An Essential Role for Interleukin 10 in the Function of Regulatory T Cells That Inhibit Intestinal Inflammation

By Chrystelle Assman, Smita Mauze, Michael W. Leach, Robert L. Coffman, and Fiona Powrie

From the Nuffield Department of Surgery, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom; the DNAX Research Institute of Molecular and Cellular Biology, Inc., Palo Alto, California 94304; and the Schering-Plough Research Institute, Lafayette, New Jersey 07848

Summary

A T helper cell type 1–mediated colitis develops in severe combined immunodeficient mice after transfer of CD45RB(high) CD4(+) T cells and can be prevented by cotransfer of the CD45RB(low) T cell population. The immune-suppressive activities of the CD45RB(low) T cell population can be reversed in vivo by administration of an anti-transforming growth factor β antibody. Here we show that interleukin (IL)-10 is an essential mediator of the regulatory functions of the CD45RB(low) population. This population isolated from IL-10-deficient (IL-10(-/-)) mice was unable to protect from colitis and when transferred alone to immune-deficient recipients induced colitis. Treatment with an anti–murine IL-10 receptor monoclonal antibody abrogated inhibition of colitis mediated by wild-type (WT) CD45RB(low) CD4(+) cells, suggesting that IL-10 was necessary for the effector function of the regulatory T cell population. Inhibition of colitis by WT regulatory T cells was not dependent on IL-10 production by progeny of the CD45RB(high) CD4(+) cells, as CD45RB(low) CD4(+) cells from WT mice were able to inhibit colitis induced by IL-10(-/-) CD45RB(high) CD4(+) cells. These findings provide the first clear evidence that IL-10 plays a nonredundant role in the functioning of regulatory T cells that control inflammatory responses towards intestinal antigens.

Key words: interleukin 10 • inflammatory bowel disease • CD4(+) T lymphocyte • regulatory T lymphocyte

The inflammatory bowel diseases encompassing Crohn's disease and ulcerative colitis are complex chronic diseases whose etiology and pathogenesis are poorly understood. Recently, several murine models of colitis have been developed which have highlighted the important role that abnormalities of the immune system, particularly those affecting T cells, may play in disease pathogenesis (for reviews, see references 1 and 2). Studies using T cell–restored immunodeficient mice have provided evidence that CD4(+) T cells play a key role in the induction and regulation of intestinal inflammation, as transfer of CD45R B(high) CD4(+) T cells from normal donors into C.B-17 severe combined immunodeficient (SCID)(1) mice led to the development of a severe inflammatory response in the colon (3, 4). Colitis was the result of the development of a Th1 response, as polarized Th1 cells were present in intestinal lesions and disease could be prevented by treatment with an anti-IFN-γ or anti-TNF mAb (5). More recently, CD45R B(high) CD4(+) T cells isolated from signal transducer and activator of transcription (Stat)-4-deficient mice, which are unresponsive to IL-12, were shown to be impaired in their ability to transfer colitis to immune-deficient recipients (6, 7). Cotransfer of the reciprocal CD45R B(low) CD4(+) T cell subset together with normally pathogenic CD45R B(high) cells prevented the development of colitis, indicating that the CD45R B(low) CD4(+) subset from normal mice contains a population of regulatory T cells capable of controlling inflammatory responses in the intestine (4). Analysis of the mechanism of immune suppression revealed a role for TGF-β but not IL-4 or IL-10, as anti-TGF-β mAb but not anti-IL-4 mAb or anti-IL-10 mAb was able to abrogate protection from colitis transferred by CD45R B(low) CD4(+) cells. Indeed, IL-4 appeared to play no demonstrable role in either the development or effector function of the regulatory T cell population, as CD45R B(low) CD4(+) cells from IL-4-deficient mice were equally as potent as wild-type.
type (WT) cells in inhibiting colitis (8). Despite the finding that administration of neutralizing anti-IL-10 mAb failed to inhibit the regulatory activity of CD45R B^low CD4^+ cells, there is evidence that IL-10 plays an important role in mucosal immune regulation, as mice with a targeted disruption of the IL-10 gene developed enterocolitis (9, 10). In addition, administration of murine rIL-10 prevented colitis in SCID mice restored with CD45R B^high CD4^+ T cells (5). Furthermore, CD45R B^high CD4^+ cells isolated from transgenic mice that expressed IL-10 under the IL-2 promoter failed to transfer colitis but rather, were able to inhibit colitis induced by WT CD45R B^high CD4^+ T cells (11). Taken together, these studies provide evidence that IL-10 is able to regulate pathogenic immune responses in the intestine; however, whether this suppression involves the development of regulatory T cells is not known.

In this study, we have analyzed the role of IL-10 in the development and effector function of regulatory T cells that control the immune response to intestinal antigens, and present evidence that IL-10 is essential for the normal functioning of these cells.

**Materials and Methods**

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Materials and Methods**

Mice. Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.

**Mice.** Specific pathogen-free BALB/c and C.B-17 SCID mice were obtained from Simonsen Laboratories or Harlan Olac and maintained in the Animal Care Facility of the DNAX Research Institute or in the Biomedical Services Unit (BMSU) at the John R Ardillie Hospital. 129/SvEv WT, 129/SvEv recombinase-activating gene (RAG)-2–deficient (RAG-2^−/−) mice were obtained from Simonsen Laboratories or Harlan Olac and presented evidence that IL-10 is essential for the normal functioning of these cells.
The findings that CD45RBlow CD4+ cells from IL-10−/− mice lacked T cells capable of regulating inflammatory responses in the intestine appears at odds with previous studies from this laboratory which showed that treatment with a neutralizing anti–IL-10 mAb failed to abrogate protection from colitis transferred by CD45RBlow CD4+ cells (8). The simplest explanation for these data is that the anti–IL-10 mAb used (JES5-2A5) in these studies failed to sufficiently neutralize IL-10. Recently, an mAb reactive with the murine IL-10R has been generated (13) and shown to efficiently neutralize the effects of IL-10. To test whether treatment with anti–IL-10R mAb was able to affect the function of the CD45RBlow population, mice restored with a mixture of CD45RBlow and CD45RBlow CD4+ cells were treated weekly with anti–IL-10, anti–IL-10R, or isotype control mAb. As can be seen in Table I (bottom), treatment with anti–IL-10R abrogated protection from colitis induced by the CD45RBlow CD4+ population, as all of the mice in these groups, treated with anti–IL-10R alone or in combination with anti–IL-10, developed colitis. Antibody treatment alone did not induce immune pathology in the absence of T cells, as unreconstituted recipients treated with anti–IL-10R did not develop colitis (data not shown). As reported previously, anti–IL-10 treatment had no effect on the immune-suppressive activities of the CD45RBlow population, as mice in this group, like mice treated with isotype control mAb, failed to develop colitis.

Colitis induced by the CD45RBlow CD4+ population was characterized by the accumulation of IFN-γ- and TNF-α-secreting Th1 CD4+ T cells in the lesions (Fig. 2 A). A similar expansion of Th1 cells was also present in the colon of mice that developed colitis after transfer of WT CD45RBlow CD4+ cells in the presence of WT CD45RBlow CD4+ cells plus anti–IL-10R or after transfer of IL-10−/− CD45RBlow cells alone. Development of disease correlated with a significant increase in the total num-

---

**Figure 1.** Representative photomicrographs of the descending colon of RAG-2−/− mice after transfer of subpopulations of CD4+ T cells from WT or IL-10−/− mice. (A) Severe colitis in a mouse injected with CD45RBlow CD4+ T cells from WT mice. (B) Normal appearance of the colon in a mouse restored with WT CD45RBlow and WT CD45RBlow CD4+ T cells, indicating that the WT CD45RBlow population is able to inhibit disease induced by WT CD45RBlow CD4+ T cells. (C) Severe colitis in a mouse cotransferred with WT CD45RBlow CD4+ T cells and IL-10−/− CD45RBlow CD4+ cells, indicating that the IL-10−/− CD45RBlow subpopulation is unable to protect from the disease. (D) Severe colitis in a mouse receiving only IL-10−/− CD45RBlow cells, indicating that this population is able to induce disease. Hematoxylin and eosin; original magnifications ×50.
Table I.  The Function of Regulatory T Cells That Control Inflammatory Responses in the Colon Is Dependent on IL-10

| Phenotype of CD4+ T cells injected | mAb treatment | No or minimal colitis (0–1) | Mild colitis (2) | Severe colitis (3–5) |
|-----------------------------------|---------------|----------------------------|-----------------|---------------------|
| RAG-2−/− recipients*              |               | 8/41 (19.5%)              | 7/41            | 26/41 (63.4%)       |
| 4 × 10^5 CD45R B<sup>high</sup>   | -             | 26/31 (83.8%)            | 3/31            | 2/31 (6.4%)         |
| + 2 × 10^3 WT CD45R B<sup>low</sup> | -             | 6/28 (21.4%)            | 4/28            | 18/28 (64.2%)       |
| 4 × 10^5 CD45R B<sup>high</sup>   | -             | 5/22 (22.7%)            | 4/22            | 13/22 (59.0%)       |
| 2 × 10^3 IL-10−/− CD45R B<sup>low</sup> | -             | 68.0%                   |                 |                     |
| C.B-17 SCID recipients†           | Isotype control | 6/7 (17.6%)            | 1/7             | 0/7                 |
| 4 × 10^5 CD45R B<sup>high</sup>   | Anti-IL-10    | 5/5 (12.5%)             | 0/5             | 0/5                 |
| 4 × 10^5 CD45R B<sup>low</sup>    | Anti-IL-10R   | 0/8 (0%)                | 0/8             | 8/8                 |
| 4 × 10^5 CD45R B<sup>high</sup>   | Anti-IL-10    | 0/5 (0%)                | 2/5             | 3/5                 |
| 2 × 10^5 CD45R B<sup>low</sup>    | Anti-IL-10R   | 0/5 (0%)                | 2/5             | 3/5                 |
| RAG-2−/− or C.B-17 SCID mice were reconstituted with sorted CD4+ T cell subsets and treated for 8 wk with antibodies as indicated (2 mg the day after T cell reconstitution and 1 mg/wk thereafter for anti-IL-10 mAb and isotype control, 1 mg the day after T cell reconstitution and 0.5 mg/wk thereafter for anti-IL-10R mAb). 8–12 wk after reconstitution, mice were killed and colonic pathology was graded on a scale of 0–5 as described in Materials and Methods.* Data from four to six independent experiments.† Statistically different (P < 0.005, Mann-Whitney test) compared with mice restored with 4 × 10^5 CD45R B<sup>high</sup>.§ Data from two experiments.
Discussion

Data presented herein provide direct evidence that IL-10 plays an obligate role in the function of regulatory T cells that control inflammatory responses in the intestine. In contrast to CD45RBlow CD4+ cells from WT mice, which inhibit colitis induced in immune-deficient mice after transfer with CD45RBhigh CD4+ T cells, the CD45RBlow CD4+ population from IL-10−/− mice failed to mediate this function. In addition, they induced severe colitis when transferred alone to immune-deficient recipients. Previous studies from this laboratory showed that TGF-β was essential for inhibition of colitis by CD45RBlow cells (8). These results, together with the find-
ings reported here, provide the first clear evidence that IL-10 and TGF-β play nonredundant roles in the functioning of regulatory T cells which control inflammatory responses towards intestinal antigens, as the neutralization or absence of one of these cytokines is sufficient to abrogate protection. Furthermore, IL-10 produced by regulatory T cells themselves is crucial for the normal functioning of these cells.

Colitis in the SCID model involves the development of Th1 cells responding primarily to intestinal flora, as transfer of CD45R B<sup>high</sup> CD4<sup>+</sup> T cells to germ-free SCID mice failed to induce disease (20). The fact that the regulatory T cells express the phenotype of antigen-experienced cells (CD45R B<sup>low</sup>) would suggest that their generation in normal mice is antigen driven; however, whether these antigens are of bacterial or self origin is not known. Recent studies of the immune response elicited by Helicobacter hepaticus infection, a bacterium that colonizes the cecum, showed that normal mice mounted an IL-10-dependent response, whereas IL-10<sup>-/-</sup> mice developed a pathogenic Th1 response towards the bacterium (21). These studies support the hypothesis that in immunocompetent hosts, enteric antigens induce IL-10-secreting T cells that are immune suppressive and prevent inflammatory responses towards intestinal antigens. Mucosal T cell unresponsiveness to enteric antigens has similarly been shown in humans to be mediated by antigen-specific CD4<sup>+</sup> T cells and production of IL-10 and TGF-β (22).

Injection of rIL-10 inhibited the development of colitis in CD45R B<sup>high</sup> CD4<sup>+</sup> T cell-restored SCID mice (5) and in IL-10<sup>-/-</sup> mice treated from weaning (10). However, the inhibitory effects of IL-10 treatment were only transitory, as colitis developed after the treatment was stopped, whereas the transfer of CD45R B<sup>low</sup> CD4<sup>+</sup> T cells provided long-lasting protection. This may reflect the capacity of regulatory T cells to provide a constant source of IL-10 upon stimulation by endogenous antigen (bacteria or self). Alternatively, they may produce or induce other regulatory molecules, such as TGF-β, which are not induced by exogenous administration of IL-10.

The finding that CD45R B<sup>low</sup> CD4<sup>+</sup> cells incapable of synthesizing IL-10 failed to inhibit colitis induced by transfer of CD45R B<sup>high</sup> cells to SCID mice illustrates that the regulatory T cell population itself is the critical source of IL-10 in this model, despite the fact that both the CD45R B<sup>high</sup> CD4<sup>+</sup> T cells and host cells in the SCID mice were capable of making IL-10. It is not clear whether the lack of regulatory T cells in IL-10<sup>-/-</sup> mice is a result of ab-

### Table II. Development of Disease Correlates with a Significant Increase in the Total Number of Leukocytes and CD4<sup>+</sup> Cells Recovered from the LP

| Phenotype of CD4<sup>+</sup> T cells used for reconstitution | mAb treatment | Total no. of LP leukocytes | Total no. of CD4<sup>+</sup> cells<sup>*</sup> |
|-------------------------------------------------------------|--------------|---------------------------|-----------------------------------------------|
| 4 × 10<sup>5</sup> WT CD45R B<sup>high</sup>                 | -            | ×10<sup>3</sup> ± SEM     | ×10<sup>3</sup> ± SEM                         |
| 2 × 10<sup>5</sup> IL-10<sup>-/-</sup> CD45R B<sup>low</sup>  | -            | 136.0 ± 34.9              | 92.7 ± 10.2                                  |
| 4 × 10<sup>5</sup> WT CD45R B<sup>high</sup> + 2 × 10<sup>5</sup> WT CD45R B<sup>low</sup> | anti-IL-10R  | 176.3 ± 104.5             | 133.3 ± 88.6                                 |
| 4 × 10<sup>5</sup> WT CD45R B<sup>high</sup> + 2 × 10<sup>5</sup> CD45R B<sup>low</sup> | Isotype      | 7.9 ± 0.9                 | 5.4 ± 0.5                                    |

RAG-2<sup>-/-</sup> or C.B-17 SCID recipients were reconstituted with cell subsets as indicated. 8–12 wk after cell reconstitution, cells from LP were isolated. Data represent the number ± SEM of two to five animals per group from two independent experiments.

*Total number of CD4<sup>+</sup> cells were determined by multiplying the number of LP leukocytes by the frequency of CD4<sup>+</sup> T cells. CD4<sup>+</sup> T cell frequencies were similar between groups ranging between 60.2 and 81.6%.

### Table III. Prevention of Colitis Is Independent of IL-10 Production by CD45R<sup>high</sup> CD4<sup>+</sup> T Cells

| Phenotype of CD4<sup>+</sup> T cells injected | N or minimal colitis (0–1) | Mild colitis (2) | Severe colitis (3–5) |
|---------------------------------------------|--------------------------|-----------------|----------------------|
| 4 × 10<sup>5</sup> IL-10<sup>-/-</sup> CD45R B<sup>high</sup> | 3/25 (12.0%)            | 5/25            | 17/25 (68.0%)        |
| 4 × 10<sup>5</sup> IL-10<sup>-/-</sup> CD45R B<sup>high</sup> + 2 × 10<sup>5</sup> CD45R B<sup>low</sup> | 26/32 (81.2%)           | 2/32            | 4/32 (12.5%)*        |

RAG-2<sup>-/-</sup> mice were reconstituted with sorted CD4<sup>+</sup> T cell subsets. 10–12 wk after reconstitution, mice were killed and colonic pathology was graded. Data are pooled from four to six independent experiments.

*Statistically different (P < 0.05, Mann-Whitney test).
normalities in the development or effector function of this population. However, the finding that treatment with anti-IL-10R was able to neutralize the immune-suppressive function of differentiated regulatory cells in cotransfers of CD45R B<sup>high</sup> and CD45R B<sup>low</sup> cells from normal mice supports the idea that IL-10 is required for the effector function. The reasons for the striking difference in effectiveness between the anti-IL-10 mAb JESS-2A5 and the anti-IL-10R antibody 1B1.2 are not clear, as both are effective neutralizing antibodies in vitro. However, the relative ineffectiveness of JESS-2A5 has been observed in other experimental situations, such as Leshmania-infected mice (M. auze, S., and R.L. Coffman, unpublished observations).

Precisely how IL-10 mediates its immune-regulatory function is not known. Inhibition of colitis mediated by IL-10–secreting CD45R B<sup>low</sup> CD4<sup>+</sup> cells was characterized by substantial reductions in total number of Th1 cells recovered from the intestine. This difference was attributable to the reduced number of CD4<sup>+</sup> cells present in the intestine (8–24-fold; Table II), as analysis of cytokine production at the single cell level revealed a similar percentage of CD4<sup>+</sup> cells capable of producing IFN-γ and TNF-α in diseased and nondiseased colons. This suggests that the major activity of IL-10–secreting regulatory T cells is to inhibit the accumulation of pathogenic Th1 cells in the intestine. Whether this is due to reduced expansion, or migration, of these cells is not known. IL-10 has been shown to mediate a range of antiinflammatory activities, including the inhibition of antigen-induced proliferation and cytokine secretion by T cells. Inhibition is thought to be mediated mainly by effects on APCs, particularly downregulation of molecules involved in T cell costimulation (23). It seems likely that IL-10–secreting regulatory T cells act to inhibit Th1 cell activation, and that IL-10 produced locally in the intestine acts on macrophages to prevent their activation and elaboration of proinflammatory molecules and chemokines, thus inhibiting T cell recruitment into the intestine. Consistent with this, mice in which macrophages and neutrophils are unable to respond to IL-10 as a result of a cell type–specific deletion of Stat-3 developed enterocolitis (24), suggesting that IL-10–mediated macrophage and neutrophil deactivation contributes to the immune-suppressive properties of IL-10 in the intestine.

Recently, regulatory cells with activities similar to those within the CD45R B<sup>low</sup> CD4<sup>+</sup> population were cloned in vitro by culturing with IL-10 (18). These cells termed T regulatory 1 (Tr-1) cells, are characterized by their ability to produce IL-10 but not IL-4 and to inhibit T cell activation in vitro and in vivo. Although immune suppression in vitro was shown to be dependent on IL-10 and TGF-β, the mechanism of action of Tr-1 cells in vivo has not been established. It is likely that regulatory T cells contained within the CD45R B<sup>low</sup> CD4<sup>+</sup> subset represent the in vivo counterpart of in vitro–derived Tr-1 cells. This is based on the fact that the function of the former population is dependent on IL-10 but not IL-4 synthesis, a defining feature of Tr-1 cells in vitro. In addition, a subset of CD45R B<sup>low</sup> CD4<sup>+</sup> T cells, identified by expression of CD38, was shown to be immune suppressive in vitro and to produce IL-10 but not IL-4 after stimulation with anti-CD3 and IL-2 (25). However, further comparison between the in vivo–derived regulatory cells described here and in vitro–derived Tr-1 cells awaits identification of cell surface markers specific for Tr-1 cells and elucidation of their mechanism of action in vivo. The finding that IL-10 leads to the differentiation of Tr-1 cells in vitro raised the possibility that part of the mechanism by which IL-10–secreting regulatory T cells contained within the CD45R B<sup>low</sup> CD4<sup>+</sup> population inhibit colitis is to induce the differentiation of the progeny of CD45R B<sup>high</sup> CD4<sup>+</sup> cells into IL-10–secreting cells. In some systems, regulatory T cells have been shown to induce the differentiation of naïve cells into cells with similar regulatory function, a phenomenon termed infectious tolerance (16, 17). However, differentiation of Tr-1 cells among the progeny of the CD45R B<sup>high</sup> population did not appear to be a crucial part of the immune-suppressive activities of the CD45R B<sup>low</sup> population, as these cells were able to inhibit colitis induced by IL-10<sup>−/−</sup> CD45R B<sup>high</sup> CD4<sup>+</sup> cells which could not differentiate into IL-10–secreting cells. However, this result does not rule out the possibility that IL-10 secretion by the CD45R B<sup>low</sup> population leads to the differentiation of the progeny of CD45R B<sup>high</sup> cells into regulatory T cells secreting other cytokines, for example TGF-β, and experiments are currently underway to test this hypothesis.

In summary, these studies identify IL-10 as an essential mediator of the function of regulatory T cells contained within the CD45R B<sup>low</sup> CD4<sup>+</sup> T cell population. Previous studies identified TGF-β as a critical component of the immune-regulatory function of these cells, and the finding that IL-10 has an identical effect is the first demonstration that both of these immune-suppressive cytokines play mandatory roles. How the functions of these two cytokines are linked is not known. The finding that IL-10<sup>−/−</sup> mice have immune pathology restricted to the intestine (9) whereas TGF-β<sup>−/−</sup> develop multiple organ disease (26) suggests that IL-10 is not required for the production of TGF-β1. However, TGF-β has been shown to induce IL-10 secretion by APCs (27), making it a possibility that TGF-β alters antigen presentation in favor of the generation of IL-10–secreting regulatory T cells. Alternatively, IL-10 and TGF-β may act entirely separately, and further experiments are required to distinguish between these possibilities.

There is now good evidence that regulatory T cells can be induced after oral exposure to antigen and that their function is dependent on TGF-β (28, 29). In addition, regulatory T cells that inhibit the development of autoimmune disease have been shown to exist naturally in mice and rats (for a review, see reference 30). However, information regarding the role of IL-10 and TGF-β in the function of these cells is incomplete, as in most cases only one or the other has been examined. Thus, TGF-β–dependent mechanisms were shown to be involved in the regulation of autoimmune nephritis (31) and thyroiditis (32), whereas inhibition of diabetes by N K1.1 T cells was dependent on
IL-4 and IL-10 (33). It remains to be established whether regulatory T cells that control inflammatory responses in the gut are the same as those shown to regulate organ-specific autoimmune disease. However, more information on the relative roles of IL-10 and TGF-β in the function of these different populations of cells will facilitate their comparison. Differentiation of IL-10–secreting TGF-β–dependent regulatory T cells is one of the host’s natural mechanisms for avoiding immune pathology. Further understanding of the antigen specificity, development, and mechanism of action of these cells is crucial for the design of effective immune interventions that seek to capitalize on this potent immune-regulatory mechanism.

We are very grateful to Dr. Don Mason for critical review of the manuscript. We are indebted to N. Ruster for excellent technical assistance with cell sorting, P. McShane for help with statistics, and C. Hetherington and staff for care of experimental animals.

F. Powrie and C. Asseman are funded by the Wellcome Trust. DNAX Research Institute is funded by the Schering-Plough Corporation.

Address correspondence to Fiona Powrie, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK. Phone: 44-1865-221482; Fax: 44-1865-768876; E-mail: fiona.powrie@nds.ox.ac.uk

Submitted: 20 April 1999 Revised: 9 July 1999 Accepted: 17 July 1999

References

1. Elson, C.O., R.B. Sartor, G.S. Tennyson, and R.H. Riddell. 1995. Experimental models of inflammatory bowel disease. Gastroenterology. 109:1344–1367.
2. Powrie, F., and M.W. Leach. 1995. Genetic and spontaneous models of inflammatory bowel disease in rodents evidence for abnormalities in mucosal immune regulation. Thet Immunol. 2:115–123.
3. Morrissey, P.J., K. Charrier, S. Braddy, D. Liggitt, and J.D. Watson. 1993. 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. J. Exp. Med. 178:237–244.
4. Powrie, F., M.W. Leach, S. Mauze, L.B. Caddle, and R.L. Coffman. 1993. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C.B-17 scid mice. Int. Immunol. 5:1461–1471.
5. Powrie, F., M.W. Leach, S. Mauze, S. M enon, L.B. Caddle, and R.L. Coffman. 1994. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RB+CD4+ T cells. Immunity. 1:553–562.
6. Simpson, S.J., S. Shah, M. Comiskey, Y.P. de Jong, B. Wang, E. Mizoguchi, A.K. Bhan, and C. Terhorst. 1998. T cell–mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (Stat)-4 pathway, but is not conditional on interferon γ expression by T cells. J. Exp. Med. 187:1225–1234.
7. Claesson, M.H., S. Bregenholt, K. Bonhagen, S. Thoma, P. Möller, M.J. Grusby, F. Leithäuser, M.H. Nissen, and J. Riemann. 1999. Colitis-inducing potency of CD4+ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB phenotype. J. Immunol. 162:3702–3710.
8. Powrie, F., J. Carlino, M.W. Leach, S. Mauze, and R.L. Coffman. 1996. A critical role for transforming growth factor β but not interleukin 4 in the suppression of T helper type 1–mediated colitis by CD45RB+CD4+ T cells. J. Exp. Med. 183:2669–2674.
9. Kühn, R., J. Löhler, D. Rennick, K. Rajewsky, and W. Müller. 1993. Interleukin-10–deficient mice develop chronic enterocolitis. Cell. 75:263–274.
10. Berg, D.J., N.J. Davidson, R. Kühn, W. Müller, S. Menon, G. Holland, L. Thompson-Snipes, M.W. Leach, and D. Rennick. 1996. Enterocolitis and colon cancer in interleukin-10–deficient mice are associated with aberrant cytokine production and CD4+ TH1-like responses. J. Clin. Invest. 98:1010–1020.
11. Hagenbaugh, A., S. Sharma, S.M. Dubinett, S.H. Wei, R. Aranda, H. Cheroute, D.J. Fowell, S. Binder, B. Tsao, R.M. Locksley, et al. 1997. Altered immune response in interleukin 10 transgenic mice. J. Exp. Med. 185:2101–2110.
12. Coffman, R.L. 1982. Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. Immunol. Rev. 69:5–23.
13. O’Farrel, A.-M., Y. Liu, K.W. Moore, and A.L.-F. Mui. 1998. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. EMBO (Eur. Mol. Biol. Organ.) J. 17:1006–1018.
14. Abrams, J.S., M.G. Roncarolo, H. Yssel, A. O’Garra, and J.E. Silver. 1992. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. Immunol. Rev. 127:5–24.
15. Openshaw, P., W.E. Murphy, N.A. Hosken, V. Maino, K. Davis, K. Murphy, and A. O’Garra. 1995. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. J. Exp. Med. 182:1357–1367.
16. Gershon, R.K., and K. Kondo. 1971. Infectious immunological tolerance. Immunology. 21:903–914.
17. Qin, S., S.P. Cobbold, H. Pope, J. Elliott, D. Kioussis, J.

1002 IL-10 and Regulatory T Cells
Davies, and H. Waldmann. 1993. “Infectious” transplantation tolerance. Science 259:974–977.
18. Groux, H., A. O’Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. de Vries, and M.-G. Roncarolo. 1993. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 389:737–742.
19. Davidson, N.J., S.A. Hudak, R.E. Lesley, S. Menon, M.W. Leach, and D.M. Rennick. 1998. IL-12, but not IFN-γ, plays a major role in sustaining the chronic phase of colitis in IL-10-deficient mice. J. Exp. Med. 181:3143–3149.
20. Aranda, R., B.C. Sydora, P.L. McAllister, S.W. Binder, H.Y. Yang, S.R. Targan, and M. Kronenberg. 1997. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4⁺, CD45RB⁺ T cells to SCID recipients. J. Immunol. 158:3464–3473.
21. Kullberg, M.C., J.M. Ward, P.L. Gorelick, P. Caspar, S. Hieny, A. Cheever, D. Jankovic, and A. Sher. 1998. Helicobacter hepaticus triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. Infect. Immun. 66:5157–5166.
22. Khoo, U.Y., I.E. Proctor, and A.J. Macpherson. 1997. CD4⁺ T cell down-regulation in human intestinal mucosa: evidence for intestinal tolerance to luminal bacterial antigens. J. Immunol. 158:3626–3634.
23. Moore, K.W., A. O’Garra, R. de Waal Malefyt, P. Vieira, and T.R. Mosmann. 1993. Interleukin-10. Annu. Rev. Immunol. 11:165–190.
24. Takeda, K., B.E. Clausen, T. Kaisho, T. Tsujimura, N. Terada, I. Förster, and S. Akira. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity 10:39–49.
25. Read, S., S. Mauze, C. Asseman, A. Bean, R. Coffman, and F. Powrie. 1998. CD38⁺ CD45R B²⁺ CD4⁺ T cells: a population of T cells with immune regulatory activities in vivo. Eur. J. Immunol. 28:3435–3447.
26. Shull, M.M., I. Ormsby, A.B. Kier, S. Pawlowski, R.J. Diebold, M. Yin, R. Allen, C. Sidman, G. Proetzel, D. Calvin, et al. 1992. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammation. Nature 359:693–699.
27. Maeda, H., H. Kuwahara, Y. Ichimura, M. Ohtsuki, S. Kurakata, and A. Shiraishi. 1995. TGF-β enhances macrophage ability to produce IL-10 in normal and tumor-bearing mice. J. Immunol. 155:4926–4932.
28. Chen, Y., V.K. Kuchroo, J.-I. Inobe, D.A. Hafler, and H.L. Weiner. 1994. Regulatory T cell clones induced by oral toleration: suppression of autoimmune encephalomyelitis. Science 265:1237–1240.
29. Neurath, M.F., I. Fuss, B.L. Kelsall, D.H. Presky, W. Waege, and W. Strober. 1996. Experimental granulomatous colitis in mice is abrogated by induction of TGF-β-mediated oral tolerance. J. Exp. Med. 183:2605–2616.
30. Mason, D., and F. Powrie. 1998. Control of immune pathology by regulatory T cells. Curr. Opin. Immunol. 10:649–655.
31. Bridoux, F., A. Badou, A. Saoudi, I. Bernard, E. Druet, R. Pasquier, P. Druet, and L. Pelletier. 1997. Transforming growth factor β (TGF-β)-dependent inhibition of T helper cell 2 (Th2)-induced autoimmunity by self-major histocompatibility complex (MHC) class II-specific, regulatory CD4⁺ T cell lines. J. Exp. Med. 185:1769–1775.
32. Read, S., S. Mauze, C. Asseman, A. Bean, R. Coffman, and F. Powrie. 1998. CD38⁺ CD45R B²⁺ CD4⁺ T cells: a population of T cells with immune regulatory activities in vitro. Eur. J. Immunol. 28:3435–3447.