering from the initial acute EAE episode (day 18 or 19), mice received SEB (25 μg) or SEA (50 μg). On the day of SE treatment and on the following 2 days, one group of mice received anti-IL-12, while a control group received rat IgG.

* Significant difference with control rat IgG treatment (p < 0.0001).
In addition, the mean (±SD) score increment was 2.61 (±1.53), as compared with 1.08 (±0.2) in the untreated mice. The difference was significant (p < 0.05 compared with mice receiving no treatment).

Also, the mean (±SD) maximal scores were enhanced: 2.71 (±1.44) as compared with 1.11 (±1.54) in mice receiving no IL-12 treatment. The difference was significant (p, 0.05 compared with mice receiving no treatment).

Inflammation

- Brain: 0.5 ± 0 vs. 0 ± 0
- Spinal cord: 2.75 ± 0.25 vs. 2.5 ± 0.29

Demyelination

- Brain: 0 ± 0 vs. 0 ± 0
- Spinal cord: 0.67 ± 0.17 vs. 1.0 ± 0.29

Table III. Histopathology of EAE in SE + control Ab and SE + anti-IL-12 Ab-treated mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inflammation</th>
<th>Demyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEA + rat IgG</td>
<td>0.5 ± 0</td>
<td>0.67 ± 0.17</td>
</tr>
<tr>
<td>SEA + α IL-12</td>
<td>0.67 ± 0.17</td>
<td>1.0 ± 0.29</td>
</tr>
<tr>
<td>SEB + rat IgG</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>SEB + α IL-12</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

*Three mouse brains or spinal cords were included in each treatment group, with the exception of spinal cords of SEA + anti-IL-12-treated mice, for which n = 2. Histopathologic evaluation was performed, and scores were given as described under Histopathology.

### Discussion

These studies indicate that IL-12 plays a role in spontaneous and SE-induced EAE relapses. In our investigation of expression of IL-12 during murine relapsing EAE in (PL/J X SL/J)F1 mice, we have found mRNA for IL-12 in the CNS only during the acute phases and its absence during remission phases (C.S.C. and A.R., unpublished observations). When we measured serum IL-12 during various stages of EAE in these mice, we did not find detectable levels of IL-12 p70. We believe that the amount of IL-12 in the serum of these animals is below the threshold of detection of our assay and will increase to detectable levels when mice are treated with SE or IL-12. The fact that neutralization of endogenous IL-12 prevented relapses argues for an important role of endogenous IL-12 in the course of EAE.

IL-12 is essential in the generation of Th1 responses and, therefore, plays a key role in the immune response to intracellular pathogens (19). Moreover, IL-12 is involved in the induction of T cell-mediated autoimmune diseases (35) including EAE (20, 21, 27). The role of IL-12 in the maintenance or recurrence of Th1 responses is also well established.

**FIGURE 4.** Representative section of spinal cord of mouse with EAE after administration of SEB + control Ab (A and B) or SEB + anti-IL-12 Ab (C). Perivascular and parenchymal inflammation and demyelination are noted in A and B, while no significant histopathology is observed in C. Similar results were obtained in mice with SEA-induced relapses. The differences between SE + control Ab- and SE + anti-IL-12 Ab-treated mice were statistically significant (p < 0.05). This section was stained with Luxol fast blue and counterstained with cresyl violet. Magnification: A and C, ×200; B, ×400.

**FIGURE 5.** Effect of IL-12 and of neutralizing anti-IL-12 mAb on the course of relapsing EAE. After recovery from the initial attack of EAE, mice were treated as indicated in the figure by the arrow and the notation “Treatment.” IL-12 mimics in part SE action by inducing immediate relapses and worsening later relapses. Anti-IL-12 Ab prevents spontaneous relapses. Results are shown as mean ± SD of clinical scores given as described in Materials and Methods. The course of EAE in mice treated with control rat IgG completely overlaps with that of mice receiving no treatment and is omitted for graphical clarity. The number of mice in each group is as follows: anti-IL-12 mAb, n = 5; rat IgG, n = 4; IL-12, n = 10; no treatment, n = 10). Further characteristics and statistical data are included in Results.
immune responses has also become a subject of recent investigations. The memory responses to certain infections appear to be IL-12-independent (36, 37). In the well-characterized model of murine leishmaniasis, we also recently demonstrated IL-12 independence of the Th1 response in the secondary infection (69). In contrast, the maintenance and chronicity of some, but not all, T cell-mediated autoimmune appearance to require IL-12. For example, the established chronic autoimmune reaction in experimental colitis is abrogated by neutralization of IL-12 (38). In contrast, however, administration of IL-12 during the established phase of autoimmune collagen-induced arthritis suppressed disease due to induction of IL-10 (39). Seder (40) has hypothesized that the differential IL-12 dependence of established Th1 responses in autoimmunity vs infectious diseases may reflect differences in the initial levels of endogenous IL-12 induction, with higher amounts of IL-12 produced during infection than during the response to an autoantigen. Because IL-12 also induces IL-10 (41, 42) in a potential autoregulatory loop, it is possible that the differential IL-12 dependence resides in the balance between IL-12 and IL-10. The role of IL-10 in EAE has been debated. A role in the recovery from EAE has been postulated based on the presence of IL-10 mRNA in the CNS of EAE animals in the recovery phase (43). With regard to the induction phase of EAE, the exogenous delivery of the cytokine in some studies was successful (44), while in other studies it was unsuccessful in preventing the disease (45). With regard to relapses, exogenous IL-10 could not prevent the SEB-induced relapses, while neutralization of the endogenous IL-10 worsened the relapses (46). This underscores the strength of cross-regulation in the endogenous cytokine network, which can determine the susceptibility, the course, and the cytokine dependence of autoimmune. Recently, a unique IL-10/IL-12 immunoregulatory circuit controlling susceptibility to EAE has been demonstrated (47), and it is likely that perturbation of this loop (for example, as in our study) by microbial products powerfully changes the outcome and manifestations of EAE.

A clear possibility to consider in the case of relapsing autoimmune is that IL-12 is important for the phenomenon of determinant (epitope) spreading. Evidence has accumulated indicating that propagation and reactivation of autoimmune diseases occur through the acquisition of autoreactivity to new self-determinants (48–50). Recently it has been shown that during the development of the determinant spreading cascade in murine relapsing EAE new sets of T cells are generated that exhibit a Th1 phenotype (50). Therefore, it is likely that the presence of IL-12 in the cytokine milieu during epitope spreading facilitates the development of these pathogenic neuautoaggressive T cells.

Optimal Th1 responses require a synergy between IL-12 and the B7-CD28 interaction (51, 52). Blockade of B7 costimulation effectively prevented epitope spreading and clinical relapses in EAE (53, 54). Therefore, it is also important to characterize the role of IL-12, the other principal component required for optimal Th1 responses, in ongoing chronic or relapsing T cell-mediated autoimmunity. Here, we show that IL-12 neutralization can also prevent spontaneous relapses in EAE. In addition, we effectively prevented the occurrence of SE-induced relapses with an anti-IL-12 Ab. Although the assessment of the longevity of the protective effect of IL-12 neutralization was not the primary objective of this study, the fact that animals given anti-IL-12 Ab did not develop relapses for a long time following treatment while untreated animals did, provides further support to the hypothesis that once epitope spreading is prevented through blockade of costimulation and/or of IL-12, relapses are also prevented.

The mechanisms of SAG-induced and spontaneous relapses may be different. Activation of the residual SAG-responsive T cells with specific Vβ TCR (for example, Vβ8 for SEB) (3) is postulated and likely to be responsible for the SE-induced relapses. Epitope spreading, as discussed above, is involved in spontaneous EAE relapses (45). Our current findings implicate IL-12 in the mechanism of both processes of T cell reactivation and corresponding clinical relapse in EAE. With respect to the SE-induced relapses, we demonstrated that SE induces IL-12 in vitro, consistent with previous results (24), and, to our knowledge, documented for the first time induction of IL-12 by SAG in vivo. Moreover, we showed that exogenous IL-12 mimicked SE action, inducing rapid relapses, resembling effects recently shown in Lewis rats (22). Because staphylococcal SAG also induce IFN-γ (55), it is possible that IFN-γ production by T cells is stimulated by SE-induced IL-12, and reciprocal stimulation of IFN-γ and IL-12 between T cells and APC is initiated. Although both SE induced similar levels of IL-12 in vitro and in vivo, the severity of SEA-induced relapses was lower than that of relapses induced by SEB. However, both SEA- and SEB-induced relapses exhibited significant dependence of this IL-12 induction. The basis of these differences is currently not completely elucidated. We used the same doses of SE that were shown in a previous study (3) to induce relapses in (PL/J × SJL/J) mice. Interestingly, in that study, SEA was also less efficient than SEB in inducing relapses. It is possible that, despite similar IL-12 inducibility, the lower frequency of SEA-responsive T cells makes this reciprocal stimulation between IL-12 and IFN-γ less efficient, which may explain the lower severity of SEA-induced relapses.

Migration of T cells to the inflammatory compartment, in this case the CNS, may also be stimulated by SE via IL-12 in a manner similar to the demonstrated IL-12 mediation of skin-homing receptor induction by staphylococcal SAG (23). This can explain the absent or minimal inflammation or demyelination in the CNS of mice given SE + anti-IL-12 compared with the inflammatory infiltrates of mice given SE + control Ab observed in our study. IL-12 up-regulates the very late Ag (VLA)-4-dependent T-cell migration (56), a phenomenon known to be required for T cell entry into the brain parenchyma (57). We also demonstrated enhanced expression of VLA-4 on murine MBP-reactive T cells after exposure to IL-12 (C.S.C. and A.R., unpublished observations). Thus, the neutralization of IL-12 may have prevented the up-regulation of VLA-4 and the reentry of autoreactive T cells into the brain. In addition, IL-12 may provide a death-preventing signal to MBP reactive T cells, as shown for other Ag-specific T cells (58).

No or very few residual inflammatory cells were seen in the CNS of mice given SE and treated with anti-IL-12 Abs. Because the time elapsed after recovery from the initial attack was relatively short, one may have expected residual inflammatory infiltrates. Their absence could be interpreted as a stimulated efflux of inflammatory cells from the CNS of mice treated with anti-IL-12 Ab similar to that postulated in the case of EAE mice treated with altered peptide ligands in which relapses were also prevented (59). Another explanation, consistent with our observation that the first bout is relatively short and mild in these mice and with the fact that the clinical residual deficit after the first bout was also mild, is that the inflammation was almost completely resolved at the time of SE administration. In support of this explanation is the fact that histologic analysis of the CNS of mice with near-complete clinical recovery shortly after the first episode is usually normal or only minimally abnormal (C.S.C., A.R., and B.H., unpublished observations).

Because spontaneous relapses may occur through epitope spreading (49), our results suggest that IL-12 plays a role in this phenomenon. Because B7 blockade also prevents spontaneous relapses, this is additional evidence for the complementarity of these
two costimulatory factors necessary for optimal Th1 responses in vivo and in vitro.

Previously, other cytokine-based immunologic manipulations have affected either SAG-induced or spontaneous EAE relapses. Administration of TGF-β (46, 60, 61), IL-10 (46), or IFN-γ (62) prevented relapses, while anti-TGF-β worsened them (63). Blockade of TNF decreased the incidence and severity of relapses (3, 63) and ameliorated EAE in a chronic/relapsing model (64). Interestingly, the cytokines that can prevent relapses antagonize IL-12, while cytokines implicated in relapse pathogenesis synergize with IL-12 (33, 65, 66). Thus, disease-suppressing effects in relapsing EAE may be mediated through IL-12 blockade, while relapses may involve IL-12 and synergistic factors.

Triggering and reactivation of autoimmunity by infectious products are documented in both spontaneous and experimental disease. Spontaneous EAE in MBP-specific transgenic mice occurred only in animals kept in a normal, pathogen-containing environment and not in animals kept in a specific pathogen-free facility (67). The effects of SAG on EAE exacerbations have been attributed either to restricted TCR-dependent mechanisms (3) or to non-specific mechanisms, which include cytokine release (4). The present study supports the latter hypothesis that SAG also employs non-specific mechanisms in reactivating EAE. The role of cytokines in the infection-induced reactivation of autoimmunity has been investigated, particularly with respect to TNF (3, 46, 68). Segal et al. (27) recently implicated IL-12 in the pathogenesis of this phenomenon as well. Our results extend these observations and bring them into the context of bacterial T cell SAG.

In conclusion, we show the ability of SE to induce IL-12 and demonstrate the involvement of IL-12 in SE-induced and spontaneous relapses of EAE. These findings may help to elucidate immunopathogenic mechanisms of relapsing autoimmunity and may provide clues to the immunopathology of MS. Neutralization of IL-12 may prove an effective therapy for autoimmunity demyelination.

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References


