T Regulatory Cells and Their Role in Autoimmune Disease Involving the Gastrointestinal Tract

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LEARNING OBJECTIVES

1. Recall how to identify T regulatory cells and the percentage of T regulatory cells among circulating lymphocytes.
2. Discuss the types of T regulatory cells in the GI tract.
3. Define immune tolerance.
4. Describe the kinetics of T regulatory cells in inflammatory bowel disease.
5. Explain the role of commensal bacteria and nutrients in regard to T regulatory cells activity.

ABSTRACT

T regulatory (Treg) cells represent a unique subset of T lymphocytes. Although they represent a small fraction of circulating T cells (< 10%) they are increasingly implicated in providing immuoregulatory function towards maintaining immune homeostasis. Treg dysregulation has been associated with autoimmune disease in general, as well as in specific organ systems. This review provides an overview of the characterization and function of Treg cells in health and disease states with an emphasis of their involvement gastrointestinal autoimmune diseases.

ABBREVIATIONS: DC – dendritic cell, GI – gastrointestinal, IBD – inflammatory bowel disease, IL-2 - interleukin 2, IPEX - immunodysregulation, polyendocrinopathy, and enteropathy X-linked, iTreg - induced T regulatory cells, nTreg - naturally occurring T regulatory cells, PBMC – peripheral blood mononuclear cells, SCFA – short chain fatty acids, Th - T helper, TGF-β - transforming growth factor, Tr1 - type 1 regulatory cells, Treg – T regulatory cells.

INDEX TERMS: Gastrointestinal (GI) tract, autoimmune disease, immunoregulatory function, immune Suppression

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INTRODUCTION

The gastrointestinal (GI) tract has the largest amount of lymphoid tissue in the body. This is likely due to the potential load of antigens it is exposed to on daily basis, ranging from commensal bacteria to food antigens.[1] A specific group of immune cells, known as T regulatory cells, maintain a state of unresponsiveness towards antigen challenge and a level of self-tolerance in the GI tract by preventing excessive undesired immune responses (immune homeostasis). Any break in the state of immune homeostasis by either genetic or environmental factors can induce an "immune-inflammatory" response in the GI tract and present clinically as an autoimmune disease.

In the early 1970s, Gershon *et al.* identified the existence of a group of T lymphocytes in mice that possessed a suppressive activity towards other immune cells.[2] Subsequent analysis revealed that these cells express CD4 and CD25 cell surface receptors.[3] In 1995, Sakaguchi *et al.* showed that CD4+ CD25+ cells constitute approximately 5-10% of circulating lymphocytes.[4] In 2001, a similar population of T cells with a comparable phenotype was identified in humans and was subsequently referred to as T regulatory (Treg) cells.[4] Treg cells represent a unique subset of T lymphocytes. Although they represent a small fraction of circulating T cells (< 10%) they are increasingly implicated in providing immunoregulatory function towards maintaining immune homeostasis.

Treg dysregulation has been associated with autoimmune disease in general as well as in specific organ systems. In humans, the protective role of T regs in autoimmune disorders has been extensively studied, by evaluating their role, via number and activity, in several immune-mediated diseases, such as multiple sclerosis, type 1 diabetes mellitus, systemic lupus erythematosus, rheumatoid arthritis, and psoriasis. The immune suppressive role of Tregs is exemplified by a reduction in the frequency of peripheral Treg cells during the relapse phase of multiple sclerosis when compared to the remission phase of the disease.[5] Likewise, the immune suppressive effects of Tregs were demonstrated when autologous expanded *ex vivo* T regulatory cells were used to treat type 1 diabetes mellitus in children and showed an increase in serum C-peptide levels, a decrease in dependence for exogenous insulin and a protective effect against the destruction of human pancreatic islets.[6]

This review provides an overview of the characterization and function of Treg cells in health and disease states with an emphasis of their involvement in inflammatory bowel disease as an example of an autoimmune disease that mainly affects the GI tract.

T Regulatory Cell Populations

Treg cells are defined as a specialized subset of T helper cells that are able to promote and maintain immune tolerance and subsequently prevent autoimmune diseases. While CD4 and CD25 (also known as interleukin 2 (IL-2) receptor) cell surface markers are both used to identify Treg cells, a major advancement in our understanding of Treg cells came after the discovery of the FOXP3 protein, a transcription factor that has been shown to be critical for both the development and function of Treg cells.[7] The importance of proper *FOXP3* gene expression is evidenced by a genetic mutation in *FOXP3* in mice that leads to suppression of Treg function and the development of lethal autoimmune diseases.[7] Likewise, in humans, a genetic mutation in *FOXP3* results in defective development of CD4/CD25+ regulatory T cells and manifests clinically by an autoimmune disorder called IPEX syndrome (immunodysregulation, polyendocrinopathy, and enteropathy X-linked). First described in 1982, IPEX usually manifests in the first few months of life, and results in death within the first two years.[8]

In general, Treg cells can be classified into two major types based on the site of their formation: naturally occurring T regulatory cells (nTreg) and adaptive or induced T regulatory cells (iTreg). nTreg cells are produced in the thymus and are thought to control reactivity toward self-antigens while iTreg cells are induced in the periphery after encountering non-self antigens from a commensal bacteria or a food antigen in a specific immune microenvironment. It is well established that both types are highly dependent on IL-2 for their proliferation and suppressive function.[9] iTreg cells include two additional subsets: type 1 regulatory cells (Tr1), which do not constitutively express FOXP3, but rather depend on the presence of IL-10 for their suppressive function and Th3 T regulatory cells that do not express CD25 and their suppressive function depends on transforming growth factor (TGF-β) secretion.[9] Table 1 outlines the differences between nTreg and iTreg cells. Finally, a recently discovered IL-10 producing double positive CD4, CD8αα (T cell receptor co-receptor), and FOXP3 negative Treg subset was identified in human colonic lamina propria with regulatory activity and found to have a role in inflammatory bowel disease.

Table 1. Differences between natural (n) and induced (i) T regulatory cells

	nTreg	iTreg
Site of origin	Thymus	Peripheral tissue
Specificity	Self-antigens	Antigens of commensal bacteria, food, and allergens
FOXP3	Expressed by all cells	Diminished in Tr1 subset
Mechanism of	Cell-cell contact	Secretion of soluble factors: IL-10 TGF-β
Suppression Markers	Helios and neuropilin 1 positive	Helios and neuropilin 1 negative

Mechanism of T Regulatory Cell Function in Immune Suppression

Several mechanisms have been identified by which Treg cells suppress other immune cells and induce tolerance. Treg cells act directly on dendritic cells (DCs) to attenuate the DCs ability for antigen presentation and the expression of co-stimulatory molecules, specifically CD80 and CD86, on their surface.[10] Additionally, Treg cells can use granzyme-A and perforin to eliminate T

effector cells by apoptosis.[11] They also exhibit their immune suppression function by secreting IL-10, TGF-β, and IL-35. IL-10 suppresses the production of pro-inflammatory cytokines by dendritic cells and macrophages in mucosa and constrains the development of Th17 cells in patients with inflammatory bowel disease. Patients with a mutation in the IL-10 gene have been shown to be more prone to Crohn's disease.[12,13] TGF-β suppresses cytotoxic T cells, as well as maintain the expression of FOXP3, thereby maintaining the function of T regulatory cells.[14] Di Sabatino *et al.* showed a down-regulation of T helper cell apoptosis, as well as up regulation of pro-inflammatory cytokines (IL-6, IL-8, IL-17, and TNFα) in human gut mucosa when anti-TGF-β antibodies were administered.[15] IL-35 is secreted by activated Tregs and is involved in protecting the colon from T-cell induced colitis in mice.[16] However, solid data on the role of IL-35 and autoimmune GI disease in humans is lacking. IL-35 could be an important therapeutic target in the future, since it can induce Treg proliferation as well as confer a regulatory function to other T effector cells, often referred to as "infectious tolerance".[17]

Certain biological and nutritional factors have been assessed for their abilities to enhance the suppressive function of Treg cells. Vitamin A and its metabolite, all-trans retinoic acid, regulate different aspects of immunity. Retinoic acid (RA) is produced by gut dendritic cells from dietary vitamin A. RA converts naïve CD4 cells to stable FOXP3 expressing cells with regulatory function.[18] Moreover, RA appears to inhibit the function of Th17 cells and reduce their inhibitory function on T regulatory cells.[19] Vitamin D is also an important nutritional factor in the function of the immune system. Accumulating evidence indicates that vitamin D affects the prevalence of multiple sclerosis, rheumatoid arthritis and diabetes.[20] In fact, previous studies in mice showed amelioration in the level of colitis after vitamin D supplementation.[21] Factors that are relevant to Treg cell function and others that inhibit them are summarized in Table 2.

T Regulatory Cells and Inflammatory Bowel Disease

Treg cells are located along the GI tract in lamina propria and they are enriched in the appendix and ascending colon of normal healthy individuals.[22] In the GI tract, Treg cells play an important role in the maintenance of immunological homeostasis. They can prevent the onset of autoimmunity by suppressing the differentiation and function of auto-reactive T helper cells (Th1 and Th2), as well as preventing inflammation in the GI tract that occurs as the result of Th17 cells functional activity.[23]

Table 2. Factors affecting T regulatory cells function in GI tract

Factors enhancing Treg function	Factors suppressing Treg function
IL-10	IL-17
TGF-β	TNF-α
IL-2	Paclitaxel
FOXP3	IL-6
Vitamin D	Tyrosine kinase inhibitors
Retinoic acid	
CTLA-4	

The two forms of inflammatory bowel disease (IBD), Crohn's disease and ulcerative colitis, are chronic relapsing conditions that affect the GI tract. Both diseases have the tendency to alternate between an active phase and a state of remission. The peak incidence of IBD occurs between 15-29 years of age. Treatment includes conservative approaches using different types of drugs among five major categories: anti-inflammatory drugs, immunosuppressants, biologic agents, antibiotics, and drugs for symptomatic relief. The aim of using such drugs is to relieve symptoms by decreasing inflammation, improving quality of life, and prevention of complications. Surgical intervention is sometimes recommended for resistant cases.

There is a general agreement that the genetic and environmental factors, dysfunctional immune responses, as well as complex interactions between the mucosal immune cells and the commensal bacteria are all involved in the pathogenesis of IBD. Treg cells are important in modulating this interaction and in maintaining a hyporesponsive state towards commensal bacterial and food antigens as well as keeping the immune system alert against invading mucosal pathogens. In fact, numerous studies on mouse models and human patients suggest that defects in Treg cells can play distinct causative roles in IBD.[24] The numerous genetic, nutritional, and microbial factors that are associated with IBD may also affect Treg cells.[25] In mice, the presence of certain bacteria, such as *Bacteroides fragilis* and groups of *Clostridia* are

important in preventing colitis through maintaining the number and function of Treg cells in the GI tract by inducing a TGF-β rich environment in the bowel.[26] In human studies, Sarrabayrouse *et al.* demonstrated that a novel Treg cell subset, characterized as IL-10 producing double positive CD4, CD8αα, and FOXP3 negative T lymphocytes, can be activated by the gut commensal bacteria, *Faecalibacterium prausnitzii.* This organism is known to be deficient in patients with inflammatory bowel disease.[27] This suggests that probiotic use may serve as a future therapeutic option for IBD.

In a recent study by Garrett *et al.*, commensal bacteria were found to secrete certain metabolites with short chain fatty acids (SCFA) namely, acetic acid, propionic acid and butyric acid, which are important in establishing mutualism between the commensal bacteria and the immune system and in maintaining immune homeostasis. Moreover, the bacterial metabolites were found to increase nTreg cells and direct them from the peripheral blood to colonic mucosa. Deficiency of SCFA was noted after directing the feces out of the colon into a stoma, inducing a clinical disease entity known as diversion colitis.[28,29] However, the relationship between the amount of SCFA and inflammatory bowel disease remains unclear.

The number of Treg cells in the peripheral blood and gut mucosa has also been studied to better understand the dynamics of Treg cells during disease relapse and remission. One study showed that CD4+CD25+ cells are reduced in the peripheral blood of IBD patients compared with normal healthy individuals.[30] Wang *et al.* analyzed the percentage of CD4+FOXP3+ Treg cells in peripheral blood mononuclear cells (PBMC) and colonic mucosa. CD4+ FOXP3+ cells were shown to be reduced among PBMC and their level in colonic mucosa increased during disease activity.[31] Maul *et al.* also reported that Treg cells decreased in the peripheral blood of patients with active IBD.[32] Several other studies have also found that the level of Treg cells in the peripheral blood was decreased with an increase in their frequency in inflamed lamina propria tissue.[29] It seems that the decrease in the number of peripheral blood T regulatory cells are inversely related to their number in colonic mucosa during disease activity. It may be that nTregs can sense the initial phase of the inflammatory disease through cell signaling and start their migration to colonic mucosa to suppress inflammation.

In an effort to develop new therapeutic strategies for patients with inflammatory bowel disease, especially those who are refractory to conventional immunosuppressive treatment, researchers introduced the concept of autologous antigen-specific regulatory T cell therapy. In a multicenter open-label study, a single injection of ovalbumin-specific Treg cells was administered to patients after collecting their cells and incubating them with ovalbumin antigen in vitro. It was postulated that priming the cells with ovalbumin in vitro induced an immunomodulatory environment in the gut since ovalbumin is a common food antigen. The study reported a 40% reduction in Crohn's disease activity.[33]

CONCLUSIONS

T reg cells provide immunomodulation of the immune response to internal and external factors in order to maintain immune homeostasis. Disruptions in immune homeostasis by either genetic or environmental factors can induce an "immune-inflammatory" response in the GI tract and present clinically as an autoimmune disease. The protective role of T reg cells in autoimmune disorders has been established by evaluating their number and activity in several immune-mediated diseases including IBD. From a clinical perspective, the future of IBD treatments may include autologous transplantation of T cells alone or in combination with certain microbiological or nutritional factors, such as commensal bacteria, vitamins, and/or select cytokines to enhance the level of immune suppression and to re-establish gut homeostasis.

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