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Regulatory T Cells in Experimental Colitis

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Abstract Induction and maintenance of peripheral tolerance are important mechanisms to maintain the balance of the immune system. Growing evidence indicates that dysregulation of mucosal T cell responses may lead to loss of tolerance to commensal flora and to the development of inflammatory bowel diseases (IBD). Many studies suggest that active suppression of enteroantigen reactive cells mediated by regulatory

T cells contributes to the maintenance of natural intestinal immune homeostasis. The use of the multiple animal models has not only improved our understanding of IBD, but also contributed to new suggestions of treatment strategies involving the use of regulatory T cells. The present review summarizes our current knowledge of regulatory T cells and their involvement in experimental IBD. The well-characterized SCID T cell transfer model and the naturally occurring regulatory $CD4^+CD25^+$ T cells are highlighted.

1 Introduction

The intestinal immune system is a very large and complex part of the immune system, which interfaces with a variety of endogenous and exogenous stimuli. The gut mucosal immune system encounters more antigens than any other part of the body and must discriminate clearly between invasive organisms and harmless antigens, such as food antigens and commensal bacteria. The mechanisms controlling the balance between tolerance and active immunity are therefore very critical, although not well understood. If the immune control mechanisms break down the consequences can be very devastating. Loss of tolerance to food antigens or commensal flora may lead to inflammatory disorders such as food allergies and inflammatory bowel disease (IBD), respectively (Bilsborough et al. 2002; Wittig et al. 2003). Induction and maintenance of peripheral tolerance are important mechanisms to maintain the balance of the immune system. Accumulating evidence suggests that apart from T cell anergy and clonal deletion by apoptosis, active suppression mediated by regulatory T cells contribute to the maintenance of natural immunological self-tolerance as well as of tolerance towards enteroantigens.

2 Inflammatory Bowel Diseases

The inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders effecting 0.3% of the Western population (Podolsky 2002). CD can affect any part of the gastrointestinal tract, from the oral cavity to the anus, whereas UC is limited to the colon and rectum. The etiology of CD and UC remains unknown, but it probably involves a combination of genetic predisposition, environmental conditions, and abnormalities in immune regulation (Chutkan 2001; Farrell et al. 2001; Podolsky 2002). In particular, the intestinal mucosal immune system

has been a major focus of research, as IBD is characterized by a hyper-reactive immune system, underlined by a heavy influx of T cells, B cells, monocytes, and neutrophils into the intestinal mucosa. On the simplest level, an imbalance between pro- and anti-inflammatory mediators leads to chronic inflammation in the gastrointestinal tract of patients with IBD. Specific cytokines important for the induction of mucosal immunity and regulation of the mucosal immune responses include the pro-inflammatory mediators IL-1, IL-6, IL-12 and TNF-α, produced by monocytes and macrophages. In addition, the $CD4^+$ T cells infiltrating the lamina propria (LP) of IBD patients display an altered cytokine profile as compared with healthy individuals. LP-derived $CD4^+$ T cells from CD patients produce increased levels of IFN-γ and IL-2, whereas LP-derived $CD4^+$ T cells from UC patients produce increased levels of IL-4 and IL-5 (Farrell et al. 2001). These observations suggest that that the immune responses are Th1 and Th2 screwed in CD and UC patients, respectively.

3
Bacterial Flora in Inflammatory Bowel Disease

Although the etiology of IBD has not been clearly linked to any specific infectious agent, it is well known from experimental models of IBD that colitis cannot be induced in animals raised under germ-free conditions (Sartor 1997; Schultz et al. 1999; Sellon et al. 1998). In the SCID transfer model of colitis, only a mild form of IBD developed after transfer of $CD4^+CD45RB^{high}$ T cells into recipients with a restricted enteric flora (Aranda et al. 1997). In addition, treatment with broad spectrum antibiotics reduces the severity of IBD (Madsen et al. 2000). These studies are consistent with a study by Duchmann et al. (1999) reporting a colonic mucosal T cell reactivity to endogenous flora in IBD patients, not present in healthy individuals. We have previously demonstrated that $CD4^+$ T cells from SCID mice with IBD, in contrast to $CD4^+$ T cells from normal BALB/c mice, respond by proliferation and cytokine secretion when exposed to enteric bacterial extracts (Brimnes et al. 2001; Gad et al. 2003). In addition, $CD4^+$ T cells from normal mice depleted in vivo or in vitro of $CD4^+CD25^+$ T cells proliferate extensively in the presence of enterobacterial antigens but are refractory to enteroantigens from the feces of germfree mice (Gad et al. 2004).

However, although the enteric flora necessary for IBD to develop has not been well characterized, a number of different bacterial species have been identified as being able to trigger the development of colitis in murine models (Rath et al. 1996; Sellon et al. 1998). One such species is the Gram-negative bacterium *Helicobacter hepaticus*. It has been shown that *H. hepaticus* can

cause colitis in immunodeficient mice (Li et al. 1998; Ward et al. 1996) and intensify colitis in immunodeficient mice reconstituted with naïve CD45RB^{high} CD4^{+} T cells (Cahill et al. 1997). In addition, transfer of CD4^{+} T cells from IL-10$^{-/-}$ mice into RAG$^{-/-}$ mice results in colitis in *H. hepaticus*-infected but not in uninfected recipients (Kullberg et al. 2002). In human studies *Mycobacteria* (Sanderson et al. 1992) and a subtype of *Escherichia coli* (Darfeuille-Michaud et al. 1998) have been found to play a pathogenic role in CD, whereas the presence of *Shingella*, *Salmonella* and *Yersinia* (Sartor et al. 1996) have been investigated as potential causal agents in UC.

Recent studies have tried to manipulate the intestinal microflora by treating with potentially protective bacteria such as lactobacilli and bifidobacteria species (Campieri et al. 2001), which are harmless components of the normal human and murine gastrointestinal microflora. It has been shown that certain probiotics can induce specific anti-inflammatory effects and they have been proposed as a therapy of colitis (Borruel et al. 2002, 2003). Consistent with this, treatment with *Lactobacillus* has shown to prevent the development of spontaneous colitis in IL-10-deficient mice (Madsen et al. 1999). The role of lactobacilli and bifidobacteria in human colitis is still not well characterized, although a significant decrease in the number of lactobacilli was found in colonic biopsies from patients with UC (Fabia et al. 1993). Clinical trials with the use of probiotics to treat patients with IBD or pouchitis proved to be quite effective (Gionchetti et al. 2000; Rembacken et al. 1999). Together, many independent studies suggest that enteric bacteria may trigger IBD, although the nature of these bacterially derived antigen(s) is unknown.

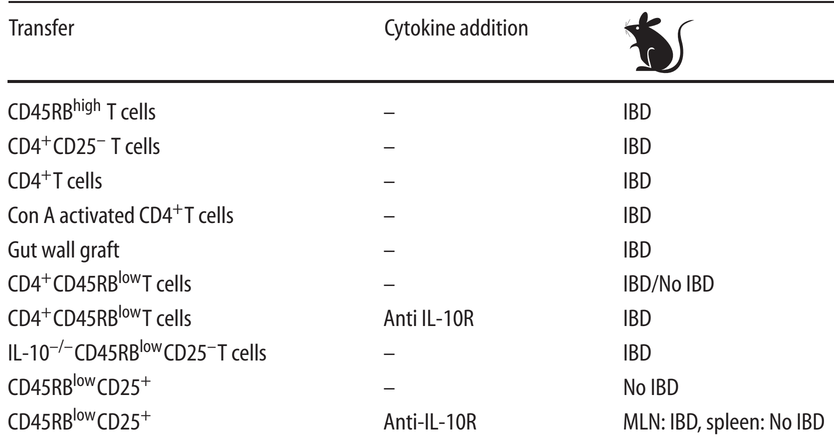
4 Animal Models of Inflammatory Bowel Disease

Studies in experimental models of mucosal inflammation have led to major new insights into the abnormalities present in human IBD as well as to new approaches in the therapy of these diseases (Hoffmann et al. 2002; Singh et al. 2001a; Strober et al. 2002). Models, based on knockout and transgenic animals, have generated the greatest interest and are commonly used. In particular, the adoptive transfer of T cells into immunodeficient mice as severe combined immune deficient (SCID) mice and recombination activation gene (RAG) deficient mice, which lack functional T and B cells, has been used to induce colitis in the recipients. The SCID transfer model will be described in more detail below, as it is one of the most widely used immunological models of inflammatory bowel disease and of major importance for the study of regulatory T cells.

4.1 The SCID Transfer Model of Colitis

In the transfer models of colitis, transfer of low numbers of $CD4^+$ T cells (Claesson et al. 1996) or a subpopulation hereof from an immunocompetent syngeneic donor mouse to an immunodeficient animal (SCID or $RAG^{-/-}$) leads to chronic and lethal colitis in the recipient. In addition, transplantation of a full gut wall graft from a normal donor mouse into the skin of a histocompatible SCID induces IBD (Rudolphi et al. 1994). While the original studies suggested that only adoptive transfer of sorted $CD45RB^{high}$ T cells leads to colitis (Morrissey et al. 1993; Powrie et al. 1993), we have repeatedly shown development of colitis following transfer to SCID mice of nonfractionated $CD4^+$ T cells (Claesson et al. 1996) and even after transfer of $CD45RB^{low}$ T cells (Claesson et al. 1999), although the onset of disease in these cases started relatively late at 12–16 weeks after transfer. In particular, in vitro activated $CD4^+$ T cells stimulated with Concanavalin A for 3 days or freshly derived $CD4^+CD25^-$ T cells are highly effective with regards to induction of colitis, which develops 6–8 weeks after transfer (Claesson et al. 1999; Liu et al. 2003; M. Gad et al., submitted). Until now transfer of naïve $CD45RB^{high}$ or Con A activated unfractionated $CD4^+$ T cells have been the best described models of colitis. However, recently the colitogenic potential of the cells within the antigen experienced $CD45RB^{low}$ T cell pool was thoroughly investigated (Asseman et al. 2003). Consistent with studies from our laboratory (Claesson et al. 1999), it was revealed that colitogenic Th1 cells are present in the antigen experienced $CD4^+CD45RB^{low}$ T cell population enriched within the $CD25^-$ subset but that the pathogenicity of these cells is controlled by IL-10. Thus, development of colitis was only seen in SCID mice after transfer of $CD4^+CD45RB^{low}$ T cells if the recipients were treated with anti-IL-10R mAb or if transferred $CD4^+CD45RB^{low}CD25^-$ T cells were derived from $IL\text{-}10^{-/-}$ mice. The pathogenic reactivity of the $CD45RB^{low}$ population was reduced when donor cells were isolated from germfree mice, indicating that the pathogenic T cells in the $CD4^+CD45RB^{low}$ T cell population represent an antigen experienced population of T cells driven by enteric bacteria in the donor mice (Asseman et al. 2003). In contrast, transfer of $CD4^+CD45RB^{high}$ T cells isolated from germfree mice to SCID mice is still able to induce colitis, suggesting that these naïve T cells differentiate into colitogenic Th1 cells upon exposure to the resident bacteria in the recipient. Attempts to induce colitis in immunodeficient animals using transfer of $CD4^+CD45RB^{low}CD25^+$ T cells have failed. However, in the absence of IL-10, pathogenic T cells have been revealed within the $CD25^+$ T cell population isolated from MLN but not from the spleen (Asseman et al. 2003), probably reflecting a higher frequency of bacteria-reactive

Table 1 The colitis inducing potency of different preparations or subsets of $CD4^+$ T cells in the SCID/$RAG^{-/-}$ transfer model



Transfer	Cytokine addition	
$CD45RB^{high}$ T cells	–	IBD
$CD4^+CD25^-$ T cells	–	IBD
$CD4^+$T cells	–	IBD
Con A activated $CD4^+$T cells	–	IBD
Gut wall graft	–	IBD
$CD4^+CD45RB^{low}$T cells	–	IBD/No IBD
$CD4^+CD45RB^{low}$T cells	Anti IL-10R	IBD
$IL\text{-}10^{-/-}CD45RB^{low}CD25^-$T cells	–	IBD
$CD45RB^{low}CD25^+$	–	No IBD
$CD45RB^{low}CD25^+$	Anti-IL-10R	MLN: IBD, spleen: No IBD

IBD can be induced in SCID/$RAG^{-/-}$ mice by transfer of different subsets of $CD4^+$ T cells, although with different kinetics. Thus mice transplanted with $CD45RB^{high}$ or $CD25^-$ T cells develop colitis after 6–8 weeks, whereas transfer of Con A activated $CD4^+$T cells induces colitis within 8–10 weeks, and transfer of unfractionated $CD4^+$ or $CD4^+CD45RB^{low}$ T cells induces colitis 12–18 weeks after transplantation. In addition, colitis is induced in SCID mice by a gut wall graft from an immunocompetent syngeneic donor. See text for references.

activated $CD25^+$ T cells in the MLN than in the spleen. In addition, different modifications of the SCID transfer model have been developed to induce colitis (Kullberg et al. 2002; Liu et al. 2003). For an overview see Table 1.

In the SCID transfer model, the transferred $CD4^+$ T cells repopulate the spleen, the mesenteric lymph nodes and the intestinal mucosa of the SCID mouse, whereas neither the thymus nor the peripheral distal lymph nodes are repopulated (Reimann et al. 1995). Subsequently, the recipients develop a lethal inflammatory bowel disease, with the main symptoms being weight loss, diarrhea and rectal prolapse (Claesson et al. 1996; Leach et al. 1996). In addition, the disease is characterized by mucosal hypertrophy, epithelial hyperplasia, decreased number of goblet cells and infiltration of the intestinal lamina propria (LP) and spleen with mononuclear cells. In severely diseased mice, transmural cell infiltration, epithelial ulceration and crypt abscesses are also seen. The histological changes are mainly found in the colon and occasionally in the small intestine (Claesson et al. 1996; Leach et al. 1996). The infiltrating mononuclear cells are dominated by the pathogenic, donor-derived $CD4^+$ T cells. These cells display surface markers consistent with

a mucosa seeking and activated memory phenotype, i.e., they are $CD69^+$, $CD25^+$, $CD44^+$, $CD45RB^{low}$, $\alpha4\beta7^+$ and L-selectin^{low} (Reimann et al. 1993). The LP $CD4^+$ T cells in SCID mice with colitis have a high turnover indicated by increased levels of both proliferation and apoptosis compared with $CD4^+$ T cells from normal mice (Bregenholt et al. 1998). In addition, these cells express a Th1 cytokine phenotype and secrete IFN-γ, TNF-α, and IL-2 (Bregenholt and Claesson 1998a).

4.2 Other Models of Colitis Caused by a Dysregulated Immune System

Genetic models as deletion of either the cytokines IL-10 (IL-$10^{-/-}$) (Rennick et al. 2000), IL-2 (IL-$2^{-/-}$) (Ehrhardt et al. 1997) or their receptors, $CRFB4^{-/-}$ (Spencer et al. 1998) and IL-$2\alpha^{-/-}$ results in colitis. Also, TCR-$\alpha^{-/-}$ (Mizoguchi et al. 1996), MHC class $II^{-/-}$ (Mombaerts et al. 1993), $G\alpha_{12}{}^{-/-}$ (Rudolph et al. 1995) as well as the HLA-B27 transgenic rat model (Taurog et al. 1994) develop mucosal inflammation.

In addition, it should be mentioned that although the role of T cells in the induction of intestinal inflammation has received much attention, it has recently been shown that innate immune mechanisms alone are able to mediate intestinal inflammation as demonstrated in $Rag^{-/-}$ mice in which exposure to *H. Hepaticus* leads to chronic colitis (Maloy et al. 2003).

5 Regulatory T Cells Prevent Colitis

General mechanisms are believed to be of importance for the prevention of autoaggression, including protection against intestinal inflammation such as T cell deletion, T cell anergy and immunological ignorance. Moreover, it appears that the lymphocyte homeostasis in normal mice is under active control by the activity of a distinct subset of regulatory T cells, which in addition may play an important role in the prevention of pathogenic immune responses towards the bacterial flora of the gut (Shevach 2002).

In T cell-deficient mice and rats, colitis induced by $CD4^+CD45RB^{high}$ T cells is prevented by co-transfer of $CD4^+CD45RB^{low}$ T cells, cells which normally do not induce disease when transferred alone (Powrie et al. 1990, 1993). Recently it was shown that the protective capacity is enriched but not exclusively present in the $CD25^+$ subset of the $CD4^+CD45RB^{low}$ T cell population (Annacker et al. 2001; Lehmann et al. 2002; Read et al. 2000). Annacker et al. (2001) observed that both the $CD4^+CD25^+CD45RB^{low}$ and $CD4^+CD25^-CD45RB^{low}$ purified cell

populations were able to confer protection of colitis induced by CD45RB^{high} cells. In another study (Lehmann et al. 2002), subdivision of integrin $\alpha_E\beta_7^+$ (CD103) cells in CD4$^+$CD25$^-$ and CD4$^+$CD25$^+$ T cells revealed that both of the $\alpha_E\beta_7^+$ subsets can protect SCID mouse recipients from colitis. In agreement with these studies, observations from a model of *H. hepaticus*-induced colitis showed that the protective CD4$^+$ T cells are contained within both the CD25 positive and negative subsets of the CD45RB^{low} T cell fraction and that the CD25$^-$ T cells are the most effective inhibitors of inflammation (Kullberg et al. 2002). We have shown that development of colitis, induced by CD4$^+$ CD25$^-$ T cells, can be prevented both by co-transfer of CD4$^+$CD25$^+$ T cells or by co-transfer of unfractionated CD4$^+$ T cells derived from a 6-day co-culture with immature dendritic cells (DCs) (M. Gad et al. submitted). Finally, also other kinds of induced Treg cells such as IL-10-secreting Tr1 and TGF-β-secreting Tr3 Treg cells prevent T cell transfer-induced colitis (Groux et al. 1997; Neurath et al. 1996). However, the relationship between these phenotypically distinct subsets of Treg cells is not known.

Since even unfractionated CD4$^+$ T cells which include 10%–15% CD25$^+$ T cells have been reported to expand and induce colitis following transfer to SCID mice (Claesson et al. 1996), it has been suggested that the presence of *H hepaticus* in the animal facilities may play a role in the development of colitis (Annacker et al. 2000; Cahill et al. 1997; Claesson et al. 1999; Foltz et al. 1998). Thus, by transfer of unfractionated CD4$^+$ T cells to immunodeficient mice the surrounding environment, i.e., the presence or absence of *H. hepaticus*, may favor the expansion of pathogenic CD4$^+$ T cells or regulatory CD25$^+$ T cells, respectively, resulting in development of colitis or absence of disease.

6 Naturally Occurring Regulatory T Cells

Recently the focus has been largely on naturally occurring CD4$^+$ T cells constitutionally expressing the α chain of the IL-2 receptor (CD25); for a review see (Shevach 2002). Even though CD25$^-$ regulatory T cells exist (Apostolou et al. 2002; Lehmann et al. 2002), the CD25 marker has been used to define the properties of regulatory cells. The regulatory population was first identified as a subset of CD4$^+$ T cells able to prevent the development of organ-specific autoimmune disease in mice thymectomized on day 3 after birth (Asano et al. 1996; Sakaguchi et al. 1995). Subsequently, the regulatory T cells have been shown to inhibit many autoimmune diseases (Shevach 2000; von Herrath et al. 2003), transfer tolerance to alloantigens (Taylor et al. 2001), hinder anti-tumor immunity (Shimizu et al. 1999) and regulate the expansion of other

peripheral $CD4^+$ T cells (Annacker et al. 2001). $CD4^+CD25^+$ regulatory T cells have also been isolated from the thymus and peripheral blood of humans and the cells have the same characteristics in mouse and man (Dieckmann et al. 2001; Jonuleit et al. 2001; Levings et al. 2001; Stephens et al. 2001). Naturally occurring regulatory $CD4^+CD25^+$ T cells are generated in the thymus (Thornton et al. 1998) and are therefore thought to recognize self-derived MHC-bound peptides. This raises the question of whether any self antigen in the thymus has the ability to generate regulatory cells. $CD4^+CD25^+$ T cells display a diverse TCR repertoire, suggesting that they undergo normal selection. Regulatory $CD4^+CD25^+$ T cells constitute around 10% of peripheral $CD4^+$ T cells (Sakaguchi et al. 1995) and are considered as resting antigen-experienced cells. Suppression mediated by the regulatory $CD4^+CD25^+$ T cells requires activation through their TCR (Dieckmann et al. 2001; Thornton et al. 1998). They are anergic in vitro as they do not proliferate or produce cytokines upon T cell receptor (TCR) stimulation. However, they regain responsiveness to TCR-mediated activation in the presence of exogenous IL-2 (Shevach 2002; Thornton et al. 1998). Although it is known that that $CD4^+CD25^+$ Treg cells suppress the transcription of the IL-2 gene in co-cultures with $CD4^+CD25^-$ responder cells (Shevach 2002), it has been suggested by Thornton and colleges (2004) that $CD4^+CD25^+$ Treg cells must respond to IL-2 before they can suppress proliferation of naïve responder cells. Somewhat unexpected from the in vitro data, recent evidence indicates that the regulatory T cells have a much more dynamic behavior than previously assumed. Thus regulatory T cells are capable of substantial antigen-induced expansion in vivo, accompanied by increased suppressive activity (Fisson et al. 2003; Klein et al. 2003; Walker et al. 2003; Yamazaki et al. 2003).

CD25 is not a very good marker for the regulatory T cells and a great effort has been made to define better markers. Numerous attempts to characterize and identify new markers revealed high expression of the negative regulator of T cell activation CTLA-4, the glucocorticoid-induced TNFR-related protein () (McHugh et al. 2002), and expression of membrane-bound tumor growth factor (TGF)-β1 after strong in vitro stimulation. Finally, the forkhead transcription factor FoxP3 which is expressed at high levels in murine regulatory $CD4^+CD25^+$ T cells, both in the thymus as in the periphery (Ramsdell 2003), but not or only weakly transcribed in naïve or recently activated $CD25^-$ T cells (Fontenot et al. 2003; Khattri et al. 2003; Ramsdell 2003) is an important marker. FoxP3-deficient mice develop massive autoimmune and inflammatory disease, whereas gene transfer of FoxP3 converts naïve $CD4^+CD25^-$ T cells into Treg cell (Fontenot et al. 2003; Khattri et al. 2003; Ramsdell 2003). However, as FoxP3 is found intracellular, it is not the best marker for functional studies.

Despite attempts to narrow the regulatory subset of cells, it was shown that $CD25^+$ cell-mediated in vitro regulation of a response to anti-CD3 was not altered much by further subdividing the cells into high and low expressers for CD62L, CD69, CD38, CD45RB (Thornton et al. 2000), or CD103 (McHugh et al. 2002). Nevertheless, in the SCID transfer model of colitis it was found that $CD4^+CD25^+$ T cells expressing $\alpha_E\beta_7$ had a higher regulatory capacity than the $\alpha_E\beta_7^-$ $CD4^+CD25^+$ T cell subset using a low regulator/target ratio, identified by a lower incidence of colitis, and lower clinical and histological colitis score in mice reconstituted with $CD4^+$ $CD45RB^{high}$ T cells and the $\alpha_E\beta_7^+$ $CD4^+CD25^+$ subset (Lehmann et al. 2002). The integrin $\alpha_E\beta_7$ is mainly expressed on intraepithelial lymphocytes residing in the gut wall and other epithelial compartments, such as skin and lung (Cerf-Bensussan et al. 1987), which suggest that $\alpha_E\beta_7$ may contribute to the regulatory function in the colitis model, e.g., by trafficking to inflamed tissues in the gut and acting directly at sites of inflammation. It should be mentioned that only $CD25^+$ $\alpha_E\beta_7^-$ cells are included among the natural Treg cells found in the thymus, whereas $CD25^+$ $\alpha_E\beta_7^+$ T cells may represent adaptive regulators which can be induced in the presence of, for example, TGF-β (Huehn et al. 2004). Thus, lately it has become clear that functionally distinct subsets of $CD4^+CD25^+$ Tregs with different phenotypes exist. It has been hypothesized by Blustone and Abbas (Bluestone et al. 2003) that there exist two subsets of $CD4^+$ Treg cells, natural and adaptive, that differ in terms of origin, specificity and mechanism of action. According to their model, natural self-antigen specific Treg develop during the normal process of T cell maturation in the thymus. In contrast, adaptive Tregs develop either from activation of natural $CD4^+CD25^+$ Treg cells or from naïve Th cells. Thus even the $CD4^+CD25^+$ Treg cells display a heterogeneous compartment of Treg cells.

7 Mechanisms of $CD4^+CD25^+$ Treg Cell-Mediated Immunosuppressive Functions

7.1 Cytokine Requirements for the Control of Colitis by $CD4^+CD25^+$ Treg

The mechanism by which regulatory T cells exert their function is currently a controversial issue. It has been demonstrated that that both TGF-β and IL-10 play important roles in regulatory T cell-induced protection of T cell-induced colitis (Asseman et al. 1999, 2003; Powrie et al. 1994; Read et al. 2000). Elevated levels of IL-10 and TGF-β mRNA were found in the $CD4^+CD25^+$ T cell subset

ex vivo (Asano et al. 1996) as well as direct secretion of these cytokines by the $CD4^+CD25^+$ T cells when stimulated in an appropriate fashion (Nakamura et al. 2001). We know that IL-10 plays an important function in the intestinal homeostasis, revealed by the fact that IL-10-deficient mice (Rennick et al. 2000) or wild type mice treated with anti-IL-10R (Asseman et al. 2003) develop chronic inflammation in the intestine and IL-10 in general plays a role as a negative regulator of the immune response. In addition, administration of exogenous IL-10 inhibits the development of colitis in SCID mice reconstituted with $CD4^+CD45RB^{high}$ T cells (Powrie et al. 1994) as well as in other models of IBD (Leach et al. 1999). Consistent with these studies, $CD4^+CD45RB^{high}$ T cells isolated from transgenic mice, which expressed IL-10 under control of the IL-2 promoter, failed to induce colitis in SCID mice and were able to inhibit the disease when transferred with $CD45RB^{high}$ $CD4^+$ T cells from normal mice. The importance of TGF-β in immune homeostasis is indicated by the fact that that TGF-β-deficient mice die within 3–5 weeks after birth due to a spontaneous autoimmune-like syndrome (Shull et al. 1992).

By adding antibodies that either block TGF-β (Powrie et al. 1996; Read et al. 2000) or the IL-10 receptor (IL-10R) (Asseman et al. 1999) to recipients of both pathogenic $CD4^+CD45RB^{high}$ T cells and regulatory $CD4^+CD45RB^{low}$ T cells, the protection against IBD is completely abrogated, suggesting that these cytokines are involved in the mechanisms of immune suppression. In addition, administration of anti-TGF-β to mice co-transferred with $CD45RB^{high}$ and $CD45RB^{low}CD25^+$ T cells led to abrogation of suppression and induction of colitis in the recipients (Read et al. 2000). Very recently, it was stated that the $CD4^+CD25^+$ T cells produce the TGF-β1 themselves, as $CD4^+CD25^+$ T cells from TGF-$\beta 1^{-/-}$ did not protect Rag-$2^{-/-}$ recipients of $CD45RB^{high}$ T cells from developing colitis (Nakamura et al. 2004). Moreover, $CD4^+CD45RB^{low}$ T cells isolated from $IL10^{-/-}$ mice failed to inhibit colitis when co-transferred with $CD4^+CD45RB^{high}$ T cells (Asseman et al. 1999). However, the requirement for IL-10 in the suppression of IBD induced by transfer of naïve $CD45RB^{high}$ T cells has until recently only been examined by using $CD45RB^{low}$ cells as a source of Treg cells (Asseman et al. 1999). In light of these observations, stating the existence of colitis-inducing T cells within the $CD45RB^{low}$ pool treated with anti- IL-10R Ab (Annacker et al. 2001; Asseman et al. 2003), it was suddenly very important to determine whether the regulation of naïve T cells by regulatory $CD4^+CD25^+$ T cells also requires IL-10. In a recent report, it was found that the $CD4^+CD25^+$ T cells isolated from IL-$10^{-/-}$ mice are still able to inhibit colitis induced by wild-type naïve $CD4^+CD45RB^{high}$ T cells in the SCID model, which importantly states that $CD4^+CD25^+$ T cells themselves, in this system, do not have to produce IL-10 (Asseman et al. 2003). However, this is in conflict with an earlier report, using the RAG-$2^{-/-}$ model

system of colitis, which states the opposite (Annacker et al. 2001). These discrepancies may simply be due to the use of immune-deficient recipients of different genetic backgrounds or different environmental conditions in the laboratories. In addition, the co-transfer of $CD4^{+}CD25^{+}$ T cells suppressed the development of colitis in SCID recipients by $CD4^{+}CD45RB^{high}$ cells, even in the presence of antil-IL-10R, although a small but significant increase in the development of colitis was seen compared with control mice, indicating the control of colitis is partly dependent on IL-10 (Asseman et al. 2003).

In contrast to this, it was found that IL-10 is very necessary for the control of colitis induced by antigen-experienced cells (Asseman et al. 2003). Transfer of $CD4^{+}CD25^{+}$ T cells prevents the development of colitis induced by IL-$10^{-/-}$ $CD45RB^{low}CD25^{-}$ T cells and anti-IL-10R treatment induces colitis in recipients of unseparated $CD45RB^{low}$ cells. Although it was found that the control of antigen-experienced colitogenic T cells was highly dependent on IL-10, it was not investigated whether IL-10 secretion in this case was required by the $CD4^{+}CD25^{+}$ T cells themselves. Hence, the role of IL-10 in vivo seems very complex, as there are different requirements for IL-10 in the regulation of naïve and antigen experienced T cells, and the IL-10 requirements depend on the genetic lesion of the recipients, i.e., SCID vs $RAG^{-/-}$. In contrast, the role of TGF-β seems simpler. Thus in summery, TGF-β is sufficient to prevent colitis induced by naïve cells (Nakamura et al. 2004; Read et al. 2000), whereas IL-10 is required to control previously activated Th1 cells (Asseman et al. 2003). In support of this, TGF-β1 was shown to inhibit resting $CD4^{+}$T cells in contrast to activated T cells, although addition of IL-10 restored TGF-β responsiveness on activated T cells (Cottrez et al. 2001), suggesting that IL-10 plays a role in potentiating the effects of TGF-β on differentiated effector T cells. Hence, both IL-10 and TGF-β seem to be involved in the function of regulatory T cells. In contrast, recombinant IL-4 had no positive effect on the health of mice injected with $CD45RB^{high}$ $CD4^{+}$ T cells (Powrie et al. 1994) and IL-$4^{-/-}CD45RB^{low}$ $CD4^{+}$ T cells were as efficient in protecting the recipient as their wild-type counterpart (Powrie et al. 1996).

It has been suggested that $CD4^{+}CD25^{+}$ Treg cells in addition to their direct inhibitory effect in vivo function indirectly by inducing the differentiation of naïve T cells into cytokine secreting Treg cells, a phenomenon termed infectious tolerance (Jonuleit et al. 2002). According to this view, the first step is a contact-dependent localized inhibitory effect, whereas the induced secondary Treg cells mediate a cell contact-independent widespread suppressive effect via cytokine secretion. This spreading of suppression from naturally occurring $CD4^{+}CD25^{+}$ cells to induced Treg cells may be a fundamental mechanism for the induction and maintenance of peripheral tolerance. Thus, the protective effect of IL-10 in some cases might depend on the ability

Table 2 Suppression of colitis by Treg cells in the SCID transfer model and the requirements of cytokines

Transfer of $CD4^+$ T cells	+Transfer of $CD4^+$ Treg cells							
+cytokine addition	$CD45RB^{low}$	$CD45RB^{low}$ $IL\text{-}4^{-/-}$	$CD45RB^{low}$ $IL\text{-}10^{-/-}$	$CD25^+$	$CD25^+$ $IL\text{-}10^{-/-}$	$CD25^+$ $TGF\text{-}\beta1^{-/-}$	Tr1+OVA	DC/$CD4^+$ Treg
$CD45RB^{high}$	No IBD	No IBD	IBD	No IBD	No IBD/IBD	IBD	No IBD	
$CD45RB^{high}$ + anti-IL-10R	IBD			No IBD/IBD			IBD	
$CD45RB^{high}$ + anti-TGF-β	IBD			IBD				
$IL\text{-}10^{-/-}CD45RB^{low}CD25^-$				No IBD				
$CD25^-$								No IBD

The co-transfer of $CD45RB^{low}$ T cells, $CD25^+$ T cells, DC/$CD4^+$ co-culture-induced Treg cells or Tr1 cells prevents induction of disease. However, the need for cytokines for control of colitis varies. See text for references.

of $CD4^+CD25^+$ Treg cells to induce other subsets of IL-10-producing Treg cells.

Table 2 shows an overview of the Treg cells used for suppression of colitis in the SCID transfer model and their requirements for cytokines to mediate suppression.

7.2
Cellular Requirements for the Regulation of Colitis by $CD4^+CD25^+$ Treg

It is well known that suppression mediated by the regulatory $CD4^+CD25^+$ T cells in vitro requires cell–cell interaction between the responder and regulatory populations and is independent of cytokines (Dieckmann et al. 2001; Jonuleit et al. 2001; Levings et al. 2001; Stephens et al. 2001; Takahashi et al. 2000; Thornton et al. 1998). A number of ligands and receptors have been suggested to be partially responsible for this inhibitory function and many of these are co-stimulatory molecules. It is widely accepted that T cell activation involves signals transduced by the TCR complex after recognition of antigen as well as from costimulatory molecules after encounter with their ligands present on APCs (Bretscher 1999). It is known that $CD4^+CD25^+$ T cells are present but in reduced numbers in both $CD28^{-/-}$ and $B7^{-/-}$ mice, suggesting that CD28-mediated co-stimulatory signals are involved in the homeostatic levels of this population in vivo (Salomon et al. 2000). In contrast to CD28, ligation of cytotoxic T-lymphocyte antigen (CTLA)-4 on the surface of activated T cells, by its ligands CD80/CD86 expressed on APCs, delivers a negative signal leading to inhibition of T cell activation (Chambers et al. 2001). Besides being expressed on activated T cells, an elevated CTLA-4 expression was found on regulatory T cells (Read et al. 2000; Takahashi et al. 2000), which suggests that the molecule might be functionally important. It has been suggested that Tregs expressing high levels of CTLA-4 may interact with APCs via B7 ligation and induce expression of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO). Expression of IDO by $CD11c^+CD8\alpha^+$ DCs in mice and $CD123^+$DCs in humans allows these DCs to suppress T cell proliferation in vitro and suppress autoimmune disorders in vivo (Mellor et al. 2003), although its role in suppression of colitis has not been investigated yet. However, there is some disagreement about the importance of CTLA-4 for the inhibitory function of regulatory T cells. Takahashi et al. (2000) have shown that the addition of anti-CTLA-4 antibody or its Fab fragment reverse suppression in co-cultures of $CD4^+CD25^+$ T cells and $CD4^+CD25^-$ T cells. Similarly, Read et al. (2000) have shown that the treatment of recipients of co-transferred $CD4^+CD45RB^{high}$ and $CD4^+$ $CD45RB^{low}CD25^+$ T cells with anti-CTLA-4 abrogated the suppression of colitis. Nevertheless, these studies

have been difficult to reproduce by other groups, including our own group, which found that suppression in vitro is not abrogated by blockade of CTLA-4 (Gad et al. 2004; Jonuleit et al. 2001; Levings et al. 2001; McHugh et al. 2002; Thornton et al. 1998). As CTLA-4 is also expressed on activated $CD4^+CD25^-$ T cells, it has been suggested that the effect of anti-CTLA-4 Ab in vitro is the result of binding to the effector $CD4^+CD25^-$ T cells since anti-CTLA4 Ab may inhibit the normal down-regulatory effects of CTLA-4 on T cell activation and raise the threshold that is required for $CD4^+CD25^+$ T cells to mediate suppression (Shevach 2002).

The importance of another costimulatory molecule Ox40 (CD134) has also been investigated for its function on regulatory T cells. The Ox40 molecule is expressed transiently on activated $CD4^+$ T cells, whereas its ligand Ox40L (CD134L) has been reported to be present on dendritic cells after activation (Annacker et al. 2002; Ohshima et al. 1997). Administration of antibody against Ox40L has been shown to prevent T cell accumulation in the intestine of $CD4^+CD45RB^{high}$ T cell restored SCID mice and abrogate the development of colitis (Malmstrom et al. 2001), suggesting that that the Ox40 molecule may also play a role in the regulation of immune reactivity by regulatory T cells. However, although 30% of resting $CD4^+CD25^+$ T cells express Ox40, administration of antibody against Ox40 could not abrogate the ability of the $CD4^+CD25^+$ T to exert suppression in response to anti-CD3 in an in vitro system (McHugh et al. 2002). As mentioned above, a novel cell surface marker, glucocorticoid-induced TNF-receptor (GITR) has been identified on resting $CD4^+CD25^+$ T cells in the thymus and in the periphery (McHugh et al. 2002; Shimizu et al. 2002). GITR ligated with agonistic antibodies on $CD4^+CD25^+$ Treg cells results in loss of suppressive activity (Shimizu et al. 2002). It has been shown that $CD4^+GITR^+$ T cells regardless of their CD25 expression can prevent colitis development. Additionally, administration of anti-GITR mAb abrogates colitis suppression in mice restored with both $CD45RB^{high}$ and $CD45RB^{low}$ $CD4^+$ T cells (Uraushihara et al. 2003). Finally, it has been shown by Nakamura et al. that stimulated $CD4^+CD25^+$ T cells express high and persistent levels of TGF-β1 on the cell surface and that suppression mediated by these regulatory cells is abolished in the presence of anti-TGF-β Ab (Nakamura et al. 2001) or rLAP (Nakamura et al. 2004). This observation, together with the fact that the suppression in vitro requires cell–cell contact suggests that membrane-bound TGF-β1 could be involved in cell-mediated immune suppression. However, we and others have not been able to reverse suppression mediated by $CD4^+CD25^+$ T cells in vitro by administration of a soluble TGF-βRII-Fc complex or by mAb (Gad et al. 2004; Piccirillo et al. 2002; Read et al. 1998; Takahashi et al. 1998). The discrepancy may be due to the fact that $CD4^+CD25^+$ T cells only produce easily detectable amounts

of TGF-β when maximally stimulated. Besides, Nakamura et al. (2001) found that only very high concentrations of anti-TGF-β mAb (50–100 µg/ml) can abrogate the suppression in vitro.

8 Other Subsets of Regulatory T Cells Involved in the Control of Colitis

Many studies suggest that SCID mice with colitis lack Treg cells. In addition to the naturally occurring regulatory $CD4^+CD25^+$ T cells, a number of different regulatory T cell populations, capable of inhibiting the response of other T cells, have been described (Cottrez et al. 2000; Gad et al. 2003; Neurath et al. 1996). Treg cells can be induced in vivo following oral exposure to antigen (Th3 cells) and in vitro after culture with antigen and IL-10 (Tr1 cells) or following co-culture of gut-derived $CD4^+$ T cells and immature DCs (Gad et al. 2003). All three subsets have been shown to prevent the development of colitis (Groux et al. 1997; Neurath et al. 1996; M. Gad et al. submitted).

The Tr1 cells are different from the classical Th1 and Th2 cells (Groux et al. 1997). They proliferate poorly, secrete neither IL-2 nor IL-4, but produce high levels of IL-10. They inhibit antigen-specific immune responses in vitro through the secretion of IL-10 and TGF-β. Besides mediating suppressing immune responses *i*n vitro, Tr1 cells were shown to be immune suppressive in vivo. In the SCID transfer model of IBD co-transfer of OVA specific Tr1 cells and pathogenic $CD4^+CD45RB^{high}$ cells prevented the induction of Th1-mediated inflammation. The in vivo function of the Tr1 cells was antigen-dependent, as only mice receiving OVA were protected from disease (Groux et al. 1997). Therefore, it was suggested that these Tr1 cells can suppress immune responses to unknown antigens by an antigen-driven bystander suppression mechanism. Similar to the observations in in vitro experiments, it was observed that the Tr1-mediated suppression was completely abrogated when mice were treated with anti-IL-10R, confirming the importance of IL-10 for the function of Tr1 cells and as a general immunomodulator of immune responses (Foussat et al. 2003).

We have investigated the capacity of isolated $CD4^+$ T cells from the colonic LP of normal mice to suppress the extensive proliferation of enterobacteria-exposed Th1 cells from SCID mice with colitis (Gad et al. 2003). We found that freshly purified LP or MLN $CD4^+$ T cells do not inhibit proliferation, whereas LP or MLN $CD4^+$ T cells co-cultured with immature DCs for 2–6 days exert strong inhibitory activity. The majority of these DC-induced Treg cells display a nonactivated phenotype, and the suppression per se is enteroantigen-independent and mediated partly by soluble factors different

from IL-10 and TGF-β. The $CD4^+$ T cells from the DC co-culture are a mixture of $CD25^+$ (10%–20%) and $CD25^-$ (80%–90%) T cells. However, in a recent report (M. Gad et al. submitted) it was revealed that all the suppressor activity both in vitro and in vivo resides in the $CD25^+$ T cell subset. The data show that the DC-induced $CD25^+$ Treg cells, in contrast to the prototype of $CD25^+$ Treg cells, display an immature phenotype and can function independently of cell activation and direct cellular contact. In addition, the DC-induced Treg cells mediate a stronger suppressive activity than the prototype $CD25^+$ regulatory T cells. Both unfractionated and $CD25^+$ DC-induced Treg cells were found to protect the recipients of $CD4^+CD25^-$ T cells in the SCID transfer model of colitis against development of colitis.

The Th3 cell is yet another type of regulatory T cell capable of inhibiting colitis induced by intraluminal exposure to TNBS (Neurath et al. 1996). Th3 cells induced during oral tolerance secrete TGFβ. However, the suppressive effect of Th3 cells is antigen nonspecific and is mediated as bystander suppression through secretion of TGF-β. It is known that Th2 conditions favor the induction of Th3 cells, whereas Th1 conditions inhibit this induction. Nevertheless, the exact cytokine milieu necessary for the induction of Th3 cells is not well understood.

9 Where Do the Treg Cells Localize and What Do They Target?

Although research has focused on the function or Treg cells in recent years, the exact mechanism by which $CD4^+CD25^+$ regulatory T cells exert their suppressive effects remain unknown. The anatomical locations in which the Treg cells act and how these cells migrate in vivo are important issues that have hardly been studied to date. Although it has been revealed that cell–cell contact but also suppressive cytokines are required for suppression, it is not known at which point in the inflammatory cascade that Treg cells work or whether they target the responder cells, the APCs or both. Neither, is it well defined whether the Treg cells inhibit the function of already activated effector cells.

It has been revealed that colitis is accompanied by an increase in the number of activated dendritic cells (DCs) in the mesenteric lymph nodes (MLN) (Malmstrom et al. 2001). As mentioned above, these DCs were found to express the costimulatory ligand CD134L and administration of anti-CD134L mAb inhibited the proliferation of T cells in the MLN and blocked the development of colitis (Malmstrom et al. 2001). Surprisingly, CD134L was found to be expressed by a proportion of DCs in the MLNs of unreconstituted SCID mice.

These activated DCs were present in a reduced number in SCID mice compared with mice restored with $CD4^+CD45RB^{high}$ T cells but it was suggested that they may provide the initial costimulatory signals that drive the $CD45RB^{high}$ cells into colitogenic Th1 cells. Importantly, mice protected from colitis by cotransfer of regulatory $CD4^+CD25^+$ T cells did not show an increase in activated $CD134L^+$ DCs in MLN, suggesting that modulation of DC function is one mechanism by which Treg cells may mediate their immune suppressive function. Whether the regulatory T cells inhibit the migration of DCs, their activation or life span still needs to be defined.

Suppression of colitis by Treg cells in vivo is characterized by a significant reduction in the number of activated Th1 cells that accumulate in the intestine (Annacker et al. 2001; Asseman et al. 1999; Mottet et al. 2003), which may be due to reduced expansion or migration of these cells. Recently, the influence of $CD4^+CD25^+$ T cells on local colitogenic T cell proliferation was examined using the $CD4^+CD45RB^{high}$ T cell transfer model of colitis (Mottet et al. 2003). In colitic SCID mice, $CD4^+CD45RB^{high}$ T cell proliferate vigorously in both MLN and LP 4 weeks after T cell transfer. It was found that $CD4^+CD25^+$ T cells transferred therapeutically at this time point proliferate vigorously in the MLN and in particular in the inflamed colonic LP a few weeks after transfer. However, the resolution of the inflammatory response, 10 weeks after the transfer of $CD4^+CD25^+$ T cells, correlates with a reduced number of proliferating pathogenic cells as well as of Treg cells. These results suggest that Treg cells control T cell effector responses not only in the lymph nodes but also in the inflamed tissue. Further, apparently the vigorous proliferation of Treg cells does not lead to loss of suppression as assessed by the resolution of inflammation (Mottet et al. 2003). Other studies agree with this assumption, as $CD4^+CD25^+$ T cells after expansion in vivo were found to be more potent suppressors in vitro (Gavin et al. 2002; Klein et al. 2003). Additionally, we have recently shown that in fully protected SCID mice co-injected with $CD25^-$ T cells and $CD25^+$ T cells, effector cells and Treg cells exist side by side. This was indicated by the fact that $CD4^+$ T cells recovered from both SCID mice with colitis and mice transplanted with $CD25^-$ T cells and Treg cells proliferate vigorously in response to enteroantigen ex vivo in contrast to unfractionated $CD4^+$ cells from normal BALB/c mice. Thus Treg cells cause neither effector cell depletion nor anergy. Finally, the regulatory $CD4^+CD25^+$ T cells were found to be in close contact with $CD11c^+$ DCs as well as pathogenic T cells in the colon and LP (Mottet et al. 2003). This location of Treg cells suggests that there is a direct physical contact between Tregs and $CD11c^+$ DCs, supporting a role for DC-Treg cell interaction.

The mechanisms for protection of colitis by Tr1 cells were also investigated (Foussat et al. 2003). $CD4^+CD45RB^{high}$ T cell-reconstituted SCID mice

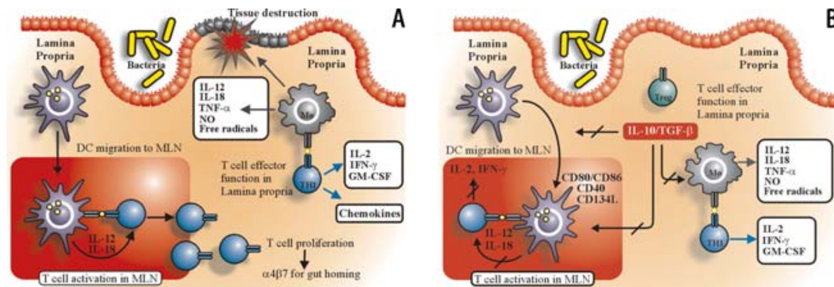


Fig. 1 A Development of intestinal inflammation. DCs sample antigens from bacterial flora, become activated and migrate to MLN. In the MLN, naïve T cells are activated in the presence of IL-12 and differentiate to Th1 cells expressing the gut-homing molecule $\alpha_4\beta_7$. The Th1 cells proliferate and enter LP, leading to recruitment of more inflammatory cells. **B** Inhibition of intestinal inflammation by regulatory T cells. It is suggested that Tregs may mediate their function at several sites. They may inhibit the migration of DCs to MLN. They may inhibit DC and T cell activation as well as T cell proliferation in the MLN by inhibiting co-stimulatory molecule expression. Finally, they may inhibit the T cell effector function in the LP by interfering with the homing capacity of activated T cells, or they may prevent the release of pro-inflammatory cytokines by macrophages, thereby inhibiting the progression of the inflammatory response. The inhibitory function of the Treg cells may be mediated either by direct cell–cell contact or by immune suppressive cytokines or both. One may hypothesize that $CD4^+CD25^+$ by infectious tolerance, via cell–cell contact, stimulates the differentiation of naïve T cell to become regulatory T cells (Tr1 or Tr3), which enhance and sustain the suppression by secretion of IL-10 and TGF-β. (Modified from Singh et al. 2001b)

co-transferred with Tr1 cells were treated with anti-IL-10R several weeks after cell transfer. The treatment completely reversed the protection of colitis up to 3 weeks after injection of Tr1 cells, which indicates that the protection of colitis is not due to a complete inhibition of the differentiation of the pro-inflammatory T cells in the colon. Indeed, some signs of inflammation were observed in the first weeks after co-transfer of pro-inflammatory $CD4^+CD45RB^{high}$ T cells and regulatory $CD4^+CD45RB^{low}$ T cells (Foussat et al. 2003), supporting the notion that Treg cells actively control inflammation.

Figure 1shows a model for the development of intestinal inflammation and how Treg cells may regulate it at several sites.

10 Antigen Specificity for Regulatory T Cells in Colitis

A very important question to answer in order to understand the precise function of regulatory T cells is their antigen specificity. It has been shown that high-affinity TCR/self peptide–MHC interactions in the thymus select for $CD4^+CD25^+$ regulatory T cells displaying immune suppressive function (Jordan et al. 2001). The thymic derived $CD4^+CD25^+$ T cells constitute a major population of Treg cells able to inhibit T cell responses both in vitro (Read et al. 1998; Thornton et al. 1998) and *i*n vivo (Read et al. 2000; Suri-Payer et al. 1998). The number of $CD4^+CD25^+$ T cells that are selected in the thymus has been shown to be proportional with the diversity of self-peptides presented in the context of MHC class II molecules on the thymic epithelium (Pacholczyk et al. 2002), suggesting that the Treg cells recognize self-antigens. As it was shown that the $CD4^+CD25^+$ T cells are a polyclonal population (Takahashi et al. 1998), it has been suggested that the Treg cells may react with a broad range of antigens.

We have demonstrated that $CD4^+CD25^+$ T cells derived from germfree mice have the ability to suppress the in vitro proliferation of $CD4^+CD25^-$ T cells stimulated with enteric bacteria (Gad et al. 2004). Consistent with our own results, $CD4^+CD45RB^{low}$ cells isolated from germfree mice can inhibit colitis (Annacker et al. 2000). We also found that the suppressive mechanism of Treg cells induced by DC/$CD4^+$ T cell co-culture is independent of exposure to the enteroantigen that stimulate the effector cells to proliferate in the absence of Treg cells (Gad et al. 2003). Thus apparently Treg cells in these model systems are not antigen experienced and in addition their specificity might not necessarily be the same as the effector cells. To date, there has been no evidence for a limited antigen repertoire of Treg cells, but most data point to the fact that $CD4^+CD25^+$ T cells require activation through their TCR in order to be suppressive, although once activated their suppressor function is completely nonspecific and does not require re-engagement of their TCR (Thornton et al. 1998, 2000). However, Treg cells might also function in an antigen specific way. Kullberg et al. (2002) used a modification of the SCID transfer model and found that Treg cells from *H. hepaticus*-infected but not from uninfected donor mice block colitis induced by *H. hepaticus*-specific effector T cells (Kullberg et al. 2002), suggesting antigen dependency. The antigen-specific protection was shown to be dependent on IL-10 both in vivo and in vitro (Kullberg et al. 2002) and the Treg cells thus resemble the IL-10 producing Ag-induced Tr1 cells more than the naturally occurring $CD25^+$ Treg cells.

11
Regulatory T Cells as a Therapeutic Agent for Inflammatory Bowel Disease

As described above, regulatory $CD4^+CD45RB^{low}$ T cells enriched within the $CD25^+$ subset as well as Tr1 cells and Treg cells from DC/$CD4^+$ T cell co-culture have been shown to prevent the development of colitis induced by transfer of $CD4^+CD45RB^{high}$ or $CD4^+CD25^-$ T cells to SCID mice (Foussat et al. 2003; Groux et al. 1997; Powrie et al. 1990, 1993; M. Gad et al. submitted). Recently, different groups have tried to cure already established colitis with regulatory T cells in the SCID transfer model (Foussat et al. 2003; Liu et al. 2003; Mottet et al. 2003). In a study by Mottet et al. (2003), immunodeficient SCID mice with clinical signs of colitis four weeks after transfer of $CD4^+CD45RB^{high}$ T cells were treated with $CD4^+CD25^+$ T cells. Histological changes, corresponding to an average colitis score of 3, confirmed the incidence of colitis at the time of treatment. In contrast to mice treated with $CD25^-$ T cell or control mice without a secondary transfer, the $CD4^+CD25^+$ T cells reduced the $CD4^+$ density in the colonic mucosa. Moreover, the transfer of the regulatory $CD4^+CD25^+$ T cells improved the clinical status, survival rate and intestinal pathology of mice with established colitis. Ten weeks after $CD4^+CD25^+$ T cell transfer, the recipient mice had almost completely recovered from colitis (Mottet et al. 2003). A second report investigated the therapeutic role of regulatory T cells in a model of colitis established by transfer of $CD4^+CD25^-$ T cells to SCID mice followed by infection of the protozoan parasite *Leishmania major* (Liu et al. 2003). Ten or 20 days after transfer of the pathogenic cells, mice were treated with freshly isolated, TGF-β-cultured or activated $CD4^+CD25^+$ T cells. In all cases, the colitis symptoms were reversed, and there were no differences in the pathology score among the mice treated with the different preparations of $CD4^+CD25^+$ T cells, indicating that it is possible to cure an established colitis. In addition, it was shown that the curative effect of the $CD4^+CD25^+$ T cell given day 21 was abolished by injecting the mice with anti-IL-10R, anti-TGF-β or anti-CTLA-4 Ab (Liu et al. 2003). These results demonstrate that the therapeutic effect of $CD4^+CD25^+$ T cell in this model is dependent on TGF-β, CTLA-4, which are in agreement with earlier prophylactic studies (Fuss et al. 2002; Read et al. 2000), and IL-10 (Annacker et al. 2001; Asseman et al. 2003). An explanation for the dependency of IL-10 in this model might be found in the authors' use of $CD4^+CD25^+$ donor T cells from both spleen and lymph nodes as $CD25^+$ T cells from MLN in the presence of anti-IL-10R may be colitogenic, as described by Asseman al. (2003). Finally, a recent study showed that Tr1 cells can also cure an ongoing colitis in the SCID transfer model even 6 weeks after transfer of the pathogenic $CD4^+CD45RB^{high}$ T cells

(Foussat et al. 2003). Treatment with Tr1 cells resulted in a very rapid remission of inflammation and mice were completely cured for colitis 3 weeks after treatment. Injection of mice with anti-IL-10R Ab abrogated the protective effect of the Tr1 cells. In contrast, Foussat et al. (2003) did not find any curative function of $CD4^{+}CD25^{+}$ T cells on colitis by looking at the colitis score 4–6 weeks after injection of the Treg cells. However, in the study by Mottet et al. (2003), the histological colonic abnormalities were not resolved before 10 weeks after $CD4^{+}CD25^{+}$ T cell transfer. Thus, $CD4^{+}CD25^{+}$ T cells seem to induce a slower remission of colitis as compared with Tr1 cells, a suggestion which supports the hypothesis of an indirect mechanism in the control of inflammation mediated by $CD4^{+}CD25^{+}$ T cells.

Taken together, the data show that adoptive transfer of regulatory T cell activity has the potential to reverse established inflammation leading to cure of colitis. However, the cured animals have not yet been observed over an extended time period and it is still unclear whether these mice can tolerate normal conventional environments including a higher risk for developing chronic infections and neoplastic diseases—issues that are of major importance for the practical use of adoptive regulatory T cell therapy in IBD.

12 Conclusion

The animal models of colitis have contributed to our understanding of the etiology, pathogenesis, immune pathology and the course of disease. Although the nature of antigens recognized by immune cells in IBD is still unknown, the triggering factors are most certainly of bacterial origin. Colitis in most experimental systems appears to be a result of a hyper-reactive Th1-mediated immune response due to lack of regulatory T cells. It is clear that Treg cells can inhibit colitis and even reverse established inflammation, leading to cure of disease. Treg cells perform multiple functions in the immune system, which altogether contribute to maintaining immune homeostasis. Recent observations open up the opportunity to use Treg cells in cellular therapy of patients with IBD. Results showing that regulatory $CD4^{+}CD25^{+}$ T cells maintain their ability to suppress after proliferation are important in the light of clinical use where induced expansion of autologous $CD4^{+}CD25^{+}$ T cells ex vivo or in vivo might be necessary. A better understanding of the physiology and pathology of regulatory T cells and the connection between its various subtypes will hopefully improve future therapies of the inflammatory diseases.

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