Harnessing Regulatory T Cells for the Treatment of Inflammatory Bowel Disease

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Abstract: Regulatory CD4$^+$ T (T_{reg}) 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 T_{reg} 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. T_{reg} cells are enriched in the intestinal lamina propria and provide a crucial function in promoting tolerance to enteric antigens while modulating tissue inflammation. Correspondingly, T_{reg} 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 T_{reg} cell biology with a focus on Foxp3$^+$ T_{reg} and type 1 regulatory (Tr1) cells and summarize the evidence for defective T_{reg} cells in experimental and human inflammatory bowel disease. The potential application of T_{reg} cells as a treatment for inflammatory bowel disease will also be discussed in the context of T_{reg} infusion therapy and the in vivo induction/expansion of intestinal T_{reg} cells.

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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]

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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 (T_{reg}) cells are an important component of the adaptive immune system that suppresses inflammation and helps to maintain homeostasis. The T_{reg} 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 T_{reg} cells and will be implemented into this review.[16] Foxp3$^+$ T_{reg} cells are one T_{reg} cell subset that constitutively expresses Foxp3 and the high-affinity α-chain of interleukin (IL)-2 receptor, CD25.[17,18] Foxp3$^+$ T_{reg} cells can arise from 2 developmentally distinct pathways in vivo: Foxp3$^+$ thymically derived (t)T_{reg} cells or Foxp3$^+$ peripherally derived (p)T_{reg} cells, which are naive CD4$^+$ T cells that upregulate Foxp3 in extrathymic tissues and become functionally suppressive.[19] Foxp3$^+$ T_{reg} 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$^+$ (i)T_{reg}

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$^+$ T_{reg} 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 T_{reg} cells contributes to the development of chronic inflammatory conditions—including intestinal inflammation. Furthermore, the adoptive transfer of T_{reg} cells helps to prevent and treat experimentally induced colitis.[23,24] These findings suggest that impairments in T_{reg} cell development or immunosuppressive functions may contribute to the pathogenesis of IBD and that T_{reg} cells may serve as a therapeutic target for IBD. This review will summarize the functions of T_{reg} cells in the intestine during steady state and inflammation as well as highlight their use as a potential therapy for IBD.

DEVELOPMENT OF FOXP3$^+$ T_{REG} AND TR1 CELLS

Central features pertaining to the development and function of T_{reg} cells are important to consider because differences in these parameters may be exploited for IBD immunotherapy. Foxp3$^+$ tT_{reg} 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 tT_{reg} 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$^+$ tT_{reg} cells was originally thought to be directed toward self-antigens. However, Pacholczyk et al[27] recently reported that high-affinity, autoreactive TCRs were not required for tT_{reg} development, and in fact, the TCR repertoire of tT_{reg} cells can react to bacterial antigens from the colon.[28] CD4 single positive thymocytes selected along the tT_{reg} 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 tT_{reg} cell development yields distinct epigenetic patterns that distinguish them from pT_{reg} or iT_{reg} cells. For example, tT_{reg} cells exhibit CpG hypomethylation of T_{reg}-associated genes and a unique methylation pattern of the T_{reg}-specific demethylation region compared with iT_{reg} and conventional T cells.[31,32] Foxp3$^+$ tT_{reg} cells may also be distinguished from extrathymic T_{reg} 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$^+$ tT_{reg} 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 tT_{reg} cells, Foxp3$^+$ T_{reg} cells can develop extrathymically 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 T_{reg} 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 T_{reg} differentiation and imprints CD4$^+$ T cells for intestinal homing through the upregulation of CCR9 and α4β7.[40,41] Intestinal DCs and macrophages express retinaldehyde dehydrogenases, the enzymes important for the biosynthesis of RA, and are adept at promoting Foxp3$^+$ T_{reg} cell differentiation.[42–45] Previous animal studies examining intestinal antigen presentation and oral tolerance provide evidence that intestinal Foxp3$^+$ pT_{reg} cells can differentiate in the mesenteric lymph nodes (mLN) on antigenic stimulation by migratory CD103$^+$ LP DCs.[46] Thereafter, a subset of activated CD4$^+$ T cells become gut-tropic Foxp3$^+$ T_{reg} cells that home to the intestinal LP and expand in response to IL-10 produced by CX3CR1$^+$ macrophages.[47]

Tr1 cells represent another subset of T_{reg} 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 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$^+$ T_{reg} cells, Tr1 cells are enriched in the intestine and GALT.[48]

Collectively, T_{reg} 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, T_{reg} 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 pT_{reg} and iT_{reg} cell differentiation.[20,21,38,53] In vivo imaging of T_{reg} 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.[54] Thus, DCs, macrophages, and other antigen presenting cells in the intestine and mLN may be important immunomodulatory targets for T_{reg} cells.[55,56] T_{reg} 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 T_{reg} cells may function to limit pro-inflammatory Th17 responses in the intestine. Additional mechanisms of T_{reg} cell-mediated suppression include cytolysis 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[63] (Fig. 1). Taken together, T_{reg} cells are capable of using a variety of immunosuppressive mechanisms to suppress inflammation and promote tolerance in the intestine.

EVIDENCE FOR DEFECTIVE T_{REG} CELLS IN IBD

The importance of T_{reg} cells in maintaining intestinal immune homeostasis is supported by numerous experimental model systems where intestinal inflammation develops in the absence of functional T_{reg} cells. The original description of T_{reg} cells preventing colitis in mice by Powrie et al,[23] and Morrissey et al,[64] established that naive $CD4^+CD45RB(hi)$ 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 $CD4^+$ T cells or $CD4^+CD45RB(lo)$ T cells. The expansion and differentiation of pro-inflammatory $CD4^+$ T cells in this model system requires the microbiota[23,65–67] suggesting that T_{reg} cells are required to suppress colitogenic $CD4^+$ T cell responses against bacterial antigens.[24,50] The importance of $Foxp3^+$ T_{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.[68–70] Mutations in the *Foxp3* gene of IPEX patients and scurfy mice lead to a global failure of $Foxp3^+$ T_{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_{reg} cells—spontaneously develop severe Th2-type inflammation in the lung and gastrointestinal tract.[71] Together, these findings demonstrate that a failure in tT_{reg} or pT_{reg} cell development is associated with immune dysregulation at mucosal surfaces.

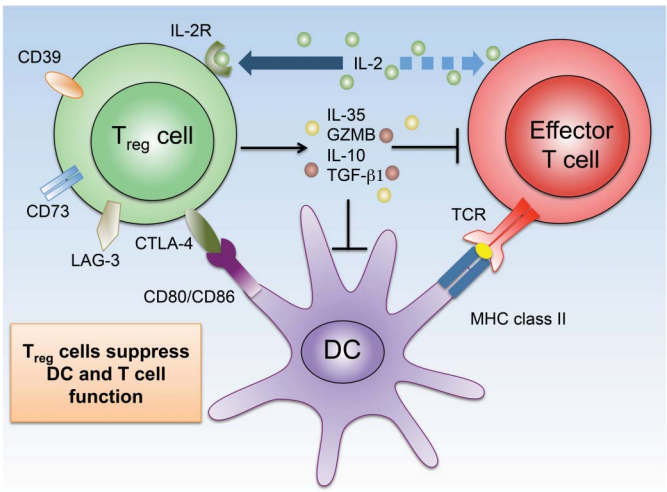

FIGURE 1. Mechanisms of T_{reg} cell–mediated suppression. T_{reg} cells inhibit pro-inflammatory cytokine production, impede antigen presentation, and/or modulate T cell survival through the secretion of immunosuppressive cytokines (IL-10, TGF-β1, and IL-35) and the engagement of immunoregulatory cell surface molecules (CTLA-4 and LAG-3) with their respective ligands on target cells. Additional mechanisms of T_{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).

T_{reg} cell deficiency and intestinal inflammation can also be instigated by a defect in T_{reg} cell survival. $Foxp3^+$ T_{reg} cells from mice deficient in IL-2, IL-2Rα, or the Wiskott–Aldrich syndrome protein (WASp) develop normally in the thymus and are functionally suppressive in vitro. However, $Foxp3^+$ T_{reg} cells from these mice exhibit decreased survival in peripheral tissues that correlates with increased susceptibility to autoimmunity and spontaneous colitis.[72–76] In line with these findings, human genetic studies have reported, *Il2*, *Il2ra*, and *Wasp* to be IBD susceptibility genes,[77–79] and patients with WAS have an increased risk of developing autoimmune disease and inflammatory conditions—including IBD.[80] Thus, poor survival of T_{reg} cells in peripheral tissues may lead to chronic intestinal inflammation.

Beyond T_{reg} cell survival, functional impairments in T_{reg} cells may also contribute to the pathogenesis of IBD. T_{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.[53,81–83] Furthermore, deletion of specific immunosuppressive mechanisms in the T_{reg} cell compartment may augment the production of proinflammatory cytokines and subsequently drive chronic inflammation. For example, $Foxp3^+$ T_{reg} cell–specific ablation of CTLA-4 leads to a lymphoproliferative disease and multiorgan autoimmunity, whereas deletion of IL-10 in $Foxp3^+$ T_{reg} cells induces microbiota-driven colitis.[84,85] In line with these findings, polymorphisms in the genes for CTLA-4 and IL-10 receptor are associated with IBD.[86–88] Although the absence or functional impairment of T_{reg} cells leads to intestinal inflammation, it is particularly important to note that $Foxp3^+$ T_{reg} cells cannot only prevent intestinal inflammation but can also treat established colitis in experimental models.[24,31,89,90] These studies demonstrate the feasibility of adoptive T_{reg} cell immunotherapy for reversing established intestinal inflammation in humans.

POTENTIAL FOR AUTOLOGOUS T_{REG} INFUSION THERAPY IN IBD

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

organ transplantation.[91] Clinical trials are also underway exploring T_{reg} cell infusion therapy in type 1 diabetes mellitus (https://clinicaltrials.gov/ct2/show/NCT01210664).

In order for infused T_{reg} cells to most effectively control the inflammation present in patients with IBD, careful consideration of T_{reg} cell purity, homing ability, antigen-specificity, and survival will likely aid in the development of a potent treatment regimen. Autologous T_{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.[92] This is not a trivial task since some cell surface markers, such as CD25, used to isolate human peripheral blood T_{reg} cells can also be expressed by activated, conventional $CD4^+$ T cells. Additionally, since naive $CD4^+$ T cells are abundant in blood, they can be isolated from peripheral blood mononuclear cells and differentiated into iT_{reg} cells when activated in the presence of TGF-β1.[93] Following isolation, tT_{reg} or iT_{reg} cells can be expanded in vivo, and the purity of the tT_{reg} and iT_{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.[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_{reg} cells should efficiently migrate to the mLN and/or intestinal LP to become optimally suppressive.[95,96] The localization of T_{reg} cells to the inflamed intestinal LP may serve to regulate ongoing proinflammatory immune responses while T_{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_{reg} cells to the mLN and intestinal LP is influenced by the expression of specific integrins and chemokine/chemokine receptor axes.[97] Efficient migration of infused T_{reg} cells to inflamed intestinal tissue may be facilitated by supplementing RA in T_{reg} cell cultures to induce upregulation of the gut homing receptors, CCR9 and α4β7.[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 $CD4^+$ T cell homing to the colon.[99] Mice deficient in GPR15 exhibited a reduction in colonic Foxp3+ T_{reg} cells that was associated with heightened pro-inflammatory responses in intestine when challenged with *Citrobacter rodentium* infection. If GPR15 also regulates migration of $CD4^+$ T cells to the human colon, exploiting factors that modulate CCR9, α4β7, and/or GPR15 expression may be important for targeting T_{reg} cells to the inflamed tissues in IBD patients. Future studies investigating homing requirements are warranted to optimize T_{reg} cell localization to the intestine during acute and chronic inflammation.

Given that TCR-mediated stimulation of T_{reg} cells is required for eliciting immunosuppressive functions, the antigen reactivity of T_{reg} cells is another important consideration for the treatment of IBD.[100,101] Although the specific antigens involved in the pathogenesis of IBD remain poorly understood, the requirement for the microbiota is well supported experimentally.[102] Animal studies demonstrating a relationship between the gut microbiota and colitis are corroborated by genetic knock-out mouse strains, such as the *Il10*$^{-/-}$ mice, and the CD45RB(hi) $CD4^+$ T cell transfer model of colitis—both of which do not develop colitis in the absence of the gut microbiota.[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 $CD4^+$ T cell clones isolated from IBD patients are reactive to specific components of the microbiota.[105,106] These data implicate a role for the microbiota in the development of IBD, and thus, T_{reg} cells reactive to bacterial and/or food antigens may be most effective in suppressing proinflammatory responses since stimulation of Treg cells is required for suppressive ability.[100,101] Interestingly, a recent phase I/IIa clinical trial demonstrated the feasibility of infusing T_{reg} cells reactive to ovalbumin (OVA), a food antigen, to treat refractory Crohn's disease.[107] In this trial, OVA-specific Tr1 cells were cloned by limiting dilution from human peripheral blood mononuclear cells and expanded ex vivo. T_{reg} cell infusion doses ranging from 10^6 to 10^9 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_{reg} cells to activate their suppressive functions in the intestines of patients with IBD.

After infusion into patients with IBD, T_{reg} cells should persist to maximize their immunosuppressive functions in the intestine. However, apoptosis of $Foxp3^+$ T_{reg} cells is increased in inflamed colonic tissue and the peripheral blood of patients with IBD indicating that T_{reg} cell survival may be impaired by pro-inflammatory cytokines.[108] This increase in apoptosis of $Foxp3^+$ T_{reg} cells was reversed in patients that responded to anti-TNF-α treatment—suggesting that blockade proinflammatory cytokines may improve T_{reg} cell survival. An alternative approach to consider for promoting T_{reg} cell survival after infusion into patients with IBD would be administration of IL-2 and folic acid (FA), which have been shown to help maintain $Foxp3^+$ T_{reg} cells in peripheral tissues.[72,109] Since CD25 and folate receptor 4 (FR4) are constitutively expressed by $Foxp3^+$ T_{reg} cells, low-dose administration of IL-2 and a diet enriched in FA may preferentially enhance survival of $Foxp3^+$ T_{reg} cells.[110–112] In fact, low-dose IL-2 was reported to increase $Foxp3^+$ T_{reg} cell proliferation, thymic export, and improve T_{reg} resistance to apoptosis.[113] In mice, FA seems to play a role in $Foxp3^+$ T_{reg} cell survival and immune homeostasis because colonic $Foxp3^+$ T_{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 $Foxp3^+$ T_{reg} cells may be improved with IL-2 and FA supplementation.

THERAPEUTIC EFFICACY OF T_{REG} CELLS IN IBD

When considering T_{reg} cells as a potential therapy for IBD, it will be valuable to know whether tT_{reg} cells, iT_{reg} cells or a combination of thereof will most effectively resolve inflammation. Several studies assessing the efficacy of tT_{reg} and iT_{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 tT_{reg} or iT_{reg} cells was sufficient to ameliorate experimental colitis.[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_{reg} cells in vivo which can affect the course of disease.[90] Thus, when testing the functional capacity of adoptively transferred tT_{reg} cells to treat established colitis in this model, it is important to appreciate that tT_{reg} cells may be working in concert with pT_{reg} cells. Interestingly, the transfer of iT_{reg} cells alone into RAG1-deficient mice was able to treat established colitis, and in fact, iT_{reg} cells were shown to have a functional advantage over tT_{reg} cells.[90,114] There is also evidence to support a functional requirement for a combination of tT_{reg} and iT_{reg} cells to treat intestinal inflammation and prevent autoimmune disease. When $CD4^+$ CD45RB(hi) T cells from *Foxp3*$^{\Delta EGFP}$mice were used to promote the development of chronic colitis in RAG1-deficient hosts, and consequently, $Foxp3^+$ pT_{reg} cells could not develop from these donor cells in vivo, the treatment of colitic mice with tT_{reg} cells alone was unable to reverse intestinal inflammation. However, a mixture of tT_{reg} and iT_{reg} cells transferred into colitic mice afforded maximal effectiveness in the treatment of intestinal inflammation—indicating that tT_{reg} and iT_{reg} cells may function together.[90] Although evidence suggests that tT_{reg} and iT_{reg} cells can treat colitis in mice, additional studies are needed to fully examine the functional relationship between tT_{reg}, pT_{reg}, and iT_{reg} cells in vivo and their efficacy for the treatment of patients with IBD.

ENHANCING THE FUNCTION OF ENDOGENOUS T_{REG} CELLS

Aside from T_{reg} infusion therapy for the treatment of IBD, the intestinal microenvironment may also be manipulated to promote pT_{reg} cell development and/or expansion of tT_{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_{reg} cell differentiation. MSCs can inhibit immune activation in vitro and ameliorate disease in different animal models of autoimmunity and inflammatory disorders—including IBD.[115,116]

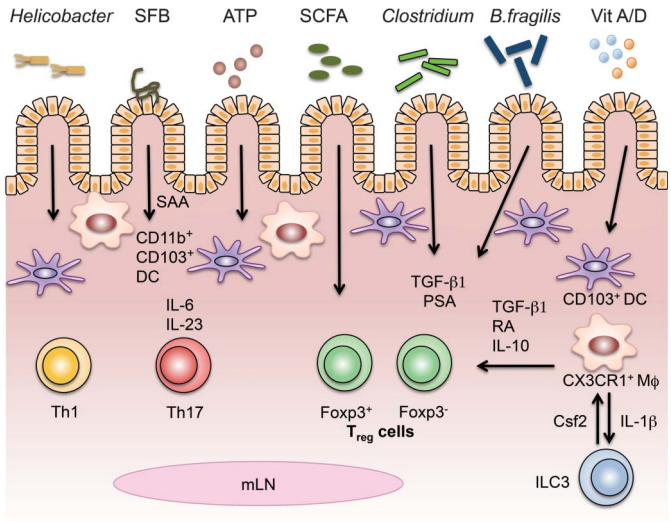

FIGURE 2. Interactions between the intestinal immune system, the gut microbiota, and dietary factors in regulating $CD4^+$ T cells in the LP. SCFAs are synthesized by the gut microbiota and regulate the development of intestinal T_{reg} cells. Colonization by a mixture of *Clostridium* strains is associated with the synthesis of SCFAs and TGF-β1 secretion, which contributes to maintaining $Foxp3^+$ T_{reg} cells in the colon, whereas polysaccharide A (PSA) derived from *B. fragilis* promotes the development of Tr1 cells. Vitamins A and D are necessary for supporting different functions of the intestinal immune system—including $Foxp3^+$ T_{reg} cell development. In response to microbial signals, intestinal macrophages secrete IL-1β that stimulates the production of colony-stimulating factor 2 (Csf2) from group 3 innate lymphoid cells (ILC3). This Csf2 derived from ILC3 recruits DCs and macrophages to the intestine, which helps to maintain $Foxp3^+$ T_{reg} cell homeostasis. 186 × 139 mm (300 × 300 DPI).

In the context of experimental colitis, the infusion of MSCs can reduce disease activity and pro-inflammatory cytokines while inducing T_{reg} cells in the mLN and colon.[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.[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_{reg} cells.[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$^+$ T_{reg} cell differentiation.[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$^+$ pT_{reg} cell development in vivo,[40] and vitamin A deficiency is associated with the induction of colitis.[124] Because patients with IBD are particularly susceptible to malabsorption, administration of vitamin A or RA may help to stimulate endogenous Foxp3$^+$ T_{reg} 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 T_{reg} cell differentiation and suppress immune responses.[125] In the intestine, vitamin D and vitamin D receptor (VDR) signaling helps to maintain epithelial integrity and ameliorate chronic colitis in mouse models.[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.[128–132] Based on these findings, supplementation of vitamin D may have therapeutic effects for patients with IBD by inducing intestinal T_{reg} cells to suppress inflammation and alleviate disease.

Microbiota

Recent studies investigating the relationship between the gut microbiota and T_{reg} cells indicate that reconstitution of IBD patients with specific commensal bacteria may help to correct dysbiosis and promote T_{reg} cell development during intestinal inflammation. In mice, *Bacteroides fragilis* and specific *Clostridia* strains have been reported to induce intestinal T_{reg} cells in the colon.[133–136] Polysaccharide A (PSA) produced by *B. fragilis* can promote the development of IL-10-producing T_{reg} cells through plasmacytoid DCs[133] and also acts on TLR2 expressed on T_{reg} cells to elicit immunosuppressive functions.[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 T_{reg} 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$^+$ T_{reg} cell development in the mouse colon.[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$^+$ T_{reg} cell differentiation and function.[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$^+$ T_{reg} cells. Accordingly, the administration of SCFAs may aid in the treatment of patients with IBD by expanding the endogenous Foxp3$^+$ T_{reg} cell compartment to suppress intestinal inflammation.

Probiotics may serve as another means to promote intestinal Foxp3$^+$ T_{reg} induction in patients with IBD. In a study by Kwon et al,[141] a probiotic mixture containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Bifidobacterium bifidium*, and *Streptococcus thermophilus* significantly increased tolerogenic DCs and T_{reg} cells in the mLN while decreasing proinflammatory cytokine expression and proliferation of splenic CD4$^+$ T cells.[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 T_{reg} cell and tolerogenic DC development. This concept of correcting dybiosis 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.[142–147]

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

T_{reg} 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 T_{reg} cell function either by inhibiting specific suppressive mechanisms or by altering the stability of the T_{reg} cell phenotype. Although effects of pro-inflammatory cytokines in modulating T_{reg} cell suppressive mechanisms have not been fully elucidated, Foxp3$^+$ T_{reg} cells are reported to lose Foxp3 expression and gain the ability to secrete proinflammatory cytokines when present in an inflammatory milieu.[82,148] Therefore, a potential risk with T_{reg} cell–based therapy for IBD may be the conversion of Foxp3$^+$ T_{reg} cells into pathogenic effector T cells in inflamed tissues. Consequently, long-term studies evaluating the plasticity and pathogenic potential of T_{reg} cells in patients with IBD are warranted. This is especially important when considering immunotherapy using iT_{reg} cells, which may be less stable than tT_{reg} cells.[31,82]

Besides altering the function and differentiation of T_{reg} cells, the inflammatory tissue environment in IBD may also decrease the responsiveness of effector T cells to apoptosis and T_{reg} 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.[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.[150–156] Additionally, $CD3^+$ T cells from the inflamed mucosa of patients with IBD express high levels of Smad7, an inhibitor of TGF-β1 signaling, that can shield pathogenic T cells against the immunosuppressive effects of TGF-β1 from T_{reg} cells.[157–159] The elevated levels of Smad7 are associated with a decrease in phosphorylated Smad3, which is a downstream signal transducer of the TGF-β1 receptor. Treatment of LP mononuclear cells from CD patients with antisense Smad7 increased phosphorylation of Smad3 and attenuated production of TNF-α and interferon-γ.[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 T_{reg} cell–mediated suppression has been demonstrated in other chronic inflammatory diseases,[161–163] and the efficacy of T_{reg} cell therapy alone would be hampered if the inflammatory milieu does indeed promote pathogenic T cells to be resistant to the suppressive mechanisms of T_{reg} cells. Elucidating additional mechanisms by which the inflammatory microenvironment can induce such resistance and translating this knowledge to develop therapies that reinstate immunoregulation and T_{reg} cell–mediated suppression would be imperative for the treatment of IBD.

CONCLUSIONS

Important insights into T_{reg} cell biology have facilitated the translation of seminal discoveries from experimental model systems to clinical trials using T_{reg} cell–based therapies for the treatment of IBD. Thus far, several clinical trials have supported the feasibility of ex vivo T_{reg} cell production and validated the safety of T_{reg} 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, T_{reg} 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 T_{reg} 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 T_{reg} cells may establish a tissue environment conducive for infused or endogenous T_{reg} cells to reinstate tolerance against the gut microbiota in patients with IBD. As additional mechanisms regulating T_{reg} cell biology are elucidated and T_{reg} cell-based therapies are optimized at the bench and in the clinic, these cells may eventually serve as a complementary treatment for IBD.

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