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CD4⁺CD25⁺ Regulatory T Cells Suppress Differentiation and Functions of Th1 and Th2 Cells, *Leishmania major* Infection, and Colitis in Mice¹

Damo Xu,^{2*} Haiying Liu,* Mousa Komai-Koma,* Carol Campbell,* Charlie McSharry,* James Alexander,[†] and Foo Y. Liew^{2*}

Regulatory T cells play a major role in modulating the immune response. However, most information on these cells centers on autoimmunity, and there is also considerable controversy on the functional characteristics of these cells. Here we provide direct *in vitro* and *in vivo* evidence that CD4⁺CD25⁺ regulatory T cells inhibit the differentiation and functions of both Th1 and Th2 cells. Importantly, CD4⁺CD25⁺ T cells suppressed the disease development of *Leishmania major* infection in SCID mice reconstituted with naive CD4⁺CD25⁻ T cells. Furthermore, CD4⁺CD25⁺ T cells inhibited the development of colitis induced by both Th1 and Th2 cells in SCID mice. Our results therefore document that CD4⁺CD25⁺ regulatory T cells suppress both Th1 and Th2 cells and that these regulatory T cells have a profound therapeutic potential against diseases induced by both Th1 and Th2 cells *in vivo*. *The Journal of Immunology*, 2003, 170: 394–399.

There is considerable current interest in the functional role of regulatory T (Treg)³ cells, which subsume the role, if not the characteristics, of the much-maligned suppressor T cells. There are currently at least three major types of Treg cells, Th3, Tr1, and CD4⁺CD25⁺ T cells, with overlapping functions (1–3). CD4⁺CD25⁺ T cells are arguably the best characterized to date. However, there is considerable controversy on the characteristics and functions of these cells, and much remains to be learned (4–7). For example, it is not clear whether CD4⁺CD25⁺ cells can suppress both Th1 and Th2 cell development and the effector functions of committed Th1/Th2 cells. To date, most studies concentrated on the role of Treg cells in autoimmunity and the functions of these cells in infection are unclear.

We have investigated the role of CD4⁺CD25⁺ T cells in the development and function of Th1 and Th2 cells. We also extended this study to examine the role of CD4⁺CD25⁺ T cells in cutaneous *Leishmania major* infection in mice, a prototypic Th1/Th2 polarization model *in vivo*. Here we report that CD4⁺CD25⁺ T cells suppressed the differentiation and functions of both Th1 and Th2 cells. Furthermore, CD4⁺CD25⁺ T cells inhibited disease development of *L. major* infection in SCID mice reconstituted with CD24⁺CD25⁻ T cells by suppressing both Th1 and Th2 cells development *in vivo*. We also showed that the SCID mice reconstituted with Th1 or Th2 cells differentiated from CD4⁺CD25⁻ T cells developed spontaneous colitis, which was prevented by the

cotransfer of CD4⁺CD25⁺ T cells. These results therefore provide direct evidence that CD4⁺CD25⁺ T cells play an important role in infection. These data may have implications in therapeutic strategy against infection and autoimmune diseases.

Materials and Methods

Mice and parasite

BALB/c mice and SCID mice (C.B-17) of the BALB/c background were obtained from Harlan Olac (Bicester, U.K.). All mice were kept at the Biological Service facilities of University of Glasgow and University of Strathclyde according to the U.K. Home Office guidelines. Mice, both male and female, were used at 6–10 wk of age. SCID mice were infected in the right hind footpad with 1 × 10⁶ stationary phase *Leishmania major* (LV39) promastigotes 1 day after receiving *i.p.* 5 × 10⁵ CD4⁺CD25⁻, CD4⁺CD25⁺ or a combination of the two cell populations. Lesion development was followed by measuring footpad swelling (the difference between the infected and the uninfected footpad) at regular intervals. The maintenance of parasite, infection, and measurement of disease progression were described previously (8). At the end of the experiment mice were sacrificed, and footpads were removed to assay for parasite load by limiting dilution (8). Draining lymph node cells were harvested and cultured (2 × 10⁶ cells/ml in culture medium) *in vitro* with frozen-thawed parasite Ag (10⁶ parasite/ml equivalent). Culture supernatant was harvested at 72 h and assayed for cytokines by ELISA. Cellular proliferation was also determined by [³H]thymidine incorporation (8). The colons of the mice were also collected and fixed with formalin for histological analysis.

Cell culture

CD4⁺ T cells were purified from BALB/c lymph nodes by negative selection using magnetic beads (MACS; Miltenyi Biotech, Auburn, CA) as described previously (9). CD4⁺ cells were then further separated into CD25⁺ and CD25⁻ populations by MACS and FACS sorter (FACSCalibur; BD Biosciences, Mountain View, CA) using PE-labeled anti-CD25 Ab (BD Biosciences). The purity of the cell preparations was determined by FACS analysis and was routinely >95%. In some experiments CD4⁺CD25⁻ T cells were cultured with soluble anti-CD3 Ab (1 μg/ml; BD PharMingen, San Diego, CA) and mitomycin C-treated BALB/c spleen cells (APC) for 3 days with or without an equal number of CD4⁺CD25⁺ T cells. Cellular proliferation and type 1 and type 2 cytokine production were analyzed by [³H]thymidine incorporation and ELISA, respectively. In some experiments CD4⁺CD25⁻ and CD4⁺CD25⁺ cells were cultured in a 96-well plate together or separated by a semipermeable membrane (Transwell; Millipore, Watford, U.K.). In other experiments CD4⁺CD25⁻ T cells were cultured with anti-CD3 Ab, anti-CD28 (1 μg/ml; BD PharMingen), and IL-12 (10 ng/ml; Genetics Institute, Cambridge, MA) plus anti-IL-4

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³ Abbreviation used in this paper: Treg, regulatory T.

Ab (1 $\mu\text{g/ml}$) for Th1 cell induction, or IL-4 (10 ng/ml) plus anti-IL-12 Ab (1 $\mu\text{g/ml}$) for Th2 cell induction (all reagents from R&D Systems, Oxon, U.K.). At the end of 3-day culture, the cells were harvested, rested for 3 days in medium containing IL-2 (10 ng/ml; BD PharMingen), and recultured as described above. After two cycles of culture, the cells were phenotyped for Th1 or Th2 by cytokine secretion and CD44 and CD62L expression. Greater than 95% of the cells were CD44⁺CD62L⁻ and produced the expected cytokines. They were adoptively transferred i.p. into SCID mice either alone or with equal numbers of freshly collected CD4⁺CD25⁺ T cells. Colitis development was then monitored by body weight and histological analysis at the end of the experiments.

CFSE analysis

CD4⁺CD25⁻ and CD4⁺CD25⁺ T cells were labeled with CFSE (Molecular Probes, Eugene, OR) according to the manufacturer's instruction. The labeled cells were cultured for 3 days as described above and were analyzed by FACS.

ELISA and Ab titration

Cytokine concentrations were determined by ELISA, using paired Abs (BD PharMingen). Detection limits were: IL-4 and IL-5, 30 pg/ml; and IL-2 and IFN- γ , 40 pg/ml. Anti-leishmanial Ab titers in the pooled sera ($n = 5$) were detected with biotin-conjugated anti-mouse IgG or IgG2a (BD PharMingen), followed by conjugated avidin peroxidase (Sigma-Aldrich, St. Louis, MO) with tetramethylbenzidine substrate (Kirkegaard & Perry, Gaithersburg, MD).

Histological examination

Colons were removed from mice 4–8 wk after T cell reconstitution and were fixed in 10% buffered formalin. Paraffin-embedded sections (6 μm) were cut and stained with H&E. Inflammation was scored as previously described (10). The sections were scanned with a Duoscan T2000XL microscope, and pictures were taken with a Fuji X digital camera (HC-300Z; Tokyo, Japan) at magnification of $\times 15$ or $\times 200$.

Statistical analysis

Statistics were determined using Minitab software for Macintosh (Cupertino, CA). The analyses were performed using Student's t test.

Results

CD4⁺CD25⁺ T cells suppress the differentiation of Th1 and Th2 cells

CD4⁺ T cells were purified from the lymph nodes of BALB/c mice by negative selection and then sorted into CD25⁺ or CD25⁻ T cells. CD4⁺CD25⁻ T cells were cultured alone or together with CD4⁺CD25⁺ T cells in the presence of soluble anti-CD3 Ab- and mitomycin C-treated spleen cells (APC providing costimulating signals). The cells were driven to Th1 or Th2 lineages by culture for 3 days with IL-12 plus anti-IL-4 Ab or IL-4 plus anti-IL-12 Ab, respectively. Cellular proliferation and cytokine production patterns were analyzed on day 3 of culture. In the absence of exogenously added cytokines, CD4⁺CD25⁻ T cells produced significant proliferation (stimulation index, 15–20), whereas CD4⁺CD25⁺ cells did not (data now shown). CD4⁺CD25⁻ T cells differentiated into Th1 and Th2 cells as expected in the presence of IL-12 and IL-4, respectively (Fig. 1). Under these culture conditions, CD4⁺CD25⁺ T cells alone did not produce significant amount of IFN- γ , IL-4, or IL-5. However, the induction of both Th1 and Th2 cells from CD4⁺CD25⁻ cells was suppressed when they were cultured together with CD4⁺CD25⁺ cells (Fig. 1a). It should be noted that CD4⁺CD25⁺ T cells alone, under these culture conditions, did not proliferate when cultured with IL-12, but proliferated significantly in the presence of IL-4. However, when cultured together in the presence of IL-4, the proliferation of both CD4⁺CD25⁻ and CD4⁺CD25⁺ cells were significantly diminished compared with the proliferation when the two populations of cells were cultured separately. Nevertheless, such proliferation of CD4⁺CD25⁺ T cells in the presence of IL-4 did not affect their ability to suppress the development of functional activity of Th2

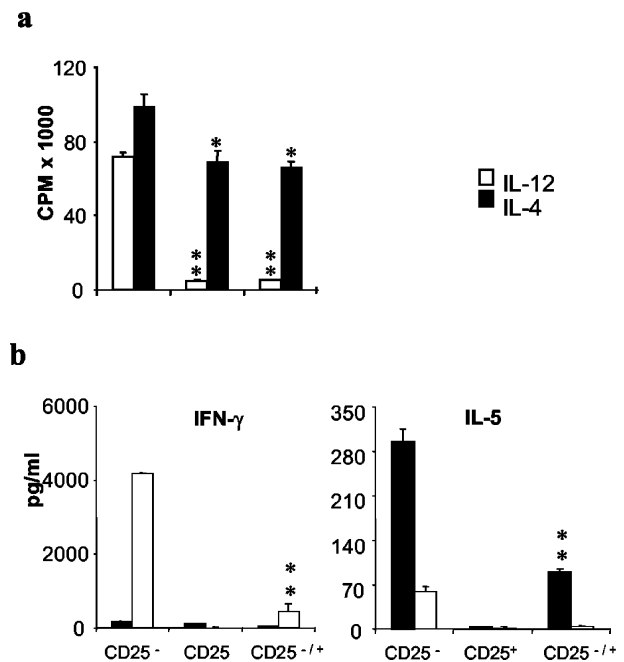


FIGURE 1. CD4⁺CD25⁺ T cells suppress the induction of Th1 and Th2 cells. CD4⁺CD25⁻ and CD4⁺CD25⁺ T cells from the lymph nodes of BALB/c mice were cultured separately or together (1:1) in the presence of anti-CD3 Ab and APC under Th1 (+IL-12) or Th2 (+IL-4) conditions. *a*, Cellular proliferation was determined by [³H]thymidine incorporation; *b*, IFN- γ and IL-5 concentrations in the culture supernatant were measured 3 days later. Data are the mean \pm SD ($n = 3$) and are representative of three experiments. *, $p < 0.05$; **, $p < 0.01$.

cells from CD4⁺CD25⁻ T cells (i.e., the production of type 2 cytokines; Fig. 1b). CFSE analysis (Fig. 2a) supports the cellular proliferation profile shown in Fig. 1a. CD4⁺CD25⁻ cells proliferated equally well in the presence or the absence of IL-4. In contrast, CD4⁺CD25⁺ cells proliferated poorly in the absence of IL-4. When cultured with CD4⁺CD25⁺ T cells, CD4⁺CD25⁻ cells showed markedly reduced proliferation compared with that when cells were cultured without CD4⁺CD25⁺ cells. This was particularly so in the absence of IL-4. When cultured with CD4⁺CD25⁻ T cells, CD4⁺CD25⁺ cells also showed significantly reduced proliferation compared with that when cells were cultured alone, even in the presence of IL-4. The percentages of dead cells after 3-day culture were similar in all cultures (20–25%). The suppression mediated by CD4⁺CD25⁺ cells required cell contact between the CD4⁺CD25⁺ cells and CD4⁺CD25⁻ cells, because no suppression was detected when these two cell populations were cultured in Transwells separated by a semipermeable membrane (Fig. 2b).

CD4⁺CD25⁺ T cells suppress Th1 and Th2 cells and modulate *Leishmania* infection and colitis in vivo

We next investigated whether the suppression of Th1 and Th2 cell development in vitro was also manifested in vivo. Cutaneous leishmaniasis in mice provides the most polarized form of Th1 and Th2 dichotomy in vivo. We therefore examined the effect of CD4⁺CD25⁺ T cells on CD4⁺CD25⁻ cells in a SCID mouse model of *L. major* infection. This study would also provide definitive information on the hitherto unknown effect of CD4⁺CD25⁺ Treg cells on infectious disease. Initially, we investigated the physiological role of CD4⁺CD25⁺ T cells on the development of *L. major* infection. SCID mice were adoptively transferred i.p. with 5×10^5 BALB/c spleen cells, depleted or undepleted of CD4⁺CD25⁺ T cells, which constitute $\sim 5\%$ of the CD4⁺ T cell population in the

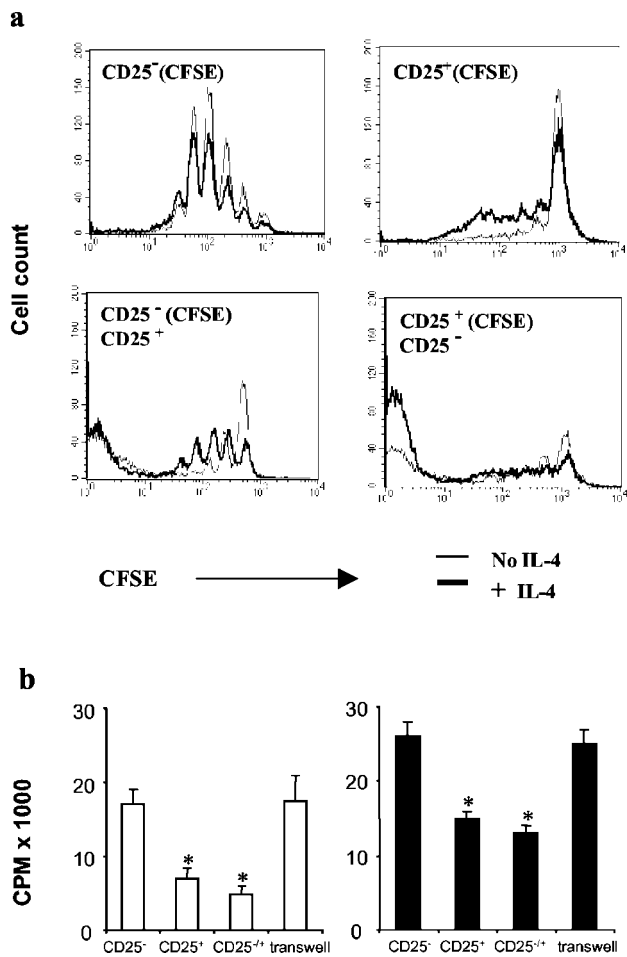


FIGURE 2. *a*, CFSE analysis shows that both CD4⁺CD25⁻ and CD4⁺CD25⁺ T cells proliferated when cultured separately or together for 3 days in the presence of IL-4 and anti-IL-12 Ab. The two upper panels show cells cultured separately in the presence (bold line) or the absence (thin line) of IL-4. The two lower panels show the cells cultured together with one of the cell types labeled with CFSE as indicated in brackets. *b*, The suppression of both Th1 and Th2 cell differentiation was dependent on cell contact between CD4⁺CD25⁺ cells and CD4⁺CD25⁻ cells, because no suppression was observed when the two cell populations were cultured separated by semipermeable membrane. Data are the mean \pm SD ($n = 3$) and are representative of three experiments. *, $p < 0.05$; **, $p < 0.01$.

spleen. The recipients were infected 1 day later with 1×10^6 *L. major* promastigotes in the footpads, and lesion development was followed at regular intervals. As expected, SCID mice did not develop local lesions over the 4-wk period after infection because the local inflammation was CD4⁺ T cell dependent (11). SCID mice reconstituted with the whole spleen cell population developed significant and progressive lesions. Interestingly, mice reconstituted with spleen cells depleted of CD4⁺CD25⁺ T cells developed even more severe lesions and higher parasite load (Fig. 3*a*). This pattern of disease development persisted for at least 60 days (data not shown), at which stage the experiments were terminated as required by the Home Office guidelines. These data therefore demonstrated that in the present experimental model, CD4⁺CD25⁺ T cells suppressed lesion development as well as parasite multiplication in SCID mice adoptively transferred with a low dose of BALB/c spleen cells. To analyze the immune mechanisms involved, some groups of mice were sacrificed on day 28 postinfection, and their lymphoid cells were cultured with leishmanial Ags in vitro. Cells from mice reconstituted with CD4⁺CD25⁺ T

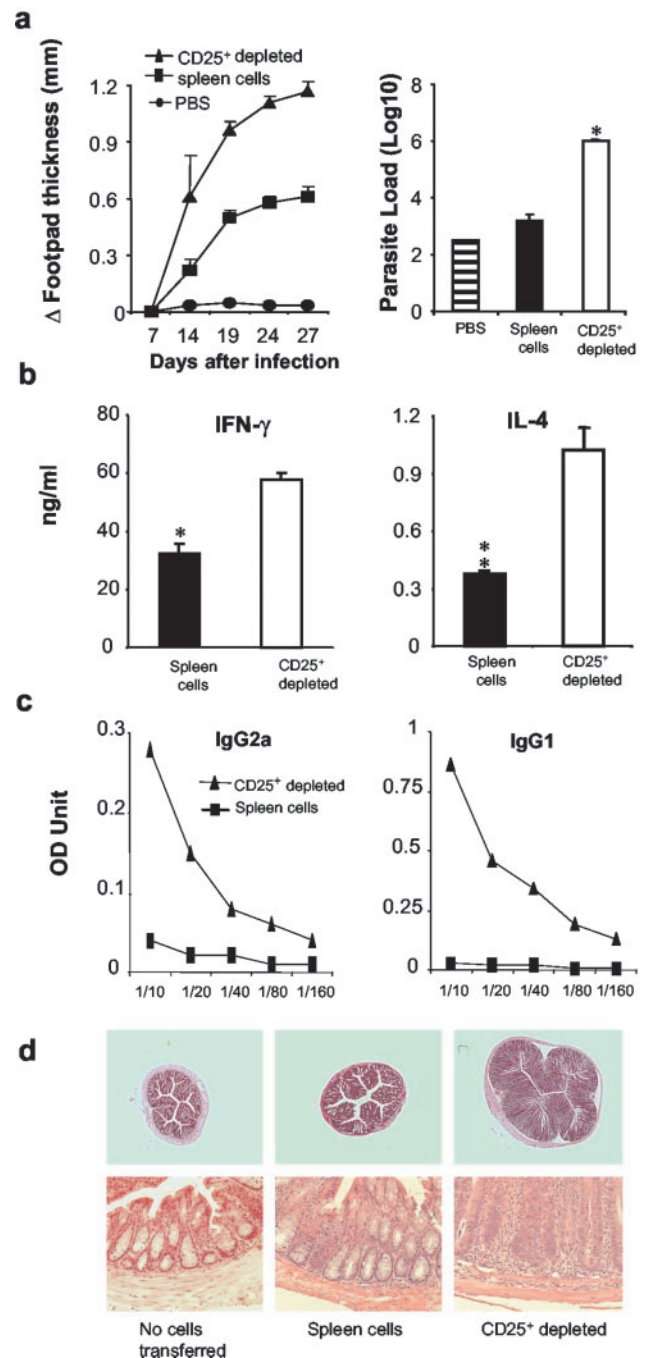


FIGURE 3. Effect of CD4⁺CD25⁺ T cell depletion on the course of *L. major* infection and colitis in SCID mice adoptively transferred with spleen cells. SCID mice were reconstituted with intact spleen cells or CD4⁺CD25⁺ T cell-depleted spleen cells and infected with *L. major* in the footpad. *a*, Lesion development and parasite loads (on day 27) were measured. *b*, IFN- γ and IL-4 in the culture supernatant of draining lymph node cells (harvested on day 27) stimulated with *L. major* Ag was measured by ELISA. *c*, Sera (day 27) of the recipient mice were pooled, and the concentrations of leishmanial-specific IgG2a and IgG1 Ab were measured. Data are the mean \pm SD ($n = 6$) and are representative of two experiments. *, $p < 0.05$; **, $p < 0.01$. *d*, Colons of the mice were removed and fixed with formalin, and sections were stained with H&E. The colons of recipients of spleen cells depleted of CD4⁺CD25⁺ T cells developed severe colitis. Results are representative of 10 mice/group. Magnification, $\times 12$ and $\times 200$.

cell-depleted spleen cells produced significantly more IFN- γ and IL-4 than cells from mice reconstituted with the intact spleen cells (Fig. 3*b*). Furthermore, mice reconstituted with CD4⁺CD25⁺ T

cell-depleted spleen cells produced substantially elevated levels of leishmanial-specific IgG1 and IgG2a Abs compared with mice reconstituted with the intact spleen cells (Fig. 3c). Importantly, SCID mice reconstituted with CD4⁺CD25⁺ T cell-depleted spleen cells developed severe colitis, whereas mice reconstituted with intact spleen cells or control unreconstituted mice did not (Fig. 3d). Mice that developed colitis began to lose weight and had diarrhea from wk 4 post-cell transfer (data not shown). These data therefore demonstrate that CD4⁺CD25⁺ T cells play an important role in suppressing T and B cell functions and controlling leishmanial infection and colitis.

We then sought direct evidence that CD4⁺CD25⁺ T cells regulate CD4⁺CD25⁻ cells in this model *in vivo*. These two populations of cells were purified from normal BALB/c mice and transferred to SCID mice separately or together (in a 1:1 ratio). Mice reconstituted *i.p.* with 5×10^5 CD4⁺CD25⁻ cells and infected with *L. major* developed progressive disease. In contrast, SCID mice similarly transferred with CD4⁺CD25⁺ cells alone exhibited minimal lesions and had 5 orders of magnitude less parasite load than mice reconstituted with CD4⁺CD25⁻ T cells. Importantly, CD4⁺CD25⁺ T cells markedly reduced the disease progression and parasite load in mice reconstituted with CD4⁺CD25⁻ T cells (Fig. 4a). This was accompanied by suppression of both IFN- γ and IL-4 production in these mice (Fig. 4b). Furthermore, SCID mice reconstituted with CD4⁺CD25⁻ T cells, but not CD4⁺CD25⁺ T cells, developed severe colitis. The disease induced by CD4⁺CD25⁻ T cells was completely abrogated by cotransfer with CD4⁺CD25⁺ T cells (data not shown). These data therefore demonstrate directly that CD4⁺CD25⁻, but not CD4⁺CD25⁺, T cells

from BALB/c mice mediated exacerbated leishmanial infection and induced rapid colitis in the infected mice. Furthermore, CD4⁺CD25⁺ T cells effectively inhibited the disease-promoting activity of CD4⁺CD25⁻ T cells.

CD4⁺CD25⁺ T cells suppress the functions of committed Th1 and Th2 cells *in vivo*

We have demonstrated the suppressive effect of CD4⁺CD25⁺ T cells on the differentiation of Th1 and Th2 cells from naive CD4⁺CD25⁻ T cells. We next investigated whether CD4⁺CD25⁺ T cells are able to regulate committed Th1 and Th2 cells. The results from this study could have important implications for the therapeutic potential of CD4⁺CD25⁺ T cells in infection and inflammatory diseases. Since CD4⁺CD25⁻ cells induced spontaneous colitis in SCID mice (Figs. 3 and 4), and it has been recently demonstrated that both Th1 and Th2 are able to induce colitis in SCID mice (12), we used a modified version of this convenient model for further investigation. This model also avoided the added complexity of infection. CD4⁺CD25⁻ T cells from BALB/c spleens were driven to Th1 or Th2 lineages by culture with anti-CD3 and anti-CD28 in the presence of IL-12 and anti-IL-4 Ab, or IL-4 and anti-IL-12 Ab, respectively. After two rounds of culture, >95% of the cells were CD44⁺CD62L⁻, a phenotype of mature T cells. The Th1 line produced IFN- γ (90.3 ng/ml) and no detectable IL-5, and the Th2 line produced IL-5 (39.1 ng/ml) and no detectable IFN- γ . The cells were injected *i.p.* (5×10^5 cells/recipient) to SCID mice with or without an equal number of CD4⁺CD25⁺ T cells from syngenic donors. The body weights of mice were monitored at regular intervals, and the animals were sacrificed 8 wk after cell transfer. Mice receiving CD4⁺CD25⁺ T cells developed normal body weight gain. In contrast, SCID mice given Th1 or Th2 cells failed to gain body weight from the onset of cell transfer and began to lose weight from wk 6. The body weight profile was similar for the Th1 and Th2 recipients. Crucially, the weight loss induced by Th1 or Th2 cells was prevented by cotransfer with CD4⁺CD25⁺ T cells (Fig. 5a). This was particularly evident in the recipients of Th2 cells, in which the reversal was apparent from wk 1 of the cell transfer. For Th1 cells this was not achieved until wk 6. At the end of the experiments (wk 8), mesenteric lymph node cells were harvested and cultured with anti-CD3 Ab, and the cytokines produced were determined. Mice receiving Th1 or Th2 cells alone retained much of the type 1 or type 2 cytokine profile, respectively. Cytokine synthesis was completely suppressed by the cotransfer with CD4⁺CD25⁺ T cells (Fig. 5b). Histological analysis of colonic tissues at wk 8 showed that the recipients of both Th1 and Th2 cells developed typical colitis pathology, which was completely reversed by CD4⁺CD25⁺ T cells (Fig. 5c). These results therefore demonstrate that 1) both Th1 and Th2 cells derived from CD4⁺CD25⁻ T cells can induce spontaneous colitis in SCID mice; 2) CD4⁺CD25⁺ T cells can suppress the functions of committed Th1 and Th2 cells; and 3) the suppressive effect of CD4⁺CD25⁺ T cells on Th2 cells appears to be more profound than that on Th1 cells in this model.

Discussion

The data presented here show that CD4⁺CD25⁺ Treg cells suppress the induction and functions of both Th1 and Th2 cells *in vitro* and *in vivo*. This finding strongly indicates a therapeutic role for CD4⁺CD25⁺ T cells in both Th1- and Th2-mediated diseases. Furthermore, these Treg cells play an influential role in infection, thereby expanding the critical functional role of these cells beyond the confines of autoimmune diseases.

Cutaneous leishmaniasis is arguably the most clear-cut example of Th1 and Th2 cell polarization *in vivo*. The outcome of infection

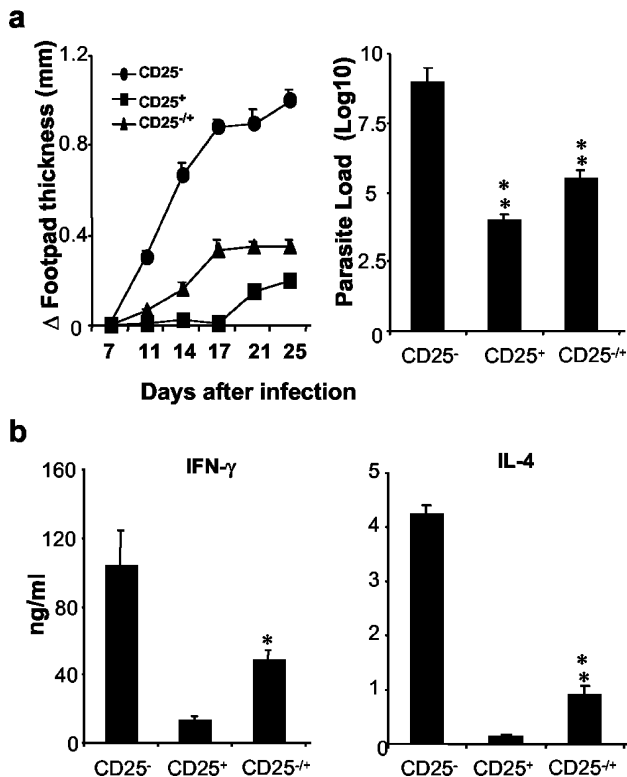


FIGURE 4. Effects of CD4⁺CD25⁺ and CD4⁺CD25⁻ T cells on the disease development of *L. major* infection in SCID mice. SCID mice were reconstituted with CD4⁺CD25⁺ T cells (CD25⁺) and CD4⁺CD25⁻ T cells (CD25⁻), alone or in combination (CD25^{+/-}), and were infected with *L. major* 1 day later. *a*, Lesion development and parasite load; *b*, cytokine productions were determined as described in Fig. 2. Data are the mean \pm SD ($n = 6$) and are representative of two experiments.

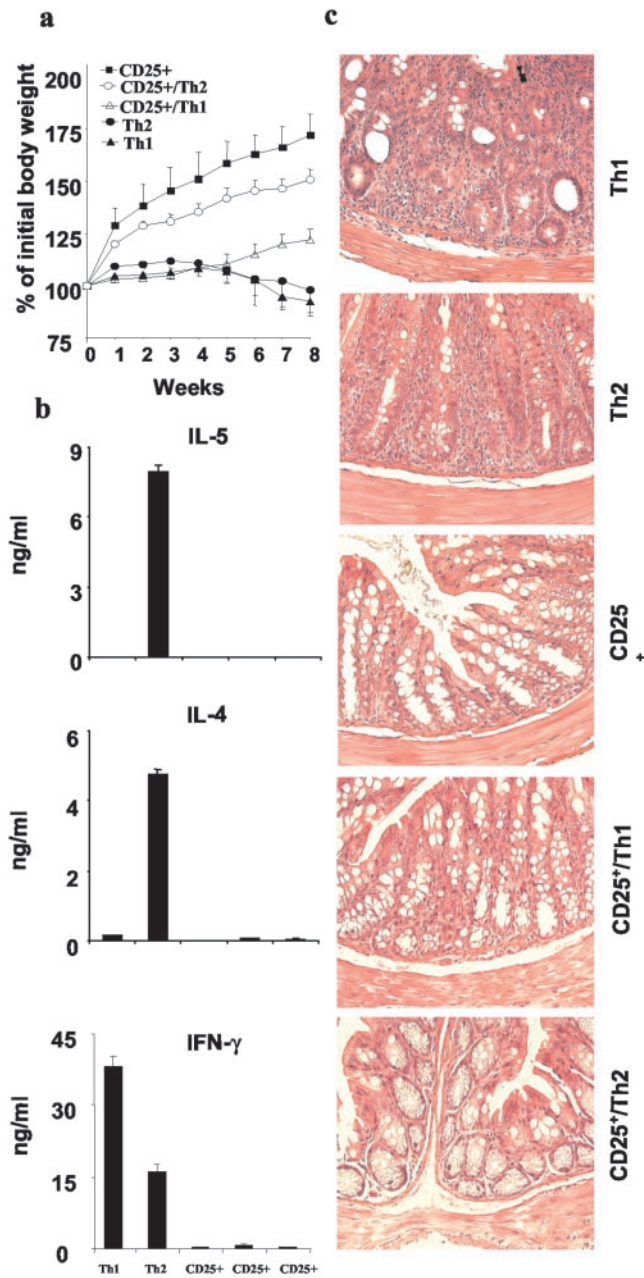


FIGURE 5. Effect of CD4⁺CD25⁺ T cells on the development of colitis in SCID mice reconstituted with committed Th1 or Th2 cells. CD4⁺CD25⁻ T cells were cultured for two rounds under the Th1 or Th2 driving conditions as described in *Materials and Methods*. The cells were then injected i.p. into SCID mice with or without CD4⁺CD25⁺ T cells. Spontaneous colitis development was followed by 1) body weight loss, and the experiment was terminated on wk 8 when some of mice developed severe diarrhea; 2) IL-5, IL-4, and IFN-γ in the culture supernatant (day 3) of mesenteric lymph node cells stimulated with anti-CD3 Ab was measured by ELISA; and 3) colonic tissues stained with H&E show that recipients of Th1 or Th2 cells developed severe colitis, which was reversed by CD4⁺CD25⁺ T cells.

is determined by the balance of Th1 and Th2 cell functions. Early studies suggested a greater heterogeneity of CD4⁺ T cells beyond the current Th1/Th2 categorization (13). In leishmaniasis, the immune response and outcome of infection may well be influenced by the presence and activation of other T cell types, including Treg cells. In 1983, a line of CD4⁺ suppressor T cells was cloned from BALB/c mice with progressive *L. major* infection and was shown

to suppress Ag-specific T cell proliferation and delayed-type hypersensitivity (14). The phenotype of these suppressor T cells was not fully characterized, but could well be the CD4⁺CD25⁺ Treg cells described here. The data reported here are the first demonstration of the regulatory role of CD4⁺CD25⁺ T cells in parasitic infection and may have wider implications in infectious diseases in general. The suppressive activity of the CD4⁺CD25⁺ T cells described here is distinct from that of the Ag-specific, IL-10-producing Tr1 cells induced during infection in mice and humans (15–17). No elevated IL-10 level was detected in the mice transferred with CD4⁺CD25⁺ T cells in our system (data not shown).

The CD4⁺CD25⁺ T cells reported here suppressed proliferation and cytokine production by both Th1 and Th2 cells in vitro and in vivo. This finding is in apparent contrast to early reports showing that, using T cells from OVA peptide-specific TCR-transgenic mice, CD4⁺CD25⁺ T cell depletion led to the preferential enhancement of Th1 cells development in vitro (18, 19). The discrepancy between this and our system may be due to the difference in cell type used (i.e., TCR vs polyclonal T cells). This may lead to a difference in the threshold of suppression between Th1 and Th2 cells. A more likely explanation, however, may be the difference in the experimental systems used. Once activated, CD4⁺CD25⁺ T cells are not Ag specific in their suppression and are likely to be nondiscriminatory against proliferating T cells, including CD8⁺ T cells (20). In the OVA TCR-transgenic system, T cells were selectively driven to the Th2 pathway in a Th2 cell-mediated airway allergic model and may thus be selectively suppressed.

Cutaneous leishmanial infection in SCID mice is critically dependent on the presence of CD4⁺ T cells (11) and the dose of cells adoptively transferred (21–23). Reconstitution of SCID mice with 10⁷ naive BALB/c spleen cells led to resistance against *L. major* infection, whereas transfer of 10⁸ of such cells rendered the mice highly susceptible to the infection. Using a low dose of cells (5 × 10⁵ cells/recipient), we have shown here that the disease development in our SCID/cell transfer model, at least during the initial 8 wk, was dependent on the presence of CD4⁺CD25⁻, but not CD4⁺CD25⁺, T cells. CD4⁺CD25⁻ T cells from the susceptible BALB/c mice developed into both Th1 and Th2 cells following leishmanial infection, with a dominant, but not exclusive, Th2 response and the development of progressive lesions. CD4⁺CD25⁺ T cells suppressed the differentiation of CD4⁺CD25⁻ T cells to both Th1 and Th2 cells and the suppression of disease development. There may be a number of interpretations for this disease outcome. 1) Th2 cells appeared to be more susceptible to the inhibitory effect of CD4⁺CD25⁺ T cells. This shift of balance in favor of Th1 cells may account for the enhanced resistance to the infection by the cotransfer of CD4⁺CD25⁺ Treg cells. The higher degree of sensitivity of Th2 cells compared with Th1 cells to the suppression of CD4⁺CD25⁺ T cells in vivo was also apparent in the colitis induced by Th1 and Th2 cells (Fig. 5). However, the different culture conditions used to drive Th1 and Th2 cells precluded a direct comparison of the relative sensitivity of these cells to the suppressive effect of CD4⁺CD25⁺ T cells in vitro. 2) An early burst of IL-4 has been shown to favor the subsequent development of Th2 cells in BALB/c mice infected with *L. major* (24). It is possible that CD4⁺CD25⁺ cells preferentially inhibited such an early event. 3) It is also possible that in BALB/c mice the determining factor in the outcome of the disease is the level of IL-4 rather than the presence of IFN-γ. Thus, the suppression of IL-4 would lead to enhanced resistance even with the concomitant decrease in IFN-γ. Whatever the explanation, it appears that CD4⁺CD25⁺ T cells are important for host resistance against cutaneous leishmanial infection in the highly susceptible BALB/c mice.

An earlier study using a high infecting dose of *L. major* (25), showed that SCID mice adoptively transferred with leishmanial-specific, IL-4-producing CD4⁺CD45RB^{low} cells developed non-healing lesions, whereas mice transferred with leishmanial-primed, IFN-producing, CD4⁺CD45RB^{high} cells produced a healing response. Furthermore, mice reconstituted with CD4⁺CD45RB^{high} cells developed severe colitis, which could be inhibited by the cotransfer with CD4⁺CD45RB^{low} cells. It is unlikely that the effect of CD4⁺CD25⁺ cells we observed here with the *Leishmania* infection was due to a low grade contamination with CD4⁺CD45RB^{high} cells. In this early study CD4⁺CD45^{high} cells were from a highly primed leishmanial-specific cell population. Naive cells were not effective in the enhancement of disease development. Furthermore, CD4⁺CD45RB^{high} cells induced spontaneous colitis in SCID mice. The CD4⁺CD25⁺ cells reported here were freshly isolated, leishmanial-naive T cells, which did not produce IFN- γ in vivo and, importantly, prevented colitis induction by CD4⁺CD25⁻ cells. Thus, the CD4⁺CD25⁺ cells used here were more akin to the CD4⁺CD45RB^{low} cells, which would be expected to counteract any small amount of contaminating CD4⁺CD45^{high} cells. However, the relationship between CD4⁺CD25⁺ and CD4⁺CD45RB^{low} Treg cells is currently unclear. CD4⁺CD45RB^{low} cells prevent colitis by IL-10 and TGF- β (10). The CD4⁺CD25⁺ T cells described here did not produce appreciable level of cytokines and performed their functions by cell contact. It will be of considerable interest to know whether CD4⁺CD45RB^{low} cells represent a subset of CD4⁺CD25⁺ regulatory T cells or belong to a distinct lineage. Contrary to earlier reports, recent investigations now show that both Th1 and Th2 cells are pathogenic in an Ag-specific model of colitis (12). In our non-Ag-specific model, colitis was induced by established Th1 and Th2 cell lines with similar kinetics and severity. Importantly, our results provide direct in vivo documentation that CD4⁺CD25⁺ T cells suppressed both Th1 and Th2 cells, resulting in the resolution of colitis. This finding strongly suggests that CD4⁺CD25⁺ T cells may have important therapeutic potential. This is supported by an early report (26) that in a murine gastritis model, an Ag-specific Th1 and a Th2 cell line could adoptively transfer the disease in *nu/nu* mice, and that the disease could be prevented by cotransfer of normal spleen cells, which presumably contained CD4⁺CD25⁺ T cells.

In summary, we demonstrate here that CD4⁺CD25⁺ regulatory T cells effectively suppress the induction and functions of Th1 and Th2 cells crucial to the outcome of infectious and inflammatory diseases. This finding strongly suggests that CD4⁺CD25⁺ T cells could play an important therapeutic role in Th1 and Th2 cell-mediated diseases.

References

- Chen, Y., V. K. Kuchroo, J. Inobe, D. A. Hafler, and H. L. Weiner. 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265:1237.
- Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155:1151.
- Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737.
- Shevach, E. M. 2000. Regulatory T cells in autoimmunity. *Annu. Rev. Immunol.* 18:423.
- Maloy, K. J., and F. Powrie. 2001. Regulatory T cells in the control of immune pathology. *Nat. Immunol.* 2:816.
- Bach, J. F. 2001. Non-Th2 regulatory T-cell control of Th1 autoimmunity. *Scand J. Immunol.* 54:21.
- Sakaguchi, S., N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda, and T. Takahashi. 2001. Immunologic tolerance maintained by CD25⁺ CD4⁺ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev.* 182:18.
- Piedrafita, D., D. Xu, D. Hunter, R. A. Harrison, and F. Y. Liew. 1999. Protective immune responses induced by vaccination with an expression genomic library of *Leishmania major*. *J. Immunol.* 163:1467.
- Openshaw, P., E. E. Murphy, N. A. Hosken, V. Maino, K. Davis, K. Murphy, and A. O'Garra. 1995. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J. Exp. Med.* 182:1357.
- Read, S., V. Malmstrom, and F. Powrie. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25⁺CD4⁺ regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192:295.
- Terabe, M., T. Kuramochi, M. Ito, T. Hatabu, C. Sanjoba, K. P. Chang, T. Onodera, and Y. Matsumoto. 2000. CD4⁺ cells are indispensable for ulcer development in murine cutaneous leishmaniasis. *Infect. Immun.* 68:4574.
- Iqbal, N., J. R. Oliver, F. H. Wagner, A. S. Lazenby, C. O. Elson, and C. T. Weaver. 2002. T helper 1 and T helper 2 cells are pathogenic in an antigen-specific model of colitis. *J. Exp. Med.* 195:71.
- Liew, F. Y. 1989. Functional heterogeneity of CD4⁺ T cells in leishmaniasis. *Immunol. Today* 10:40.
- Liew, F. Y. 1983. Specific suppression of responses to *Leishmania tropica* by a cloned T-cell line. *Nature* 305:630.
- Doetze, A., J. Satoguina, G. Burchard, T. Rau, C. Loliger, B. Fleischer, and A. Hoerauf. 2000. Antigen-specific cellular hyporesponsiveness in a chronic human helminth infection is mediated by T(h)3/T(r)1-type cytokines IL-10 and transforming growth factor- β but not by a T(h)1 to T(h)2 shift. *Int. Immunol.* 12:623.
- McGuirk, P., C. McCann, and K. H. Mills. 2002. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J. Exp. Med.* 195:221.
- MacDonald, A. J., M. Duffy, M. T. Brady, S. McKiernan, W. Hall, J. Hegarty, M. Curry, and K. H. Mills. 2002. CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected persons. *J. Infect. Dis.* 185:720.
- Suto, A., H. Nakajima, S. I. Kagami, K. Suzuki, Y. Saito, and I. Iwamoto. 2001. Role of CD4⁺ CD25⁺ regulatory T cells in T helper 2 cell-mediated allergic inflammation in the airways. *Am. J. Respir. Crit. Care Med.* 164:680.
- Kagami, S., H. Nakajima, A. Suto, K. Hirose, K. Suzuki, S. Morita, I. Kato, Y. Saito, T. Kitamura, and I. Iwamoto. 2001. Stat5a regulates T helper cell differentiation by several distinct mechanisms. *Blood* 97:2358.
- Piccirillo, C. A., and E. M. Shevach. 2001. Cutting edge: control of CD8⁺ T cell activation by CD4⁺CD25⁺ immunoregulatory cells. *J. Immunol.* 167:1137.
- Holaday, B. J., M. D. Sadick, Z. E. Wang, S. L. Reiner, F. P. Heinzl, T. G. Parslow, and R. M. Locksley. 1991. Reconstitution of *Leishmania* immunity in severe combined immunodeficient mice using Th1- and Th2-like cell lines. *J. Immunol.* 147:1653.
- Varkila, K., R. Chatelain, L. M. Leal, and R. L. Coffman. 1993. Reconstitution of C.B-17 *scid* mice with BALB/c T cells initiates a T helper type-1 response and renders them capable of healing *Leishmania major* infection. *Eur. J. Immunol.* 23:262.
- Powrie, F., R. L. Coffman, and R. Correa-Oliveira. 1994. Transfer of CD4⁺ T cells to C.B-17 SCID mice: a model to study Th1 and Th2 cell differentiation and regulation in vivo. *Res. Immunol.* 145:347.
- Launois, P., I. Maillard, S. Pingel, K. G. Swihart, I. Xenarios, H. Acha-Orbea, H. Diggelmann, R. M. Locksley, H. R. MacDonald, and J. A. Louis. 1997. IL-4 rapidly produced by V β 4 V α 8 CD4⁺ T cells instructs Th2 development and susceptibility to *Leishmania major* in BALB/c mice. *Immunology* 6:541.
- Powrie, F., R. Correa-Oliveira, S. Mauze, and R. L. Coffman. 1994. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4⁺ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J. Exp. Med.* 179:589.
- Suri-Payer, E., A. Z. Amar, R. McHugh, K. Natarjan, D. H. Margulies, and E. M. Shevach. 1999. Post-thymectomy autoimmune gastritis: fine specificity and pathology of anti-H/K ATPase-reactive T cells. *Eur. J. Immunol.* 29:669.