Persistence of Pathogenic CD4+ Th1-Like Cells In Vivo in the Absence of IL-12 but in the Presence of Autoantigen

Kenneth Hong, Ellen L. Berg and Rolf O. Ehrhardt

*J Immunol* 2001; 166:4765-4772; doi: 10.4049/jimmunol.166.7.4765

http://www.jimmunol.org/content/166/7/4765
Persistence of Pathogenic CD4+ Th1-Like Cells In Vivo in the Absence of IL-12 but in the Presence of Autoantigen

Kenneth Hong,* Ellen L. Berg, † and Rolf O. Ehrhardt††

Despite recent successful treatment of murine autoimmune disease with anti-IL-12 mAb, it has not yet been addressed whether anti-IL-12 mAb can also be effective in late stages of disease and whether it can provide lasting protection against recurrence, especially during continued presence of autoantigen. We used a newly developed psoriasis model in scid/scid mice, which allows easy tracking of pathogenic T cells, to show that when anti-IL-12 mAb is given for 2 wk (1 mg/wk) in the late stage of severe disease, inflammation is greatly reduced, as measured by ear thickness and histology (scores, 1.1 ± 0.1 vs 2.0 ± 0.4). Moreover, prolonged treatment (4 wk) of chronic psoriatic mice with high doses of mAb (1 mg/wk; prolonged active anti-inflammatory treatment (PAAIT)) results in the almost complete resolution of lesions (scores, 0.3 ± 0.1 vs 2.7 ± 0.2). Surprisingly, however, despite these significant treatment results, the psoriasis-like lesions return soon after the anti-IL-12 mAb treatment is discontinued. This rapid relapse of disease may be attributed to large populations of activated CD4+ T cells present in the lymph nodes of PAAIT animals still expressing an effector/memory phenotype (CD45RBlow, L-selectinlow). Upon stimulation in vitro such PAAIT lymph node cells secrete high amounts of IFN-γ (129 ng/ml); when transferred into naive scid/scid animals they are able to rapidly induce disease without costimulation. Our data indicates an alternative IL-12-independent pathway for pathogenic Th1-like cells in vivo during the chronic phase of disease that allows these cells to persist and maintain their pathogenicity in the draining lymph tissue of the autoimmune site. The Journal of Immunology, 2001, 166: 4765–4772.

The pathologic mechanism of psoriasis has been very difficult to identify due to the complex nature of this autoimmune disease. However, it has been linked directly to the infiltration of CD4+ T cells in humans and mice (1–4). Various animal models have been developed to study the mechanism of psoriasis, including a recent minor haplotype T cell transfer model in scid/scid mice (1, 3). In these studies it was noted that among other inflammatory mediators, IFN-γ and IL-12 are highly expressed by cells directly isolated from the skin lesions of mice. Furthermore, transfer studies with IFN-γ-deficient T cells have provided evidence that IL-12 acts independently of IFN-γ, and while IFN-γ contributes to the severity of the disease, IFN-γ is essential for neither the induction nor the maintenance of disease (3, 5). Numerous publications have provided evidence that the administration of anti-IL-12 mAb can either prevent or ameliorate early autoimmune-like conditions in rodents (3, 6–15). These studies have indicated that anti-IL-12 mAb treatment may be superior to anti-TNF-α mAb treatment or other treatment strategies, because neutralization of IL-12 leads to the down-regulation of more than one inflammatory mediator (3, 15–17) or, perhaps, to apoptosis of the effector CD4+ T cells themselves (18, 19). That anti-IL-12 mAb treatment is able to eliminate pathogenic Th1-like cells contradicts studies indicating that IL-12 is crucial only for the expansion of Th1-like cells (7). These studies raise the important question of whether Th1-like cells can maintain their pathogenicity in vivo in the absence of IL-12, especially in the presence of autoantigen. In other words, can a pathogenic Th1-like response be maintained in vivo in the presence of continued TCR stimulation, but in the absence of IL-12?

In this study we used a recently described scid/scid psoriasis model (3) to take advantage of the fact that monitoring of disease can be performed easily in this model (i.e., without sacrificing the animal), and thus disease progression can be measured over extended periods of time. In addition, this model is advantageous for studying chronic inflammatory disease treatment because the autoantigen, provided by the minor haplotype mismatch, driving the pathogenic T cells is ever-present. This is in contrast to other models where the autoantigen is removed rather quickly by the endothelioreticular system (14, 20). Third, CD4+ T cells transferred into scid/scid mice can be easily traced, because the scid/scid mouse lacks intrinsic T cells. Thus, any treatment strategy that is able to reduce or eliminate activated CD4+ cells should be easily identified by analyzing CD4+ cells in the diseased tissues as well as in primary and secondary lymphoid tissues.

Here we report that treatment of murine psoriasis with anti-IL-12 mAb can successfully ameliorate late stages of severe chronic disease, but to our surprise fails to eliminate or down-regulate activated Th1-like cells residing in the draining lymph nodes (LN)2 and spleen, even after prolonged administration of mAb. Overall, these studies indicate that established Th1-mediated chronic inflammation can be maintained by an IL-12-independent mechanism and argue for the identification of other targets for the treatment of psoriasis and other autoimmune conditions.

Materials and Methods

Mice

Female BALB/c mice (donor mice) were purchased from The Jackson Laboratory (Bar Harbor, ME), and C.B-17/Icr scid/scid (recipient) mice were purchased from Taconic Farms (Germantown, NY). All mice were

*Protein Design Labs, Inc., Fremont, CA 94555; and †BioSeek, Inc., Burlingame, CA 94010

Received for publication July 12, 2000. Accepted for publication January 24, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Address correspondence and reprint requests to Dr. Rolf O. Ehrhardt, BioSeek, Inc., 863-C Mitten Road, Burlingame, CA 94010, E-mail address: rehrhardt@bioseekinc.com

2 Abbreviations used in this paper: LN, lymph node(s); MHV, mouse hepatitis virus; PAAIT, prolonged active anti-inflammatory treatment.
housed in a specific pathogen-free environment at the Protein Design Labs animal facility and were used between 4 and 12 wk of age. Sentinel mice were used to screen for the following pathogens: mouse hepatitis virus (MHV), Sendai virus, pneumonia virus of mice, Rovivirus serotype 3, Theil-ler’s murine encephalomyelitis virus, *Mycoplasma pulmonis*, and parovirus. Random screens of mice for pinworms were also conducted. MHV was detected once during the work reported here; the affected mice were euthanized, and remaining sources of MHV were eradicated by conventional procedures. None of the other pathogens listed above were detected at any time.

Induction of psoriasiform lesions

The scid/scid CD4+CD45RBlo cells were induced by transfer model used here has previously been described (3). Briefly, splenocytes were collected from 6- to 12-wk-old donor mice (BALB/c), and CD4+ T cells were enriched by a positive selection procedure. The resulting CD4+ enriched population was >90% pure by flow cytometric analysis. Cells labeled with anti-CD4-FITC (9004D; PharMingen, San Diego, CA) and anti-CD45RB-PE (01145A; PharMingen) were sorted using a FACStar (Becton Dickinson, San Jose, CA) cell sorter. Double-positive cells (CD4+CD45RB+) were collected, selecting cells that expressed high levels of CD45RB (the brightest 45%). The collected cell population was >95% pure and viable and was injected s.c. (near base of tail) into C.B-17scid/scid mice, aged 4–6 wk (3 × 10⁵ cells each in 200 μL). An i.p. injection of 20 μg of LPS (L-212, Sigma, St. Louis, MO) and 10 ng of IL-12 p70 (19361V; PharMingen) was given on day 1 following T cell transfer, and an additional dose of 10 ng of IL-12 was administered on day 3, as this cotreatment procedure was found to result in an increased disease penetrance and severity of psoriasis (3).

Lymphocyte cell isolation

Animals from all experimental groups were sacrificed, and spleens and LN were collected in cold sterile PBS. Cell populations from both tissues were recovered by mechanical homogenization. RBC lysis buffer (R7757; Sigma) was used to remove RBC from splenocyte suspensions. In cell transfer studies 5 × 10⁵ cells of whole LN cell suspension were injected i.p. into naive (nondiseased) healthy scid/scid mice.

In vitro stimulation of lymphocytes and detection of cytokines

LN or splenic lymphocytes were resuspended at 10⁶/mL in RPMI 1640 medium supplemented with 10% FBS and 5 × 10⁻⁵ M 2-ME, 2 mM l-glutamine (Life Technologies, Gaithersburg, MD), 10 U/mL penicillin and 100 μg/mL streptomycin (Life Technologies), and 10 mM HEPES. A total of 200 μL of these suspensions were then placed in 96-well tissue culture plates (3072, Falcon; Becton Dickinson) and incubated for 48 h with anti-CD3 (clone 145-2C11; provided by J. Tso, Protein Design Labs, CA) and anti-IL-12 mAb (3, 7, 9–11, 14, 17, 18). This dose was also selected because it was found to be successful in resolving the clinical and histological signs of disease.

Cell surface FACS staining and analysis

One million viable (trypan blue exclusion) lymphocytes in 100 μL were treated with 0.5 μg/mL Fc block (anti-mouse CD16/32 (FcγRI/II receptor), 01241A, PharMingen, clone 2.4 G2) to reduce nonspecific Fc receptor-dependent staining, then stained for 20 min with 0.5 μg of one or more of the following FITC- or PE-conjugated mAbs: anti-mouse L-selectin (01265B; PharMingen), anti-mouse CD4 (L3T4; PharMingen), or anti-mouse CD45RB (16A; PharMingen).

Histopathologic analysis

Necropsies were performed on mice within 2 wk of the last treatment except where noted. Tissue samples from the ear were collected, fixed in paraformaldehyde solution, and submitted to Comparative Bioscience (Sunnyvale, CA) for section preparation. To record disease severity, semi-quantitative histological scores from 0 to 4 were given based on the severity of inflammation from three different cross-sections of the tissue, namely, the base, the middle area, and the tip region of the ear. The histopathological scores were then averaged from all three sections of each tissue, and the scores were reported as the average of all mice examined. Histological evaluation was blindly conducted by two independent investigators. Histological scoring for the skin was as follows: 0 = no signs of inflammation; 1 = low focal areas of infiltration; 2 = low level of mononuclear cell infiltration, mild thickening of epidermis, and mild to moderate acanthosis; 3 = high level of mononuclear cell infiltration, high vascular density, and thickening of the epidermis (acanthosis, rete pegs and hyperplasia of epidermis and keratinocytes, microabscesses, thinning of the granular cell layer); and 4 = very extensive infiltration in epidermis and dermis, very high vascular density, extreme thickening of epidermis, pustule formation, and destruction of granular cell layers.

Results

Anti-IL-12 mAb treatment significantly ameliorates disease even in the late, severe, and chronic stage of disease

Previous studies have demonstrated that anti-IL-12 mAb can prevent the induction of psoriasis-like disease in the scid/scid transfer model (3). In the current study we used this model to assess the ability of anti-IL-12 mAb to affect moderate to severe established disease (9 wk after cell transfer) to answer the question of whether IL-12 is essential for the maintenance of chronic pathogenic Th1-like cells, especially in the presence of persistent Ag (autoantgens). Hence, we only used mice that displayed chronic severe stages of psoriasis (ear thickness >35 μm for >3–5 wk compared with normal ear thickness of 20–22 μm). Of note, the ear thickness of untreated diseased mice remained >35 μm for >18 wk without self-resolution, demonstrating that any improvement in skin lesions is attributable to the actual treatment given in this study.

Initially, anti-IL-12 mAb (1 mg/mouse) was injected i.p. into chronically diseased mice at wk 9. Surprisingly, immediate improvements in ear thickness were observed within 1 wk of the first injection (Fig. 1A). By the second week, 1 wk after the second injection of anti-IL-12 mAb, the average ear thickness of treated animals was reduced by a total of 4.8 ± 1.8 μm, and this improvement was observed in four of four mice (100%). The control groups continued to demonstrate persistent chronic lesions, which, in fact, continued to increase in severity during the course of this study (Fig. 1A). The untreated group had an average increase in ear thickness of 10.3 ± 3.2 μm at the end of the treatment period, while the isotype-treated group had an average increase in ear thickness of 4.8 ± 3.8 μm. These data are representative of four separate treatment experiments, all of which are summarized in Table I.

To evaluate whether these rapid clinical improvements were also reflected in the histologic improvements of the skin, we collected skin biopsies 14 days after the second anti-IL-12 mAb injection. Histologic analysis revealed that the treated mice had significantly improved histopathology compared with the untreated
and isotype-treated group, including thinner epidermis (less acanthosis), reduction of hyperplasia, and improvement of the thickened stratum corneum with fewer focal mounds of parakeratosis. Also, the intraepithelial pustules that are regularly found in severely diseased mice were absent from all anti-IL-12 mAb-treated mouse biopsies. The biopsies were scored 0–4 (criteria given in Materials and Methods), and as shown in Fig. 1B, the anti-IL-12 mAb-treated animal group had an average histology score of 1.1 ± 0.2 (n = 4) compared with the control groups (untreated and isotype-treated; n = 4 each group), which had average scores of 2.0 ± 0.2 and 2.6 ± 0.2, respectively (p < 0.001). The above results were corroborated by extracting cells via tissue digestion and subsequent FACS analysis, demonstrating that virtually no CD3+ and CD4+ T cells (<1%) were left in the tissue of anti-IL-12-treated mice (data not shown). Thus, anti-IL-12 mAb treatment during established chronic skin inflammatory disease was able to reduce disease expression significantly, as measured by both skin thickness and skin histology, even in the continued presence of autoantigens.

**Discontinuation of anti-IL-12 mAb treatment leads to rapid recurrence of disease even after prolonged mAb treatment**

The data collected in the initial experiments indicated that anti-IL-12 mAb is able to ameliorate psoriatic lesions over the short term; however, it remained unclear whether anti-IL-12 mAb treatment cured Th1-mediated inflammatory responses over the long term. To determine the degree of protection of anti-IL-12 mAb treatment against the recurrence of psoriatic lesions, we continued to measure disease development for an extended period of up to 6 wk following mAb treatment. Surprisingly, all animals that showed initial significant disease regression had a recurrence of disease appearing as early as 3 wk after the final injection of mAb. As shown in Fig. 2A, animals that received two injections of anti-IL-12 mAb exhibited a dramatic reduction in ear thickness of 17 ± 2.5 μm 2 wk after the final injection (wk 12). However, this resolution of the disease reversed rapidly, as shown by a 13 ± 6.2 μm increase in average ear thickness from 12–15 wk, which was maintained until 17 wk (Fig. 2A) and later in other experiments.

Of note, the parallel behavior of both arms (control and anti-IL-12-treated) in these experiments during wk 12–17 is expected, because after discontinuation of therapy both groups are left under the same conditions, i.e., no Ab, but the autoantigen is still present (minor haplotype Ag). This ensures disease progression at a similar rate, because the autoantigen is expressed at similar levels in both groups. It is also clear that the anti-IL-12-treated group is moving closer to the control group, because the SDs (not the means) of both groups become greater with time.

The recurrence of disease was also confirmed by histological analysis of skin tissue biopsies taken at both 12 and 17 wk for comparison (2 and 7 wk after the final administration of mAb). At wk 12 the average histology score of anti-IL-12 mAb-treated mice (Fig. 2B) was 1.0 ± 0.6, compared with the average histology

### Table I. Anti-IL-12 mAb treatment is able to resolve severe chronic psoriasis

<table>
<thead>
<tr>
<th></th>
<th>Average Ear Thickness</th>
<th>Average Ear Thickness</th>
<th>Average Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(week 9)</td>
<td>Post Treatment Period</td>
<td>(week 12–14)</td>
</tr>
<tr>
<td>Diseased untreatedb</td>
<td>40.1 ± 6.8c</td>
<td>40.9 ± 3.2</td>
<td>2.5 ± 0.09</td>
</tr>
<tr>
<td>Isotype-treatedd</td>
<td>39.5 ± 1.9</td>
<td>43.1 ± 2.2</td>
<td>2.9 ± 0.23</td>
</tr>
<tr>
<td>Anti-IL-12 mAb-treatede</td>
<td>45.8 ± 2.6</td>
<td>33.7 ± 1.9</td>
<td>0.8 ± 0.15</td>
</tr>
</tbody>
</table>

*Ear thickness of both right and left ears was measured using mechanical spring caliper. Reported as average of four independent experiments.

*Mice with psoriatic lesions were left untreated to ensure that lesions were chronic in the absence of isotype control (n = 24).

*Isotype-treated animals received weekly 1-mg injections of purified rat IgG for 2 or 4 wk (n = 11).

*Anti-IL-12 mAb was administered weekly 1 mg/mouse for 2 or 4 wk (n = 20).
score of 1.5 ± 0.4 at 17 wk, suggesting that a new influx of inflammatory cells enters the disease site after discontinuation of IL-12-neutralizing mAb treatment. In comparison, the histology score of untreated animals was 2.8 ± 0.3. Interestingly, although the recurrence of psoriasiform lesions resulted in an average ear thickness comparable to that of untreated diseased mice, the histology score was less severe in the relapse group. This was due to a moderately lower number of infiltrating mononuclear cells and a lower frequency of micropustules, although the acanthosis in the relapsed animals remained severe. The latter observation could be largely due to the fact that there might be a residual anti-IL-12 effect present after the discontinuation of therapy (especially within the 2-wk period after discontinuation) because of the relatively long half-life of the Ab (~10–14 days).

To address the possibility that anti-IL-12 mAb treatment failed to eliminate pathogenic T cells due to insufficient treatment time, we increased the duration/dose of treatment by 100%. Animals in this study were 14 wk post-T cell transfer, and all had ear thickness >40 μm (very severe disease). One group of animals received two additional injections of 1 mg of anti-IL-12 mAb/mouse/wk for a total of four injections over a period of 4 wk (prolonged active anti-inflammatory treatment (PAAIT)). As shown in Fig. 3, mice that received the extended treatment achieved showed improved ear thickness for the entire treatment period, further indicating that the anti-IL-12 mAb treatment is an effective therapeutic drug candidate for even very severe established disease. As expected, the longer treatment schedule also resulted in a greater reduction in average ear thickness (ear thickness reduced by 21 ± 2.3 μm) 1 wk after the fourth injection of anti-IL-12 (see also Fig. 3) compared with that after 2 wk. Very remarkably, treatment in these mice resulted in the almost complete resolution of psoriatic lesions, at least as indicated by skin thickness. However, consistent with the previous shorter treatment protocol, ear thickness once again began to increase within 2 wk (wk 12) following the last injection of anti-IL-12 mAb (Fig. 3).

The above data indicate that severe psoriasiform lesions, while almost completely resolved in the presence of prolonged high doses of anti-IL-12 mAb, returns soon after anti-IL-12 administration is discontinued. Thus, protection from the recurrence of psoriatic lesions and thus from the influx and/or expansion of pathogenic Th1-like cells is dependent on the continued neutralization of IL-12.

Anti-IL-12 treatment, including PAAIT, fails to eliminate pathogenic Th1-like cells

The data clearly indicate that PAAIT can resolve psoriasiform lesions late in the course of chronic disease, but, surprisingly, this treatment does not provide extended protection against recurrence. One explanation for such rapid recurrence of disease could be that the inflammatory cells (e.g., Th1-like cells) are, in fact, residing in sanctuary sites such as draining LN and/or spleen during the anti-IL-12 mAb treatment period. To determine whether activated/pathogenic T cells at such sanctuary locations are present, we collected cells from the spleen and LN of the animals that had received PAAIT. As shown in Tables II and III, the number of...
CD4+ T cells found in the spleens of treated mice (1.4 ± 0.5 million) was only slightly decreased from that in untreated diseased mice (1.8 ± 0.2 million). In addition, the majority of the CD4+ T cells possessed an activated profile, as 81% of these cells from treated animals were CD45RBlo, and 77% were L-selectinlow. These percentages were strikingly similar to the percentages of activated T cells in diseased animals that had not been treated (72 and 81%; Table III). The lack of change in the activation profile of T cells from treated and untreated mice indicates that anti-IL-12 treatment does not influence the number or activation of inflammatory T cells in the LN and spleens of psoriatic animals.

Cells isolated from LN could not be quantified due to unequal numbers of LN collected from each mouse. However, the proportion of CD4+ T cells in the LN of treated mice compared with that in untreated diseased mice was again similar (51 ± 10 and 64 ± 3%, respectively). Also, draining LN-residing T cells had similar activation profiles among all treatment groups. The majority of T cells in untreated diseased mice compared with treated mice were CD45RBlow (92 and 85%, respectively) and L-selectinlow (84 and 82%; see also Table III). In fact, the ratio of CD45RBlow to CD45Rblohigh cells was consistently greater than 2:1 in both LN and spleen, indicating the presence of a majority of activated T cells. These data clearly suggest that a significant number of activated T cells survive in vivo even in the presence of high amounts of neutralizing IL-12 mAb.

The failure of anti-IL-12 mAb to eliminate activated CD4+ effector T cells raises the question of whether these activated T cells are pathogenic (e.g., able to induce psoriasis). To determine whether the activated T cells in LN and spleen of PAAIT mice were associated with the pathogenic Th1-like phenotype, cells were isolated from the secondary lymphoid organs and stimulated in vitro with anti-CD3 and anti-CD28 for 2 days. The supernatants were collected and tested by ELISA for the presence of secreted IFN-γ and IL-4. As shown in Table IV, the level of IFN-γ secreted from cells isolated from the LN of PAAIT animals after 4 wk of treatment was extremely high and similar to the IFN-γ level in cells isolated from untreated diseased mice (>100 ng/ml; Table IV). In previous studies (3) significant levels of IL-4 were also detected in the inflammatory skin T cells of diseased mice. Similar levels of IL-4 secretion were found in both treated and untreated diseased mice. Interestingly, the ratio of IFN-γ/IL-4 was similar in all groups (treated, isotype, and untreated), indicating that anti-IL-12 mAb treatment fails to induce a shift toward a potentially therapeutic Th2-dominant ratio.

Transfer of LN CD4+ T cells isolated from animals that received PAAIT is able to induce psoriasis

In this final study we sought to determine whether T cells isolated from the LNs of PAAIT-treated animals possessed the ability to cause disease. Thus, 0.5 million LN cells from PAAIT animals were injected into 4- to 6-wk-old naive undiseased scid/scid recipients. The LN inflammatory cell mixture contained only ~20,000 CD4+ T cells as calculated by FACS analysis, and the negative control spleen group consisted of ~5 × 10^6 spleen cells. (Of note: we choose spleen cells as a negative control because scid/scid animals inherently do not exhibit LN cells and only developed LNs in these studies as a result of the drainage from the adjacent inflammatory tissue that was, in turn, induced through the transfer of BALB/c spleen cells.) As shown in Fig. 4, mice that received PAAIT LN cells developed chronic inflammation in the skin, beginning as soon as 5 wk after transfer, as determined by skin thickness. Ear thickness increased in three of four mice to >25 μm (average, 30 ± 4.7 μm at wk 9). This finding was supported by a high histology score of the skin tissue of 2.4 ± 0.4 (wk 10). Interestingly, in these studies mice were not given any additional immunostimulatory coinjections (e.g., LPS and staphylococcal enterotoxin B) as in the original studies (3), indicating that these cells, in fact, consist of activated and skin-specific pathogenic T cells. In sum, the T cells found in the draining LN of PAAIT-treated mice preserve their pathogenicity and homing characteristics in vivo in the presence of neutralizing anti-IL-12 mAb.

Discussion

In the present study, the ability to establish a murine Th1-mediated skin disease by transferring naive T cells into a scid/scid mouse

Table IV. Cytokine profile of cells from LN and spleen of PAAIT treated psoriatic mice (n = 6 mice per group)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IFN-γ (ng/ml)</th>
<th>IL-4 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated LN</td>
<td>113.6 ± 3.6d</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>Isotype-treated LN</td>
<td>100.1 ± 5.4</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>Anti-IL-12-treated LN</td>
<td>129.0 ± 5.5</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>Anti-IL-12 mAb-treated spleen</td>
<td>106.1 ± 3.7</td>
<td>92 ± 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>IFN-γ (ng/ml)</th>
<th>IL-4 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated LN</td>
<td>113.6 ± 3.6d</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>Isotype-treated LN</td>
<td>100.1 ± 5.4</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>Anti-IL-12-treated LN</td>
<td>129.0 ± 5.5</td>
<td>103 ± 12</td>
</tr>
<tr>
<td>Anti-IL-12 mAb-treated spleen</td>
<td>106.1 ± 3.7</td>
<td>92 ± 8</td>
</tr>
</tbody>
</table>

* Cells represent cell populations pooled from six mice recovered from LN and spleen and cultured for 3 days using anti-CD28 and anti-CD3 conventional stimulation methods.

* Secreted IFN-γ release was measured in supernatant of cultured cells, measured by ELISA in ng/mL. Data represent an average of two experiments.

* Error = ±SD, values were calculated using Soft-Max software.

* Values represent percentages from one of two experiments, calculated using CellQuest (Becton Dickinson) software (n = 5 or 6 mice per group).
humans (3, 21, 22). The crucial roles in psoriasis both in animal models and possibly in cating that additional factors are responsible for the perpetuation of and by transfer studies. Overall, the above data suggest that neu-
tissues of spleen and draining LN. Furthermore, these T cells
skin lesions could be reduced and nearly eliminated even in the
3), we demonstrate that the clinical and histological signs of the
prevented with the use of a neutralizing mAb against IL-12 (3).
Here, we describe the effect of neutralizing IL-12 in the chronic
late stages of murine Th1-mediated psoriasis and studies to deter-
mine the role of this cytokine in the maintenance of pathogenic Th1-like cells. Using a previously established psoriasis model (1, 3), we demonstrate that the clinical and histological signs of the skin lesions could be reduced and nearly eliminated even in the severe, late chronic stage of disease by neutralizing IL-12. Mice that received systemic administration of anti-IL-12 mAb showed a significant decrease in skin thickness, with a reduction of both aca
th and infiltration of mononuclear cells at the primary disease site. We further show that T cells, while absent from the disease site, are not eliminated from treated animals even from PAAIT animals, but are instead found in the secondary lymphoid tissues of spleen and draining LN. Furthermore, these T cells proved to be activated and pathogenic by the cell surface activation markers CD45RB and L-selectin, by a Th1-like cytokine profile, and by transfer studies. Overall, the above data suggest that neu-
neutralization of IL-12 after disease has begun does not lead to the elimination or down-regulation of all Th1-like cells in vivo, indic-
that additional factors are responsible for the perpetuation of Th1-like cells in vivo.

Th1-like cells and, in particular, IL-12 have been shown to play crucial roles in psoriasis both in animal models and possibly in humans (3, 21, 22). The scid/CD45RB<sup>high</sup> transfer model of psoriasis has several advantages over other models of chronic inflammatory diseases. It is induced by a traceable population of CD4<sup>+</sup>/CD45RB<sup>high</sup> T cells in a host mouse that has no indigenous immune system. Compared with other models the cell transfer does not involve genetic mutations that render the mouse unable to regulate the immune system. In addition, the disease progress is easy to monitor, and the extended chronic duration allows for long time-course treatment studies. Moreover, the desirable chronic trait is mostly due to the T cell response to the ever-present minor haplotype mismatch acting as a model autoantigen (1). These fac-
tors offer advantages over most existing models of chronic Th1-
mediated disease that use haptenizing agents that are eventually removed from the host animal, thus making it difficult to interpret treatment effects over long periods of time. Lastly, the psoriasis that is induced in this study is monitored by measuring the thick-
ening of the skin (ears) and histological hallmarks (hyperkeratosis, parakeratosis) in the skin tissue, which are remarkably similar to those in the human condition (3).

Previous studies have shown that IL-12 has important proin-
flammatory functions, as it plays a key role in the differentiation of naive T cells into IFN-γ-producing Th1-like cells, which, in turn, can induce a multitude of autoimmune diseases (10, 23–25). The regulation of autoimmune diseases during the late chronic stage by this cytokine, e.g., the indirect or direct influence of IL-12 on the maintenance and/or influx of mononuclear cells including the Th1-
like population, remains largely unclear. This is of particular in-
terest in the clinical setting, because the question arises of whether the administration of anti-IL-12 is able to control these cells and/or eliminate the pathogenic culprit, e.g., the effector T cell, altogether.

In this study one of the most notable findings is that the severe T cell-induced chronic psoriasis-like lesions are resolved quickly upon treatment with anti-IL-12 mAb. However, such resolution is nonlasting, as the inflammation returns within weeks after the re-
mob of the Ab, suggesting that IL-12 must be present to maintain mononuclear infiltration, including Th1-like cells at the autoim-
mune site. Interestingly and surprisingly, while the inflammatory process resolves at the autoimmune site in mice treated with anti-
IL-12, inflammatory Th1-like cells are found in abundance in the draining LN and spleens of these animals. In another report sug-
gest IL-12 dependence of pathogenic Th1-like cells in vivo (26), the authors observed that although Th1-like responses to exogenous Ags were significantly reduced in wild-type and IL-12-
deficient NOD mice the grades of insulinitis and insulin-dependent diabetes mellitus were similar. Interestingly, in these mice the au-
ors also observed a significant change in the tissue distribution of Th1-like cells.

Several mechanisms could be responsible for the tissue-selective inflammation-promoting effect of IL-12. Inflammatory cells, including Th1-like cells, could undergo only selective apoptosis in the skin upon treatment, but not in the draining LN or spleen of these mice. However, this is unlikely because this would argue for the presence of differential environments, one that supports apo-
thesis and not proliferation of Th1-like cells and vice versa. In this regard it is important to mention that others have reported that anti-IL-12 mAb is able to promote Fas-mediated apoptosis of Th1-
like T cells in the trimethoprazene sulphonic acid-induced model of colitis (19). Thus, it might be possible that anti-IL-12 mAb could have differential effects in various local environments.

Another possible mechanism that could result in a reduced num-
ber of skin inflammatory mononuclear cells after anti-IL-12 treat-
ment is the down-regulation of cell adhesion molecules. In this regard several studies suggest that in psoriasis, proinflammatory cytokines such as IL-12 and TNF-α must remain at high levels to support the induction of a multitude of adhesion molecules that include ICAM, VCAM, and E- and P-selectins (27, 28). In partic-
ular, the finding that IL-12 has a promoting effect on the expres-
sion of P-selectin ligands on Ag-activated T cells (28) comple-
mements evidence that activated CD4<sup>+</sup> T cells can only migrate into the skin if they express E- and P-selectin ligands (29). These data also are consistent with our previous findings using the CD45RB psoriasis transfer model, where Th1-like cells that express E/P-
selectin ligand are preferentially found in the skin, but not in the colon, and are capable of inducing psoriasis, but not colitis, in

![FIGURE 4.](http://www.jimmunol.org/) Redistribution of Th1-like cells in the absence of IL-12.

Cells isolated from LN of PAAIT animals cause psoriatic lesions upon transfer without coinjections of IL-12 and/or LPS. From one representative experiment, the average ear thickness of naïve scid/scid mice that received unsorted LN cells derived from PAAIT animals (■) and unsorted spleen cells from normal undiseased BALB/c mice (●; negative control). LN recipient mice received s.c. injections of 5 × 10<sup>5</sup> LN cells (n = 4). Spleen recipient mice received s.c. injections of 5 × 10<sup>5</sup> spleen cells (n = 4). Psoriatic lesions were observed beginning 8 wk after transfer of LN cells isolated from PAAIT-treated mice.
adoptive transfer studies (30, 31). Moreover, Austrup et al. showed that only Th1-like cells, but not Th2-like cells, can enter into cutaneous Th1-type inflammatory sites, presumably through E- and P-selectin-dependent mechanisms (32). These findings are further supported by evidence that the recruitment of pathogenic T cells into peripheral inflammatory sites, including adjuvant-induced arthritis, can be prevented using anti-E- and P-selectin mAbs (33, 34). Additionally, activated T cells up-regulate their expression of E- and P-selectin ligand as observed in a sensitized skin model, and the presence of a combination of anti-E- and P-selectin Abs was able to block the migration of such T cells into the skin (29). Other studies, which examined the mechanism of E- and P-selectin ligand up-regulation, showed that when CD4+ T cells are activated in the presence of IL-12, levels of FucT-VII mRNA and binding to E- and P-selectin are significantly increased (35, 36). Thus, the down-regulation of E/P-selectin (ligand) on endothelial and inflammatory T cells through the neutralization of IL-12 might contribute to the redirection of pathogenic cells away from inflammatory sites into the draining LN.

Most T cells isolated from animals treated with anti-IL-12 mAb expressed very low levels of L-selectin. This is not unexpected, because it has been shown that upon cell activation, naive CD4+ mouse T cells shed L-selectin, and it has been suggested that certain memory cells are associated with the L-selectin− subset (37, 38). Moreover, others have demonstrated that the expression of L-selectin on Th1-like cells is dependent on the presence of IL-12 (39). Given the key role played by IL-12 in the differentiation of naive T cells into the Th1-type response, the observation that IL-12 can also regulate L-selectin expression has implications for the migration of Th1-like effector cells both through the lymphatic system and to sites of inflammation. In this regard our adoptive transfer studies add support to the idea that L-selectin might not be necessary for the migration of Th1-like cells into the skin. However, this is in contrast to studies suggesting that expression levels of L-selectin are particularly important for the homing of memory cells to the skin (40).

Recently, the novel cytokine IL-18 or IFN-γ-inducing factor has been described as a growth and differentiation factor for Th1-like cells, thus sharing some of the biological activities of IL-12. IL-18 has been shown to induce the production of IFN-γ while inhibiting the production of IL-10, but surprisingly, despite the similarities with IL-12, has not been shown to drive de novo Th1-type development; instead, it promotes the expression of existing live cells (41).

Based on these in vitro observations of IL-18, it is likely that IL-18 may play a key role in the maintenance of Th1-like cells in vivo. Thus, it is likely that some or all subsets of Th1-like T cells can maintain their Th1-like character and pathogenicity in the absence of IL-12, and thus studies examining the effect of combined IL-12 and IL-18 neutralization are necessary in the future. An indication of the importance of the combined effect of IL-12 and IL-18 in the pathogenesis of autoimmunity might be given in a recent study by Takeda et al., in which it was shown that in combined IL-12- and IL-18-deficient mice, Th1-like and NK cell development was significantly more impaired, but was not completely abolished, than that in either single cytokine-deficient mouse alone (42). Thus, although IL-18 might play an important synergistic role in Th1-like development, it is most likely that factors other than IL-18 and IL-12 are involved in the in vivo differentiation of Th1-like cells.

In summary, the data demonstrate the effect of anti-IL-12 mAb in severe cases of chronic progressive murine psoriasis induced by CD4/45RBhigh cell transfer. The remarkable abrogation of skin inflammation of these mice and the similarity of histology observed in humans suggest that anti-IL-12 mAb treatment has potential therapeutic value in human patients with this disease, but possibly only when continuously administered. This strategy may become increasingly effective when combined with apoptotic treatment strategies. In this regard it will be interesting to determine under which conditions, if at all, neutralization of IL-12 can lead to the elimination of chronic Th1-like cells at the disease site and also, importantly, at the Th1-like cell sanctuary sites.

Acknowledgments

We thank Dickie Polakoff for performing the cell sorting.

References

tol. 8:429.
tigen-induced and spontaneous relapses of experimental autoimmune encephalo-
munol. 158:566.
munol. 111:377.
12. Neurath, M. F., K. Vermeire, T. Mitera, H. Heremans, S. Huang, and A. Billian. 1998. Anti-IL-12 antibody prevents the development and progression of collag-
perimentally induced colitis with trinitrobenzene-sulfonic acid in rats. Int. J. Exp. Pathol. 77:175.
kinin-11 therapy selectively downregulates type I cytokine proinflammatory path-


