Harnessing Regulatory T Cells for the Treatment of Inflammatory Bowel Disease

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Abstract: Regulatory CD4+ T (Treg) cells are comprised of a heterogeneous population of cells that play a vital role in suppressing inflammation and maintaining immune tolerance. The immunoregulatory function of Treg cells is especially important in the intestine where the mucosa is exposed to a diverse array of foreign antigens—including those derived from food and commensal bacteria. Treg cells are enriched in the intestinal lamina propria and provide a crucial function in promoting tolerance to enteric antigens while modulating tissue inflammation. Correspondingly, Treg cell dysfunction is associated with a breakdown in intestinal tolerance and the induction of aberrant immune responses that may contribute to the pathogenesis of inflammatory bowel disease. This review will provide a brief overview of Treg cell biology with a focus on Foxp3+ Treg and type 1 regulatory (Tr1) cells and summarize the evidence for defective Treg cells in experimental and human inflammatory bowel disease. The potential application of Treg cells as a treatment for inflammatory bowel disease will also be discussed in the context of Treg infusion therapy and the in vivo induction/expansion of intestinal Treg cells.

Key Words: intestine, regulation, lymphocyte, colitis

The intestinal immune system plays an important role in promoting tolerance to the plethora of antigens that interface with the mucosa while protecting against colonization and invasion by pathogens. As an important organ for the absorption of nutrients, the intestine also harbors trillions of bacteria that collectively comprise the microbiota.1,2 The mucus that covers the epithelium helps to establish a physical barrier between the host and the microbiota, but nonetheless, the intestinal epithelium and underlying immune cells are exposed to foreign antigens from the environment—including those from food and bacteria.3,4 Consequently, the intestinal immune system must properly distinguish between antigens originating from innocuous sources and those from pathogens and elicit appropriate tolerogenic or proinflammatory immune responses. By balancing host defense and tolerance to enteric antigens, immune homeostasis is maintained in the intestine through a network of interactions involving the microbiota, epithelium, and immune cells in the lamina propria (LP) and gut-associated lymphoid tissues (GALT).5

A breakdown of the tolerance established by the intestinal immune system results in dysregulated immune responses against the microbiota and chronic intestinal inflammation as seen in inflammatory bowel disease (IBD).6 The term IBD collectively refers to Crohn’s disease (CD) and ulcerative colitis, which are chronic, relapsing inflammatory disorders of the gastrointestinal tract. The pathogenesis of IBD is thought to arise from a complex set of interactions involving genes, environment, microbiota, and the immune system. Research investigating the mechanisms contributing to IBD has elucidated key features that include a dysfunctional mucus and intestinal epithelial barrier and alterations in the microbiota composition (dysbiosis), leading to uncontrolled innate and adaptive immune responses.7–15 The inappropriate immune response toward the microbiota underscores the importance of proper immunoregulation and tolerance in the intestine.

Regulatory T (Treg) cells are an important component of the adaptive immune system that suppresses inflammation and helps to maintain homeostasis. The Treg compartment comprises a heterogeneous population in terms of development, phenotype, and suppressive functions. Recently, a system of nomenclature has been proposed to describe these various Treg cells and will be implemented into this review.16 Foxp3+ Treg cells are one Treg cell subset that constitutively expresses Foxp3 and the high-affinity α-chain of interleukin (IL)-2 receptor, CD25.17,18 Foxp3+ Treg cells can arise from 2 developmentally distinct pathways in vivo: Foxp3+ thymically derived (iTreg) cells or Foxp3+ peripherally derived (p)Treg cells, which are naive CD4+ T cells that upregulate Foxp3 in extrathymic tissues and become functionally suppressive.19 Foxp3+ Treg cells may also be induced in vitro from naive CD4+ T cells in specific culture conditions containing transforming growth factor-β1 (TGF-β1) and are referred to as in vitro-induced Foxp3+ (iTreg) cells.

Received for publication November 24, 2014; Accepted January 9, 2015.
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Supported by National Institutes of Health Grants 1R01DK097256 (to T.L.D.) and 1F30DK097904-03 (to D.G.).
The authors have no conflicts of interest to disclose.
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DOI 10.1097/MIB.0000000000000343
Published online 19 March 2015.
cells. Type 1 regulatory (Tr1) cells represent another subset that is characterized primarily by their robust production of IL-10 and lack of Foxp3 expression.20

Foxp3+ Treg and Tr1 cells are enriched in the intestinal LP and GALT where they function to maintain immune tolerance to enteric antigens.21,22 Animal studies have demonstrated that a deficiency or dysfunction of Treg cells contributes to the development of chronic inflammatory conditions—including intestinal inflammation. Furthermore, the adoptive transfer of Treg cells helps to prevent and treat experimentally induced colitis.23,24 These findings suggest that impairments in Treg cell development or immunosuppressive functions may contribute to the pathogenesis of IBD and that Treg cells may serve as a therapeutic target for IBD. This review will summarize the functions of Treg cells in the intestine during steady state and inflammation as well as highlight their use as a potential therapy for IBD.

DEVELOPMENT OF FOXP3+ TREG AND TR1 CELLS

Central features pertaining to the development and function of Treg cells are important to consider because differences in these parameters may be exploited for IBD immunotherapy. Foxp3+ tTreg cells arise as a distinct lineage of CD4 single positive thymocytes following high-affinity interactions between the T cell receptor (TCR) and major histocompatibility complex (MHC) and costimulatory signals.25,26 Thymic selection of tTreg cells is distinct from conventional T cells in that high-affinity interactions with self-antigens and MHC does not invoke apoptosis through negative selection but instead promotes survival, a process termed “agonist selection.”26 Accordingly, the TCR reactivity of Foxp3+ tTreg cells was originally thought to be directed toward self-antigens. However, Pacholczyk et al27 recently reported that high-affinity, autoreactive TCRs were not required for tTreg development, and in fact, the TCR repertoire of tTreg cells can react to bacterial antigens from the colon.28 CD4 single positive thymocytes selected along the tTreg cell lineage become CD25+Foxp3+ precursor cells that subsequently upregulate Foxp3 in response to cytokines activating the STAT5 signaling pathway.29,30 The intracellular signaling events during tTreg cell development yields distinct epigenetic patterns that distinguish them from pTreg or iTreg cells. For example, tTreg cells exhibit CpG hypomethylation of Treg-associated genes and a unique methylation pattern of the Treg-specific demethylation region compared with iTreg and conventional T cells.31,32 Foxp3+ tTreg cells may also be distinguished from extrathymic Treg cells based on their higher expression of Helios, an Ikaros transcription factor family member, and the cell surface molecule, Neuropilin-1.33,34 After thymic selection and egress, Foxp3+ tTreg cells seed peripheral tissues including the intestine beginning at postnatal day 3 in mice,35 whereas in humans, Foxp3+ cells have been observed in the LP of the small and large intestines as early as 23 weeks of gestation.36

In contrast to tTreg cells, Foxp3+ Treg cells can develop extra-thymically when naive CD4+ T cells are activated in the appropriate milieu, such as that in the intestinal LP and GALT, resulting in the upregulation of Foxp3 and the gain of immunosuppressive functions. One cytokine important for extrathymic Treg cell differentiation is TGF-β1, which is secreted by epithelial cells and T cells in the intestine.37–39 Also produced in the intestine is retinoic acid (RA), a vitamin A metabolite, that potentiates extrathymic Treg differentiation and imprints CD4+ T cells for intestinal homing through the upregulation of CCR9 and z4β7.40,41 Intestinal DCs and macrophages express retinaldehyde dehydrogenases, the enzymes important for the biosynthesis of RA, and are adept at promoting Foxp3+ Treg cell differentiation.42–45 Previous animal studies examining intestinal antigen presentation and oral tolerance provide evidence that intestinal Foxp3+ pTreg cells can differentiate in the mesenteric lymph nodes (mLN) on antigen stimulation by migratory CD103+ LP DCs.46 Thereafter, a subset of activated CD4+ T cells become gut-tropic Foxp3+ Treg cells that home to the intestinal LP and expand in response to IL-10 produced by CX3CR1+ macrophages.57

Tr1 cells represent another subset of Treg cells that are characterized by robust production of IL-10, low expression of IL-2, poor proliferative capacity, and lack of Foxp3 expression.20 Unlike T-helper (Th)1 and Th2 cells, Tr1 cells express low levels of interferon-γ and do not subsequently express IL-4. Initially, in vitro experiments demonstrated the differentiation of naive CD4+ T cells into Tr1 cells when activated in the presence of IL-10; however, both IL-10 and TGF-β1 are important in regulating their development in vivo.22 Phenotypically, CD49b and lymphocyte activation gene 3 (LAG-3) are preferentially co-expressed on Tr1 cells in humans and mice, and similar to Foxp3+ Treg cells, Tr1 cells are enriched in the intestine and GALT.48

Collectively, Treg cells possess different immunosuppressive functions and play an important role in regulating the intestinal immune system. Through the secretion of anti-inflammatory cytokines and the engagement of immunoregulatory cell surface molecules, Treg cells inhibit proinflammatory cytokine production, downregulate costimulatory molecules on antigen presenting cells, and modulate T cell proliferation and differentiation.49–52 Specific cytokines, including TGF-β1, IL-10, and IL-35, serve not only to dampen the immune response but are also involved in pTreg and iTreg cell differentiation.50,51,52 In vivo imaging of Treg cells has highlighted the propensity of these cells to engage in stable, long-lasting interactions with DCs in lymph nodes, which results in the inhibition of CD4+CD25+ T cell priming.53 Thus, DCs, macrophages, and other antigen presenting cells in the intestine and mLN may be important immunomodulatory targets for Treg cells.54–56 Treg cells may also express cell surface CD39 and CD73, which can catalyze ATP, ADP, and AMP to produce the immunosuppressive metabolite, adenosine.57–59 The function of CD39 and CD73 is particularly relevant in the intestine where ATP produced by host cells and the microbiota can support the development of colitogenic Th17 cells.60 Accordingly, catabolism of ATP by Treg cells may function to limit pro-inflammatory Th17 responses in the intestine. Additional mechanisms of Treg cell-mediated suppression include cytolyis of effector T cells mediated by the granzyme–perforin pathway.61,62 and deprivation of proliferating T cells from pro-survival cytokines, like IL-2, that is...
associated with the induction of colitogenic T cell apoptosis\textsuperscript{63} (Fig. 1). Taken together, T\textsubscript{reg} cells are capable of using a variety of immunosuppressive mechanisms to suppress inflammation and promote tolerance in the intestine.

**EVIDENCE FOR DEFECTIVE T\textsubscript{REG} CELLS IN IBD**

The importance of T\textsubscript{reg} cells in maintaining intestinal immune homeostasis is supported by numerous experimental model systems where intestinal inflammation develops in the absence of functional T\textsubscript{reg} cells. The original description of T\textsubscript{reg} cells preventing colitis in mice by Powrie et al.\textsuperscript{23} and Morrissey et al.\textsuperscript{64} established that naive CD4\textsuperscript{+}CD45RB(hi) T cells induced chronic colitis when transferred into lymphopenic (SCID- or RAG-deficient) mice and that disease could be prevented by cotransfer of either total CD4\textsuperscript{+} T cells or CD4\textsuperscript{+}CD45RB(lo) T cells. The expansion and differentiation of pro-inflammatory CD4\textsuperscript{+} T cells in this model system requires the microbiota,\textsuperscript{23,65-67} suggesting that T\textsubscript{reg} cells are required to suppress colitogenic CD4\textsuperscript{+} T cell responses against bacterial antigens.\textsuperscript{24,50} The importance of Foxp3\textsuperscript{+} T\textsubscript{reg} cells in maintaining immune homeostasis in immunologically replete hosts is exemplified by patients with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) and scurfy mice.\textsuperscript{68-70} Mutations in the Foxp3 gene of IPEX patients and scurfy mice lead to a global failure of Foxp3\textsuperscript{+} T\textsubscript{reg} cell development and subsequent autoimmune destruction of various organs including the skin, endocrine glands, and intestines. Additionally, mice with a deletion in the conserved noncoding sequence 1 of the Foxp3 locus—such that they have impairments in the generation of pT\textsubscript{reg} cells—spontaneously develop severe Th2-type inflammation in the lung and gastrointestinal tract.\textsuperscript{71} Together, these findings demonstrate that a failure in iT\textsubscript{reg} or pT\textsubscript{reg} cell development is associated with immune dysregulation at mucosal surfaces.

T\textsubscript{reg} cell deficiency and intestinal inflammation can also be instigated by a defect in T\textsubscript{reg} cell survival. Foxp3\textsuperscript{+} T\textsubscript{reg} cells from mice deficient in IL-2, IL-2R\alpha, or the Wiskott–Aldrich syndrome protein (WASp) develop normally in the thymus and are functionally suppressive in vitro. However, Foxp3\textsuperscript{+} T\textsubscript{reg} cells from these mice exhibit decreased survival in peripheral tissues that correlates with increased susceptibility to autoimmunity and spontaneous colitis.\textsuperscript{72-76} In line with these findings, human genetic studies have reported, H2, H2ra, and Wasp to be IBDSusceptibility genes.\textsuperscript{77-79} and patients with WAS have an increased risk of developing autoimmune disease and inflammatory conditions—including IBD.\textsuperscript{80} Thus, poor survival of T\textsubscript{reg} cells in peripheral tissues may lead to chronic intestinal inflammation.

Beyond T\textsubscript{reg} cell survival, functional impairments in T\textsubscript{reg} cells may also contribute to the pathogenesis of IBD. T\textsubscript{reg} cells from mice deficient in cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), IL-35, IL-10, or LAG-3 are unable to effectively suppress T-cell proliferation in vitro and cannot prevent chronic T cell–mediated colitis in vivo.\textsuperscript{53,81-85} Furthermore, deletion of specific immunosuppressive mechanisms in the T\textsubscript{reg} cell compartment may augment the production of proinflammatory cytokines and subsequently drive chronic inflammation. For example, Foxp3\textsuperscript{+} T\textsubscript{reg} cell–specific ablation of CTLA-4 leads to a lymphoproliferative disease and multorgan autoimmunity, whereas deletion of IL-10 in Foxp3\textsuperscript{+} T\textsubscript{reg} cells induces microbiota-driven colitis.\textsuperscript{84,85} In line with these findings, polymorphisms in the genes for CTLA-4 and IL-10 receptor are associated with IBD.\textsuperscript{56-68} Although the absence or functional impairment of T\textsubscript{reg} cells leads to intestinal inflammation, it is particularly important to note that Foxp3\textsuperscript{+} T\textsubscript{reg} cells cannot only prevent intestinal inflammation but can also treat established colitis in experimental models.\textsuperscript{24,31,39,90} These studies demonstrate the feasibility of adoptive T\textsubscript{reg} cell immunotherapy for reversing established intestinal inflammation in humans.

**POTENTIAL FOR AUTOLOGOUS T\textsubscript{REG} INFUSION THERAPY IN IBD**

Many experimental studies have demonstrated that T\textsubscript{reg} cells are potently immunosuppressive, and their dysfunction can lead to the development of chronic inflammatory disorders and autoimmune disease. If T\textsubscript{reg} cells are indeed defective in patients with IBD, a potential therapeutic approach would be to correct the deficiency or dysfunction through autologous T\textsubscript{reg} cell infusion. The feasibility of using T\textsubscript{reg} cell immunotherapy to treat established inflammation in humans is supported by the efficacy of autologous T\textsubscript{reg} cell infusion for graft-versus-host disease after

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**FIGURE 1.** Mechanisms of T\textsubscript{reg} cell–mediated suppression. T\textsubscript{reg} cells inhibit pro-inflammatory cytokine production, impede antigen presentation, and/or modulate T cell survival through the secretion of immunosuppressive cytokines (IL-10, TGF-β1, and IL-35) and the engagement of immunoregulatory cell surface molecules (CTLA-4 and LAG-3) with their respective ligands on target cells. Additional mechanisms of T\textsubscript{reg} cell–mediated suppression include the conversion of ATP to adenosine by CD73 and CD39, IL-2 deprivation of effector T cells, and granzyme (GZMB)-or perforin-dependent killing of responder T cells. 186 × 139 mm (300 × 300 DPI).
Clinical trials are also underway exploring T\textsubscript{reg} cell infusion therapy in type 1 diabetes mellitus (https://clinicaltrials.gov/ct2/show/NCT01210664).

In order for infused T\textsubscript{reg} cells to most effectively control the inflammation present in patients with IBD, careful consideration of T\textsubscript{reg} cell purity, homing ability, antigen-specificity, and survival will likely aid in the development of a potent treatment regimen. Autologous T\textsubscript{reg} cells isolated from the human peripheral blood mononuclear cell fraction and prepared for infusion must be highly pure to ensure that other immune cells are not a source of contamination.\textsuperscript{92} This is not a trivial task since some cell surface markers, such as CD25, used to isolate human peripheral blood T\textsubscript{reg} cells can also be expressed by activated, conventional CD4\textsuperscript+ T cells. Additionally, since naïve CD4\textsuperscript+ T cells are abundant in blood, they can be isolated from peripheral blood mononuclear cells and differentiated into iT\textsubscript{reg} cells when activated in the presence of TGF-β.\textsuperscript{93} Following isolation, iT\textsubscript{reg} or iT\textsubscript{reg} cells can be expanded in vivo, and the purity of the iT\textsubscript{reg} and iT\textsubscript{reg} cells can be verified by assessing Foxp3 expression and the methylation status of the TDSR prior to infusion. Similarly, Tr1 cells have been cloned from the blood and expanded in vivo using feeder cells transfected to express anti-CD3, CD80, CD58, IL-2, and IL-4.\textsuperscript{94} Following expansion, the purity of Tr1 cells can be verified using flow cytometry and enzyme-linked immunosorbent assay for IL-10 production.

To maximize the therapeutic efficacy, infused T\textsubscript{reg} cells should efficiently migrate to the mLN and/or intestinal LP to become optimally suppressive.\textsuperscript{95,96} The localization of T\textsubscript{reg} cells to the inflamed intestinal LP may serve to regulate ongoing proinflammatory immune responses while T\textsubscript{reg} cells in the mLN may function to inhibit antigen presentation and the amplification of T and B cell-mediated priming in the draining LN. The homing of T\textsubscript{reg} cells to the mLN and intestinal LP is influenced by the expression of specific integrins and chemokine/chemokine receptor axes.\textsuperscript{97} Efficient migration of infused T\textsubscript{reg} cells to inflamed intestinal tissue may be facilitated by supplementing RA in T\textsubscript{reg} cell cultures to induce upregulation of the gut homing receptors, CCR9 and α4β7.\textsuperscript{98} Interestingly, RA has been primarily described for lymphocyte homing to the small intestine and recent data suggest that the G-coupled protein receptor 15 (GPR15) is important for CD4\textsuperscript+ T cell homing to the colon.\textsuperscript{99} Mice deficient in GPR15 exhibited a reduction in colonic Foxp3\textsuperscript+ T\textsubscript{reg} cells that was associated with heightened pro-inflammatory responses in intestine when challenged with Citrobacter rodentium infection. If GPR15 also regulates migration of CD4\textsuperscript+ T cells to the human colon, exploiting factors that modulate CCR9, α4β7, and/or GPR15 expression may be important for targeting T\textsubscript{reg} cells to the inflamed tissues in IBD patients. Future studies investigating homing requirements are warranted to optimize T\textsubscript{reg} cell localization to the intestine during acute and chronic inflammation.

Given that TCR-mediated stimulation of T\textsubscript{reg} cells is required for eliciting immunosuppressive functions, the antigen reactivity of T\textsubscript{reg} cells is another important consideration for the treatment of IBD.\textsuperscript{100,101} Although the specific antigens involved in the pathogenesis of IBD remain poorly understood, the requirement for the microbiota is well supported experimentally.\textsuperscript{102} Animal studies demonstrating a relationship between the gut microbiota and colitis are corroborated by genetic knock-out mouse strains, such as the Il10\textsuperscript{-/-} mice, and the CD45RB(hi) CD4\textsuperscript+ T cell transfer model of colitis—both of which do not develop colitis in the absence of the gut microbiota.\textsuperscript{103,104} Furthermore in the context of active colitis, immune reactivity to the microbiota has been observed as antibodies generated against flagellin are detected in mice and humans, and CD4\textsuperscript+ T cell clones isolated from IBD patients are reactive to specific components of the microbiota.\textsuperscript{105,106}

These data implicate a role for the microbiota in the development of IBD, and thus, T\textsubscript{reg} cells reactive to bacterial and/or food antigens may be most effective in suppressing proinflammatory responses since stimulation of T\textsubscript{reg} cells is required for suppressive ability.\textsuperscript{100,101} Interestingly, a recent phase I/IIa clinical trial demonstrated the feasibility of infusing T\textsubscript{reg} cells reactive to ovalbumin (OVA), a food antigen, to treat refractory Crohn’s disease.\textsuperscript{107} In this trial, OVA-specific Tr1 cells were cloned by limiting dilution from human peripheral blood mononuclear cells and expanded ex vivo. T\textsubscript{reg} cell infusion doses ranging from 10\textsuperscript{5} to 10\textsuperscript{6} cells were well tolerated, and after infusion, patients were provided an OVA-enriched diet. A clinically significant improvement was observed in 40% of the patients with a peak effect at week 5 post-infusion. These results highlight the potential for using known antigens to provide TCR-mediated stimulation of infused T\textsubscript{reg} cells to activate their suppressive functions in the intestines of patients with IBD.

After infusion into patients with IBD, T\textsubscript{reg} cells should persist to maximize their immunosuppressive functions in the intestine. However, apoptosis of Foxp3\textsuperscript+ T\textsubscript{reg} cells is increased in inflamed colonic tissue and the peripheral blood of patients with IBD indicating that T\textsubscript{reg} cell survival may be impaired by pro-inflammatory cytokines.\textsuperscript{108} This increase in apoptosis of Foxp3\textsuperscript+ T\textsubscript{reg} cells was reversed in patients that responded to anti-TNF-α treatment—suggesting that blockade proinflammatory cytokines may improve T\textsubscript{reg} cell survival. An alternative approach to consider for promoting T\textsubscript{reg} cell survival after infusion into patients with IBD would be administration of IL-2 and folate acid (FA), which have been shown to help maintain Foxp3\textsuperscript+ T\textsubscript{reg} cells in peripheral tissues.\textsuperscript{72,109} Since CD25 and folate receptor 4 (FR4) are constitutively expressed by Foxp3\textsuperscript+ T\textsubscript{reg} cells, low-dose administration of IL-2 and a diet enriched in FA may preferentially enhance survival of Foxp3\textsuperscript+ T\textsubscript{reg} cells.\textsuperscript{110-112} In fact, low-dose IL-2 was reported to increase Foxp3\textsuperscript+ T\textsubscript{reg} cell proliferation, thymic export, and improve T\textsubscript{reg} resistance to apoptosis.\textsuperscript{113} In mice, FA seems to play a role in Foxp3\textsuperscript+ T\textsubscript{reg} cell survival and immune homeostasis because colonic Foxp3\textsuperscript+ T\textsubscript{reg} cells from mice fed a diet deficient in FA were highly proliferative and exhibited an increased sensitivity to apoptosis. Furthermore, supplementation with FA protected mice from experimental colitis as survival was higher and disease activity was reduced when compared with mice fed a FA-deficient diet. Because patients with IBD are susceptible to nutrient deficiencies that coincide with the pro-inflammatory milieu in the intestine, the survival of Foxp3\textsuperscript+ T\textsubscript{reg} cells may be improved with IL-2 and FA supplementation.
THERAPEUTIC EFFICACY OF T\text{REG} CELLS IN IBD

When considering T\text{REG} cells as a potential therapy for IBD, it will be valuable to know whether iT\text{REG} cells, tT\text{REG} cells or a combination of thereof will most effectively resolve inflammation. Several studies assessing the efficacy of iT\text{REG} and tT\text{REG} cells to treat chronic colitis using the CD4+ CD45RB(hi) T cell transfer mouse model have provided important insight into this issue. Adoptive transfer of either iT\text{REG} or tT\text{REG} cells was sufficient to ameliorate experimental colitis.\textsuperscript{24,31,89} However, the caveat of using wild-type, naive CD4+ T cells in the T cell transfer model of colitis is that a small fraction of these cells differentiate into pT\text{REG} cells in vivo which can affect the course of disease.\textsuperscript{90} Thus, when testing the functional capacity of adaptively transferred iT\text{REG} cells to treat established colitis in this model, it is important to appreciate that iT\text{REG} cells may be working in concert with pT\text{REG} cells. Interestingly, the transfer of iT\text{REG} cells alone into RAG1-deficient mice was able to treat established colitis, and in fact, iT\text{REG} cells were shown to have a functional advantage over iT\text{REG} cells.\textsuperscript{90,114} There is also evidence to support a functional requirement for a combination of iT\text{REG} and iT\text{REG} cells to treat intestinal inflammation and prevent autoimmune disease. When CD4+ CD45RB(hi) T cells from Foxp3\textsuperscript{EGFP} mice were used to promote the development of chronic colitis in RAG1-deficient hosts, and consequently, Foxp3+ pT\text{REG} cells could not develop from these donor cells in vivo, the treatment of colitic mice with iT\text{REG} cells alone was unable to reverse intestinal inflammation. However, a mixture of iT\text{REG} and iT\text{REG} cells transferred into colitic mice afforded maximal effectiveness in the treatment of intestinal inflammation—indicating that iT\text{REG} and iT\text{REG} cells may function together.\textsuperscript{90} Although evidence suggests that iT\text{REG} and iT\text{REG} cells can treat colitis in mice, additional studies are needed to fully examine the functional relationship between iT\text{REG}, pT\text{REG}, and iT\text{REG} cells in vivo and their efficacy for the treatment of patients with IBD.

ENHANCING THE FUNCTION OF ENDOGENOUS T\text{REG} CELLS

Aside from T\text{REG} infusion therapy for the treatment of IBD, the intestinal microenvironment may also be manipulated to promote pT\text{REG} cell development and/or expansion of iT\text{REG} cells through the administration of mesenchymal stem cells (MSCs), supplementation of vitamins A and D, and/or reconstitution of the microbiota with specific commensal bacteria (Fig. 2). The anti-inflammatory cytokines and metabolites generated as a result of these treatment modalities can also act on different innate and adaptive immune components to quell intestinal inflammation.

Mesenchymal Stem Cells

MSCs are nonhematopoietic progenitor cells that are capable of differentiating into various cell types and possess immunoregulatory properties important for suppressing inflammation and inducing T\text{REG} cell differentiation. MSCs can inhibit immune activation in vitro and ameliorate disease in different animal models of autoimmunity and inflammatory disorders—including IBD.\textsuperscript{115,116}

In the context of experimental colitis, the infusion of MSCs can reduce disease activity and pro-inflammatory cytokines while inducing T\text{REG} cells in the mLN and colon.\textsuperscript{117,118} Given these promising experimental results, the efficacy of MSC infusion is now being investigated in clinical trials for IBD. The low immunogenicity of MSCs due to poor MHC class II expression and the ability to isolate and expand these cells from various tissues, such as the bone marrow and adipose tissue, supports the feasibility of MSC infusion therapy in translational medicine.\textsuperscript{119} Thus far, clinical studies have reported MSC infusion therapy to be safe for patients with CD. One study investigating autologous MSC infusion for fistulizing CD observed complete closure in 7 of 12 patients, which was associated with a reduced disease activity, and an induction in circulating and mucosal T\text{REG} cells.\textsuperscript{120} With these promising results, additional clinical trials are underway investigating MSC infusion therapy for CD and ulcerative colitis (www.clinicaltrials.gov).

Dietary Factors

Vitamin A is an important dietary factor that can modulate intestinal immune cell function. In the intestine, vitamin A is metabolized to RA, which has pleiotropic effects on intestinal immune cells and regulates processes including gut homing of
lymphocytes, intestinal IgA production, development of specific DC subsets, and Foxp3+ Treg cell differentiation.\textsuperscript{41–43,98,121–123} The breadth of intestinal immune cell functions affected by RA indicates that vitamin A metabolism is important for immune homeostasis. Indeed, impairments in RA signaling results in reduced intestinal Foxp3+ Treg cell development in vivo,\textsuperscript{40} and vitamin A deficiency is associated with the induction of colitis.\textsuperscript{124} Because patients with IBD are particularly susceptible to malabsorption, administration of vitamin A or RA may help to stimulate endogenous Foxp3+ Treg cell differentiation and correct a dysregulated intestinal immune system.

In addition, vitamin D is a precursor for calcitriol (1,25 dihydroxy-vitamin D), which maintains calcium and phosphate balance, regulates bone formation, and more recently has been shown to promote Treg cell differentiation and suppress immune responses.\textsuperscript{125} In the intestine, vitamin D and vitamin D receptor (VDR) signaling helps to maintain epithelial integrity and ameliorate chronic colitis in mouse models.\textsuperscript{126,127} Epidemiological and genetic studies in humans also support a relationship between vitamin D and IBD. Low serum vitamin D in patients with IBD is linked to higher disease risk and morbidity, and polymorphisms in the Vdr gene are associated with IBD.\textsuperscript{128–132} Based on these findings, supplementation of vitamin D may have therapeutic effects for patients with IBD by inducing intestinal Treg cells to suppress inflammation and alleviate disease.

**Microbiota**

Recent studies investigating the relationship between the gut microbiota and Treg cells indicate that reconstitution of IBD patients with specific commensal bacteria may help to correct dysbiosis and promote Treg cell development during intestinal inflammation. In mice, Bacteroides fragilis and specific Clostridia strains have been reported to induce intestinal Treg cells in the colon.\textsuperscript{133–136} Polysaccharide A (PSA) produced by B. fragilis can promote the development of IL-10-producing Treg cells through plasmacytoid DCs\textsuperscript{135} and also acts on TLR2 expressed on Treg cells to eliciting immunosuppressive functions.\textsuperscript{134,137} The administration of PSA into mice was sufficient to prevent and cure experimental colitis, which highlights the possibility of using specific bacterial components for expanding intestinal Treg cells and inhibiting established colitis. Additionally, a mixture of 17 Clostridia strains derived from the commensal human microbiota was discovered to produce short-chain fatty acids (SCFAs), such as butyrate, that were associated with the secretion of TGF-β1 by the intestinal epithelium and Foxp3+ Treg cell development in the mouse colon.\textsuperscript{136} Mice colonized by the Clostridia mixture were protected in 2 models of experimental colitis, and these results indicate that reconstitution of IBD patients with specific commensal bacteria or their metabolites may be a novel therapeutic approach to treat intestinal inflammation. Indeed, several recent reports have shown that the SCFAs generated by the microbiota mediate epigenetic modifications that promote Foxp3+ Treg cell differentiation and function.\textsuperscript{138–140} Administration of specific SCFAs alone or in combination provided protection against experimental colitis in mice, and this was associated with a decrease in pro-inflammatory cytokine production and the induction of colonic Foxp3+ Treg cells. Accordingly, the administration of SCFAs may aid in the treatment of patients with IBD by expanding the endogenous Foxp3+ Treg cell compartment to suppress intestinal inflammation.

Probiotics may serve as another means to promote intestinal Foxp3+ Treg induction in patients with IBD. In a study by Kwon et al.,\textsuperscript{141} a probiotic mixture containing Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus reuteri, Bifidobacterium bifidum, and Streptococcus thermophilus significantly increased tolerogenic DCs and Treg cells in the mLN while decreasing pro-inflammatory cytokine expression and proliferation of splenic CD4+ T cells.\textsuperscript{141} Mice treated with this probiotic mixture were also protected against trinitrobenzene sulfonic acid (TNBS)-induced colitis. Together, these findings indicate that reconstitution of IBD patients with specific commensal bacteria and/or probiotics may help to correct dysbiosis and ameliorate dysregulated immune responses by promoting intestinal Treg cell and tolerogenic DC development. This concept of correcting dysbiosis with reconstitution of the gut microbiota from healthy individuals is currently being applied in the context of pseudomembranous colitis mediated by Clostridium difficile. Fecal transplants from healthy donors into C. difficile–infected patients has improved clinical outcome and is now under investigation for treating IBD.\textsuperscript{142–147}

**RESISTANCE TO T_{REG}-MEDIATED SUPPRESSION IN IBD?**

Treg cell dysfunction in patients with IBD can also arise as a result of the inflammatory milieu in the intestine. Proinflammatory cytokines and metabolites produced by immune cells may inhibit Treg cell function either by inhibiting specific suppressive mechanisms or by altering the stability of the Treg cell phenotype. Although effects of pro-inflammatory cytokines in modulating Treg cell suppressive mechanisms have not been fully elucidated, Foxp3+ Treg cells are reported to lose Foxp3 expression and gain the ability to secrete proinflammatory cytokines when present in an inflammatory milieu.\textsuperscript{82,148} Therefore, a potential risk with Treg cell–based therapy for IBD may be the conversion of Foxp3+ Treg cells into pathogenic effector T cells in inflamed tissues. Consequently, long-term studies evaluating the plasticity and pathogenic potential of Treg cells in patients with IBD are warranted. This is especially important when considering immunotherapy using iTreg cells, which may be less stable than Treg cells.\textsuperscript{31,82}

Besides altering the function and differentiation of Treg cells, the inflammatory tissue environment in IBD may also decrease the responsiveness of effector T cells to apoptosis and Treg cell–mediated suppression in IBD. The proinflammatory cytokines IL-6, IL-12, and TNF-α that are secreted during intestinal inflammation can inhibit apoptosis, which may allow pathogenic CD4+ T cells to persist and perpetuate the dysregulated immune response.\textsuperscript{149} Treatment of patients with neutralizing antibodies to these proinflammatory cytokines have yielded promising results.
and infliximab, an anti-TNF biologic, has become an FDA-approved drug for both ulcerative colitis and CD.\textsuperscript{150–156} Additionally, CD3\textsuperscript{+} T cells from the inflamed mucosa of patients with IBD express high levels of Smad7, an inhibitor of TGF-\textbeta1 signaling, that can shield pathogenic T cells against the immunosuppressive effects of TGF-\textbeta1 from Trg cells.\textsuperscript{157–159} The elevated levels of Smad7 are associated with a decrease in phosphorylated Smad3, which is a downstream signal transducer of the TGF-\textbeta1 receptor. Treatment of LP mononuclear cells from CD patients with antisense Smad7 increased phosphorylation of Smad3 and attenuated production of TNF-\textalpha and interferon-\gamma.\textsuperscript{160} These results were also observed in mouse models of experimental colitis using TNBS and oxazolone. Oral administration Smad7 antisense oligonucleotides ameliorated disease making Smad7 an attractive therapeutic target. Resistance of pathogenic T cells to Trg cell–mediated suppression has been demonstrated in other chronic inflammatory diseases,\textsuperscript{161–163} and the efficacy of Trg cell therapy alone would be hampered if the inflammatory milieu does indeed promote pathogenic T cells to be resistant to the suppressive mechanisms of Trg cells. Elucidating additional mechanisms by which the inflammatory microenvironment can induce such resistance and translating this knowledge to develop therapies that reestablish immunoregulation and Trg cell–mediated suppression would be imperative for the treatment of IBD.

**CONCLUSIONS**

Important insights into Trg cell biology have facilitated the translation of seminal discoveries from experimental model systems to clinical trials using Trg cell–based therapies for the treatment of IBD. Thus far, several clinical trials have supported the feasibility of ex vivo Trg cell production and validated the safety of Trg cell infusion therapy with promising results. Recent developments in mucosal immunology pertaining to host–microbe interactions have highlighted potential new approaches for IBD therapy and mechanistic insights into the environmental role of diet, the microbiota and their derivatives, and probiotics in maintaining gastrointestinal health. Given the complex, multifactorial pathogenesis of IBD, Trg cell-based therapies may have the potential to significantly improve the mortality and morbidity associated with IBD when utilized in conjunction with other treatment modalities to correct the dysbiosis and inflammatory milieu. For example, an expanded approach for the treatment of IBD could theoretically entail Trg cell infusion in combination with manipulation of the microbiota and neutralization of specific proinflammatory cytokines. Correction of the dysbiosis and attenuation of the inflammatory milieu during delivery of Trg cells may establish a tissue environment conducive for infused or endogenous Trg cells to reestablish tolerance against the gut microbiota in patients with IBD. As additional mechanisms regulating Trg cell biology are elucidated and Trg cell-based therapies are optimized at the bench and in the clinic, these cells may eventually serve as a complementary treatment for IBD.

**REFERENCES**

1. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A.* 1998;95:6578–6583.

2. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486:207–214.

3. Pelaseyed T, Bergstrom JH, Gustafsson JK, et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Inmunol Rev.* 2014;260:8–20.

4. Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat Rev Immunol.* 2014;14:141–153.

5. Maynard CL, Elson CO, Hatton RD, et al. Reciprocal interactions of the intestinal microbiota and immune system. *Nature.* 2012;489:231–241.

6. Guarnier F. What is the role of the enteric commensal flora in IBD? *Inflamm Bowel Dis.* 2008;14(suppl 2):S83–S84.

7. Van Limbergen J, Radford-Smith G, Satansg J. Advances in IBD genetics. *Nat Rev Gastroenterol Hepatol.* 2014;11:372–385.

8. Danese S, Sans M, Fiocchi C. Inflammatory bowel disease: the role of environmental factors. *Autoimmun Rev.* 2004;3:394–400.

9. McGuckin MA, Eri R, Simms LA, et al. Intestinal barrier dysfunction in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2009;15:100–113.

10. Li J, Butcher J, Mack D, et al. Functional Impacts of the intestinal microbiome in the pathogenesis of inflammatory bowel disease. *Inflamm Bowel Dis.* 2015;21:139–153.

11. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol.* 2010;28:573–621.

12. Long M, Li X, Wegener Parfrey L, et al. The feasibility of ex vivo Treg cell therapy for the treatment of IBD. *Inflamm Bowel Dis.* 2014;20:1820–1827.

13. Tomita T, Kanai T, Fujii T, et al. Continuous generation of colitogenic CD4+ T cells in persistent colitis. *Eur J Immunol.* 2008;38:1264–1274.

14. Abbas AK, Benoist C, Bluestone JA, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol.* 2013;14:307–308.

15. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003;299:1057–1061.

16. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol.* 2003;4:330–336.

17. Saigusa K, Hisamatsu T, Hando T, et al. Classical Th1 cells obtain colitogenicity by co-existence of RORgammat-expressing T cells in experimental colitis. *Inflamm Bowel Dis.* 2014;20:1820–1827.

18. Tomita T, Kanai T, Fujii T, et al. Continuous generation of colitogenic CD4+ T cells in persistent colitis. *Eur J Immunol.* 2008;38:1264–1274.

19. Abbas AK, Benoist C, Bluestone JA, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol.* 2013;14:307–308.

20. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003;299:1057–1061.

21. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol.* 2003;4:330–336.

22. Cuortto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity.* 2009;30:626–635.

23. Groux H, O’Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature.* 1997;389:737–742.

24. Huber S, Gagliani N, Esplugues E, et al. Th17 cells express interleukin-10 receptor and are controlled by Foxp3+ and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity.* 2011;34:554–565.

25. Maynard CL, Harrington LE, Janowski KM, et al. Regulatory T cells expressing interleukin 10 receptor and are controlled by Foxp3+ and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity.* 2011;34:554–565.

26. Aschenbrenner K, D’Cruz LM, Vollmann EH, et al. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Air1+ medullary thymic epithelial cells. *Nat Immunol.* 2007;8:931–941.

27. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B17 scid mice. *Int Immunol.* 1993;5:1461–1471.

28. Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol.* 2003;170:3939–3943.

29. Aschenbrenner K, D’Cruz LM, Vollmann EH, et al. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Air1+ medullary thymic epithelial cells. *Nat Immunol.* 2007;8:351–358.

30. Jordan MS, Boesteau A, Reed AJ, et al. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat Immunol.* 2001;2:301–306.

31. Pacholczyk R, Kern J, Singh N, et al. Nonself-antigens are the cognate specificities of Foxp3+ regulatory T cells. *Immunity.* 2007;27:493–504.
28. Cebula A, Seweryn M, Rempala GA, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. Nature; 2013; 497:258–262.
29. Lio CW, Hsieh CS. A two-step process for thymic regulatory T cell development. Immunity; 2008;28:100–111.
30. Burchill MA, Yang J, Wang KB, et al. Linked T cell receptor and cyto- kinase signaling govern the development of the regulatory T cell repertoire. Immunity; 2008;28:112–121.
31. Ohkura N, Hamaguchi M, Morikawa H, et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity; 2012;37:785–799.
32. Flossa S, Freyer J, Siewert C, et al. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol. 2007;5:e38.
33. Thornton AM, Korty PE, Tran DQ, et al. Neurotrophin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. J Exp Med. 2012;209:1713–1722.
34. Sakaguchi S, Fukuma K, Kuribayashi K, et al. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J Exp Med. 1985;161:72–87.
35. Weitkamp JH, Rudzinski E, Koyama T, et al. Ontogeny of FOXP3(+) regulatory T cells in the postnatal human small intestinal and large intestinal lamina propria. Pediatr Dev Pathol. 2009;12:443–449.
36. Antonioli L, Pacher P, Vizi ES, et al. CD39 and CD73 in immunity and inflammation. PLoS Biol. 2008;28:112.
37. Deaglio S, Dwyer KM, Gao W, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204:1257–1265.
38. Bollrath J, Powrie FM. Controlling the frontier: regulatory T-cells and their role in intestinal homeostasis. Nat Immunol. 2006;7:83–92.
39. Ramalingam R, Larmonier CB, Thurston RD, et al. Dendritic cell-specific disruption of TGF-beta receptor II leads to altered regulatory T-cell gene expression and phenotype and spontaneous multorgan autoimmunity. J Immunol. 2012;189:3878–3893.
40. Burchill MA, Yang J, Wang KB, et al. Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. Immunity; 2008;28:112–121.
41. Iwata M, Hirakiyama A, Eshima Y, et al. Retinoic acid imprints gut-homing specificity on T cells. Immunity; 2004;20:257–263.
42. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, et al. A functionally distinct subset of human foetal T cells mediates inhibition of adaptive immune responses. Nature. 2012;480:449–453.
43. Sun CM, Hall IA, Blank RB, et al. Expression of Helios, an Ikaros family member, marks a new subset of regulatory T cells. J Exp Med. 2004;199:1401–1408.
44. Chen W, Jin W, Hardegen N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of Foxp3. J Exp Med. 2003;198:1875–1886.
45. Feagins LA. Role of transforming growth factor-beta in inflammatory bowel disease and colitis-associated colon cancer. Inflamm Bowel Dis. 2010;16:1963–1968.
46. Worbs T, Bode U, Yan S, et al. Foxp3+ regulatory T cells control mucosal TH2 inflammation in mice. J Exp Med. 2007;209:1713–1722.
47. Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. J Exp Med. 2004;199:1401–1408.
48. Sun CM, Hall IA, Blank RB, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med. 2010;16:1963–1968.
49. Ziegler C, Hansel H, Scholz T, et al. Thymus-derived regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Immunity; 2007:27:655–666.
50. Castaneda R, Takahashi T, Strober W, et al. Dendritic cell-mediated induction of regulatory T cells in the intestine. Nature. 2007;455:808–812.
51. González DC, Lu LF, Quezada SA, et al. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory T cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol. 2005;174:1783–1786.
52. Cao X, Cai SF, Fehninger TA, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity. 2007;27:237–244.
53. Pandiyan P, Zheng L, Ishihara S, et al. CD4+CD25+FOXP3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol. 2007;8:1353–1362.
54. Morrissey PJ, Charrier K, Braddy S, et al. CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. J Exp Med. 1993;178:237–244.
55. Oosting AV, Pavlick KP, Bharwani S, et al. T cell-mediated induction of the small and large intestine in immunodeficient mice. Am J Physiol Gastrointest Liver Physiol. 2006;290:G109–G119.
56. Feng T, Wang L, Schoeb TR, et al. Microbiota innate stimulation is a prerequisite for T cell spontaneous proliferation and induction of experimental colitis. J Exp Med. 2010;209:1321–1332.
57. Kiefer WC, Troy A, Burghardt JT, et al. Recent immune status determines the source of antigens that drive homeostatic T cell expansion. J Immunol. 2005;174:3158–3163.
58. Bennett CL, Christie J, Ramsdell F, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27:20–21.
59. Wildin RS, Ramsdell F, Peake J, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001;27:18–20.
60. Brunow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27:68–73.
61. Josefowicz SZ, Niew RE, Kim HY, et al. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012; 482:395–399.
62. Fontenot JD, Rasmussen JP, Gavin MA, et al. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol. 2005;6:1142–1151.
63. D’Cruz LM, Klein L. Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. Nat Immunol. 2005;6:1152–1159.
64. Humblet-Baron S, Sather B, Anover S, et al. Wiskott-Aldrich syndrome protein is required for regulatory T cell homeostasis. J Clin Invest. 2007; 117:407–418.
65. Maragoni F, Trifari S, Scaramuzza S, et al. WASP regulates suppressor activity of human and murine CD4(+)CD25(+)Foxp3(+) natural regulatory T cells. J Exp Med. 2007;204:369–380.

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76. Maillard MH, Cotta-de-Almeida V, Takeshima F, et al. The Wiskott-Aldrich syndrome protein is required for the function of CD4(+)CD25(+) Foxp3(+) regulatory T cells. J Exp Med. 2007;204:381–391.

77. Parkes M, Satsangi J, Jewell D. Contribution of the IL-2 and IL-10 genes to inflammatory bowel disease (IBD) susceptibility. Clin Exp Immunol. 1998;113:28–32.

78. Bouzid D, Amouri A, Fourati H, et al. Polymorphisms in the IL2RA and IL2RB genes in inflammatory bowel disease risk. Genet Test Mol Biomarkers. 2013;17:833–839.

79. Viklund IM, Kuznetsov NV, Lobberg R, et al. Identification of a new WASP and FKBP-like (W AFL) protein in inflammatory bowel disease: a potential marker gene for ulcerative colitis. Int J Colorectal Dis. 2008;23:921–930.

80. Schurman SH, Candotti F. Autoimmunity in Wiskott-Aldrich syndrome. Curr Opin Rheumatol. 2003;15:446–453.

81. Read S, Malmstrom V, Powie R. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)/CD4(+) regulatory cells that control intestinal inflammation. J Exp Med. 2000;192:295–302.

82. Schmitt EG, Haribhai D, Williams JB, et al. IL-10 produced by induced regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012;189:5635–5648.

83. Huang CT, Workman CJ, Flies D, et al. Role of LAG-3 in regulatory T lymphocytes is increased in chronic inflammatory bowel disease and reversed by anti-TNFalpha treatment. Gut. 2011;60:1345–1353.

84. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3+ regulatory T cell function. J Immunol. 2013;190:1157–1211.

85. Haribhai D, Lin W, Edwards B, et al. A central role for induced regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012;189:5635–5648.

86. Ng SC, Tsoi KK, Kamm MA, et al. Genetics of inflammatory bowel disease in interleukin-10 knockout mice. Am J Pathol. 2009;178:2665–2676.

87. Moran CJ, Walters TD, Guo CH, et al. IL-10R polymorphisms are associated with very early-onset ulcerative colitis. Inflamm Bowel Dis. 2013;19:115–123.

88. Glover EO, Kotlarz D, Boztug K, et al. Induced regulatory T cells that suppress graft-versus-host disease. Proc Natl Acad Sci U S A. 2011;108:9269–9278.

89. Mora JR, Iwata M, Eksteen B, et al. Generation of gut-homing regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012;189:5635–5648.

90. Koreth J, Matsuoka K, Kim HT, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med. 2011;365:2055–2066.

91. Saadoun D, Rosenzweig M, Joly F, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. N Engl J Med. 2011;365:2067–2077.

92. Yangaguchi T, Hirota K, Nagahama K, et al. Control of immune responses by antigen-specific regulatory T cells expressing the follicle receptor. Immunity. 2007;27:145–159.

93. Matsuoka K, Koreth J, Kim HT, et al. Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. Sci Transl Med. 2013;5:179ra143.

94. Valatas V, He J, Rivollier A, et al. Host-dependent control of early regulatory and effector T-cell differentiation underlies the genetic susceptibility to RAG2-deficient mouse strains to transfer colitis. Mucosal Immunol. 2013;6:601–611.

95. Ben-Ami E, Berrih-Aknin S, Miller A. Mesenchymal stem cells as an immunomodulatory therapeutic strategy for autoimmune diseases. Autoimmun Rev. 2011;10:410–415.

96. Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. Annu Rev Pathol. 2011;6:457–478.

97. Gonzalez MA, Gonzalez-Rey E, Rico L, et al. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterology. 2009;136:978–989.

98. Tanaka F, Tominaga K, Ochi M, et al. Exogenous administration of mesenchymal stem cell ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. Life Sci. 2008;83:771–779.

99. Ren G, Chen X, Dong F, et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med. 2012;1:51–58.

100. Ciccioccopello R, Bernardo ME, Sgarella A, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn’s disease. Gut. 2011;60:788–798.

101. Levine AG, Arvey A, Jin W, et al. Continuous requirement for the TCR in regulatory T cell function. Nat Immunol. 2014;15:1070–1078.

102. Stepankova R, Powrie F, Kofronova O, et al. Segmented nuclear factor-kappaB and collagen formation. J Nutr. 2004;134:1157–1160.

103. Lu L, Zhou X, Wang J, et al. Characterization of protective human CD4(+)CD25(+)Foxp3(+) regulatory T cells generated with IL-2, TGF-beta and retinoic acid. PLoS One. 2010;5:e15150.

104. Reifen R, Nur T, Ghebermeskel K, et al. Vitamin A deficiency exacerbates inflammation in a rat model of colitis through activation of nuclear factor-kappaB and collagen formation. J Nutr. 2002;132:2743–2747.

105. Mouil VP, Ananthakrishnan AN. Review article: vitamin D and inflammatory bowel diseases. Aliment Pharmacol Ther. 2014;39:125–136.
212. Liu, W., Chen, Y., Golan, M.A., et al. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. J Clin Invest. 2013;123:3983–3996.

213. Bruce, D., Yu, S., Ooi, J.H., et al. Converging pathways lead to overproduction of IL-17 in absence of vitamin D signaling. Int Immunol. 2011;23:519–528.

214. Khalili, H., Huang, E.S., Ananthakrishnan, A.N., et al. Geographical variation and incidence of inflammatory bowel disease among US women. Gut. 2012;61:1686–1692.

215. Ananthakrishnan, A.N., Khalili, H., Higuchi, L.M., et al. Higher predicted vitamin D status is associated with reduced risk of Crohn’s disease. Gastroenterology. 2012;142:482–489.

216. Jorgensen, S.F., Hvas, C.L., Agholt, J., et al. Active Crohn’s disease is associated with low vitamin D levels. J Crohns Colitis. 2013;7:e407–e413.

217. Xue, L.N., Xu, K.Q., Zhang, W., et al. Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn’s disease: a meta-analysis. Inflamm Bowel Dis. 2013;19:54–60.

218. Eloranta, J.J., Wenger, C., Mwinyi, J., et al. Association of a common vitamin D-binding protein polymorphism with inflammatory bowel disease. Pharmacogenet Genomics. 2011;21:559–564.

219. Dasgupta, S., Erturk-Hasdemir, D., Ochoa-Reparaz, J., et al. Plasmacytoid dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both innate and adaptive mechanisms. Cell Host Microbe. 2014;15:413–423.

220. Round, J.L., Lee, S.M., Li, J., et al. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011;332:974–977.

221. Round, J.L., Mazmanian, S.K. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci U S A. 2010;107:12204–12209.

222. Atarashi, K., Tanoue, T., Oshima, K., et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013;500:232–236.

223. Shen, Y., Giardino Torchia, M.L., Lawson, G.W., et al. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. Cell Host Microbe. 2012;12:509–520.

224. Furusawa, Y., Obata, Y., Fukuda, S., et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504:446–450.

225. Arpaia, N., Campbell, C., Fan, X., et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013;504:451–455.

226. Smith, P.M., Howitt, M.R., Panikov, N., et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341:569–573.

227. Kwon, H.K., Lee, C.G., So, J.S., et al. Generation of regulatory dendritic cells and CD4+Foxp3+ T cells by probiotics administration suppresses immune disorders. Proc Natl Acad Sci U S A. 2010;107:2159–2164.

228. Bakken, J.S., Borody, T., Brandt, L.J., et al. Treating Clostridium difficile infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol. 2011;9:1044–1049.

229. Brandt, L.J., Reddy, S.S. Fecal microbiota transplantation for recurrent clostridium difficile infection. J Clin Gastroenterol. 2011;45(suppl):S159–S167.

230. Rehlik, F., Surawicz, C.M., Stollman, N. Fecal flora reconstitution for recurrent Clostridium difficile infection: results and methodology. J Clin Gastroenterol. 2010;44:567–570.

231. Yoon, S.S., Brandt, L.J. Treatment of refractory/recurrent C. difficile-associated disease by donated stool transplanted via colonoscopy: a case series of 12 patients. J Clin Gastroenterol. 2010;44:562–566.

232. Aas, J., Gessert, C.E., Bakken, J.S. Recurrent Clostridium difficile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. Clin Infect Dis. 2003;36:580–585.

233. Kao, D., Hotte, N., Gilleveyt, P., et al. Fecal microbiota transplantation inducing remission in Crohn’s colitis and the associated changes in fecal microbial profile. J Clin Gastroenterol. 2014;48:625–628.

234. Zhou, X., Bailey-Bucktrout, S.L., Jekel, J.T., et al. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nat Immunol. 2009;10:1000–1007.

235. Mudter, J., Neurath, M.F. Apoptosis of T cells and the control of inflammatory bowel disease: therapeutic implications. Gut. 2007;56:293–303.

236. Ito, H., Takazoe, M., Fukuda, Y., et al. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn’s disease. Gastroenterology. 2004;126:989–996; discussion 947.

237. Mannon, P.J., Fuss, I.J., Mayer, L., et al. Anti-interleukin-12 antibody for active Crohn’s disease. N Engl J Med. 2004;351:2069–2079.

238. Sandborn, W.J., Gasink, C., Gao, L.L., et al. Ustekinumab induction and maintenance therapy in refractory Crohn’s disease. N Engl J Med. 2012;367:1519–1528.

239. Abtaya, R., Zimmer, M., Bartsch, B., et al. Antibodies against tumor necrosis factor (TNF) induce T-cell apoptosis in patients with inflammatory bowel diseases via TNF receptor 2 and intestinal CD14(+) macrophages. Gastroenterology. 2011;141:2026–2038.

240. van Dullemen, H.M., van Deventer, S.J., Hommes, D.W., et al. Treatment of Crohn’s disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). Gastroenterology. 1995;109:129–135.

241. Danese, S., Colombel, J.F., Peyrin-Biroulet, L., et al. Review article: the role of anti-TNF in the management of ulcerative colitis—past, present and future. Aliment Pharmacol Ther. 2013;37:855–866.

242. Pizarro, T.T., Cominelli, F. Cytokine therapy for Crohn’s disease: advances in translational research. Anna Rev Med. 2007;58:433–444.

243. Monteleone, G., Boirivant, M., Pallone, F., et al. TGF-beta1 and Smad7 in active Crohn’s disease. Gut. 2007;56:293–303.

244. Fantini, M.C., Rizzo, A., Fina, D., et al. Smad7 controls resistance of colonic T cells to regulatory T cell-mediated suppression. Gastroenterology. 2006;130:1308–1316.

245. Monteleone, G., Kumberova, A., Croft, N.M., et al. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. J Clin Invest. 2001;108:601–609.

246. Boirivant, M., Pallone, F., Di Giacinto, C., et al. Inhibition of Smad7 with antisense oligonucleotide facilitates TGF-beta1-mediated suppression of colitis. Nat Immunol. 2008;9(suppl 1):S50–S53.

247. Longhi, M.S., Hussain, M.I., Miny, R.R., et al. Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. J Immunol. 2006;176:4484–4491.

248. Miyara, M., Amoura, Z., Parizot, C., et al. The immune paradox of sarcoidosis and regulatory T cells. J Exp Med. 2006;203:359–370.

249. Alvarado-Sanchez, B., Hernandez-Castro, B., Portales-Perez, D., et al. Regulatory T cells in patients with systemic lupus erythematosus. J Autoimmun. 2006;27:110–118.