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$CD4^+CD25^+$ Regulatory T Cells Cure Murine Colitis: The Role of IL-10, TGF-β, and CTLA4[1]

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$CD4^+CD25^+$ Regulatory T Cells Cure Murine Colitis: The Role of IL-10, TGF-β, and CTLA4[1]

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Regulatory T cells are critical in regulating the immune response, and therefore play an important role in the defense against infection and control of autoimmune diseases. However, a therapeutic role of regulatory T cells in an established disease has not been fully established. In this study, we provide direct evidence that $CD4^+CD25^+$ regulatory T cells can cure an established, severe, and progressive colitis. SCID mice developed severe colitis when adoptively transferred with naive $CD4^+CD25^-$ T cells and infected with the protozoan parasite *Leishmania major*. The disease development can be completely halted and symptoms reversed, with a healthy outcome, by transferring freshly isolated or activated $CD4^+CD25^+$ T cells from syngeneic donors. The therapeutic effect of the regulatory T cells was completely blocked by treatment of the recipients with anti-IL-10R, anti-CTLA4, or anti-TGF-β Ab. However, the resurgence of colitis under these treatments was not accompanied by the reactivation of Th1 or Th2 response nor was it correlated to the parasite load. These results therefore demonstrate that $CD4^+CD25^+$ T cells are therapeutic and that the effect is mediated by both IL-10/TGF-β-dependent and independent mechanisms. Furthermore, colitis can manifest independent of Th1 and Th2 responses. *The Journal of Immunology*, 2003, 171: 5012–5017.

There is considerable current interest in the functional role of suppressor or regulatory T cells (Treg).[3] There are at least three major types of Treg cells, Th3, Treg 1, and $CD4^+CD25^+$ T cells with overlapping functions (1–3). These cells have been implicated in avoidance of autoimmune diseases (4, 5), as well as in defense against infection by perpetuating persistent infection so as to maintain long-term resistance against reinfection (6). It has also been suggested that Treg cells may be important in tumor progression (7). $CD4^+CD25^+$ T cells are arguably the best characterized. They are naturally generated in thymus and constitute 5–10% of peripheral $CD4^+$ T cells in naive animals and humans. However, a number of key questions concerning the molecular characteristics of their suppressive function and the therapeutic potential of this population of Treg cells remain to be addressed. One such question is whether $CD4^+CD25^+$ Treg cells can cure or reverse an ongoing inflammatory disease. A positive answer to this question would add considerable therapeutic potential to this subset of T cells.

We have investigated the role of $CD4^+CD25^+$ T cells in regulating the development of inflammatory colitis, a common pathology of inflammatory bowel disease (IBD) that affects 1 of 1000 people in the western world. The colitis model used in this study is induced by the effector $CD4^+CD25^-$ T cells in SCID mice. The disease could be considerably accelerated by concomitant s.c. infection of the recipients with the protozoan parasite *Leishmania major*. The colitis developed about 1 wk after cell transfer, and the disease could be completely reversed and normal health restored by transferring $CD4^+CD25^+$ T cells even up to 21 days after the disease onset. Furthermore, the curative activity of the Treg cells was completely abolished by anti-IL-10R, anti-CTLA4, or anti-TGF-β Ab. These results therefore demonstrate directly that $CD4^+CD25^+$ Treg cells have therapeutic potential against an established inflammatory disease and that IL-10, CTLA4, and TGF-β are closely involved in the suppressive effect in vivo.

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, Oxon, U.K.). All mice were kept in the Biological Service facilities in the University of Glasgow according to the U.K. Home Office guidelines. Both male and female mice were used at 6–10 wk of age. SCID mice were injected i.p. with (5×10^5 cells per mouse) $CD4^+CD25^-$ cells, $CD4^+CD25^+$ cells, or a combination (equal number) of the two cell populations. One day later, the animals were infected in the right hind footpad with 1×10^6 stationary phase *L. major* (LV39) promastigotes. The maintenance of parasite, infection, and measurement of disease progression were described previously (8). To induce experimental colitis, SCID mice were reconstituted i.p. with freshly isolated $CD4^+CD25^-$ T cells with or without *Leishmania* infection. To test the therapeutic effect of $CD4^+CD25^+$ T cells, colitis was established in SCID mice, as described above, and then injected i.p. with 5×10^5 freshly isolated or activated $CD4^+CD25^+$ T cells at various times after the transfer of $CD4^+CD25^-$ T cells. In some experiments, the recipient mice were also treated with specific Abs. Anti-IL-10R Ab (kindly provided by K. Moore, DNAX, Palo Alto, CA; 1 mg/mouse/injection) was injected weekly, anti-CTLA4 (clone UC10-4F10-11; American Type Culture Collection (ATCC), Manassas, VA; 0.25 mg/mouse/injection) was given twice per week, or anti-TGF-β Ab (clone 1D11.16; ATCC; 1 mg/mouse/injection) was injected weekly. All Abs were injected i.p. for 3 wk in mice transferred with $CD4^+CD25^+$ and/or $CD4^+CD25^-$ T cells on the day of cell transfer. Control mice were injected with normal rat IgG (1 mg/mouse/injection; Sigma-Aldrich, Poole, U.K.). All mice were monitored closely for body weight change and for general health status, according to the U.K. Home Office guidelines for animal experimentation. Experiments were terminated when the mice developed severe diarrhea and were incontinent or lost up to 20% of their initial body weight. At the end of the experiments, mice were sacrificed and mesenteric lymph nodes were harvested and cultured (2.5×10^5 cells/ml in culture medium) in vitro with plate-bound anti-CD3

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[3] Abbreviations used in this paper: Treg, regulatory T cell; IBD, inflammatory bowel disease.

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Ab (5 μg/ml; BD PharMingen, Oxford, U.K.). Culture supernatant was harvested at 72 h and assayed for cytokines by ELISA, using paired Abs (BD PharMingen). Detection limits were: IL-4, 5 pg/ml; IFN-γ, 40 pg/ml. Cellular proliferation was also determined by [^{3}H]thymidine incorporation. Colons of the mice were also collected and fixed for histological analysis.

Cell culture

CD4$^+$ T cells were purified from BALB/c lymph nodes by negative selection (9) using CD4$^+$ isolation kit (MACS; Miltenyi Biotec, Auburn, CA). CD4$^+$ cells were then further separated into CD25$^+$ and CD25$^-$ populations by MACS and FACS sorter (FACSCalibur; BD Biosciences, San Jose, CA) using PE-labeled anti-CD25 Ab (BD PharMingen) and anti-PE Ab conjugated with Microbeads (Miltenyi Biotec). The purity of the cell preparations was determined by FACS analysis and was routinely >95%. In some experiments, CD25$^+$ T cells were activated with plate-bound anti-CD3 (5 μg/ml) Ab, anti-CD28 (1 μg/ml) Ab, and IL-2 (10 ng/ml) with or without TGF-β (1 ng/ml) for 3 days. The activated cells were then rested in the presence of 10 ng/ml of IL-2 for another 3 days before being washed and transferred into recipient mice.

Histological examination

Colons were removed from mice at different times after T cell reconstitution and fixed in 10% buffered Formalin. Paraffin-embedded sections (6 μm) were cut and stained with H&E. Inflammation was scored, as described previously (10). Each sample was graded from a scale of 0–4: 0 = normal; 1 = mild epithelial hyperplasia; 2 = pronounced hyperplasia and significant inflammatory infiltrates; 3 = severe hyperplasia and infiltration with significant decrease in goblet cells; 4 = severe hyperplasia, severe transmural inflammation, ulceration, crypt abscesses, and substantial depletion of goblet cells. Ceca and colons were assessed separately, and at least three separate sections from each sample were scored blind. The sections were scanned with a Duoscan T2000XL microscope, and pictures were taken with a Fuji X digital camera (HC-300Z) at a magnification of $\times$100.

Statistical analysis

Statistics were performed using Mintab software for the Macintosh. The analyses were performed using Student's *t* test.

Results

The colitis model in SCID mice

We first established a mouse colitis model that is amenable to the modulatory effect of CD4$^+$CD25$^+$ T cells. In a recent report (11), we showed that SCID mice adoptively transferred with spleen cells depleted of CD4$^+$CD25$^+$ T cells developed severe colitis following s.c. infection with the protozoan parasite, *Leishmania major*. We therefore first defined the cell types and the conditions leading to the colitis, which will serve as a model for inflammatory disease in vivo. CD4$^+$CD25$^+$ and CD4$^+$CD25$^-$ T cells were purified from the spleen and lymph nodes of naive BALB/c mice and then adoptively transferred to SCID mice (C.B-17) of the BALB/c background. Some of the recipients were infected with *L. major* in the footpad 1 day after the cell transfer. Colitis development was followed for the next 8 wk. SCID mice transferred with CD4$^+$CD25$^+$ T cells did not develop any sign of colitis, with normal body weight gain with or without *L. major* infection (Fig. 1). In contrast, SCID recipients of CD4$^+$CD25$^-$ T cells developed IBD. This was particularly severe in mice with concomitant *L. major* infection, exhibiting loose stools and marked weight loss 2 wk after cell transfer and showing clear colonic enlargement with pronounced epithelial hyperplasia by 3 wk after cell transfer (Fig. 1, *b* and *c*). It should also be noted that SCID mice adoptively transferred with CD4$^+$CD25$^-$ T cells and infected with *L. major* developed significant footpad lesions that peaked at 3 wk, but progressively declined and reached a low, but steady level by 7 wk after infection (Fig. 1*d*). In contrast, *L. major*-infected SCID mice injected with CD4$^+$CD25$^+$ T cells did not develop significant footpad lesions until 4 wk after infection, but the lesions progressed inexorably by 8 wk after infection (Fig. 1*d*). The footpad lesion size is directly correlated to the parasite load in the footpad

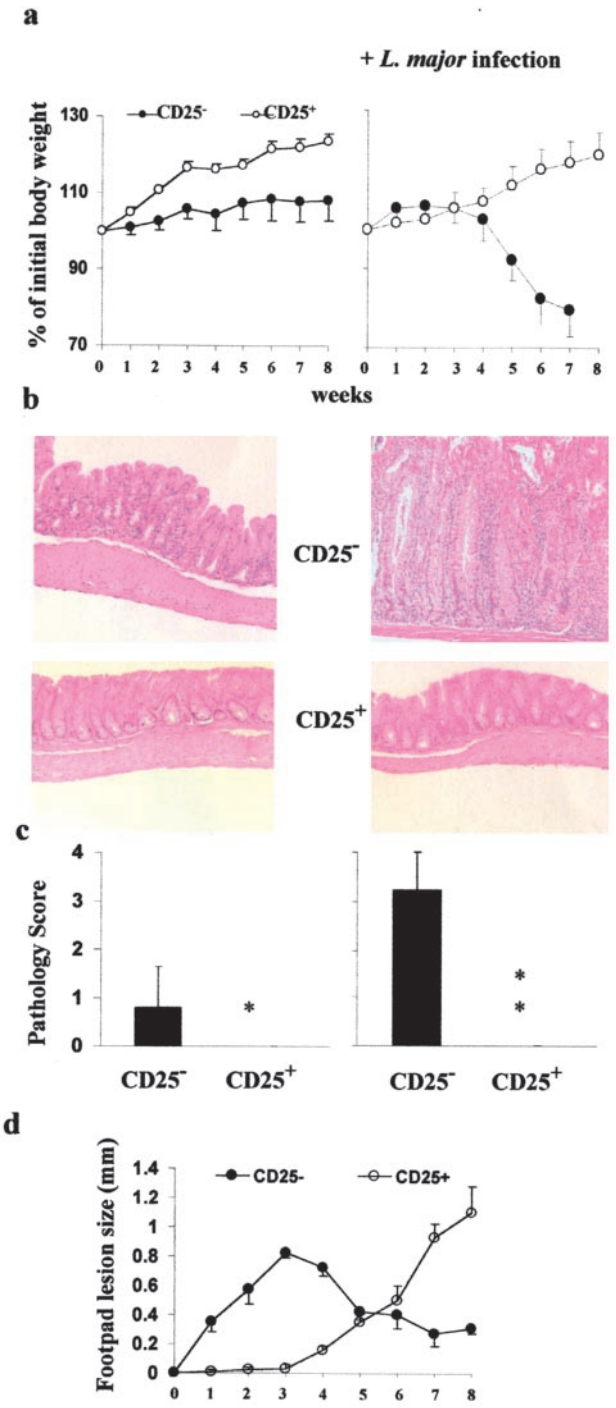

FIGURE 1. The colitis model used in the study. Naive CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells were purified from the spleens and lymph node of normal BALB/c mice and injected i.p. (5×10^5 cells/mouse) into SCID mice. Some of the mice (shown in the *right panel*) were infected in the hind footpad with 1×10^6 stationary phase *L. major* promastigotes 1 day after the cell transfer. The mice were monitored for disease development, including footpad swelling and body weight. At the end of the experiment, when the diseased mice had lost ~20% of their initial body weight, the mice were sacrificed and histopathology of the colon was determined (as described in Ref. 10). *a* and *c*, Results are mean ± SD, $n = 6$, and are representative of three experiments. *b*, A representative histology of the colonic section from each group of mice ($n = 5$). Mice were also monitored for *Leishmania* infection (*d*). The lesion size was measured, as described previously (8), and expressed as the difference in footpad thickness between the infected and uninfected hind footpad. The lesion size consistently correlated with the parasite load in the footpad. Results are mean ± SEM, $n = 6$, and are representative of three independent experiments.

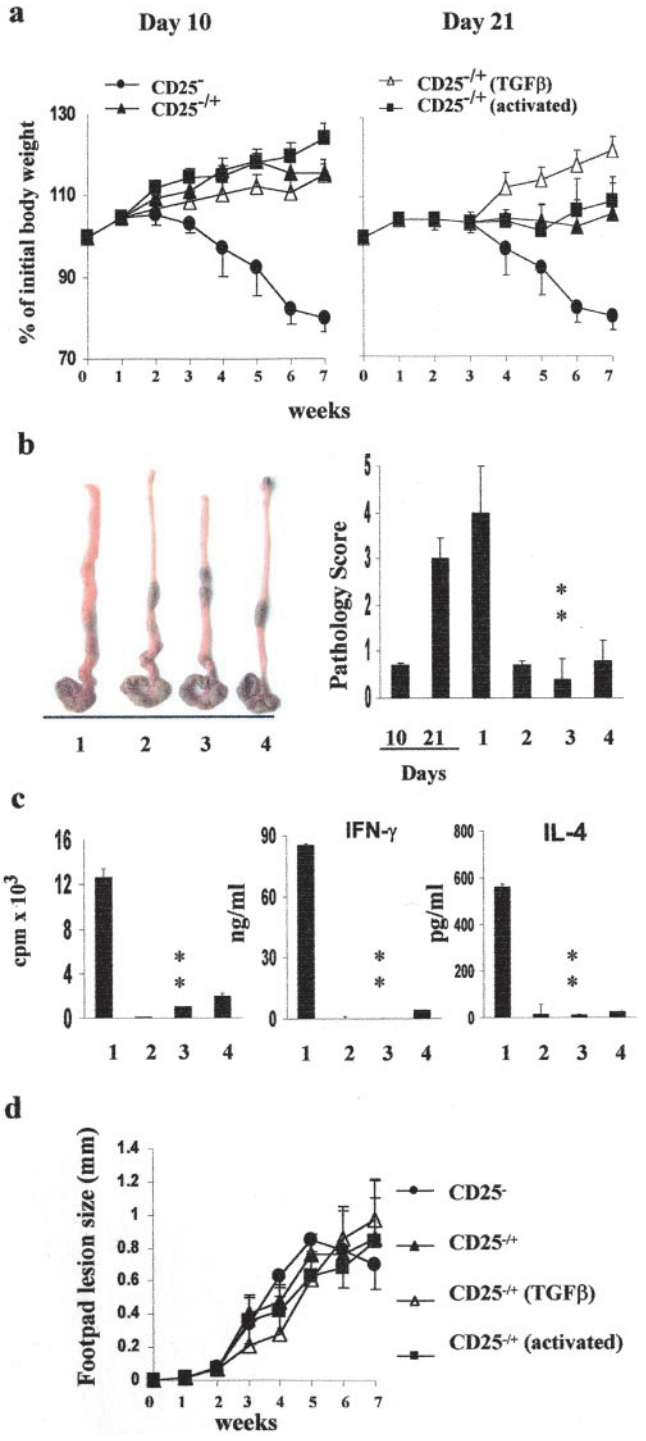

FIGURE 2. Cure of colitis by $CD4^+CD25^+$ T cells. SCID mice were adoptively transferred with $CD4^+CD25^-$ T cells and infected with *L. major*. Ten or twenty-one days later, the diseased mice were injected with: 1) PBS ($CD25^-$), or 2) freshly purified, 3) TGF-β-cultured, or 4) anti-CD3-activated $CD4^+CD25^+$ T cells ($CD25^{+/-}$). The mice were monitored closely for disease development, including: *a*, body weight, and *b*, histological examination of the colon at the end of the experiment. The pathology score of mice at days 10 and 21 just before transfer of $CD4^+CD25^+$ cells was also shown (*b*, *right panel*). Mesenteric lymph node cells were collected at the end of experiments and cultured in vitro with plate-bound anti-CD3 Ab. Cellular proliferation and IFN-γ and IL-4 production were determined (*c*). Data are mean ± SD, $n = 6$, and are representative of two experiments. **, $p < 0.01$ for groups 2–4 compared with group 1. Mice were also monitored for *Leishmania* infection (*d*), as in Fig. 1 above. Data are mean ± SEM, $n = 6$, and are representative of two experiments.

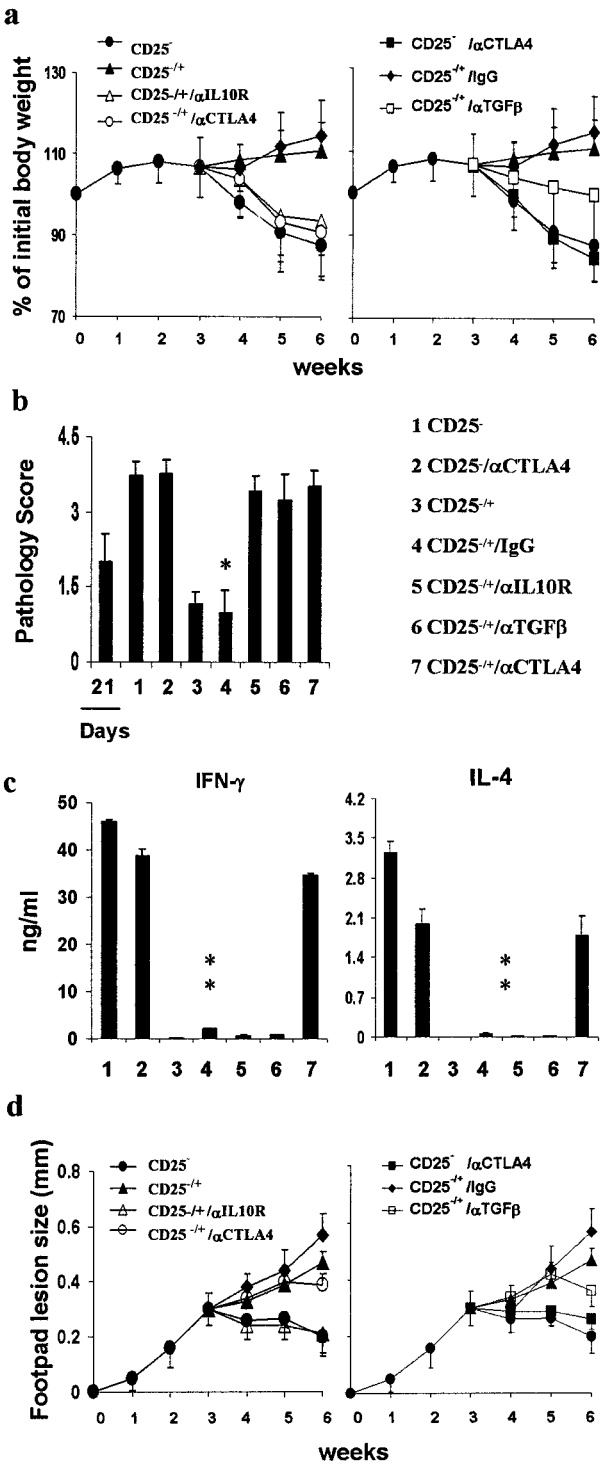

FIGURE 3. The therapeutic effect of $CD4^+CD25^+$ T cells are IL-10, TGF-β, and CTLA4 dependent. SCID mice were adoptively transferred with $CD4^+CD25^-$ T cells and infected with *L. major*. Twenty-one days later, the mice were injected either with control PBS ($CD25^-$) or with $CD4^+CD25^+$ T cells ($CD25^{+/-}$). Some of the mice were also treated with anti-IL-10R, anti-TGF-β, or anti-CTLA4 Ab, as described in *Materials and Methods*. Disease development was monitored for: *a*, body weight, and at the end of the experiment, *b*, colonic pathology, and *c*, cytokine production by mesenteric lymph node cells cultured with plate-bound anti-CD3. The pathology score of mice at day 21 before $CD4^+CD25^+$ T cell transfer was also shown (*b*). Data are mean ± SD, $n = 5$, and representative of two experiments. *, $p < 0.05$ for groups 3 and 4 compared with group 1; **, $p < 0.01$ for groups 3–6 compared with group 1. Mice were also monitored for *Leishmania* infection (*d*), as described in Fig. 1 above. Data are mean ± SEM, $n = 5$, and are representative of two experiments.

(data not shown). By wk 8 of infection, the parasite load of the mice transferred with $CD4^+CD25^+$ cells was 6.5 $\log_{10}$, while that of mice transferred with $CD4^+CD25^-$ cells was 2.0 $\log_{10}$. This pattern of infection development is in apparent contrast to the disease progression reported earlier (11). However, in this earlier report, the lesions were measured up to only 27 days after *L. major* infection, and this disease pattern was reproduced in the present report. The disease development in this model at the later stages was similar to that reported by Belkaid et al. (12). These results show that naive $CD4^+CD25^-$ T cells can induce spontaneous colitis in SCID mice and that the disease could be markedly accelerated by *Leishmania* infection. We therefore decided to use this severe colitis model to investigate the therapeutic potential of $CD4^+CD25^+$ regulatory T cells on inflammatory disease.

Therapeutic effect of $CD4^+CD25^+$ T cells in colitis

$CD4^+CD25^+$ T cells were purified from naive BALB/c mice and were used freshly isolated or cultured with plate-bound anti-CD3 Ab (activated) with or without TGF-β. The cells were then washed, rested, and injected i.p. into SCID mice that had received similar number of naive $CD4^+CD25^-$ T cells and were infected with *L. major* 10 or 21 days previously. Mice receiving $CD4^+CD25^-$ T cells alone developed severe colitis by 21 days, with a clear onset of disease by 10 days after cell transfer. This was clearly demonstrated by body weight loss and colon pathology (Fig. 2*b*). Remarkably, SCID mice given the $CD4^+CD25^-$ T cells and then injected with $CD4^+CD25^+$ T cells, as late as day 10 and even day 21 after the first cell transfer ($CD4^+CD25^-$), began to gain weight almost immediately after receiving the $CD4^+CD25^+$ T cells (Fig. 2*a*). This recovery was accompanied by curing of diarrhea and remission of epithelial hyperplasia, which had a pathology score of 3 (of maximum of 4) (Fig. 2*b*). By day 56, when the mice receiving $CD4^+CD25^-$ T cells alone were euthanased because of excessive pathology, those mice treated on day 10 with $CD4^+CD25^+$ T cells had colonic appearance indistinguishable from normal mice. There was no significant difference among recipients of freshly isolated, TGF-β-cultured, or activated $CD4^+CD25^+$ T cells. For mice treated on day 21 with $CD4^+CD25^+$ T cells, TGF-β-cultured cells were more efficient than freshly isolated or activated $CD4^+CD25^+$ T cells in reversing the body weight of the disease mice. However, there was no difference in the pathology score among the mice treated with these three different preparations of $CD4^+CD25^+$ T cells (Fig. 2*b*). At the end of the experiment (day 56), mesenteric lymph node cells were cultured with plate-bound anti-CD3 Ab, and T cell proliferation and IFN-γ and IL-4 production were determined. T cells from mice transferred with $CD4^+CD25^-$ T cells alone proliferated vigorously and produced significant amounts of IFN-γ and IL-4. In contrast, cells from mice transferred with the $CD4^+CD25^-$ cells and treated with $CD4^+CD25^+$ T cells 10 or even 21 days later produced little or no proliferation or IFN-γ and IL-4 synthesis (Fig. 2*c*). These results therefore demonstrate clearly that $CD4^+CD25^+$ T cells are capable of curing an established wasting inflammatory disease. This was accompanied by a profound suppression of $CD4^+CD25^-$ T cell reactivity in vivo. The mice were also monitored for *Leishmania* infection (Fig. 2*d*). Mice injected with $CD4^+CD25^+$ cells 10 days after $CD4^+CD25^-$ cell plus infection developed lesion size indistinguishable from mice not receiving $CD4^+CD25^+$ cells up to 7 wk after infection, although by wk 5 the lesion in the $CD4^+CD25^-$ alone group began to regress. There is thus no direct correlation between the parasite load and severity of colitis.

Mechanism of the therapy with $CD4^+CD25^+$ T cells

We next investigated the mechanisms involved in the therapeutic action of $CD4^+CD25^+$ T cells. SCID mice transferred with $CD4^+CD25^-$ T cells and infected with *L. major* were given $CD4^+CD25^+$ cells 21 days later. Some of the mice were also injected with Abs against IL-10R, TGF-β, or CTLA4. Control mice were either untreated or injected with normal IgG. SCID mice given $CD4^+CD25^-$ T cells alone developed colitis, as expected, and the disease outcome was not influenced by treatment with normal IgG or anti-CTLA4 Ab. $CD4^+CD25^+$ T cells markedly reversed the disease progression, as expected. However, the curative activity of the $CD4^+CD25^+$ cells was completely abolished by injecting the mice with anti-IL-10R, anti-CTLA4, or anti-TGF-β Ab (Fig. 3). These results therefore demonstrate clearly that the therapeutic effect of $CD4^+CD25^+$ T cells in this model is critically dependent on IL-10, TGF-β, and CTLA4. Finally, we investigated cytokine production by the mesenteric lymph node cells of the mice (harvested at the end of experiment on day 49) when activated with anti-CD3 Ab in vitro. Cells from mice transferred with $CD4^+CD25^-$ T cells alone produced substantial amounts of IFN-γ and IL-4. These were profoundly suppressed by the transfer of $CD4^+CD25^+$ T cells on day 21. The suppression mediated by $CD4^+CD25^+$ T cells was not affected by the treatment of the mice with anti-IL-10R or anti-TGF-β (Fig. 3*c*). In contrast, anti-CTLA4 Ab treatment reversed the suppressive effect of $CD4^+CD25^+$ T cells on IFN-γ and IL-4 synthesis. However, anti-CTLA4 Ab had little or no effect on the $CD4^+CD25^-$ T cells alone (Fig. 3, *b* and *c*). It should also be noted that in the present model system, anti-glucocorticoid-induced TNFR family-related gene Ab (kindly provided by S. Sakaguchi, University of Kyoto, Kyoto, Japan) (13) had little or no influence on the therapeutic effect of $CD4^+CD25^+$ T cells in vivo (data not shown). We also monitored the *Leishmania* infection (Fig. 3*d*). As expected, mice injected with $CD4^+CD25^-$ cells and infected with *L. major* developed lesion that progressed to wk 3, then began to regress. However, the lesion continued to progress when the mice were transferred with $CD4^+CD25^+$ cells on day 21 after infection. This lesion progression was not affected by the treatment of control IgG or anti-CTLA4 Ab, but reversed by anti-IL-10R Ab and partially halted by anti-TGF-β Ab.

Discussion

The etiology and pathogenesis of IBD are poorly understood. IBD is generally believed to be a Th1-mediated inflammatory disease. However, Th2 cells have been shown to exacerbate colitis (14), and both Th1 and Th2 cells could adoptively transfer colitis in SCID mice in an Ag-specific (15) and a nonspecific manner (11). It has also been reported that colitis could be accelerated by *Leishmania* infection in dogs (16), but the mechanism involved remains unknown. Our data demonstrated that cutaneous *Leishmania* infection markedly exacerbated the colitis induced by $CD4^+CD25^-$ T cells in SCID mice, with increased disease incidence (from 50 to 100%) and severity (clinical score from 1 to 3/4). The kinetics and course of the disease are similar to the colitis induced by $CD45RB^{low}$ cells (17). In the absence of *L. major* infection, $CD4^+CD25^-$ T cells were also able to induce colitis, but at a significantly slower rate (Fig. 1*a*). This milder form of colitis could also be effectively reversed by $CD4^+CD25^+$ T cells (data not shown). We decided to use the *Leishmania* model to demonstrate the therapeutic value of $CD4^+CD25^+$ Treg cells in an acute and severe form of colitis.

Data reported in this work demonstrate directly that $CD4^+CD25^+$ T cells can cure an established progressive inflammatory

disease. Parallel with these results, it was recently reported that $CD4^+CD25^+$ T cells could also cure an IBD in *rag1*$^{-/-}$ mice induced by $CD4^+CD45RB^{low}$ T cells (18). We further advanced this observation by demonstrating that the therapy is critically associated with IL-10 and TGF-β and CTLA4. Despite the general belief that $CD4^+CD25^+$ Treg cells suppress $CD4^+CD25^-$ T cells by a cell contact-dependent mechanism in vitro, there is growing evidence that cytokine-dependent pathways are critical in the suppressive function of $CD4^+CD25^+$ T cells in vivo (19). The suppressive cytokines, IL-10 or TGF-β, may be produced by $CD4^+CD25^+$ T cells or indirectly by $CD4^+CD25^-$ T cells through infectious tolerance mechanism (20). It is important to note that although curing of the colitis by $CD4^+CD25^+$ T cells was accompanied by profound suppression of proliferation and cytokine production, the blocking of the therapeutic effect of $CD4^+CD25^+$ T cells by the anti-IL-10R and anti-TGF-β Ab did not involve the reactivation of IFN-γ/IL-4 productions by $CD4^+CD25^-$ T cells (Fig. 3). This result has two important implications: 1) $CD4^+CD25^+$ T cells could suppress $CD4^+CD25^-$ T cells via an IL-10- and TGF-β-independent pathway in vivo, because the suppression persisted in the presence of neutralizing doses of anti-IL-10R and anti-TGF-β Abs. 2) Colitis could persist in the absence of type 1 or 2 cytokines, because severe pathology continued in the absence of detectable levels of IFN-γ and IL-4. Interpretation 2 is consistent with previous reports showing that IFN-γ did not play a major role in the pathogenesis (21) or in sustaining (22) colitis. The mechanism for the pathogenesis of colitis is currently unknown, but it is clearly controllable by IL-10 and TGF-β. This finding demonstrates the considerable functional overlap among $CD4^+CD25^+$ Treg cells, Treg 1 cells (IL-10 producing) (3), and Th3 cells (TGF-β-producing) (1) cells.

$CD4^+CD25^+$ T cells are the only $CD4^+$ cells in the naive mouse that express the CTLA4 Ag (10, 15, 23, 24). It has been demonstrated that anti-CTLA4 Ab abrogated $CD4^+CD25^+$ T cell-mediated suppression in vitro and treatment of normal adult mice with anti-CTLA4-induced gastritis (23). Similarly, the protective effect of $CD4^+CD45RB^{low}$ T cells in colitis induced by $CD4^+CD45RB^{high}$ T cells was abolished by treatment of the animals with anti-CTLA4 Ab in SCID mice (10). In contrast, the suppressive effect of $CD4^+CD25^+$ T cells on $CD4^+CD25^-$ T cells in murine gastritis was apparently not affected by the presence of anti-CTLA4 Ab (24). In the colitis model reported in this work, the suppressive effect of $CD4^+CD25^+$ T cells was abolished by anti-CTLA4 Ab, consistent with the earlier observations for an important role of CTLA4 Ag in autoimmune diseases in vivo. However, the mechanism by which CTLA4 influences the suppressive effect of $CD4^+CD25^+$ T cells remains unclear. As CTLA4 is up-regulated following T cell activation, it is uncertain as to the target of the Ab. It is possible that anti-CTLA4 Ab blocked CTLA4 on the Treg cells. Alternatively, the Ab could activate $CD4^+CD25^-$ T cells. This seems unlikely because anti-CTLA4 Ab treatment of mice transferred with $CD4^+CD25^-$ T cells alone did not affect the pathogenicity of these cells. Instead, the Ab modestly down-regulated IL-4 production by the transferred $CD4^+CD25^-$ T cells. However, the possibility that anti-CTLA4 Ab may render the $CD4^+CD25^-$ T cells refractory to the suppressive effect of the Treg cells by masking their CTLA4 Ag cannot be excluded.

We also monitored the progression of *Leishmania* infection throughout the experiments. The effect of $CD4^+CD25^+$ cell transfer on the infection depends on the timing of the transfer relative to $CD4^+CD25^-$ cell reconstitution of the SCID mice. SCID mice reconstituted with $CD4^+CD25^+$ T cells alone developed minimal lesion for the first 3 wk after infection. Thereafter, the lesion progressed inexorably. In contrast, SCID mice transferred with $CD4^+CD25^-$ T cells developed significant lesion for the first 3 wk and then regressed and maintained at a low level. The lesion size directly correlated with parasite loads. This pattern of infection is consistent with previous reports (11, 12). The reason for this biphasic response is currently unclear and is beyond the scope of this report. When $CD4^+CD25^+$ cells were transferred 10 days after $CD4^+CD25^-$ cells (freshly isolated, or activated with anti-CD3 Ab in vitro, or cultured with TGF-β), the infection that developed was indistinguishable from that of mice injected with $CD4^+CD25^-$ cells alone for the first 5 wk; thereafter, the infection in the $CD4^+CD25^+$ cell recipients continued to progress, while that of the mice receiving $CD4^+CD25^-$ cells alone began to regress. In contrast, when $CD4^+CD25^+$ cells were transferred 21 days after $CD4^+CD25^-$ cells, the infection continued to progress, while that of mice injected with $CD4^+CD25^-$ cells alone began to regress. The relation between the degree of *Leishmania* infection and severity of colitis appears to be complex. For example, whereas both anti-IL-10R and anti-CTLA4 Ab treatment abrogated the colitis-protective effect of $CD4^+CD25^+$ cells, only anti-IL-10R Ab treatment significantly reduced the infection-promoting effect of $CD4^+CD25^+$ cells. This lesion-reduction effect of anti-IL-10R Ab is consistent with a recent report (25). Furthermore, although anti-TGF-β Ab significantly reduced the severity of colitis, the Ab also reduced the *Leishmania* infection, albeit delayed (Fig. 3*d*). The effect of anti-TGF-β Ab on *Leishmania* infection is again consistent with a previous report (26). Thus, it is unlikely that the curative effect of $CD4^+CD25^+$ cells is due to an indirect effect on *Leishmania* infection.

In summary, data reported in this work directly demonstrate the therapeutic role of $CD4^+CD25^+$ T cells in severe inflammatory colitis. Furthermore, we show that the therapeutic effect is critically dependent on IL-10, TGF-β, and CTLA4. The results also suggest that expansion of autologous $CD4^+CD25^+$ T cells in vitro, followed by reinfusion of these cells, may hold considerable therapeutic potential in treating severe clinical chronic inflammatory diseases.

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