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CD4⁺ T Cells Mediate Murine Syngeneic Graft-versus-Host Disease-Associated Colitis¹

J. Scott Bryson,²*,† Lining Zhang,‡,§ Sarah W. Goes, † C. Darrell Jennings,§ Betty E. Caywood,* Sonia L. Carlson,§ and Alan M. Kaplan†

Syngeneic graft-vs-host disease (SGVHD) is an inducible syndrome that develops following syngeneic bone marrow transplantation (BMT) and treatment with a short course of the immunosuppressive agent cyclosporin A (CsA). Following cessation of CsA, this inducible disease is characterized by weight loss, diarrhea, and development of inflammation in the colon and liver. Although nonspecific effector cells and Th1 cytokines have been shown to participate in disease induction, the role of T cells has not been fully elucidated. Initial studies demonstrated significant increases in CD4⁺ T cells, but not other T cell populations in the colons of diseased animals relative to transplant control animals. To demonstrate a functional linkage between increases in colonic CD4⁺ T cells and disease induction, in vivo T cell depletion studies were performed. Beginning on the day of bone marrow transplantation, groups of control and CsA-treated animals were treated with mAb against either CD4 or CD8 for 21 days. Treatment with anti-CD4, but not anti-CD8, eliminated clinical symptoms and colon pathology. Interestingly, neither anti-CD4 nor anti-CD8 therapy affected the development of liver pathology associated with SGVHD. These findings demonstrated that CD4⁺ T cells initiate development of the intestinal inflammation associated with murine SGVHD. The Journal of Immunology, 2004, 172: 679–687.

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a functional link between the increase in colonic CD4+ and development of murine SGVHD.

Materials and Methods

Mice

C3H/HeN mice were purchased from Harlan Sprague-Dawley (Indianapolis, IN) at 20–21 days of age and were used within 1 wk of arrival. Mice were housed in sterile microisolator cages (Lab Products, Maywood, NJ) and fed autoclaved food and acidified water ad libitum.

Induction of SGVHD

BM was isolated from the femurs and tibias of syngeneic age-matched mice. The donor BM suspensions were prepared in RPMI 1640 (Cellgro, Herndon, VA) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin. The resulting cell suspensions were depleted of Thy-1+ cells using mAb to Thy-1.2 (HO-13-4) and Low Tox M rabbit complement (Cedarlane Laboratories, Westbury, NY), as previously described (4). Reusing mAb to Thy-1.2, animals were treated with 0.83% Tris-buffered NH4 Cl. IEL were then isolated according to a modification of the method of Lefrancois and Lycke (31). Briefly, the contents were flushed from the colon with CMF solution (HBSS, Ca2+/Mg2+ free, 100 mM HEPES (Sigma-Aldrich), 250 mM sodium bicarbonate (Fisher Scientific, Pittsburgh, PA), and 2% FBS (HyClone, Logan, UT)). The tissue was then placed into a flask and shaken for 30 min at 37°C. The cell containing supernatant was removed and transferred into 50-ml centrifuge tubes and put on ice. CMF-DTT-EDTA was added to the tissue and was incubated an additional 30 min, as described. The cells were pooled within a group and concentrated by centrifugation and resuspended in RPMI 1640 (Cellgro) containing penicillin (100 U/ml)-streptomycin (100 µg/ml) (Life Technologies-Invitrogen, Carlsbad, CA). The resuspended cells were then passed over a 0.2-g nylon wool (Robbins Scientific, Sunnyvale, CA) column and eluted with RPMI 1640. Following concentration by centrifugation, the cells were resuspended in 44% Percoll cushion in a 15-ml polycarbonate centrifuge tube. The tubes were centrifuged (2000 rpm) for 20 min at room temperature. The IEL were removed from the 44/67% Percoll interface and washed with RPMI 1640. The resulting cell population was counted using typing blue exclusion and analyzed by flow cytometry, as described below.

Flow cytometry analysis

Isolated lymphoid cells from the periphery or intestinal tract were placed in staining buffer (PBS containing 1% FCS, 0.1% NaN3). To reduce nonspecific staining, cells were incubated with Ab against CD16/CD32 (2.4G2; BD Pharmingen, San Diego, CA) and were stained with fluorochrome-conjugated mAb against various lymphoid surface markers. Conjugated mAb against CD4 (CT-CD4) and CD8 (CT-CD8a) were purchased from Caltag (Burlingame, CA), while conjugated mAb against CD3 (145-2C11), αβ TCR (H57-597), γδ TCR (GL3), CD69 (H1.2F3), and CD3(1) (MEL-14) were purchased from BD Pharmingen. These mAb were used for two- and three-color analysis using a BD Biosciences FACSCalibur flow cytometer (San Jose, CA).

In vivo T cell depletion following induction of SGVHD

Mice were lethally irradiated, reconstituted with syngeneic T cell-depleted BM, and treated with diluent or CsA, as described. Beginning on the day of BMT, mice were treated i.p. with 500 µg/day of anti-CD4 (GK1.5; ascites) or anti-CD8 (53-6.72; ammonium sulfate precipitate of culture supernatant) for 7 days, then every other day for an additional 14 days. Following cessation of CsA and mAb therapy, the animals were monitored for the development of clinical symptoms of SGVHD. In some experiments, groups of control, CsA-treated, and mAb-treated control and CsA-treated mice were euthanatized; the spleen and IEL were isolated; and the level of T cell depletion was determined by flow cytometry. Finally, normal animals were also treated with anti-CD4 and anti-CD8 mAb daily for 1 wk, and peripheral lymphoid cells and IEL were analyzed by flow cytometry.

Histologic evaluation of SGVHD

Tissue samples were taken from euthanatized mice at the indicated times post-CsA therapy and immediately placed in 10% buffered formaldehyde. Fixed tissues were embedded in paraffin, cut into 4- to 6-μm tissue sections, mounted on glass slides, then stained with a standard H&E procedure. All tissue sections were analyzed blind without the knowledge of the treatment category of the animal and graded for inflammation caused by SGVHD using a previously published grading scale (colon: 0, none; 1+, sparse cells; 2+, definite scattered single-cell necrosis in crypts; 3+, several necrotic cells in gland, crypt abscesses present; 4+, confluent destruction of glands) (32). To perform immunohistochemistry, tissue samples were placed in tissue-freezing medium (Triangle Biomedical Sciences, Durham, NC) and snap frozen in liquid N2. Tissues were cut into 6 to 10-μm sections. After fixing to microscope slides, the tissues were stained with primary mAb against CD4 (GK1.5; BD Pharmingen) and CD8 (53-6.72; BD Pharmingen). The cells were visualized using HRP-conjugated F(ab)c2 anti-rat secondary Abs (Jackson Immunoresearch Laboratories, West Grove, PA) and dianaminobenzidine (Sigma-Aldrich).

Statistical analysis

Statistical differences between control and SGVHD samples were determined using Student’s t test, Fisher’s exact test (induction), and log rank test. Differences ≤0.05 were considered statistically different.

Results

Alterations in T cell phenotype in IEL of SGVHD mice

Previous studies have demonstrated that lymphocytes are increased in the colonic lesions associated with SGVHD (2, 5, 13, 14). To monitor the cells that make up the in situ inflammatory response in the colon of SGVHD mice, frozen tissue sections from control and diseased animals (3 wk post-CsA therapy) were analyzed by immunohistochemistry for the presence of CD4+ and CD8+ T cells (Fig. 1). Consistent with previously published data (2, 5, 13, 14), increased lymphocytic infiltration was observed in the colons of SGVHD animals. Specifically, there was an increase in the number of densely staining CD4+ T cells in the mucosa of diseased (Fig. 1B) vs control animals (Fig. 1A). Little change in CD8+ IEL was observed (data not shown). These findings demonstrated that increased numbers of CD4+ T cells were present in the mucosa of the SGVHD colons.

Based on the findings that the number of CD4+ T cells in the colons of SGVHD animals was increased, additional experiments were initiated to qualitatively and quantitatively analyze the inflammatory infiltrate of colons from normal, BMT control, and SGVHD mice post-CsA therapy. Colons were obtained 2–4 wk post-CsA from age-matched normal, control BMT, and CsA-treated SGVHD mice, and the IEL were isolated. Cells within the lymphocyte gate were analyzed by flow cytometry for various T cell markers. As shown in Fig. 2A, there were significant increases in αβ+, CD4+ T cells in IEL cells isolated from the colon of SGVHD mice relative to transplant control animals (p = 0.0104) at 2–4 wk post-CsA (5–7 wk post-BMT). The increased percentage of CD4+ T cells also translated into increased numbers of...
these cells. In the individual experiments in which IEL were isolated, the number of cells recovered was as much as 2.5-fold more from the colons of diseased animals than from BMT controls. However, within the gated populations, the percentages of CD3+/H11001, αβ+, and γδ+ cells in normal, control, and SGVH normal, control, and SGVH mice (Fig. 2B) were similar. Thus, changes within the various treatment groups were not due to skewing or enrichment of specific IEL populations during the IEL isolation protocol. In addition, there was a ~2-fold increase in CD4+ IEL T cells that expressed the CD69 activation Ag in IEL that were isolated from diseased vs control animals (data not shown). Similar changes in CD4+ cells were also observed in two experiments in which lamina propria lymphocytes (LPL) were analyzed as well (64 vs 33% αβ CD4+ SGVH vs control BMT LPL). Conversely, no differences were observed in CD8+ IEL (Fig. 2C) (p = 0.8742). It should also be noted that the percentage of CD4−CD8− T cells in IEL isolated from control vs diseased animals increased (data not shown). The increased percentages of these double-negative cells appear to account for the remainder of the T cells in the IEL population from control animals. Finally, when the IEL isolated from BMT control and SGVH mice were analyzed by RT-PCR for the presence of Th1 and Th2 cytokine mRNA, IEL from the SGVH animals only produced mRNA for the Th1 cytokines IFN-γ and TNF-α (data not shown). No mRNA for Th2 cytokines (IL-4, isolated IL-10) was detected in either group.

Finally, it had been hypothesized by Matsuda et al. (33) that during the development of colitis, naive T cells are activated in the MLN before migration to the colon. When MLN cells were analyzed, there were significantly reduced percentages of CD4+ (Fig. 3) (p < 0.001) and CD8− (data not shown) T cells in the MLN isolated from SGVH animals. Similar numbers of LN cells were isolated from each group (data not shown). Interestingly, >75% of the CD4+ T cells from SGVHD mice had lost expression of CD62L compared with 25% of cells obtained from control BMT animals (p = 0.0024). These results suggested that while there were fewer CD4+ T cells in the lymph nodes that drain the intestinal tract of SGVH mice, a high percentage of CD4+ T cells in the draining LN was activated and increased numbers of CD4+ T cells were present in the colons.

In vivo anti-T cell therapy did not remove IEL from colon

The ability to detect increased numbers and percentages of CD4+ T cells in IEL isolated from SGVH vs control and normal animals was intriguing, especially in light of previous data by Flanagan et al. (16) that demonstrated that T cell depletion in the immediate post-CsA therapy period did not affect the induction of SGVH. However, in that study, T cell depletion was monitored in the periphery, with efficient depletion being observed using an anti-T cell mixture containing mAb against CD4 and CD8. It was
possible that the depletion protocol did not effectively deplete T cells from the colon of BMT- and CsA-treated mice.

To address this issue, experiments were performed to determine whether T cells were depleted from the periphery and colon of normal C3H/HeN mice treated with anti-T cell mAb (anti-CD4 and anti-CD8), as previously described (16). Normal mice were treated for 7 days with anti-T cell mAb and spleen cells and colonic IEL were isolated, stained for T cell markers, and analyzed by flow cytometry (Fig. 4). Treatment of these animals with anti-T cell mAb depleted completely splenic CD4+ and CD8+ T cells, as previously described (16) (Fig. 4). However, the percentages of CD4+ and CD8+ T cells from the colons of normal control and mAb-treated normal animals were identical (Fig. 4). These findings suggested that the rat anti-T cell mAb did not penetrate the gut epithelium and that it is likely that in previous T cell depletion studies T cells were not depleted from the colon, the major target organ of SGVHD (16).

Effect of T cell depletion on the development of SGVHD

The above findings demonstrated that activated CD4+ cells were present in the colon during active disease, but it was not clear as to when the T cells began to accumulate within the colon of CsA-treated animals. Preliminary studies suggested that increased numbers of CD4+ T cells migrated into the colon of CsA-treated animals as early as day 21 post-BMT, at which time an approximate 2-fold increase in CD4+ IEL was observed in diseased vs control animals (data not shown). Based on these findings, additional in vivo cell depletion studies were designed, taking into account the enhanced migration of T cells into the colon during the CsA treatment period. Anti-CD4 or anti-CD8 therapy was initiated in control and CsA-treated animals beginning at the time of BMT. The animals were given 500 μg of mAb daily for 1 wk, then every other day through the end of CsA treatment. It was hypothesized that if the CD4+ T cells were critical for the disease process, then migration of these cells to the colon after thymic emigration could be prevented by systemic anti-CD4 therapy. Both CD4+ (>90%) (Fig. 5) and CD8+ (>75%) (data not shown) T cells were depleted efficiently from the periphery and IEL of control and CsA-treated mAb-treated animals relative to non-mAb-treated controls at day 21 post-BMT. Thus, mAb therapy during the post-BMT period prevented T cells from populating the colon before disease development. A significant reduction in disease induction 3 wk after cessation of CsA and mAb therapy was observed between the CsA-treated SGVHD animals (75%) and the CsA anti-CD4-treated mice (15%) (Fig. 6A; p < 0.0001). In addition, a significantly greater percentage of the CsA-treated animals developed clinical symptoms of disease by 3 wk post-CsA treatment relative to the transplant controls (p < 0.0001). Treatment with anti-CD8 mAb during the CsA therapy period had no effect on the induction of SGVHD (p = 0.7472) relative to CsA-treated SGVHD animals at 3 wk post-CsA. There was also a significant increase in the induction in disease between the anti-CD8-CsA-treated and anti-CD4-CsA-treated animals (p < 0.0001), demonstrating that CD4+, but not CD8+ T cells were involved in the development of SGVHD.

Because treatment with anti-CD4 mAb beginning on the day of BMT for 21 days (CsA treatment period) significantly inhibited the development of SGVHD through 3 wk post-CsA, it follows that such treatment should also alter the pathology associated with SGVHD at this time point. As shown in Fig. 7, anti-CD4 therapy virtually eliminated the development of the inflammatory lesions that are typically seen in the colon of diseased animals (2, 4, 16). Colonic mucosal inflammatory infiltrate consistent with SGVHD (Fig. 7B) was observed in CsA-treated animals 3 wk post-CsA relative to control animals (Fig. 7A). In contrast, treatment with anti-CD4 resulted in a near complete reduction of this inflammation (Fig. 7B vs C). Anti-CD8 treatment had no effect on SGVHD-associated pathology of the colon (Fig. 7D). In addition to the colon, significant pathology also occurs in the livers of
SGVHD mice (3). Interestingly, when the livers of SGVHD and anti-CD4-CsA-treated mice were analyzed histologically, significant inflammation was observed in the SGVHD (Fig. 7F) and both anti-CD4- (Fig. 7G) and anti-CD8-CsA-treated mice (Fig. 7H) relative to control BMT animals (Fig. 7E).

When the tissue samples were quantitatively graded for SGVHD-associated pathology (32), a significant reduction in the SGVHD grade was observed in the colons of anti-CD4-CsA-treated animals when compared with CsA- or anti-CD8-CsA-treated animals (Fig. 8A) \( (p < 0.0001) \). Conversely, when the livers of these animals were analyzed and graded for liver pathology associated with SGVHD, the grade of SGVHD was similar, regardless of whether the CsA-treated animals were given anti-CD4 or anti-CD8 therapy (Fig. 8B). Thus, there appears to be a dichotomy in the role for CD4\(^+\) T cells in the development of inflammation in the various target tissues associated with SGVHD. Liver disease developed under conditions that inhibited the induction of SGVHD-mediated colitis.

Finally, an interesting finding in these studies was that with time after cessation of CsA and mAb therapy, the anti-CD4-CsA-treated animals began to develop clinical symptoms by ~4 wk post-CsA mAb therapy (Fig. 6B). Although the anti-CD4 therapy during the CsA treatment period inhibited the induction of SGVHD during the early post-CsA treatment time frame, new effector cells appear to have migrated to the colon once anti-CD4 mAb therapy stopped, resulting in a delayed development of SGVHD. In fact, while the numbers of anti-CD4-CsA-treated animals that developed SGVHD increased with time, the induction curve was significantly delayed compared with that observed for the CsA-treated \( (p = 0.0432) \) animals. The median induction time (MIT) for the anti-CD4-CsA-treated \( (MIT = 50\) days) was also delayed over that observed for the CsA \( (MIT = 38\) days)- and anti-CD8-CsA-treated mice \( (MIT = 40\) days) as well. The percentage of induction of the anti-CD4-CsA-treated animals increased such that by 80 days after BMT, the percentages of induction among the CsA-treated, anti-CD4-CsA-treated, and anti-CD8-CsA-treated animals were identical. Together, the data presented demonstrate that during SGVHD, while peripheral T cells are reduced following lethal irradiation, BMT, and CsA therapy relative to control animals, it appears that CD4\(^+\) T cells preferentially localize to the colon, and through depletion studies it was shown that these cells are necessary for the development of SGVHD.

**Discussion**

The immune mechanisms responsible for the development of murine CsA-induced SGVHD have not been fully elucidated. Non-specific effector cells and Th1 cytokines have been associated with this inducible disease. Data presented in this manuscript demonstrated that increases in CD4\(^+\), but not other T cells were observed in the colons of diseased animals. Furthermore, there was an increase in activated CD4\(^+\) T cells in the lymph nodes that drain the intestinal tract. Finally, data demonstrated that in vivo depletion of CD4\(^+\), but not CD8\(^+\) T cells, during the immediate post-BMT period (days 0–21) significantly inhibited disease. Thus, this study is the first to demonstrate conclusively that CD4\(^+\) T cells participate in the induction of colitis that is associated with murine SGVHD.

SGVHD was first described by Glazier et al. (34) in the rat, and extensive work has been performed to determine the immune processes involved in the development of this disease. The induction of a graft-vs-host disease-like syndrome in mice following lethal irradiation and CsA therapy was first described by Cheney and Sprent (1). Subsequently, it was shown that induction of SGVHD in the mouse was strain specific (2, 13) and was regulated by
Thy-1<sup>+</sup> cells that were present in the BM (4, 5). Studies by Flanagan et al. (16, 20) demonstrated that Th1 cytokines and NK1.1<sup>+</sup> cells participated in the pathogenic process. Neutralization of IL-12 and TNF-α after cessation of CsA therapy eliminated the induction of SGVHD. The significant role of IL-12 in SGVHD suggested that activated macrophages and their products, including cytokines (16, 20) and reactive nitrogen species (21), were critical to the disease process. In vivo depletion of NK1.1<sup>+</sup> cells in the immediate post-CsA period inhibited disease and eliminated IFN-γ mRNA from the colon of treated animals, suggesting a role for NK cells in the production of IFN-γ during SGVHD (16). It was interesting, however, that in the same study, in vivo depletion of T cells after CsA therapy did not affect disease induction. These studies were in conflict with those in the rat model, demonstrating a clear role for T cells (8, 34–39).

It was shown previously by Bucy et al. (5) that increased numbers of T cells were present in the colons of BALB/c mice following lethal irradiation and CsA therapy. Furthermore, adoptive transfer of spleen cells from these animals when transferred into secondary recipients induced lesions. Data from this laboratory have also demonstrated increased numbers of lymphoid cells, as well as T cells in the colon by histological (2, 4, 16, 20, 21) and immunohistochemical techniques (Fig. 1). Because of the conflicting data relating to the role of T cells in this model, studies were
undertaken to address further the role of T cells in murine SGVHD.

Due to immunohistochemical analysis demonstrating increased numbers of CD4<sup>+</sup> T cells in the colons of affected animals, IEL were isolated from the colon and a phenotypic analysis was performed. The numbers and percentages of αβ<sup>+</sup>CD4<sup>+</sup> T cells were increased in IEL (LPL) isolated from SGVHD vs BMT control or normal animals. Increases in CD8<sup>+</sup> or γδ<sup>+</sup> T cells were not observed. Based on these findings, the inability to deplete colonic T cells with anti-CD4 mAb treatment after cessation of CsA was surprising (16). Although in vivo T cell depletion completely eliminated T cells from peripheral lymphoid organs, the mAb treatment did not eliminate T cells from the colon of treated animals. In contrast, a CD4<sup>+</sup> T cell depletion protocol that began on the day of BMT and continued throughout the CsA treatment period depleted CD4<sup>+</sup> T cells and the development of SGVHD measured 3 wk post-CsA. Depletion of CD8<sup>+</sup> T cells had no effect.

It is interesting, however, that anti-CD4 therapy did not completely inhibit the development of SGVHD colitis over the entire time period of a given experiment (80 days). A delayed increase in the number of animals with clinical symptoms developed in the anti-CD4-CsA-treated animals and ultimately reached the levels of CsA-treated animals not treated with anti-CD4. The delayed development of SGVHD colitis may be due to the continued production of CD4<sup>+</sup> T cells with the capability of responding to Ag present in the target organs of this disease. Wu and Goldschneider (40) have demonstrated in a rat model of CsA-induced autoimmunity that CD8<sup>+</sup> autoeffector cells were detectable within the first week of CsA therapy and those cells continued to be detectable in the periphery up to 15 days after cessation of CsA therapy. Prolonged changes in the expression of MHC molecules by thymic epithelial and dendritic cells (41) following CsA therapy could result in alterations in positive and negative selection (11, 12) with continued production of cells capable of mediating SGVHD-induced colitis. Alternatively, it has been shown that CsA therapy reduces significantly the numbers of T cells that emigrate from the thymus following BMT (11). It is necessary that continued immune surveillance be present to prevent escape of pathogens from the intestinal tract. Thus, it is likely that the CD4<sup>+</sup> T cells that are involved in the induction of SGVHD-induced colitis may migrate to the colon during CsA therapy so that T cell homeostasis is re-established in the colon following BMT. Upon cessation of CsA, cells that have homed to the gut respond to either self or bacterial Ags, and the development of SGVHD occurs. In the presence of CsA and anti-CD4 therapy, a prolongation in T cell deficiency above that which occurs following CsA therapy alone occurs. Following therapy, it is possible that naïve T cells that recently emigrated from the thymus may continue to migrate to the colon and respond to target Ags involved in disease pathogenesis, resulting in the delayed development of SGVHD-induced colitis. Given either of these scenarios, continued anti-CD4 therapy after cessation of CsA would most likely eliminate the delayed disease induction observed in these studies.

At this time, it is unclear as to the different outcomes that arise when two different species of rodents are lethally irradiated, reconstituted with syngeneic BM, and treated with a short course of the immunosuppressant CsA. In its acute form, SGVHD in the rat is predominantly mediated by CD8<sup>+</sup> CTLs with pathology predominantly located in the skin and liver (34). Later, progression to a chronic graft-vs-host disease-like disease with alopecia and scleroderma requires the involvement of autoreactive CD4<sup>+</sup> T cells (42, 43). In contrast, murine SGVHD is characterized by extensive inflammation of the colon and liver with involvement of the skin in selected strains (4). As described in this study, in the predominant T cell involved in the induction and development of murine SGVHD-mediated colitis appears to be CD4<sup>+</sup>. At this time, the specificity of these cells is unknown. However, it is interesting to note the striking similarities between murine SGVHD and various models of murine colitis. Colitis has been shown to develop spontaneously in several mutant mouse strains, in animals that have deficiencies in various cytokine or T cell receptors, following adoptive transfer of naïve CD4<sup>+</sup> T cells into immunodefficient animals or following treatment with chemical agents (reviewed in Ref. 44). IL-12, Th1 cytokines, and CD4<sup>+</sup> T cells are predominant participants in many of these models. Similar findings have been observed in the murine model of SGVHD (16, 20). Thus, common mechanisms may be at work in the murine colitis and post-CsA SGVHD animals, leading to the similar pathology associated with these disparate models of intestinal inflammation.

**FIGURE 8.** SGVHD grade was decreased in the colon, but not liver of anti-CD4-CsA-treated animals. Tissue sections (Fig. 7) were isolated 2 wk after cessation of CsA therapy; sections of colon (A) and livers (B) were removed from euthanatized animals and stained using H&E staining procedures. Pathology grading of tissues from control, SGVHD, anti-CD4-CsA-treated, or anti-CD8-CsA-treated animals was performed, as previously described (32). Data presented represent mean ± SEM, with n representing the number of animals analyzed in each treatment group.
An unexpected finding of this study was the dichotomy between colon and liver pathology associated with SGVHD. Although colon pathology was transiently inhibited, liver disease was unaffected by CD4 depletion. These studies suggest that different effector populations may be responsible for liver and colon pathology. During IL-12-induced liver inflammation, increases in mononuclear cells (CD8+ T cells, NK cell macrophages, and Kupffer cells) were observed (45, 46). A large percentage of lymphocytes detected in the livers of IL-12-treated animals are NK cells and NK T cells (47). Given that SGVHD mice have increased IL-12 production (16), it is likely that increases in NK, NK T cells, macrophages, and Kupffer cells are also increased as well. In this study, we demonstrated that in vivo depletion of CD4+ or CD8+ had no effect on liver pathology in the SGVHD model. As preliminary, unpublished data demonstrated increases in macrophages and NK T cells in the livers of SGVHD mice, these cells, as opposed to CD4+ T cells, are likely to be involved in SGVHD-mediated liver pathology.

Although it is clear that CD4+ T cells participate in the development of SGVHD, the specificity of these cells is not known. T cells that have increased reactivity to enteric Ags are present in the spleen and MLN in a spontaneous model of colitis that develops in C3H/HeJ Bir mice (30, 48, 49). Moreover, enteric-reactive T cell lines can induce colitis when adoptively transferred into secondary recipients (48). Preliminary data have demonstrated that splenic T cells from SGVHD mice have increased capacity to proliferate against enteric Ag-pulsed APCs or APC from the MLN isolated from SGVHD, but not control animals (unpublished data). Similarly, it has been suggested by Matsuda et al. (33) that antigenic stimulation of pathogenic T cells in a CD4+ T cell transfer model of colitis occurs outside the intestine in the spleen or MLN, before T cell migration into the colon. Furthermore, it has been shown that dendritic cells from the intestinal mucosa can migrate to peripheral lymphoid sites outside of the intestinal tract (50). Thus, it is likely that APC present in the MLN of SGVHD animals may present intestinal Ag, resulting in enhanced stimulation of SGVHD T cells. These preliminary studies described above also demonstrate that SGVHD effector cells can be detected in the periphery when the appropriate source of APC (enteric Ag-pulsed or MLN APC from diseased animals) is used. Clearly, further work will be required to define the specificity of CD4+ T cells present in SGVHD animals.

It is also interesting to note that at the time of cessation of CsA therapy, increases in the percentages of CD4+ T cells can already be observed in the colons of SGVHD animals. This finding, along with the data demonstrating that T cell depletion during the 21-day CsA treatment period and not post-CsA therapy (16) inhibited SGVHD-induced colitis, suggests that mechanisms are present during the CsA treatment period that allow for either enhanced migration of T cells to the colon of CsA-treated animals or the expansion of effector T cells in the colon of treated animals in a CsA-resistant manner. It will be interesting to follow the accumulation of T cells during CsA therapy, relative to BMT control animals and to determine whether these cells are activated during this time of CsA-mediated immune suppression. Mechanisms have been described to activate T cells in a CsA-resistant manner and include signaling through CD28/B7 interactions (51–53) and via IL-12/IL-18 (54) and may participate in the induction of murine SGVHD. Thus, this manuscript has for the first time demonstrated a role for CD4+ T cells in the development of murine SGVHD. The specificity and the processes that lead to accumulation and activation of these cells in the colons of CsA-treated animals are currently under investigation.

References


CD4⁺ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype. J. Immunol. 162:3702.


