Colitis-Inducing Potency of CD4⁺ T Cells in Immunodeficient, Adoptive Hosts Depends on Their State of Activation, IL-12 Responsiveness, and CD45RB Surface Phenotype

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Colitis-Inducing Potency of CD4+ T Cells in Immunodeficient, Adoptive Hosts Depends on Their State of Activation, IL-12 Responsiveness, and CD45RB Surface Phenotype

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We studied the induction, severity, and rate of progression of inflammatory bowel disease (IBD) induced in SCID mice by the adoptive transfer of low numbers of the following purified BALB/c CD4+ T cell subsets: 1) unfractionated, peripheral, small (resting), or large (activated) CD4+ T cell populations; 2) fractionated, peripheral, small, or large, CD45RBhigh or CD45RBlow CD4+ T cell populations; and 3) peripheral IL-12-unresponsive CD4+ T cell populations from STAT-4-deficient mice. The adoptive transfer into SCID host of comparable numbers of CD4+ T cells was used to assess the colitis-inducing potency of these subsets. Small CD45RBhigh CD4+ T lymphocytes and activated CD4+ T blasts induced early (6–12 wk posttransfer) and severe disease, while small resting and unfractionated CD4+ T cells or CD45RBlow T lymphocytes induced a late-onset disease 12–16 wk posttransfer. SCID mice transplanted with STAT-4−/− CD4+ T cells showed a late-onset IBD manifest >20 wk posttransfer. In SCID mice with IBD transplanted with IL-12-responsive CD4+ T cells, the colonic lamina propria CD4+ T cells showed a mucosa-seeking memory/effector CD45RBlow Th1 phenotype abundantly producing IFN-γ and TNF-α. In SCID mice transplanted with IL-12-unresponsive STAT-4−/− CD4+ T cells, the colonic lamina propria, mesenteric lymph node, and splenic CD4+ T cells produced very little IFN-γ but abundant levels of TNF-α. The histopathologic appearance of colitis in all transplanted SCID mice was similar. These data indicate that CD45RBhigh and CD45RBlow, IL-12-responsive and IL-12-unresponsive CD4+ T lymphocytes and lymphoblasts have IBD-inducing potential through of varying potency. The Journal of Immunology, 1999, 162: 3702–3710.

H uman inflammatory bowel disease (IBD) is a chronic and severe condition of unknown etiology and pathogenesis (reviewed in Ref. 1). Different animal models are available to study its induction, development, and control by new therapeutic approaches (2–5). A severe and lethal chronic IBD can be induced in immunodeficient mice reconstituted with low numbers of immunocompetent, syngeneic CD4+ T cells (reviewed in Ref. 6). It is not clear if different murine CD4+ T cell subsets express different colitis-inducing potency. This is related to the question of whether a factor imbalance in the local or systemic cytokine homeostasis dominates in colitis (reviewed in Ref. 7–10). In the present study, we transplanted severely immunodeficient C.B-17 scid/scid (SCID) mice with syngeneic, small, or activated CD4+ T cells that were either unfractionated or fractionated into the CD45RBhigh and CD45RBlow subset. In addition, we used IL-12-unresponsive CD4+ T cells from BALB/c STAT-4-deficient (STAT-4−/−) donor mice to reconstitute SCID mice. We monitored disease development using clinical, immunological, and histopathological parameters.

CD45RBhigh CD4+ T cells from normal rats have the potential to induce autoimmune diseases in congenic, immunodeficient hosts (11–13). CD45RBhigh CD4+ T cells from healthy animals thus seem to express an autogamous potential that can be revealed in vivo. This concept has been applied to the study of murine IBD. It has been shown that CD45RBhigh CD4+ T cells from immunocompetent mice induce clinical and histopathological signs of colitis after transfer into a histocompatible, immunodeficient host. In this system, peripheral CD45RBhigh CD4+ T cells from BALB/c donor mice induced a colitis after adoptive transfer into C.B-17 scid/scid hosts, while nonfractionated CD4+ T cells or CD45RBlow CD4+ T cells did not (14–20).

Our groups observed the development of a progressive and lethal IBD in SCID mice reconstituted with nonfractionated, peripheral CD4+ T cells (containing mixtures of CD45RBlow and CD45RBhigh cells) from different lymphoid tissues of immunocompetent, histocompatible donor mice (21–25). We used H-2d SCID mice of the BALB/c genetic background and H-2b SCID mice of the C57BL/6 genetic background. We demonstrated that a gut wall graft from a normal, immunocompetent donor mouse put heterotopically into the skin of histocompatible SCID mice induces an IBD (21). In this system, CD45RBlow memory/effector CD4+ T cells were shown to migrate preferentially from the graft into the gut lamina propria, mesenteric lymph nodes (mLN), and spleen of the immunodeficient host. Furthermore, a colitis developed in SCID hosts after transfer of CD45RBlow CD4+ T cells.
from either spleen, mLNs, or colonic lamina propria of transplanted SCID mice with IBD (25), the colonic lamina propria of aging nude mice (without IBD) (26), or spleen, mLNs or gut lamina propria of SCID mice (without IBD) expressing a rearranged, transgenic TCR-β (22). It was unclear if the CD45RB$^+$ surface phenotype, the activation state, or the Th1 phenotype conferred an IBD-inducing potential to the CD4$^+$ T cells under these experimental conditions. Recently, we demonstrated that activated CD4$^+$ T blasts exhibit a more potent IBD-inducing potential than nonactivated CD4$^+$ T lymphocytes (27).

In this model, the inflamed colon is infiltrated by rapidly proliferating Th1 CD4$^+$ T blasts producing the proinflammatory cytokines IFN-γ and TNF-α (26, 28, 29). IFN-γ-deficient CD4$^+$ T cells have lost the potential to induce IBD (30). The main inducer of IFN-γ is IL-12 (31). The binding of IL-12 to IL-12R generates IFN-γ-inducing signals, the intracellular transduction of which depends on the STAT-4 protein (32, 33). To study if effective induction of IFN-γ is an essential prerequisite for the induction of IBD in this model, we transferred CD4$^+$ T cells from STAT-4-deficient BALB/c donor mice into SCID hosts to assay their IBD-inducing potential.

Materials and Methods

Mice and induction of IBD

Female mice of the H-2$^b$ inbred strains BALB/cJ and C.B-17 scid/scid (SCID) were bred under standard pathogen-free conditions in the animal studies, we used immunocompetent BALB/c (C57BL/6J) or congenic C.B-17 R. A. Phillips (Toronto, Canada). STAT-4$^{-/-}$ BALB/c mice obtained from the Harvard animal colony (34) were bred in Ulm. In previous transfer systems, we used immunocompetent BALB/c$^{+/-}$ (dm2) or congenic C.B-17$^{+/-}$ donor mice. In the experiments described in this paper, we used BALB/c mice with our data comparing with published experiments using CD45RB$^{+/-}$CD4$^+$ T cell transfers into SCID mice (14–20). SCID mice were transplanted i.p. at 4–6 wk of age. We did not detect differences in these three transfer systems, which are all based on the BALB/c genetic background (data not shown). Transplanted mice were monitored for weight loss, rectal prolapse, rectal bleeding, and diarrhea. Mice were sacrificed for histological and cytological examinations when they exhibited more than or equal to two of the following signs of disease: 1) a loss of >15% of their adult body weight (compared with a group of nontransplanted SCID mice), 2) the development of a large (>3 mm) rectal prolapse, or 3) extensive diarrhea or bloody stools. Donor mice and sentineel mice in our colony are routinely screened for serum Abs against mouse hepatitis virus. No evidence for infection of mice with this pathogen was detected in the last 5 years.

Preparation of CD4$^+$ T cells for adoptive transfer

Spleen cells or mLNs were aseptically prepared. CD4$^+$ T blasts were generated from lymphoid cells depleted of CD8$^+$ T cells by treatment with anti-CD8 Ab and low-toxicity rabbit complement (catalog no. CL3051, Cedarlane, Hornby, Canada) following the manufacturer’s instructions. Cells (1 × 10^6 cells/ml) were stimulated with 2 μg/ml Con A for 3 days. Harvested cells were >95% CD4$^+$ T blasts and <1% CD8$^+$ T blasts. The cells were washed and cultured for a further 2 days without mitogen. For separating CD4$^+$ T cells and their CD45RB$^{+/-}$ and CD45RB$^{+}$ subsets, the anti-CD45RB mAb 16A or 23G2 (catalog nos. 01142D and 01532D, PharMingen, Hamburg, Germany) and the anti-CD4 mAb GK1.5 (both rat IgG2a Abs, PharMingen) were used. Cells were washed, suspended in PBS/0.3% w/v BSA, labeled with 0.5 μg/10^6 cells of the phycoerythrin (PE)-conjugated anti-CD4 mAb GK1.5 and the biotin-conjugated anti-CD45RB mAb 16A or (biotinylated anti-CD4 mAb GK1.5 and FITC-conjugated anti-CD8 mAb 11B12) for 20 min at 4°C, and washed. Cells were stained with FITC- or PE-conjugated streptavidin as the second layer. Isolation of lymphoid cell populations from transplanted SCID mice

Transplanted SCID mice or normal BALB/c control mice were sacrificed by cervical dislocation. Colonic lamina propria lymphocytes (LPL) and single-cell suspensions from spleen, peritoneal cavity, and mLNs were prepared as described (35, 36). The surface phenotype of isolated cells was always analyzed within a few hours after cell preparation.

Immunofluorescence staining and flow cytometry (FCM) analyses

For FCM marker studies, cells were suspended in PBS/0.3% w/v BSA supplemented with 0.1% w/v sodium azide. Nonspecific binding of Abs to Fc receptors was blocked by preincubating cells with the mAb 2.4G2 (catalog no. 01241D, PharMingen) directed against the FcγRII/II CD16/CD32 (1 μg mAb/10^6 cells/100 μl). Cells were incubated with 0.5 μg/10^6 cells of the relevant mAb for 30 min at 4°C and washed. In most experiments, cells were subsequently incubated with a second-step reagent for 15 min at 4°C. Three-color FCM analyses were performed on a FACSscan (Becton Dickinson). The forward-angle light scatter was used as an additional parameter to facilitate exclusion of dead cells and aggregated cell clumps. The following reagents and mAbs were used: FITC-conjugated or biotinylated anti-CD3 e mAb 145-2C11 (catalog nos. 01084D and 01082D, FITC-conjugated anti-CD4 (L7/4) mAb H12.19 (catalog no. 010904D, bio- tinylated anti-CD4 (L7/4) mAb RM4-5 (catalog no. 01092D), FITC-conju- gated anti-CD4 (Pgp-1) mAb IM7 (catalog no. 01224D), biotinylated or FITC-conjugated anti-CD45RB mAb 23G2 (catalog nos. 01532D and 01534D), biotinylated anti-CD2 mAb RM2-5 (catalog no. 01172D), biotinyl- ated anti-CD28 mAb 37.51 (catalog no. 01672D), PE-conjugated anti-CD49d (α4, integrin chain) mAb R1-2 (catalog no. 01275B), biotinylated anti-CD62L (L-selectin) mAb MEL-14 (catalog no. 01292D), biotinylated anti-CD69 mAb H1.2F3 (catalog no. 01502D), and PE-, chyrome-, and FITC-conjugated streptavidin (catalog nos. 13025D, 13038A, and 13024D) (all obtained from PharMingen). SA-Red670 was obtained from Life Technologies (catalog no. 19543-024; Berlin, Germany). PE-conjugated anti-CD4 (L7/4) mAb GK1.5 (catalog no. 01447) and FITC-conjugated or biotinylated anti-CD8ε (Ly-2) mAb 53–67 (catalog no. 01351) were obtained from Becton Dickinson.

FCM detection of cytoplasmic cytokine expression by CD4$^+$ T cells

To control for cytokine expression patterns, we generated Th1 and Th2 CD4$^+$ T blasts in vitro. Lymphoid cell populations were depleted of CD8$^+$ T cells by treatment with anti-CD8 Ab and complement. Cells (10^6 cells/ml) were stimulated with 2 μg/ml Con A and either 200 U/ml recombinant mouse IFN-γ (catalog no. 1276905, Boehringer Mannheim, Mannheim, Germany), 2 ng/ml recombinant mouse IL-12 (catalog no. 9361V, Phar- Mingen) and the anti-IL-4 mAb 11B11 (2 μg/ml) to expand Th1 CD4$^+$ T blasts, or 10 ng/ml recombinant mouse IL-4 (catalog no. MIL-4–C, Genzyme, Rüsselsheim, Germany), 20 U/ml recombinant human IL-2 (a generous gift from Chiron, Ratingen, Germany), and the anti-IFN-γ mAb XMG1.2 (2 μg/ml) to expand Th2 CD4$^+$ T blasts. Cells harvested after 3 days of culture contained >85% CD4$^+$ T blasts and <1% CD8$^+$ T blasts. These cells were washed and cultured for a further 2 days without mitogen in the presence of the indicated cytokine supplements.

Cytoplasmatic cytokine expression was studied as reported previously (28, 29). In brief, the (Th1 or Th2) CD4$^+$ T blasts, mLNs, spleenocytes, and colonic LPL (from normal mice or transplanted SCID mice) were stimulated with 50 μg/ml PMCA and 500 ng/ml ionomycin in the presence of 10 μg/ml of phorbol myristat A in RPMI 1640/10% FCS for 5 h at 37°C with 5% CO₂ (4 μg/ml) stop/0.6 ml culture medium; Cytofix/Cytoperm Plus, catalog no. 2076KK, PharMingen). Cells were harvested, washed twice in staining buffer (PBS without Mg$^{2+}$/Ca$^{2+}$, 0.3% w/v BSA, 0.1% w/v sodium azide), incubated (15 min, 4°C) with purified 2.4G2 Ab to block nonspecific binding of Ab to Fc receptors, washed with staining buffer, resuspended in staining buffer, and surface stained with anti-CD3 and anti-CD4 Ab. Cells were washed with staining buffer, labeled with the second-step reagent, and washed twice. Cells were then resuspended in 100 μl of Cytofix/Cytoperm solution for 20 min at 4°C and washed twice in 1 ml 1× Perm/Wash solution (PharMingen). Fixed and permeabilized cells were resuspended in 50 μl of 1× Perm/Wash solution and stained with 1 μg mAb/10^6 cells of either FITC-conjugated anti-IL-4 mAb 11B11 (catalog no. 18914A), FITC-conjugated anti-IL-2 mAb 54B6 (catalog no. 18004A), FITC-conjugated anti-IL-10 mAb JES5-16E3 (catalog no. 18434A), FITC-conjugated anti- TNF-α mAb MP6-XT22 (catalog no. 18134A), FITC-conjugated anti-
Freshly isolated CD4^+ T cell populations from spleen or mLNbs of young BALB/c donor mice contained 3–5% (spleen) or 1–8% (mLN) T blasts. Furthermore, we polyclonally activated these CD4^+ T cells in vitro in some experimental groups to generate CD4^+ T blasts. SCID mice were transplanted with 3 × 10^5 purified, splenic CD4^+ T cells from age- and sex-matched BALB/c donor mice that were either small lymphocytes or mitogen-stimulated large lymphoblasts (n = 12). The weight of these mice was monitored weekly. The percentage changes in body weight (g) ± SEM of the two experimental groups are plotted.

Histological examinations
Immediately after death, autopsies of the mice were performed. For histological examination, tissue samples of multiple segments of the gastrointestinal tract were taken. There were no gross pathologic changes in nontransplanted age- and sex-matched SCID mice. In contrast, transplanted animals showed the typical swelling of the colon of various extent and degree. These tissue samples were formalin-fixed and stained with hematoxylin-eosin for histology.

Results
Development of colitis in SCID mice transplanted with small (resting) or large (activated) CD4^+ T cells
Freshly isolated CD4^+ T cell populations from spleen or mLNbs of young BALB/c donor mice contained 3–5% (spleen) or 1–8% (mLN) T blasts. Furthermore, we polyclonally activated these CD4^+ T cells in vitro in some experimental groups to generate CD4^+ T blasts. SCID mice were transplanted with 3 × 10^5 purified small (resting) CD4^+ T cells or large (mitogen-activated) CD4^+ T blasts. These T cell populations contained a mixture of CD45RB^high and CD45RB^low cells. The injected small (resting) CD4^+ T cells populations contained a contaminating fraction of 1–5% T blasts; the injected large (mitogen-activated) CD4^+ T blast population contained a contaminating fraction of 10–12% small CD4^+ T cells. At the end of the observation period of 20 wk, all transplanted SCID mice had developed diarrhea and had lost >20% of their adult body weight (the transplanted mice were compared with an age-matched group of nontransplanted SCID mice) (Fig. 1). There was variability in the severity and progression of the disease between individual transplanted SCID mice, but mice transplanted with CD4^+ T blasts developed a more severe IBD earlier posttransplantation, confirming our previous report (27). Th1 CD4^+ T blasts (generated in the presence of IL-12, IFN-γ, and anti-IL-4 Ab) or Th2 CD4^+ T blasts (generated in the presence of IL-4 and anti-IFN-γ Ab) showed similar IBD-inducing potential in vivo after transfer into SCID hosts, suggesting that this phenotype is not stable in 5-day mitogen-driven cultures (data not shown). Increasing the number of transferred small lymphocytes or large blasts (to 1–50 × 10^5 cells per mouse) did not lead to an accelerated appearance or increased severity of colitis (data not shown).

Adoptive transfer of CD45RB^high and CD45RB^low CD4^+ T cells into SCID hosts
A majority of freshly isolated CD4^+ T cells express the CD45RB^high surface phenotype (70–80%), while a minor fraction (10–20%) was CD45RB^low (Fig. 2). In mitogen-stimulated CD4^+ T blast populations, 50–70% expressed the CD45RB^high and 15–30% the CD45RB^low phenotype. CD4^+ T cells expressing either the CD45RB^high or the CD45RB^low phenotype were isolated by FACS (Fig. 2). The purity of the sorted cell populations was assessed in realanyses and was found to be $>$98% for CD4^+ CD45RB^high and CD4^+ CD45RB^low cells. SCID mice were reconstituted with 3 × 10^5 small CD4^+ T cells or large CD4^+ T blasts expressing either the CD45RB^high or the CD45RB^low phenotype.

SCID mice transplanted with small CD45RB^high CD4^+ T cells developed diarrhea and showed weight losses of >10% at 6–8 wk posttransplantation (Fig. 2). Thus, disease manifestations appeared early, and the progression of the disease in individual animals tended to run a more uniform and faster course than in SCID mice transplanted with unfractionated CD4^+ T cells. SCID mice transplanted with small CD45RB^low CD4^+ T cells developed a late-onset diarrhea and a >10% weight loss 14–15 wk posttransplantation (Fig. 2, A and B).

SCID mice were further transplanted with fractionated, histo-compatible large CD45RB^high or CD45RB^low CD4^+ T blasts (Fig. 2, C and D). In these groups, the adoptive hosts showed signs of IBD with weight losses of >10% at 9–10 wk (CD45RB^high blasts) and 14–15 wk (CD45RB^low blasts) posttransplantation. Thus, SCID mice reconstituted with CD4^+ CD45RB^high T cells developed an early and severe IBD, while SCID mice transplanted with CD4^+ CD45RB^low T cells developed a delayed-onset and slowly progressing but equally lethal disease.

Adoptive transfer of CD4^+ T cells from IL-12-unresponsive STAT-4^−/− donor mice into SCID hosts induces a colitis
CD4^+ T cells were isolated from spleen or mLNbs of BALB/c STAT-4^−/− donor mice. Mitogen-induced CD4^+ T blasts from these “knockout” mice stimulated in vitro with varying concentrations of recombinant mouse IL-12 produced 10^2- to 10^4-fold less IFN-γ (data not shown). SCID mice were transplanted with small CD4^+ T cells or large CD4^+ T blasts from STAT-4^−/− donor mice and were followed for 6 mo (Fig. 3). Whereas control SCID mice transplanted with an equal number of CD4^+ T cells from STAT-4^−/− BALB/c mice showed rapid IBD development, SCID mice injected with STAT-4^−/− CD4^+ T cells gained weight and looked healthy for 16–18 wk posttransplantation but developed clinical signs of colitis thereafter (Fig. 3). This included diarrhea and rectal prolapse in some of the animals. Thus, IL-12 unresponsive CD4^+ T cells induce a late-onset, slowly progressive colitis associated with low local and systemic IFN-γ levels (see below).
Histopathology of CD4\(^+\) T cell-induced colitis in SCID mice

SCID mice transplanted with small CD4\(^+\) T cells or CD4\(^+\) T blasts of either the CD45RB high or the CD45RB low phenotype that are either IL-12 responsive or IL-12 unresponsive developed a pancolitis. The histological appearance and severity of the inflammatory reactions in the large intestine of the transplanted mice was comparable in all groups of transplanted SCID mice, irrespective of the CD45RB\(^{\text{high}}\) vs CD45RB\(^{\text{low}}\) phenotype or the resting vs activated functional state of the transferred CD4\(^+\) T cells. The onset of the disease and its progression differed between the groups. The colon wall showed erosions of the mucosal surface, a thickening of the lamina propria with a diffuse inflammatory infiltrate of mononuclear and polymorphonuclear cells, and a marked distortion of the crypt architecture with a reduced number of goblet cells (Fig. 4, compare A–E with F). Occasional crypt abscesses were found with accumulations of neutrophils in the lumen of the crypts.

Inflammation of the colon in SCID mice transplanted with CD4\(^+\) T cells from STAT-4\(^{-/-}\) or STAT-4\(^{+/+}\) BALB/c donor mice showed epithelial proliferation, crypt elongation, and mononuclear infiltration in the lamina propria, while the epithelial lining appeared to be intact and no crypt abscesses or polymorphonuclear cell infiltration were observed (Fig. 4).

Accumulation of Th1 CD4\(^+\) T cells in CD4\(^+\) T cell-transplanted SCID mice with colitis

The CD3\(^+\) CD4\(^+\) T cells repopulating the SCID host expressed the CD45RB\(^{\text{low}}\) CD44\(^{\text{high}}\) CD2\(^{\text{high}}\) CD28\(^{\text{high}}\) surface phenotype of memory T cells (Fig. 5). These CD4\(^+\) T cells expressed only low levels of CD62L (L-selectin), a marker of naïve T cells. Most CD4\(^+\) T cells found in transplanted SCID mice expressed CD49d, the \(a_\alpha\)-integrin chain. Virtually all CD3\(^+\) CD4\(^+\) T cells expressed the CD95 (Fas) molecule. A similar marker profile was found on the surface of CD4\(^+\) T cells isolated from the spleen, the peritoneal cavity, the mLNs, and the gut lamina propria of transplanted SCID mice (data not shown). No differences were observed in the surface...
A similar pattern of the prevalence of CD4+ T cells was seen in the mLNs and spleen of transplanted SCID mice with colitis (Fig. 6). The colonic lamina propria CD4+ T cells of diseased SCID mice transplanted with either small CD4+ T cells or large CD4+ T blasts of the CD45RBhigh or the CD45RBlow phenotype expressed indistinguishable phenotypes. Thus, irrespective of the phenotype of the inducing CD4+ T cell populations (small resting T lymphocytes vs large activated T blasts; CD45RBhigh vs CD45RBlow surface phenotype), the development of colitis in this model was associated with the accumulation of in situ activated CD4+ T cells of the Th1 phenotype in the colonic lamina propria. This confirms findings in many different mouse colitis models (38–45).

Accumulation of TNF-α but not IFN-γ-producing CD4+ T cells in STAT-4−/− CD4+ T cell transplanted SCID mice with colitis

Only a small fraction of IFN-γ-expressing CD4+ T cells were found in the colonic lamina propria of diseased SCID mice reconstituted with CD4+ T cells from STAT-4−/− donor mice (Fig. 6). The low fraction of IFN-γ-expressing CD4+ T cells was comparable to the fraction present in the normal colonic mucosa of healthy, immunocompetent specific pathogen-free BALB/c mice (Fig. 6). In contrast, the increased fraction of TNF-α-expressing CD4+ T cells in the colonic lamina propria of diseased SCID mice reconstituted with CD4+ T cells from STAT-4−/− donor mice were similar to those found in diseased SCID mice transplanted with various CD4+ T cell subsets from normal BALB/c donor mice (Fig. 6). The fractions of IL-2-, IL-4-, and IL-10-secreting CD4+ T cells in the colonic lamina propria, mLNs, and spleen in SCID mice with colitis transplanted with BALB/c-derived STAT-4−/− or STAT-4−/− CD4+ T cell subsets were similar (Fig. 6).
Thus, in different tissues of SCID mice transplanted with CD4⁺ T cells from either STAT-4⁺/⁺ or STAT-4⁻/⁻ CD4⁺ T cells, the fractions of TNF-α-, IL-2-, IL-4-, and IL-10-producing CD4⁺ T cells were similar. The only difference was the low fractions of IFN-γ-expressing CD4⁺ cells in all tested organs from diseased SCID mice transplanted with STAT-4⁻/⁻ CD4⁺ T cells.

The serum levels of IFN-γ and TNF-α were increased >10-fold in all diseased SCID mice transplanted with STAT-4⁻/⁻ CD4⁺ T cells (Fig. 7) compared with levels in normal BALB/c mice. In SCID mice transplanted with STAT-4⁻/⁻ CD4⁺ T cells, the serum level of TNF-α was more than fivefold above that of BALB/c mice.

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The serum levels of IFN-γ and TNF-α were increased >10-fold in all diseased SCID mice transplanted with STAT-4⁻/⁻ CD4⁺ T cells (Fig. 7) compared with levels in normal BALB/c mice. In SCID mice transplanted with STAT-4⁻/⁻ CD4⁺ T cells, the serum level of TNF-α was more than fivefold above that of BALB/c mice.
mice, while the level of IFN-γ was low and not significantly different from that of BALB/c mice (Fig. 7). We were not capable of measuring IL-12, neither in diseased nor in healthy mice. Therefore, the pattern of changes of IFN-γ and TNF-α in serum levels of observed corresponded to that seen with CD4+ T cells from different lymphoid tissues of the respective groups (compare Figs. 6 and 7). The data confirm our reports that Th1 CD4+ T cell reactivity in diseased SCID mice is not restricted to the inflamed colon but detectable in all lymphoid organs of the transplanted animal (29).

Discussion

It was mainly through the development of adoptive T cell transfer systems in SCID mice that hypotheses on the pathogenesis of IBD were focused on CD4+ T cells. Different groups reported that activated memory effector Th1-type CD4+ T cells accumulate in the colonic lamina propria and the epithelium of transplanted SCID mice (14–18, 20–25). Similarly, Th1 CD4+ T cells have been implicated in the pathogenesis of IBD in genetically engineered mice (38, 42–44, 46–57). The dominant role of Th1 cells as the disease-inducing cell was supported by the successful therapy of colitis with reagents that suppress Th1-like CD4+ T cell reactivity (42, 58). In addition, bacterial colonization and/or bacterial products of the large intestine seem to be important for disease manifestation. The data from different experimental models are thus compatible with the hypothesis that strong Th1 CD4+ responses in the colonic lamina propria and epithelium caused by bacterial Ags play a central role in the pathogenesis of colitis.

The CD45 surface phenotype of CD4+ T cells that induce colitis in the adoptive SCID host seems to be important. Different groups reported that transfer of CD45RBhigh CD4+ T cells into SCID hosts induces an early and severe IBD (15–20, 40, 59). We have shown that transfer of unfraccionated, peripheral CD4+ T cells (that contain both CD45RBhigh and CD45RBlow T cells) into histocompatible SCID mice induces an IBD with diarrhea and weight loss (21–27, 29, 60). The transfer of CD45RBhigh T cells induces weight losses of >10% and severe diarrhea manifesting 6–8 wk posttransplantation, whereas the appearance of the disease and its progression are delayed after the transfer of unfraccionated CD4+ T cells with body weight losses and diarrhea manifesting as late as 12–18 wk posttransfer. The data shown in this paper are in agreement with this picture. Small (resting) CD45RBhigh CD4+ T cells efficiently induced an IBD manifesting at 6–8 wk posttransplantation, but small CD45RBlow CD4+ T cells also induced an IBD that develops more slowly, first becoming apparent 14–15 wk posttransplantation. When large (activated) CD4+ T blasts were transferred, the difference in the IBD-inducing potential of the CD45RBhigh and CD45RBlow CD4+ T blast subsets is lower than that of unfraccionated blast cells, although there is a higher IBD-inducing potential in CD45RBhigh when compared with the CD45RBlow CD4+ T blast cell subset. Thus, it appears that in activated CD4+ T blasts some kind of collaboration between CD45RBhigh and CD45RBlow CD4+ T blast subsets exists in the induction of the disease. It is unclear why small CD45RBhigh CD4+ T cells or activated CD4+ T blasts express an enhanced IBD-inducing potential when compared with small CD45RBlow or nonfraccionated CD4+ T cells. Evidence has been presented that activated mucosal T cells play a role in the pathogenesis of human enteropathy (61). Small CD45RBhigh CD4+ T cells may more readily transform into blast cells in the adoptive SCID host than small CD45RBlow CD4+ T cells, which would explain the observed differences.

Little is known about the cytokines involved in the initiation of CD4+ T cell-induced IBD induction. There is a consensus that disease manifestation is associated with a Th1 CD4+ T cell reactivity (23, 24, 27, 28, 38–45, 62). Either IFN-γ or TNF-α or both may be major driving forces that imprint a Th1 phenotype onto an ongoing mucosal immune response. A predominant pathogenic role of TNF-α in experimental colitis in mice has been demonstrated (63). Local IFN-γ secretion seems equally important in inducing the typical histopathological lesions of murine IBD as demonstrated by the absence of pathology in SCID mice transplanted with syngeneic IFN-γ-deficient CD4+ T cells (Ref. 30 and S.B. and M.H.C., unpublished observations). IFN-γ expression depends on IL-12. Signal transduction of the IL-12R triggered by its cytokine ligand depends on STAT-4, which binds to and activates the IFN-γ-promoter (64). Our data show that CD4+ T cells from STAT-4−/− (IL-12-nonresponsive) donor mice are severely deficient in producing IFN-γ but can still induce a colitis after adoptive transfer into the immunodeficient host. In agreement with these data, a slowly progressing colitis was observed in recombination-activating gene-deficient (RAG−/−) hosts reconstituted with STAT-4−/− CD45RBhigh CD4+ T cells or in RAG−/− hosts reconstituted with CD45RBhigh CD4+ T cells and repeatedly injected with anti-IL-12 Ab (65). The appearance of IBD and its progression are delayed under these conditions, but clinical and histopathological disease manifestation are readily evident. Manifestation of IBD could be due to the low IFN-γ activity still present or to the unimpaired TNF-α activity. It seems unlikely that the very low level of IFN-γ detectable in SCID mice transplanted with STAT-4−/− CD4+ T cells are the main driving force behind the disease process, but the low doses of this cytokine may still play a role in synergism with TNF-α. Taken together, the data presented in this paper indicate that a pathogenic role of IFN-γ reactivity dependent on IL-12-induced STAT-4-mediated signaling can at least be partly substituted by other proinflammatory cytokines such as TNF-α.

Although adoptive CD4+ T cell transfer models seem to be the simplest in vivo models for the study of the pathogenesis of IBD and to fulfill some essential requirements for a reductionist experimental approach, they have left many questions yet unanswered. The questions regarding the relative efficiency of different CD4+ T cell subsets to home to the gut, the factors that regulate the polarization of the CD4+ T cell response in the colonic lamina propria,
the regulation of the decision between clonal expansion and “ac-
tivation-induced cell death” (apoptosis) of CD4+ T blasts in col-
lonic lamina propria immune responses (27), and CD4+ T cell-
dependent mechanisms that induce mucosal lesions in colitis are largely unresolved. The SCID model may allow us to experiment-
tally address some of these questions.

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