Suppression of CD4+ T Lymphocyte Effector Functions by CD4+CD25+ Cells In Vivo

Bruno Martin, Alice Banz, Boris Bienvenu, Corinne Cordier, Nicole Dautigny, Chantal Bécourt and Bruno Lucas

J Immunol 2004; 172:3391-3398; doi: 10.4049/jimmunol.172.6.3391
http://www.jimmunol.org/content/172/6/3391

Why The JI?

• Rapid Reviews! 30 days* from submission to initial decision
• No Triage! Every submission reviewed by practicing scientists
• Speedy Publication! 4 weeks from acceptance to publication

*average

References
This article cites 46 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/172/6/3391.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Suppression of CD4+ T Lymphocyte Effector Functions by CD4+CD25+ Cells In Vivo

Bruno Martin,* Alice Banz,2† Boris Bienvenu,* Corinne Cordier,† Nicole Dautigny,† Chantal Bécourt,* and Bruno Lucas3*  

CD4+CD25+ regulatory T cells have been extensively studied during the last decade, but how these cells exert their regulatory function on pathogenic effector T cells remains to be elucidated. Naïve CD4+ T cells transferred into T cell-deficient mice strongly expand and rapidly induce inflammatory bowel disease (IBD). Onset of this inflammatory disorder depends on IFN-γ production by expanding CD4+ T cells. Coinjection of CD4+CD25+ regulatory T cells protects recipient mice from IBD. In this study, we show that CD4+CD25+ regulatory T cells do not affect the initial activation/proliferation of injected naïve T cells as well as their differentiation into Th1 effectors. Moreover, naïve T cells injected together with CD4+CD25+ regulatory T cells into lymphopenic hosts are still able to respond to stimuli in vitro when regulatory T cells are removed. In these conditions, they produce as much IFN-γ as before injection or when injected alone. Finally, when purified, they are able to induce IBD upon reinjection into lymphopenic hosts. Thus, prevention of IBD by CD4+CD25+ regulatory T cells is not due to deletion of pathogenic T cells, induction of a non reactive state (anergy) among pathogenic effector T cells, or preferential induction of Th2 effectors rather than Th1 effectors; rather, it results from suppression of T lymphocyte effector functions, leading to regulated responses to self. The Journal of Immunology, 2004, 172: 3391–3398.

The existence of a subpopulation of T cells that specialize in the suppression of immune responses was originally postulated in the early 1970s (1). However, interest in such cells was gradually lost because the molecular mechanisms responsible for such suppressive phenomena were difficult to characterize. Recently, advances in the identification of CD4+ T cell subpopulations, together with the use of genetically modified mice, have led to a renaissance of this field; the concept of regulatory T cells has been reborn as an additional mechanism to explain the maintenance of peripheral self-tolerance, alongside T cell deletion and T cell anergy (2–7). The notion of regulatory T cells is conceptually attractive because it explains how tolerance can be adaptively transferred by T cells in some experimental systems, how pathological responses to self and harmless foreign Ags are prevented, and how host bystander tissue insult is avoided during normal immune responses.

Physiologically generated CD4+CD25+ cells are the most widely studied type of regulatory T cells. These cells inhibit a wide range of autoimmune and inflammatory manifestations such as gastritis, oophoritis, orchitis, thyroiditis, inflammatory bowel disease (IBD), and spontaneous autoimmune diabetes (8–11). Despite numerous studies, the mechanisms by which regulatory T cells exert their function are unclear. Some studies have shown that regulation is dependent in vivo on the production of suppressive cytokines such as IL-10 and TGF-β and cell surface molecules such as CTLA-4 (12–18). In vitro experiments aimed at further dissecting the mechanisms by which T cells exert their regulatory function have given controversial results. Indeed, in contrast to in vivo studies, neither soluble cytokines nor CTLA-4 seem to be required for the suppressive effects of CD4+CD25+ cells in vitro (19–22).

Despite numerous studies, it remains to be shown how regulatory T cells exert their suppressive function on pathogenic effector T cells. The conflict between in vitro and in vivo data now makes it mandatory to study regulatory functions in vivo, using experimental models. Naïve CD4+ T cells transferred into T cell-deficient mice expand strongly and rapidly induce IBD (23). This inflammatory disorder depends on IFN-γ production by expanding CD4+ T cells (24). Coinjection of CD4+CD25+ regulatory T cells protects recipient mice by inhibiting IFN-γ production by pathogenic effector CD4+ T cells (18, 25–27), but the precise mechanism by which they act remains to be elucidated. Indeed, prevention of IBD could be due to deletion of pathogenic T cells, induction of a non reactive state (anergy) among pathogenic effector T cells, preferential induction of Th2 effectors rather than Th1 effectors or permanent action (direct or indirect (through APCs)) of regulatory T lymphocytes on effector T cells (suppression).

Materials and Methods

Mice

C57BL/6 mice (Thy1.2; H-2b) and BALB/c mice (H-2d) were from Centre d’élevage Janvier (Le Genest Saint Isle, France), and C57BL/6 CD3ε-deficient mice (CD3ε” mice) (28) were from Centre de Développement des Techniques Avancées pour l’Experimentation Animale (Orléans, France). C57BL/Ba mice (Thy1.1, H-2b) were maintained in our own animal facilities.

Copyright © 2004 by The American Association of Immunologists, Inc.
Adoptive transfer of T cells

Lymph node cells were depleted of macrophages, granulocytes, and CD8+ T cells by incubating them first with anti-CD11b (Mac-1) Ab, anti-GR1 (8C5) Ab, and anti-CD8 (Ly-2) Ab, and then with magnetic beads coupled to anti-rat Ig (Dynal, Great Neck, NY). B cells were removed using magnetic beads coupled to anti-mouse Ig (Dynal). Purified CD4+ T cells from C57BL/6 mice (Thy1.2) were labeled with biotinylated anti-CD25 (clone PC61). CD4+ CD25+ T cells were then positively selected using MACS streptavidin microbeads (Miltenyi Biotec, Paris, France). CD4+ CD25- T cells were usually 90–95% pure. Purified CD4+ T cells from C57BL/6Ba mice (Thy1.1) were labeled with biotinylated anti-CD25 (clone PC61) and PE anti-CD44 (clone 1M7). CD4+ CD25+ CD44- naive T cells were then purified by sorting in a FACSVantage flow cytometer (BD Biosciences, Mountain View, CA).

CD4+ naive T cells (1 × 10⁷) were injected i.v., with or without purified CD4+ CD25+ T cells (0.2 × 10⁶), into C57BL/6 CD3e-deficient mice. The spleen and lymph nodes of these mice were recovered, pooled for cell preparation, and analyzed at various times after CD4+ T cell transfer.

In some experiments (Fig. 7), naive CD4+ T cells (Thy1.1) were injected, alone or together with CD4+ CD25+ regulatory T cells (Thy1.2), before being purified as described above. Thy1.2+ cells were removed by incubating purified CD4+ T cells first with anti-Thy1.2 Ab (clone 53-2.1) and then with magnetic beads coupled to anti-rat Ab. Purified Thy1.2+ CD4+ T cells (0.5 × 10⁷) were then retransferred into CD3e-deficient mice.

Histology

Colon were removed from mice 4 wk after transfer and fixed in PBS containing 10% formaldehyde. Five-micrometer paraffin-embedded sections were cut and stained with H&E.

Cell surface staining and flow cytometry

Lymph nodes and spleens were pooled; homogenized in PBS, 5% FCS, and 0.2% NaN3 with a nylon cell strainer (Falcon, Franklin Lakes, NJ); and distributed in 96-well U-bottom microplates (4 × 10⁶ cells per well). Staining was performed on ice for 30 min per step.

Abs were purchased from BD PharMingen (San Diego, CA) unless otherwise indicated. The following Abs combinations were used: for four-color analysis, PE anti-Thy1.2, anti-CD19, anti-CD44, FITC anti-TCR, anti-GR1, PerCP anti-CD4, anti-CD8, and biotinylated anti-CD25, anti-CD25, anti-CD44, anti-CD45 or anti-CD69 with allophycocyanin-streptavidin development (BD PharMingen). Four-color immunofluorescence was analyzed using a FACSCalibur cytometer (BD Biosciences). List-mode data files were analyzed using CellQuest software (BD Biosciences).

Intracytoplasmic staining

Peripheral T cells were recovered at different time-points after transfer and then stimulated 6 h with PMA and ionomycin in the presence of brefeldin A. After surface staining, cells were fixed and permeabilized, and then intracytoplasmic staining was performed using PE anti-IL-2 (JES6-5H4), anti-IFN-γ (XMG1.2) and anti-IL-4 (11B11). PE rat IgG1 was used as the isotype control. Abs were purchased from BD PharMingen.

Bromodeoxyuridine (BrdU) labeling

One milligram of BrdU (Sigma-Aldrich, St. Louis, MO) was injected i.p., twice at a 30-min interval. To detect and characterize DNA-synthesizing cells, spleens and lymph nodes were removed 30 min after the second injection and pooled for cell preparation. Surface-stained cells were fixed and permeabilized in PBS containing 1% paraformaldehyde plus 0.01% Tween 20 for 48 h at 4°C and then submitted to the BrdU DNase detection technique as described in Ref. 29, using FITC-conjugated anti-BrdU Ab (BD Biosciences).

Proliferation and ELISA

A total of 10⁷ purified CD4+ T cells was cultured in RPMI Glutamax 1640 medium (Life Technologies-Invitrogen Corporation, U.K.), 10% FCS, 2 mM l-glutamine and antibiotics, with 5 × 10⁵ irradiated (2000 rad) peritoneal macrophages from H-2b (C57BL/6) or H-2d (BALB/c) mice in 96-well U-bottom microplates (Falcon). Supernatants were collected 96 h after the beginning of culture. IFN-γ production was assessed by ELISA.

Results

CD4+ CD25+ regulatory T cells do not affect the initial proliferation/expansion of naive CD4+ T cells

CD4+ T cells from C57BL/Ba mice (Thy1.1) were purified by depletion of B cells, macrophages, granulocytes, and CD8+ T cells using magnetic beads; naive CD4+ T cells were then electronically sorted on the basis of their nonexpression of CD25 and low or absent expression of CD44, an activation marker expressed at high densities on effector/energetic/memory/regulatory T cells (Fig. 1A). CD4+ CD25+ T cells from C57BL/6 mice (Thy1.2) were purified by depletion of B cells, macrophages, granulocytes and CD8+ T cells, followed by positive selection of CD25-expressing cells (Fig. 1B).

A total of 1 × 10⁶ purified naive CD4+ T cells were transferred with or without 0.2 × 10⁶ purified CD4+ CD25+ T cells (to respect the normal proportion of CD25+ cells among CD4+ T cells, i.e., between 10 and 20% depending on the mouse strain) to T cell-deficient mice (CD3e-deficient mice; Fig. 1C; groups B and C). Total CD4+ T cells were also injected (Fig. 1C; group A).

FIGURE 1. CD4+ CD25+ regulatory T cells prevent wasting disease induced by transfer of naive CD4+ T cells in CD3e-deficient mice. A, CD4+ T cells from C57BL/Ba mice (Thy1.1) were purified by negative selection of non-CD4+ T cells and naive CD4+ T cells, which were electronically sorted on the basis of their expression of CD25 and their low or absent expression of CD44. B, CD4+ CD25+ T cells from C57BL/6 mice (Thy1.2) were positively selected on the basis of their CD25 expression. C, CD3e-deficient mice were injected i.v. with total CD4+ T cells (group A), purified naive CD4+ T cells (CD4+ CD25+; group B) or both naive and CD25+ CD4+ T cells (group C). D, Body weight was monitored during the first 4 wk after CD4+ T cell transfer. Results are representative of four independent experiments (three mice per group and per experiment).
Mice injected with naive CD4⁺ T cells alone lost 20–40% of their initial weight 4 wk after transfer (Fig. 1, D and E). This loss of weight was associated with poor general health, severe diarrhea, and rectal prolapse. More precisely, these mice developed IBD characterized by extensive mononuclear cell infiltrates, depletion of mucin-secreting cells, ulcers, and pronounced epithelial cell hyperplasia (Fig. 2). As already described by other groups (23), coinjection of CD4⁺CD25⁻ cells together with naive CD4⁺ T cells prevented wasting and histological signs of inflammation (Figs. 1E and 2). Similarly, mice injected with total CD4⁺ T cells showed no signs of IBD, demonstrating that a normal proportion of CD4⁺CD25⁺ regulatory T cells was sufficient to protect mice from the disease (Fig. 1, D and E).

The use of Thy1 as an allotypic marker to discriminate between injected naive CD4⁺ T cells and CD4⁺CD25⁺ regulatory T cells allowed us to study the absolute number and cycling status of...

FIGURE 2. CD4⁺CD25⁺ regulatory T cells prevent IBD induced by transfer of naive CD4⁺ T cells in CD3-e-deficient mice. CD3-e-deficient mice were injected i.v. with purified naive CD4⁺ T cells (A–C) or both naive and CD25⁺CD4⁺ T cells (D–F). Mice were killed 4 wk post-transfer and paraffin-embedded sections of distal colon stained with H&E. Magnifications were the same for each row, ×14 (A and D) and ×70 (B, C, E, and F).

FIGURE 3. CD4⁺CD25⁺ regulatory T cells do not affect the initial proliferation/expansion of naive CD4⁺ T cells. CD3-e-deficient mice were injected i.v. with total CD4⁺ T cells, purified naive CD4⁺ T cells (CD4⁺CD44⁺CD25⁻; Thy1.2⁻) or both naive (Thy1.2⁺) and CD25⁺ (Thy1.2⁺) CD4⁺ T cells. Lymph nodes and spleens were recovered, pooled, and used to prepare single cell suspensions at various times after transfer. A, The absolute numbers of CD4⁺ Th1.2⁺ TCR⁺ cells (as a function of their Thy1.2 expression), CD19⁺ cells and GR1⁺ cells were then determined. Lymph nodes and spleens of control mice (normal C57BL/6 mice) contained 28 × 10⁶ ± 1 × 10⁶ CD4⁺ TCR⁺ cells, 67.4 × 10⁶ ± 3.8 × 10⁶ CD19⁺ cells and 1.8 × 10⁶ ± 0.3 × 10⁶ GR1⁺ cells. B, Mice were injected twice with BrdU before being sacrificed. FSC/BrdU dot-plots are shown for CD4⁺Th1.2⁺ cells from group B and C mice as a function of time after transfer. C, FSC fluorescence histograms of CD4⁺Th1.2⁺ cells from group B and C mice are shown in comparison with FSC fluorescence histograms of control CD4⁺ T cells from normal C57BL/6 mice. Results are representative of at least two independent experiments (three mice per group and per experiment).
injected with total CD4
/H11001 strongly during the first 2 wk, independently of regulatory T cell coinjection (Fig. 3A, group B; group C: Thy1.2– cells). More precisely, expansion was maximum in all groups between the first and the second week. Indeed, two days after transfer, CD4+ T cell recovery was around 20% of the absolute number of injected cells, i.e., around 0.2 × 10^6 CD4+ T cells (data not shown). Expansion was only up to 2-fold during the first week whereas absolute numbers of recovered CD4+ T cells increased by 50-fold between the first and the second week. The proliferation rate (BrDU incorporation) and cell size (FSC) of recovered naive CD4+ T cells were similar in the two groups 1 and 2 wk after transfer (Fig. 3, B and C; Thy1.2– cells). Only when they were injected alone (group B) did naive T cells continue to expand, giving rise to a CD4+ T cell pool five times larger than that obtained when regulatory T cells were coinjected (group C: Thy1.2– cells) or when total CD4+ T cells were injected (group A). Accordingly, naive CD4+ T cells exhibited a higher proliferation rate and contained a higher proportion of blast cells 3 wk after transfer when injected alone than when coinjected with CD4+/CD25+ regulatory T cells (Fig. 3, B and C).

In all groups, the absolute number of granulocytes increased strongly during the first 2 wk posttransfer (Fig. 3A). Only in mice injected with total CD4+ T cells (group A) did the absolute number of peripheral granulocytes then return to normal, suggesting that a subset of CD4+ T cells other than CD25+ regulatory T cells participates in the control of inflammation. The absolute numbers of B lymphocytes as determined by CD19 staining varied similarly with time in all groups (Fig. 3A).

CD4+CD25+ regulatory T cells modulate the phenotype of expanding naive CD4+ T cells

The expression kinetics of surface molecule expression by expanding naive CD4+ T cells started to diverge as early as 1 wk after transfer according to whether or not regulatory T cells had been coinjected. Indeed, 1 and 2 wk after transfer, CD45 expression by naive CD4+ T cells increased less strongly when CD4+/CD25+ regulatory T cells were coinjected. Moreover, naive CD4+ T cells exhibited stronger CD45 expression, at all time-points after transfer, when they were co-injected with CD25+ regulatory T cells. No such differences were observed concerning the expression of activation markers (CD25, CD44, CD45RB, CD62L, CD69), costimulatory molecules (CD28, CD152), adhesion molecules (CD11a, CD54), or the TCR itself (Fig. 4 and data not shown).

Thus, although the extent of naive CD4+ T cell proliferation during the first 2 wk after transfer was independent of CD4+/CD25+ regulatory T cell cotransfer, their phenotype was already modulated by CD4+/CD25+ regulatory T cells.

CD4+CD25+ regulatory T cells do not interfere with the differentiation of expanding naive CD4+ T cells into Th1 effector/memory T cells

At different time-points after transfer, peripheral T cells were recovered and their capacity to produce IL-2, IFN-γ, and IL-4 (Fig. 5) was estimated ex vivo. More precisely, peripheral T cells were submitted to a short (6 h) stimulation by PMA and ionomycin in the presence of brefeldin A followed by intracytoplasmic staining of their cytokine production. Previous data have shown that regulatory CD4+CD25+ T cells did not interfere with T cell stimulation by PMA and ionomycin (19). Therefore, such a protocol allowed us to determine the differentiation status of expanding naive CD4+ T cells according to whether or not regulatory T cells were coinjected.

Independently of regulatory T cell coinjection, injected naive CD4+ T cells (Thy1.1) exhibited a similar pattern of cytokine production at all tested time-points after their transfer (Fig. 5). Only 4 wk after transfer did naive CD4+ T cells injected alone produce significantly less IL-2 than naive CD4+ T cells co-injected together with regulatory CD4+CD25+ T cells. Such a result certainly reflected the poor general health of mice injected only with naive CD4+ T cells at this time-point (Figs. 1E and 2) and therefore the effect of stress on immune cells. More importantly, in both groups B and C, most expanding naive T cells differentiated into IFN-γ-producing cells, i.e., into Th1 effector/memory T cells. No significant IL-4 production was detected in both groups (Fig. 5B). Thus, no evidence of a shift from a Th1 to a Th2 cytokine pattern induced by regulatory CD4+CD25+ T cells can be characterized in.
CD4^+CD25^+ regulatory T cells do not interfere with the differentiation of expanding naive CD4^+ T cells. CD3e-deficient mice were injected i.v. with purified naive CD4^+ T cells (Thy1.1^+; group B) or with both naive (Thy1.1^+ and CD25^+ (Thy1.2^+)) CD4^+ T cells (group C). Lymph nodes and spleens were recovered, pooled, and used to prepare single cell suspensions at various times after transfer. Cells were cultured 6 h in the presence of PMA, ionomycin, and brefeldin A. Then they were stained for surface expression of Thy1.1, TCR, and CD4 followed by intracytoplasmic staining of IL-2, IL-4, or IFN-γ.

A. IL-2 and IFN-γ production by CD4^+TCRhiThy1.1^+ cells from group B and C mice are shown in comparison with control CD4^+ T cells from normal C57BL/6 mice, 1 wk after transfer. B. IL-2, IL-4, and IFN-γ production by CD4^+TCRhiThy1.1^+ cells from group B and C mice are shown as a function of time after their transfer. CD4^+ T cells from normal C57BL/6 mice were used as controls. Three mice per group have been analyzed for 1 and 2 wk after transfer, six mice in two independent experiments for 4 wk after transfer.

**Discussion**

Several mechanisms might explain how CD4^+CD25^+ regulatory T cells prevent autoimmune and inflammatory disorders, such as induction of anergy (31) or clonal deletion (32) of pathogenic T cells. The ability of H-2^d transferred CD4^+ T cells to respond to H-2^d APCs was evaluated 4 wk after transfer, when the absolute number of recovered CD4^+ T cells reached a plateau in all groups. When injected alone, expanding naive CD4^+ T cells proliferated and produced IFN-γ to the same extent as control CD4^+ T cells (Fig. 6; group B). By contrast, no response was observed when total CD4^+ T cells were injected, or when CD4^+CD25^+ regulatory T cells were coinjected at the same time as naive CD4^+ T cells, suggesting that, in these two groups (A and C), following their expansion, naive CD4^+ T cells became anergic. Nevertheless, the ability of naive CD4^+ T cells to respond to H-2^d APCs was restored by removing CD4^+CD25^+ regulatory T cells before culture. Indeed, in these conditions, they proliferated and produced IFN-γ (Fig. 6; group C: Thy1.2^+ ) and IL-2 (data not shown) to the same extent as control CD4^+ T cells. Thus, naive CD4^+ T cells co-injected with CD4^+CD25^+ regulatory T cells do not completely lose their ability to respond to antigenic stimulation but are rather subject to suppression as long as they are together with CD4^+CD25^+ regulatory T cells.

To determine whether this also applied to pathogenic CD4^+ T cells responsible for IBD, naive CD4^+ T cells (Thy1.2^+), injected alone or together with CD4^+CD25^+ regulatory T cells, were purified 4 wk later and then retransferred into empty hosts (Fig. 7A). Naive CD4^+ T cells injected first alone induced rapidly and uniformly IBD upon retransfer (Fig. 7B; group B’ vs group B). For their part, naive CD4^+ T cells co-injected first with CD4^+CD25^+ regulatory T cells also induced IBD upon retransfer although less rapidly that what could be observed with naive CD4^+ T cells injected first alone (Fig. 7B; group C’ vs group B’). When we assessed the ability of retransferred CD4^+ T cells to respond to H-2^d APCs 4 wk after transfer, no significant differences were observed between the two groups (Table I).
FIGURE 6. CD4+CD25+ regulatory T cells suppress functional capacities of expanding naive CD4+ T cells. The ability of H-2b transferred CD4+ T cells to respond to H-2b or H-2d APCs was evaluated 4 wk after transfer. CD4+ T cells from all mouse groups were purified by negative selection of non-CD4+ T cells. Thy1.2+ CD4+ T cells from group B mice were purified by negative selection of Thy1.2+ T cells. CD4+ T cells from normal C57BL/6 mice were used as controls. Purified CD4+ T cells (10^5) were then incubated with 5 x 10^6 peritoneal macrophages from H-2b or H-2d mice. Supernatants were collected after 96 h of culture. IFN-γ production was assessed by ELISA. [3H]Thymidine was added to the culture at the same time and proliferation was measured 16 h later. Results are representative of three independent experiments.

FIGURE 7. Pathogenic T cells still transfer IBD once CD4+CD25+ regulatory T cells have been removed. Thy1.2+ CD4+ T cells from group B and C mice were purified by negative selection of non CD4+ T cells and of Thy1.2+ T cells. A, CD3ε-deficient mice were injected i.v. with 0.5 x 10^6 purified Thy1.2+ CD4+ T cells from group B mice (group B') or from group C mice (group C'). B, Injected mice were weighed 4 wk after CD4+ T cell transfer. Results are representative of two independent experiments.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4+ Thy1.2- Group B</th>
<th>CD4+ Thy1.2- Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>B'</td>
<td>0.5x10^6</td>
<td>No</td>
</tr>
<tr>
<td>C'</td>
<td>No</td>
<td>0.5x10^6</td>
</tr>
</tbody>
</table>

Table I. Functional capacities of expanding naive CD4+ T cells upon retransfer

<table>
<thead>
<tr>
<th>APCs</th>
<th>b/b</th>
<th>c/c</th>
<th>d/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responding cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferation (cpm)</td>
<td>6,472 ± 4,927</td>
<td>3,848 ± 160</td>
<td>15,052 ± 2,278</td>
</tr>
<tr>
<td>IFN-γ (ng/ml)</td>
<td>0.59 ± 0.20</td>
<td>1.02 ± 0.90</td>
<td>6.30 ± 0.93</td>
</tr>
</tbody>
</table>

*CD4+ T cells from the indicated mouse groups were purified by negative selection of non-CD4+ T cells. Purified CD4+ T cells (10^5) were then incubated with 5 x 10^6 peritoneal macrophages from H-2b or H-2d mice. Supernatants were collected after 96 h of culture. IFN-γ production was assessed by ELISA. [3H]Thymidine was added to the culture at the same time and proliferation was measured 16 h later.
transferred into lymphopenic mice differs according to whether or not they exhibit IBD upon retransfer to lymphopenic hosts. Moreover, we found no evidence for inflammatory and autoimmune diseases.

Fluctuations and/or cellular therapy protocols aimed at preventing in vivo and protecting from inflammation were observed in mice. We thank B. Faideau, F. Lepault, and C. Pe for critical reading of the manuscript and A. Benraiss for his invaluable help in histology.

Acknowledgments

We thank B. Faideau, F. Lepault, and C. Pénit for critical reading of the manuscript and A. Benraiss for his invaluable help in histology.

References


