# CD4+ T Cells That Express High Levels of CD45RB Induce Wasting Disease When Transferred into Congenic Severe Combined Immunodeficient Mice. Disease Development Is Prevented by Cotransfer of Purified CD4+ T Cells

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## Summary

Purified CD4+ lymph node T cells were sorted into two populations on the basis of their expression of CD45RB (CD45RBhi and CD45RBlo) and injected into congenic severe combined immunodeficient (SCID) mice. After a period of time that was dependent on the number of cells injected, the SCID mice that received CD45RBhi/CD4+ T cells developed a wasting disease that was not seen in SCID mice that received the CD4+/CD45RBlo cells or whole lymph node cells. At death, SCID mice that received the CD4+/CD45RBhi cells had increased spleen and lymph node cellularity compared with normal SCID mice and SCID mice that received the CD4+/CD45RBlo T cells. The spleen and lymph node contained CD4+ cells and neither CD8+ nor surface immunoglobulin M–positive cells, plus a population of cells that did not express any of those markers. At necropsy, the SCID mice that received the CD4+/CD45RBhi cells had significant hyperplasia of the intestinal mucosa with significant lymphoid cell accumulation in the lamina propria. Interestingly, mice that received mixtures of whole lymph node or purified CD4+ cells with CD4+/CD45RBhi cells did not develop weight loss, indicating that the unseparated CD4+ population contained cells that were capable of regulating the reactivity of the CD4+/CD45RBhi cells.

Autoaggressive immunological reactivity can be driven by CD4+ thymus-derived lymphocytes. For instance, it has been demonstrated that experimental autoimmune encephalomyelitis and diabetes can be induced in normal animals by injecting them with CD4+ T cell clones or lines derived from animals with autoimmune disease of that particular tissue (1–5). Also, T cell reactivity to self-antigens can be demonstrated in normal animals by immunizing them with a closely related antigen in a unique way or by depleting them of a regulatory population (6–8). These data indicate that T cells with specificity for self-antigens exist normally, but that their reactivity is controlled by immunoregulatory mechanisms.

It is well appreciated that thymus-derived lymphocytes can be categorized according to the cell surface antigens they express. This has allowed the classification of class I or II MHC-recognizing T cells based on their expression of CD8 or CD4 (9). Also, recent data indicate that virgin and memory T cells can be distinguished by their expression of other cell surface markers such as CD44 or CD45 (10–12). The ability to as-

sociate T cell function with the expression of a unique array of cell surface determinants is useful in studying the function of these populations in isolation as well as in defined combinations. For instance, Powrie and Mason (13) have separated CD4+ T cells based on their expression of CD45R and injected the resultant subpopulations into congenic, athymic (nude) animals. They found that nude rats injected with congenic CD45Rhi/CD4+ T cells developed wasting disease characterized by inflammatory infiltrates in many organs. Rats injected with unfractionated CD4+ cells (a mixture of CD45Rhi and CD45Rlo cells) did not develop wasting disease, suggestive of an immunoregulatory mechanism that acted to prevent the development of autoimmune disease.

Here we describe similar findings as a result of injecting CD4+/CD45RBhi and CD4+/CD45RBlo T cells into congenic SCID mice. In SCID mice that received the CD4+/CD45RBhi cells, severe intestinal lesions developed that were not observed in the mice that received the CD4+/CD45RBlo

cells. In addition, SCID mice that received unseparated CD4$^+$ cells or a mixture of CD4$^+$ T cells and CD4$^+$/CD45RB$^{hi}$ T cells developed no intestinal lesions. Thus, this model system allows the assessment of a unique autoreactivity in one T cell subpopulation as well as the study of the immunoregulatory processes that control such reactivity.

## Materials and Methods

*Mice.* Normal C.B-17 and congenic C.B-17 (*scid/scid*) mice were obtained from Taconic Farms, Inc. (Germantown, NY) (14). The mice were maintained in specific pathogen-free conditions and fed autoclaved food and water. The animals used in these studies were female and between 7 and 18 wk of age.

*mAbs and Immunofluorescent Staining.* The mAbs used in this study were: CD4-PE (clone GK1.5); CD8-FITC (clone 53.8.16) (both obtained from Becton Dickinson & Co., Sunnyvale, CA); CD45RB-FITC (clone 23.G.2) (15); and B220-FITC (clone RA3-6B2), (both obtained from PharMingen, San Diego, CA). Goat anti–mouse IgM-PE was obtained from Southern Biotechnology Co. (Birmingham, AL). For immunofluorescent staining, cells were incubated with the directly conjugated mAbs in a staining buffer containing anti-FcRγII (2.4G2 mAb) and 0.5% normal mouse sera (to minimize Fc receptor–mediated binding) for 30 min at 4°C. The cells were washed twice and analyzed. Cells stained with FITC- or PE-conjugated mouse or rat IgG2 (PharMingen, San Diego, CA) were used as negative controls. For assessment of B cells numbers, cells were incubated with 1% normal goat serum for 15 min, washed, and incubated with the PE-conjugated goat anti–mouse IgM sera. The cells were washed and incubated for 15 min with 1% normal mouse serum, and washed and incubated for 30 min with FITC-B220 mAb.

*Flow Cytometry.* Immunofluorescent analysis was performed on a FACScan® flow cytometer (Becton Dickinson & Co.). Negative controls consisting of cell preparations stained with FITC- and PE-conjugated rat IgG2a were used to set background gates. Cells stained with single-color reagents were used to set the appropriate compensation levels. For analysis, at least 10,000 events were analyzed. Cell sorting was performed on a FACStar Plus® (Becton Dickinson & Co.).

*Preparation of CD4$^+$/CD45RB$^+$ Cells.* Lymph nodes (axillary, inguinal, and mesenteric) from normal C.B-17 mice were dissected and teased into a cell suspension. After washing, the cells were incubated in a mixture of culture supernatants from the following hybridomas: 83-12-5 (anti-Lyt-2.2; a gift from Dr. J. Bluestone, University of Chicago, Chicago, IL) and 25-9-17 (anti-I-A$^d$; American Type Culture Collection, Rockville, MD) for 30 min at 4°C. After washing, the cells were incubated with rabbit complement (diluted 1:15, 1 ml/10$^7$ cells; Pel-Freeze Biologicals, Rogers, AR) for 30 min at 37°C. After washing, viable cells were purified on a discontinuous density gradient (Lympholyte-M; Cedarlane Laboratories, Hornsby, Ontario, Canada). The cells were stained with anti-CD4 and anti-CD45RB mAbs as described above.

*Histology.* Small pieces of tissue were fixed in phosphate-buffered 10% formalin. The tissue was embedded in paraffin, sectioned, and stained with hematoxylin/eosin.

*Experimental Design.* Sorted or whole populations of lymph node CD4$^+$ T cells were washed, resuspended in cold PBS, counted, and injected intravenously via the retro-orbital sinus in lightly anesthetized C.B-17 SCID mice. The recipient mice were between 6 and 8 wk of age. The mice were individually marked and weighed. Body weights were measured weekly thereafter. The

body weight data are presented as "percent initial body weight," which is the weight of the mouse at the time indicated divided by the weight of the mouse on the day of transfer. The data presented are from individual experiments, unless otherwise indicated, that have been repeated at least three times with similar results. Statistical significance between the groups was assessed using the Student's *t* test.

## Results

*CD45RB$^{hi}$ but Not CD45RB$^{lo}$ CD4$^+$ T Cells Induce Wasting Disease When Injected into Congenic C.B-17 SCID Mice.* Purified CD4$^+$ lymph node T cells were sorted according to their expression of CD45RB (Fig. 1) and injected into congenic SCID mice, and their body weights were measured weekly. In Fig. 2, it can be seen that SCID mice that received 2 × 10$^5$ whole CD4$^+$ cells or CD4$^+$ cells expressing low levels of CD45RB continued to gain weight over the course of the experiment, whereas the SCID mice that received an identical number of CD45RB$^{hi}$ cells developed a wasting syndrome after a latent period of 20–30 d. The weight loss was progressive, irreversible, and eventually resulted in the death of the animal (data not shown). The weight loss was associated with noticeable diarrhea in the SCID mice that received the CD4$^+$/CD45RB$^{hi}$ T cells. The relationship between the number of CD45RB$^{hi}$ cells and the development of the wasting disease was determined by injecting different numbers of CD4$^+$/CD45RB$^{hi}$ cells into SCID mice. As can be seen in Fig. 3, there was a direct relationship between the number of CD4$^+$/CD45RB$^{hi}$ T cells transferred and the period of time until the development of weight loss. Injection of 10$^6$ cells resulted in the development of disease 3 wk after transfer, while injection of 2 × 10$^5$ cells induced weight loss starting ∼5 wk after transfer. Even injection with as few as 40,000 cells resulted in significant weight loss 7 wk after transfer.
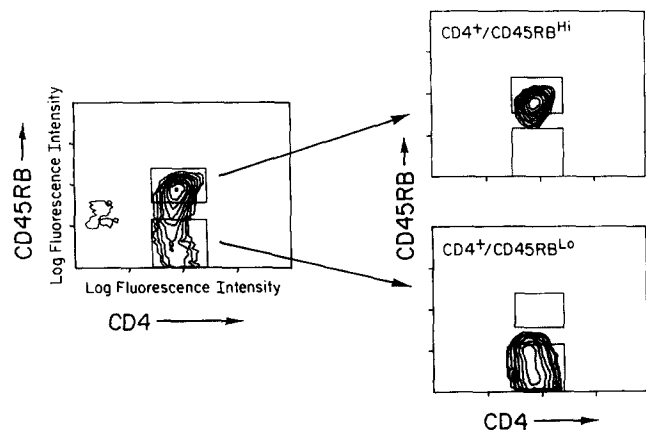


**Figure 1.** Immunofluorescent profiles of lymph node T cells stained with anti-CD4 and anti-CD45RB and the sorted subpopulations. CD4$^+$ lymph node T cells from C.B-17 mice were prepared as described in Materials and Methods, stained with anti-CD4 and anti-CD45RB mAbs, and sorted into the depicted subpopulations.
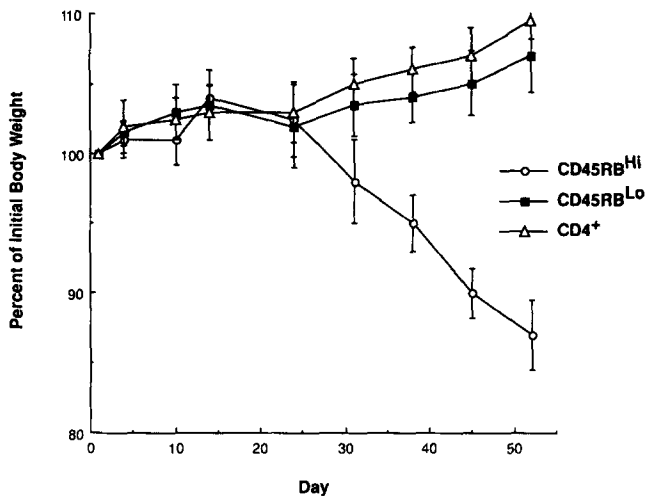
**Figure 2.** Body weights of C.B-17 SCID mice injected with congenic CD45RB$^{hi}$ or CD45RB$^{lo}$/CD4$^+$ T cells or unsorted CD4$^+$ T cells. The CD4$^+$ T cells were prepared as described and sorted into CD45RB$^{hi}$ or CD45RB$^{lo}$ subpopulations. 2 × 10$^5$ cells were injected intravenously into C.B-17 SCID mice. The CD4$^+$ population consisted of stained (anti-CD4 and anti-CD45RB mAbs) but unsorted CD4$^+$ T cells. There were three mice per group. The initial body weights averaged 17.6 g. The difference in body weight between the SCID mice that received the CD4$^+$/CD45RB$^{hi}$ cells and CD45RB$^{lo}$ cells was significant ($p < 0.05$) on days 38, 45, and 52.

*Analysis of SCID Mice That Received CD4$^+$/CD45RB$^{hi}$ or CD4$^+$/CD45RB$^{lo}$ T Cells.* The cellularity and phenotypic composition of the spleen and lymph nodes of the SCID mice that received congenic transfers of CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ T cells was determined. SCID mice that received either 2 × 10$^5$ CD4$^+$/CD45RB$^{hi}$ or CD4$^+$/CD45RB$^{lo}$

were killed 6 wk after transfer. At this time the body weight of SCID mice that received the CD45RB$^{hi}$ cells averaged 87% of their initial body weight, while the body weight of SCID mice that received the CD45RB$^{lo}$ cells averaged 108% of the initial body weight (data not shown). The cellularity and phenotype of the spleens and lymph nodes of these animals are shown in Table 1. Here it can be seen that SCID mice that received the CD45RB$^{hi}$ cells had increased splenic and lymph node cellularity as compared with SCID mice that had received the CD45RB$^{lo}$ cells and normal SCID mice. The cell numbers presented are from pooled lymph nodes (axillary, inguinal, and mesenteric), but in other experiments where lymph node cellularity was assessed individually, the mesenteric lymph node accounted for >80% of the total lymph node cellularity. The lymph node cellularity from unmanipulated SCID mice is negligible in our colony. Also, in our study of mice, which received different populations of CD4$^+$ cells, thymic cellularity was never increased over that of unmanipulated SCID mice (data not shown). Phenotypically, there were significant numbers of CD4$^+$ cells in the SCID mice that received either the CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ cells, and the percentages were always greater in the mice that received the CD4$^+$/CD45-RB$^{hi}$ cells. In these mice there were negligible numbers of CD8$^+$ or surface (s)IgM$^+$ cells. Representative histograms depicting the CD4/CD8 profiles from the lymph node cells of these mice are shown in Fig. 4. The identity of the remaining cells, which were the majority of the spleen and a significant proportion of the lymph nodes, has not yet been determined. Also, it is of interest to note that although equal numbers (2 × 10$^5$) of CD4$^+$/CD45RB$^{hi}$ or CD4$^+$/CD45RB$^{lo}$ cells were transferred into the SCID mice in the experiments shown in Table 1, the total number of CD4$^+$
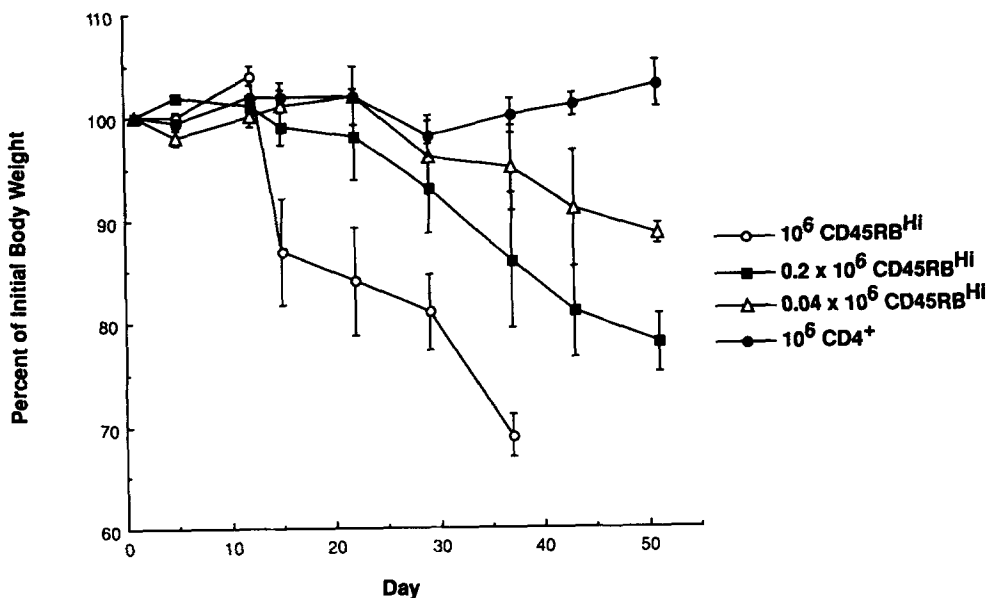


**Figure 3.** Body weights of C.B-17 SCID mice injected with various numbers of congenic CD4$^+$/CD45RB$^{hi}$ cells or unsorted CD4$^+$ T cells. The CD4$^+$ T cells were prepared as described and the CD45RB$^{hi}$ subpopulation was purified by sorting. C.B-17 SCID mice were injected intravenously with the indicated number of CD4$^+$/CD45RB$^{hi}$ T cells or 10$^6$ purified, stained, but unsorted CD4$^+$ T cells. There were four mice per group. The initial body weight of the SCID mice averaged 18.2 g. Compared with the mice that received the CD4$^+$ cells, the body weights of the mice that received 10$^6$ CD45RB$^{hi}$ cells were significantly different on days 15–51. For the mice that received 0.2 × 10$^6$ cells the differences were significant on days 43 and 51. For the mice that received 0.04 × 10$^6$ cells the difference was significant on day 51 only.
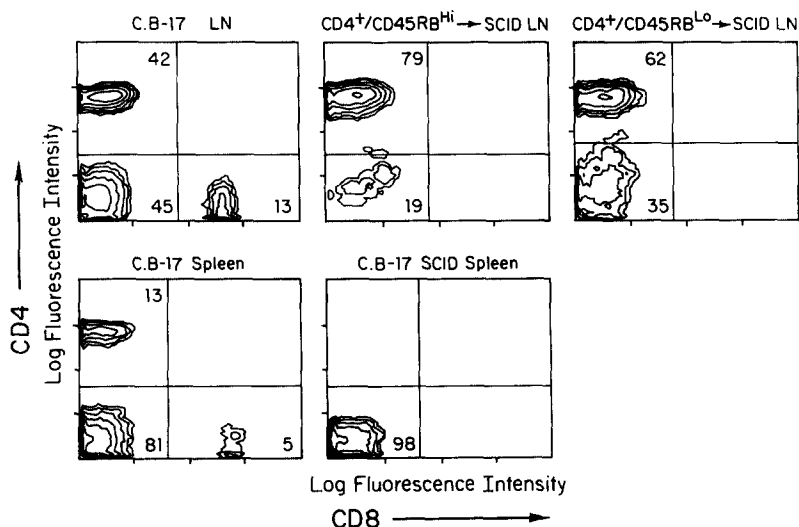
**Figure 4.** CD4 and CD8 immunofluorescent profiles in C.B-17 SCID mice that had received congenic CD45RB$^{hi}$ or CD45RB$^{lo}$ CD4$^+$ T cells. C.B-17 SCID mice were injected with 2 × 10$^5$ sorted CD45RB$^{hi}$ or CD45RB$^{lo}$ CD4$^+$ T cells. After 6 wk, the mice were killed. The data are representative histograms.

cells recovered from the spleens and lymph nodes of these mice indicated that the CD4$^+$ cells had expanded significantly (Table 2). The number of CD4$^+$ cells recovered from the mice that received the CD45RB$^{hi}$ cells was about fivefold greater than that recovered from the mice that received the CD45RB$^{lo}$ cells and ~40-fold greater than the number originally injected (2 × 10$^5$). Over many experiments, the number of CD4$^+$ cells harvested from the SCID mice that received the CD4$^+$/CD45RB$^{hi}$ T cells was always greater than the number harvested from the mice that received the CD4$^+$/CD45RB$^{lo}$ T cells. The CD45RB phenotype of these cells was also determined (Fig. 5). Here it can be seen that the CD4$^+$ cells from the lymph nodes of the SCID mice were invariably CD45RB$^{lo}$ regardless of their phenotype at the time of transfer. This was also true of the CD4$^+$ cells present in the spleen (data not shown). Also, these cells were CD3$^+$/TCR $\alpha/\beta^+$, IL-2R$^-$, and MEL-14$^{lo}$. There were no cells positive for expression of TCR $\gamma/\delta$ in the spleen or lymph nodes of these mice (data not shown).

*Injection of CD4$^+$/CD45RB$^{hi}$ T Cells Mixed with Unfractionated CD4$^+$ Cells Prevents the Development of Wasting Disease.* The previous observation that SCID mice that were

injected with stained but unsorted CD4$^+$ cells did not develop wasting disease (Figs. 2 and 3) suggested that the CD45RB$^{lo}$ cells were exerting a regulatory influence on the CD45RB$^{hi}$ cells that prevented the development of wasting disease. This was further assessed by injecting equal numbers of sorted CD4$^+$/CD45RB$^{hi}$ cells and purified, unstained CD4$^+$ cells. As can be seen in Fig. 6, SCID mice that were injected with CD4$^+$/CD45RB$^{hi}$ cells developed the typical wasting disease, however, the SCID mice that received the same number of CD4$^+$/CD45RB$^{hi}$ cells mixed with an equal number of purified, unstained CD4$^+$ cells did not develop wasting disease.

*Histopathology of Tissues from SCID Mice Injected with CD4$^+$/CD45RB$^{hi}$ T Cells.* To determine the cause of the wasting disease in SCID mice that received CD4$^+$/CD45RB$^{hi}$ cells, the mice were killed after statistically significant weight loss developed and histological evaluation of tissue sections from lung, liver, kidney, spleen, and large and small intestine was performed. Of these tissues only the intestines had demonstrable lesions (Fig. 7). At gross examination, both the small and large intestine were diffusely thickened and semirigid in the SCID mice that received the

**Table 1.** *Cellularity and Phenotype of C.B.-17 SCID Mice Injected with CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ Congenic T Cells*

| | Spleen | | | | Lymph node | | | |
|---|---|---|---|---|---|---|---|---|
| | Cellularity | CD4$^+$ | CD8$^+$ | sIgM$^+$ | Cellularity | CD4$^+$ | CD8$^+$ | sIgM$^+$ |
| | × 10$^{-6}$ | % | % | % | × 10$^{-6}$ | % | % | % |
| C.B-17 | 112.4 (5.3) | 14.3 (2.3) | 4.6 (1.8) | 65.3 (4.6) | 36.9 (3.7) | 48.2 (2.9) | 14.1 (2.1) | 29.8 (2.6) |
| C.B-17 SCID | 9.6 (1.8) | <0.1 | <0.1 | <0.1 | ND | ND | ND | ND |
| CD45RB$^{hi}$→SCID | 57.2 (8.7) | 8.6 (3.7) | <0.1 | <0.1 | 3.9 (1.2) | 69.3 (7.1) | <0.1 | <0.1 |
| CD45RB$^{lo}$→SCID | 18.3 (3.2) | 3.5 (0.8) | <0.1 | <0.1 | 1.8 (0.9) | 48.7 (3.1) | <0.1 | <0.1 |

The data represent the arithmetic average (SEM) of the indicated groups of mice. The data are pooled from two experiments with a total of six animals per group. Animals were assessed individually. Immunofluorescent staining and flow cytometry were done as described.
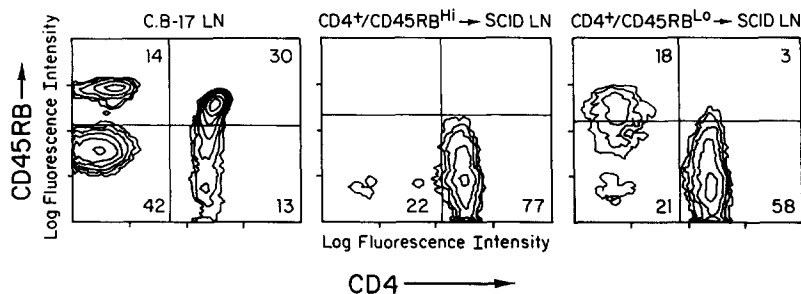
**Figure 5.** CD4 and CD45RB immunofluorescent profiles of lymph node cells from control C.B-17 or C.B-17 SCID mice that had been injected with congenic CD45RB$^{hi}$ or CD45RB$^{lo}$/CD4$^+$ T cells. The data are derived from the same experiment as shown in Fig. 4. The data are representative histograms.

CD4$^+$/CD45RB$^{hi}$ cells. Microscopic examination of the small intestine revealed normal villous structures, but multifocal accumulations of moderate numbers of lymphocytes and macrophages in the lamina propria and, rarely, in the submucosa. The large intestine was markedly hyperplastic due to a four to five times increase in mucosal thickness relative to the control. This was attributable to a combination of increased numbers of intestinal epithelial cells and supporting stromal elements as well as diffuse dense accumulations of lymphocytes and macrophages (along with clusters of neutrophils) within the lamina propria. Also, contributing to this change was mild hyperplasia of the muscularis mucosa and increased prominence of the submucosa due to accumulation of mixed inflammatory cells and dilated lymphatics. The overall increase in thickness and density of the intestinal wall resulted in a significant narrowing of the intestinal lumen. Sections from the intestines of the SCID mice that received either CD4$^+$/CD45RB$^{lo}$ cells or a mix of whole CD4$^+$ T cells and sorted CD4$^+$/CD45RB$^{hi}$ cells revealed generally similar but substantially less severe histological lesions.
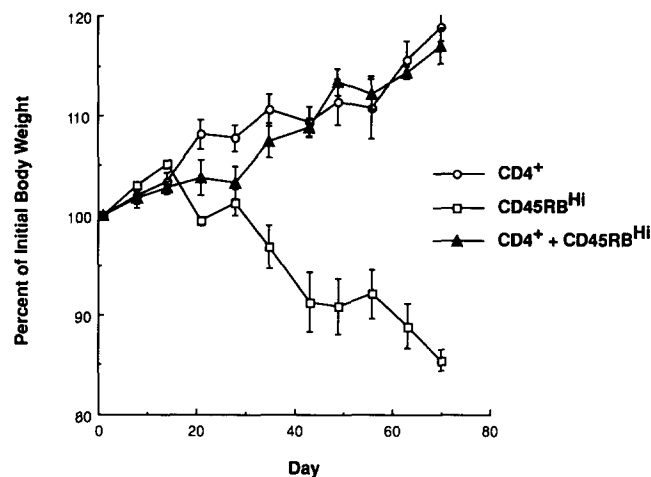
## Discussion

The data presented here described distinct functional capabilities between subpopulations of CD4$^+$ T cells separated on the basis of their expression of CD45RB. Thus, CD4$^+$/CD45RB$^{hi}$ T cells, when transferred into a congenic SCID

**Table 2.** *Total Number of CD4$^+$T Cells in SCID Mice That Were Injected with CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ T Cells*

| | CD4$^+$ cells | | |
|---|---|---|---|
| | Spleen | Lymph node | Total |
| | | $\times 10^{-6}$ | |
| CD45RB$^{hi}$→SCID | 4.92 | 2.71 | 7.63 |
| CD45RB$^{lo}$→SCID | 0.63 | 0.88 | 1.51 |

The values are the absolute numbers of CD4$^+$ T cells as calculated from the data in Table 1 by multiplying the average percent positive cells by the average cellularity and dividing by 100.

host, induce a wasting disease with a variable latency period that is dependent on the number of cells transferred. Neither identical numbers of whole lymph node cells nor purified CD4$^+$/CD45RB$^{lo}$ cells induced weight loss. The number of CD4$^+$ cells recovered from the SCID mice that had received either CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ cells was significantly greater than the number injected, suggesting that they had undergone significant expansion. This number does not include lymphocytes from the intestinal mucosa, where histologically significant numbers appear to be present. The cells recovered from SCID mice that had been injected with either CD4$^+$/CD45RB$^{hi}$ or CD45RB$^{lo}$ T cells were invariably CD45RB$^{lo}$. This indicates that the initial CD45RB$^{hi}$ cells decreased their expression of CD45RB. This is consistent with an existing state of activation and expansion in the mice since it has been shown that the levels of CD45RB decrease upon activation (11, 16). We have not definitively proven



**Figure 6.** Body weights of SCID mice that were injected with CD4$^+$/CD45RB$^{hi}$ cells, CD4$^+$ cells, or a mixture of the two populations. SCID mice were injected with 2 × 10$^5$ purified CD4$^+$/CD45RB$^{hi}$ cells, 2 × 10$^5$ purified, unstained CD4$^+$ cells, or a mixture of equal numbers of CD4$^+$/CD45RB$^{hi}$ cells and purified, unstained CD4$^+$ cells (2 × 10$^5$ each for a total of 4 × 10$^5$ cells per animal). There were four mice per group. The average body weight of the SCID mice on day 0 was 17.8 g. The differences in body weight between the SCID mice that received the CD45RB$^{hi}$ cells and the purified CD4$^+$ cells or the mixture were statistically significant ($p < 0.05$) on day 42 through the termination of the experiment.

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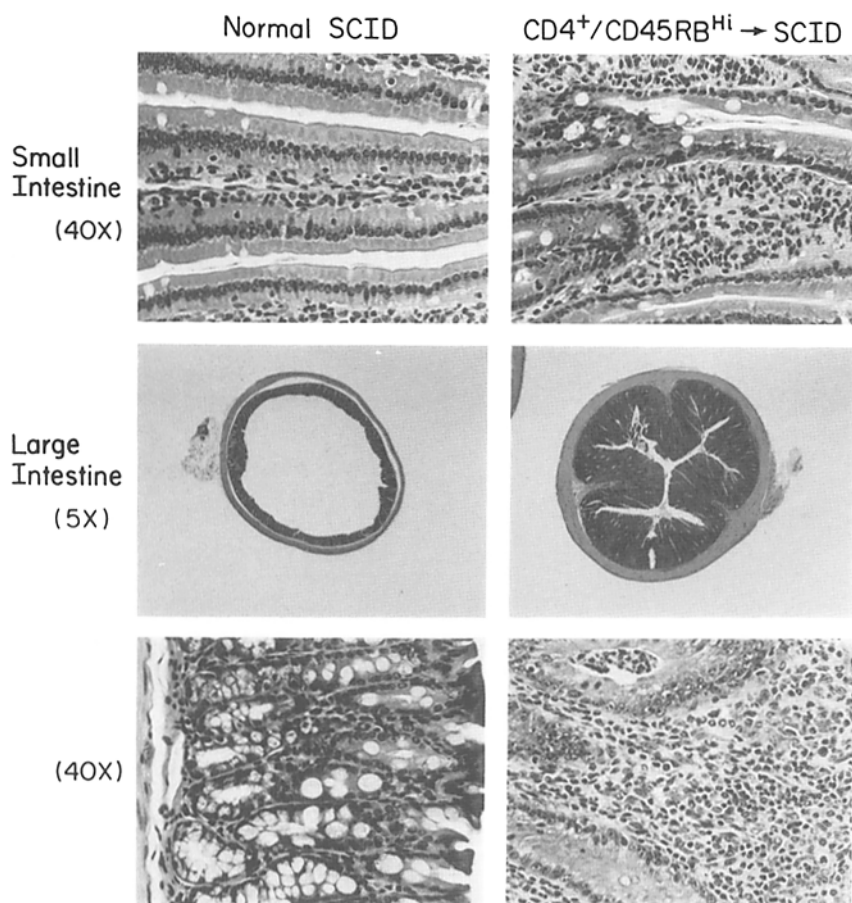
**Figure 7.** Intestinal tissue from normal SCID mice or SCID mice that were injected with CD4+/CD45RBhi cells. Photomicrographs are of representative sections from small and large intestines of normal SCID mice and SCID mice injected with CD4+/CD45RBhi cells 6 wk earlier. The magnifications are shown.

that the CD4+ cells harvested after transfer into the SCID mice are all of donor origin. However, we believe that a significant component of host origin is unlikely because: (a) the SCID mice in our colony have a very low incidence of leakiness (17); (b) expansion of host cells would have to be specifically in the CD4+ population since there were negligible numbers of CD8+ and sIgM+ cells in the mice; and (c) at no time point was there any evidence of thymic repopulation.

The transfer of CD4/CD45RBhi cells into congenic SCID mice resulted in a significant hyperplasia of the intestinal epithelium that was seen to a much lesser extent when either CD4+/CD45RBlo cells or normal lymph node cells were transferred into the SCID mice. The intestinal mucosa in the mice that received CD4+/CD45RBlo or CD4+ cells was slightly affected and this may explain the occasional, slight, but temporary decrease in the body weight of these mice (for instance, Fig. 2, day 24; Fig. 3, day 30). Thus, the mechanism that counteracts the stimulation of significant intestinal epithelial cell growth may allow or even require some level of autoreactivity.

The epithelial lining of the intestinal tract is normally in a continual state of renewal that is characterized by a high rate of cell turnover. However, the growth of intestinal epithelial cells can change in response to different stimuli such

as inflammation or food deprivation (reviewed in reference 18). The drastic hyperplasia seen in the SCID mice that received the CD45RBhi/CD4+ T cells would lead to the postulate that these cells are somehow driving the increased proliferation of the intestinal epithelial cells. It has been shown that T cell–derived products can stimulate intestinal epithelial cell proliferation (19) and, indeed, T cell activation in graft-vs.-host reaction in mice leads to significant changes in the intestinal epithelium that include an initial period of epithelial hyperplasia followed by a destructive enteropathy characterized by villous atrophy (20, 21). These results further demonstrate that activated T cells can induce pathological changes in the intestinal mucosa. It is also of interest to note that in the SCID mice that received the CD4+/CD45RBhi cells, the histological changes that we have observed are characterized by epithelial and crypt hyperplasia. We have not observed a destructive enteropathy that is characteristic of graft-vs.-host disease and inflammatory bowel disease.

It is not known if the lesions we have observed in these mice are an expression of an autoimmune disease, that is, an immune response to a self-antigen, or an immune reaction to environmental antigens. Since the pathology we observed is limited to an area continually in contact with antigen (e.g., intestinal bacteria), it is possible that the T cell reactivity is

driven by these antigens. The gut-associated lymphoid tissue is a complex mixture of different cells types and unique tissue architecture (22, 23). Normally, lymphoid cells contained within the intestinal epithelial layer, the lamina propria, and peyer's patches function to maintain the integrity of the gut epithelial barrier and to respond to pathogens and parasites that might be present within the intestinal lumen. In the SCID mouse, intestinal epithelial lymphocytes have been described, but these cells are negative for CD3 expression as well as V$\gamma$7 mRNA (24). Thus, in SCID mice the contingent of lymphoid elements present in the gut is not similar to that in normal mice and it is not known what effect this has on the integrity of the epithelial barrier. Evidence exists that in mice treated with immunosuppressive agents the rate of bacterial translocation is increased (25). Others have shown that suppressing immune function (by antithymocyte Ig treatment) in mice leads to increased survival of bacteria that had translocated to the mesenteric lymph node (26). This was decreased by treating the mice with IL-2. Also, the number of TCR $\alpha/\beta^+$ T cells present in the intestinal epithelial layer of germ-free animals was increased after bacterial colonization of the gut, suggesting a positive role of these cells in maintaining the epithelial barrier (27). Thus, the absence of the normal array of gut-associated lymphoid tissue in the SCID mouse may result in greater levels of bacterial translocation or survival. Consequently, the introduction of CD4$^+$/CD45RB$^{hi}$ cells may lead to an augmented, unregulated reaction toward higher levels of luminal-derived bacteria or bacterial products. Cytokines produced by these T cells might then stimulate intestinal epithelial hyperplasia.

Also, it is known that infection of mice with *Citrobacter freundi* induces colonic epithelial hyperplasia, indicating that an abnormal or altered intestinal bacterial flora can induce similar histological changes (28). Characterization of intestinal bacteria from these mice failed to reveal the presence of *C. freundi*. In addition, the colony in which these mice were housed has consistently tested negative for *C. freundi*. Thus, the exact stimulus for the changes seen in the SCID mice that received the CD4$^+$/CD45RB$^{hi}$ cells is unknown, however, it does not appear to be associated with a unique infectious agent.

These studies also revealed an immunoregulatory process in that whole CD4$^+$ cells when cotransferred in equal numbers with the CD45RB$^{hi}$ cells prevented the development of the wasting disease. It is possible that the immunoregulatory population is contained in the CD45RB$^{lo}$ population since the transfer of stained, but unsorted, CD4$^+$ T cells, which can be considered a mixture of CD45RB$^{hi}$ and CD45RB$^{lo}$ cells, did not induce wasting disease. This would be consistent with the findings of Powrie and Mason (13) who found that the transfer of unseparated CD4$^+$ T cells did not induce autoimmune disease.

The ability of one T cell population to modulate the autoreactivity of a second population has been observed in other systems as well. For instance, the development of multiorgan autoimmunity in adult mice that were neonatally thymectomized can be prevented by the transfer of normal adult T cells (29). The transfer of normal CD4$^+$ cells has been shown to inhibit the development of diabetes in NOD mice and BB rats (30–32). PVG.RT1$^u$ rats developed diabetes after thymectomy and repeated low dose $\gamma$ irradiation, and disease development could be inhibited by injecting the rats with congenic CD4$^+$/CD45RC$^{lo}$ T cells (33). Also, the development of fatal autoimmune disease in irradiated, bone marrow–reconstituted rats given cyclosporine could be prevented by the cotransfer of lymphoid cells from normal donors (34). To date, the mechanism of protection conferred by the administration of normal cells in these different systems is unknown, and though it is not clear if the pathology described here in the SCID mice receiving CD4$^+$/CD45RB$^{hi}$ cells represents reactivity to an autoantigen, it is clear that whole CD4$^+$ cells can counteract this reactivity.

Since it is known that CD4$^+$/CD45RB$^{hi}$ and CD45RB$^{lo}$ T cells produce different cytokines after stimulation, it is possible that pathology and the immunoregulation seen in the experiments shown here reflect a difference in the types or amounts of cytokines produced by the different T cell subpopulations (11, 12). For instance, upon activation, the CD45RB$^{hi}$ cells could produce cytokines that stimulate epithelial cell hyperplasia and, in the absence of counterregulation, results in compromised intestinal function with consequent diarrhea and weight loss. In contrast, the immunoregulatory population might produce a distinct profile of cytokines that either negates the stimulatory effect of those produced by the CD45RB$^{hi}$ cells on the intestinal epithelial cells or prevents the CD45RB$^{hi}$ cells from producing those cytokines. Studies to determine the types of cytokines produced in the lymph node, lamina propria, and intestinal epithelial lymphocytes from SCID mice that received the various T cell subpopulations are in progress.

In summary, the results presented here, in which the transfer of congenic CD4$^+$/CD45RB$^{hi}$ into SCID mice produced a wasting syndrome characterized by severe hyperplasia of the intestinal epithelial cells, and the prevention of the syndrome by cotransferring whole CD4$^+$ cells, describe a unique system for the investigation of intestinal immunity and immune regulation.

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