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The role of IL-17-producing Foxp3+ CD4+ T cells in inflammatory bowel disease and colon cancer

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Abstract

The intestinal epithelium and underlying lamina propria contain T cells that play important roles in maintaining colonic homeostasis. These T cells mediate substantial and specific regulation to ensure that pathogenic microorganisms are eliminated while commensal bacteria are tolerated. There is considerable evidence supporting the notion that the altered ratio between $Foxp3^+CD4^+$ T regulatory cells and T effector cells in the colonic microenvironment might contribute to the initiation and progression of inflammation and eventually development of colon cancer. Recent findings on the heterogeneity and plasticity of T regulatory cells, such as the identification of $IL\text{-}17^+Foxp3^+CD4^+$ and the $ROR\gamma t^+Foxp3^+CD4^+$ subsets, in patients with colorectal inflammation and cancer have provided a new twist in our understanding of the pathogenesis of colonic diseases. Phenotypic and functional properties of IL-17-producing $Foxp3^+CD4^+$ T cells as well as the significant implications of these cells in the initiation and progression of colorectal diseases are discussed in this review.

Keywords

T regulatory cells; Th17; IL-17; RORγt; Inflammatory bowel disease; Colon cancer

1. Introduction

Regulatory T (T_{Reg}) cells expressing the transcription factor forkhead box P3 (Foxp3) are naturally present in the immune system. T_{Reg} cells suppress the activation, proliferation and effector functions of a wide range of immune cells, including $CD4^+$ and $CD8^+$ T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells *in vitro* and in *vivo* [1]. T_{Reg} cells have key roles in the prevention of autoimmune responses and the development of immunopathology as well as in the maintenance of homeostasis. As a double-edged sword, T_{Reg} cells can also suppress antitumor immune responses and can favor tumor progression. The unique functional properties of T_{Reg} cells make these cells a primary target in the search for new cell-based immunotherapeutic approaches. However, recent studies showed that T_{Reg} cells are not a homogeneous and terminally differentiated

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cell population. Instead, these cells are heterogeneous in gene expression, phenotype and function. In addition, T_{Reg} cells are strongly affected by other immune components including effector cells and innate immune cells during initiation and progression of disease. Thus, to achieve the translational goal of applying T_{Reg} cells in the treatment of diseases, understanding of the T_{Reg} cell immunobiology in the context of various tissues and pathogenesis of various diseases is required. Lymphocytes of mucosal tissues form a relatively autonomous immune subsystem, with specialized adaptations appropriate for this particular microenvironment [2-4]. The intestinal mucosa is normally maintained in a state of controlled inflammation in which equilibrium exists between protective immunity and tolerance to self-antigen and commensal bacteria [5]. Protective immunity against different classes of pathogens depends on the generation of distinct types of immune responses mediated and coordinated by effector cells specifically T_H1, T_H2 and T_H17. On the other hand, T_{Reg} cells are involved in the maintenance of tolerance. A series of observations suggest that T_{Reg} cells correlate with poor prognosis in many cancer types, including breast, lung, melanoma, and ovarian carcinoma [6] due to their suppressive effects on anti-tumor immunity. However, several studies have shown that T_{Reg} cells are protective in cancer by virtue of their ability to control inflammation in an IL-10 dependent manner [7]. In fact, Foxp3 expression has been indicative of better prognosis in gastric cancer, head and neck, and breast cancer [8-10]. Recent identification of IL-17-producing $Foxp3^+CD4^+$ T cells in IBD, colon cancer and polyposis might provide a potential explanation for the conflicting observations regarding the role of T_{Reg} cells in cancer. Furthermore, the advancements in understanding T_{Reg} immunobiology locally and systemically and the influence of inflammatory microenvironments on the differentiation of effector cells and on the stability of T_{Reg} cells would improve the use of T_{Reg} as cell-based immunotherapy.

2. Heterogeneity of T_{Reg} cells

$Foxp3^+CD4^+$ T_{Reg} cells are not homogeneous in gene expression, phenotype and suppressive function. The transcription factor Foxp3, a master control gene for the development and function of both mouse and human T_{Reg} cells [11-14], is currently the definitive marker of T_{Reg} cells. However, Foxp3 can be transiently expressed in activated human T cells. This transient Foxp3 expression in T cells does not enable suppression but, instead, makes separation of T_{Reg} cells from activated T effector cells difficult to perform. Therefore, studies to determine the frequency of various T cell subsets in diseases require further scrutiny and mandate the use of multiple parameters instead of Foxp3 alone to define T_{Reg} cells. In spite of multiple attempts for the identification of the proper marker combination including CD25, CD127 and CD62L to delineate human T_{Reg} cells, the identification and purification of human T_{Reg} remains a challenge. Recent studies have shown that CD45RA or CD45RO, which are mutually exclusive, are particularly useful markers when combined with CD25 and/or Foxp3 [15]. $CD45RA^+Foxp3^+$ naïve T_{Reg} cells, are present in peripheral blood and prevalent in cord blood [16, 17] and are considered the human counterparts of mouse natural T_{Reg} cells that develop in the thymus. Naïve T_{Reg} cells proliferate after *in vitro* stimulation via their TCR and are highly resistant to apoptosis [18], in contrast to $CD45RO^+Foxp3^+$ T_{Reg} cells, which tend to be hyporesponsive and apoptotic after activation *in vitro*. Effector $CD45RO^+Foxp3^+$ T_{Reg} cells, which derive mainly from naïve T_{Reg} cells, as well as $CD45RA^+Foxp3^+$ naïve T_{Reg} cells have potent *in vitro* suppressive activity [15]. In contrast to the human system, naïve mouse $CD4^+$ T cells lack transient and promiscuous expression of Foxp3 after activation. But naive mouse T cells readily convert to $Foxp3^+$ T_{Reg} (iT_{Reg}) cells following *in vitro* stimulation with TGF-β or retinoic acid [19]. These induced T_{Reg} cells share many phenotypic markers and regulatory functions natural T_{Reg} cells, but do not exhibit the signature gene transcription profile of natural T_{Reg} cells [20]. Instead, the gene profile of induced T_{Reg} cells is Foxp3 independent [15].

3 Characterization of *in vitro* induced IL-17-producing Foxp3$^+$CD4$^+$ T cells

T_{Reg} cells are well known to produce IL-10 and TGF-β during activation and these cytokines are considered to be part of the mechanisms via which T_{Reg} cells mediate their suppressive function [21]. Recent findings reveal that T_{Reg} cells are capable of producing IL-17. There is an intimate link between T_H17 cells and T_{Reg} cells. Exposure of antigen-activated naïve T cells to TGF-β *in vitro* results in transcriptional up-regulation of both Foxp3 and RORγt [22]. Furthermore, T cells co-expressing Foxp3 and RORγt have been identified *in vivo* in both mice and humans [23]. Foxp3 is able to physically bind to RORγt and to inhibit the transcriptional activity of RORγt thereby blocking IL-17 production. Therefore, in steady state conditions, T_{Reg} cells are unable to produce IL-17 [22]. A number of research groups have reported that during activation in the presence of appropriate inflammatory stimuli *in vitro*, both human and murine T_{Reg} cells display a IL17$^+$Foxp3$^+$CD4$^+$ phenotype and can produce IL-17 [24-27]. Recent studies including our own work have identified that IL-1β is a critical mediator in the conversion of T_{Reg} cells into IL-17-producing cells *in vitro*. In the presence of IL-1β, T_{Reg} cells express increased levels of RORγt and are able to secrete IL-17 in response to T cell receptor (TCR)-mediated stimulation. The activation of MAPK pathways via IL-1β/IL-1R might have an active role in this differentiation process [24]. We have determined that Runx1, a transcription factor required for the expression of Foxp3 and RORγt, is highly expressed in IL-17-prducing Foxp3$^+$CD4$^+$ cells compared to Foxp3$^+$CD4$^+$ T cells, and silencing of Runx1 with small interfering RNA significantly reduced the numbers of IL-17-producing cells induced by IL-1β [28]. Thus, sustained expression of Runx1 in T_{Reg} cells by IL-1β might be responsible for the conversion of T_{Reg} cells into IL-17-producing RORγt$^+$Foxp3$^+$CD4$^+$ cells. Although these *in vitro* generated IL-17-producing Foxp3$^+$CD4$^+$ T cells retain phenotypic properties of T_{Reg} cells, including expression of CD25, CTLA-4 and GITR, these cells represent a distinct subset of T_{Reg} cells. IL-17-producing Foxp3$^+$CD4$^+$ T cells express increased levels of ICOS compared to T_{Reg} cells, and more importantly, display potent suppressive activity and are capable of inducing apoptosis of responder T cells [28].

As mentioned above, Foxp3$^+$$T_{reg}$ cells represent a heterogeneous population consisting of committed T_{Reg} cells and of a minor subpopulation that retains developmental plasticity [29]. It is tempting to speculate that this plasticity-retaining subset gives rise to T_{Reg} cells capable of producing IL-17. Several attempts have been made to identify the origin of IL-17-producing Foxp3$^+$CD4$^+$ T cells. Most of the studies have demonstrated that effector CD45RO$^+$ T_{Reg} cells but not CD45RA$^+$ naïve T_{Reg} cells are capable of producing IL-17 upon TCR stimulation in the presence of the combination of IL-1β, IL-2, IL-23 and IL-21 [23]. CD45RA$^+$ naïve T_{Reg} cells retain stable CpG methylation across the *RORC* locus even upon prolonged *ex vivo* expansion and as a consequence, they display only a marginal tendency to express RORγτ and to develop into IL-17-producing cells. In contrast, stimulation-induced DNA demethylation of *RORC* occurs selectively in effector CD45RA-T_{Reg} cells [30]. IL-17-producing Foxp3$^+$CD4$^+$ T cells like T_H17 cells express CCR6, a trait of tissue-homing effector T cells [31, 32], suggesting that CCR6 combined with other markers such as CD45RA, CD45RO and Foxp3 could be used to identify IL-17-producing T_{Reg} cells *in vitro* and *in vivo*. In addition, studies by Raffin et al. have determined that IL-1 receptor type 1 (IL-1R1) positive effector T_{Reg} cells can become IL-17-secreting effectors in response to TCR-mediated stimulation in the presence of IL- 1β, suggesting that *ex vivo* expression of IL-1R1 in human T_{Reg} cells identifies an early intermediate in the conversion of IL-17-producing cells from T_{Reg} cells [33].

4. Roles of T_{Reg} cells and T_H17 cells in inflammation and colon cancer

The intestinal tract contains large numbers of immune cells involved in the encounter with microbial antigens. Under normal conditions, the lumen of the intestine is covered by a layer of intestinal epithelial cells (IECs), which are joined together by tight junctions forming a protective layer impeding bacterial invasion [34]. Immune responses in the colon take place in three distinct compartments: the lamina propria, the isolated lymphoid follicles (ILFs) scattered throughout the colon, and the mesenteric lymph nodes. The latter two sites may play important roles in initiating immune responses and in dictating quantitative and qualitative features of the responses. Cells in the lamina propria are involved in antigen sampling, and this is the site in which effector cells accumulate during inflammation [35].

T_{Reg} cells actively suppress enteroantigen-reactive cells and contribute to the maintenance of intestinal immune homeostasis. Distinct T_{Reg} cell subsets coexist in the intestinal mucosa and mesenteric lymph nodes. Studies have shown that commensal bacteria can direct the development of $Foxp3^+$ T_{Reg} cells with a unique ‘inducible” genetic signature, suggesting the existence of a mechanism via which microbiota can actively promote mucosal tolerance by inducing the differentiation of conventional T cells into $Foxp3^+$ T_{Reg} cells [36]. In fact, it is widely accepted that ‘inducible’ rather than ‘natural’ T_{Reg} cells are particularly effective in maintaining immune homeostasis of the intestine (Figure 1). The use of murine models of inflammatory bowel disease (IBD) has indicated that these IL-10-producing inducible T_{Reg} cells play an important role in preventing or limiting colitis [37]. The precise origin of these inducible T_{Reg} cells remains unclear.

In mucosal surfaces, T_H17 cells are thought to protect the host from infections, whereas several types of T_{Reg} cells keep effector T cell populations in check and prevent T cell-mediated destruction of intestinal tissue [38-41]. There is compelling evidence that Crohn's disease (CD) is driven by T_H17 cells [42]. CD, one type of IBD, is believed to be the result of an aberrant response of the gut-associated lymphoid tissue to bacterial and/or dietary antigens. Increased serum IL-17 levels have been observed in patients with CD compared to healthy individual. In addition, IL-17 mRNA expression and the number of $IL\text{-}17^+$ T cells were also increased in the inflamed mucosa from patients with active CD [43]. However, blocking IL-17 is ineffective for the treatment of CD, suggesting that although IL-17 is involved in the pathogenesis of CD, IL-17 itself is unlikely to serve as a therapeutic target for CD [44]. Increased numbers of T_H17 cells were also observed in the intestinal mucosa in patients with ulcerative colitis (UC), another well-characterized type of IBD [45]. It is unclear whether T_H17 cells play the same role in the pathogenesis of these two types of IBD or whether these two forms of IBD share a common mechanism underlying the accumulation of T_H17 cells as a consequence of the inflammation process in the intestinal mucosa. Of note, concomitantly with the increased frequency of T_H17 cells, there is also an increased number of T_{Reg} cells in the lamina propria, mesenteric lymph nodes, and inflamed intestinal mucosa of IBD patients [46]. Although the presence of IL-17, T_H17 cells and T_{Reg} cells in the inflamed intestine is well established, their dynamics and contribution to the IBD disease remain elusive.

T_{Reg} cells are an obstacle for immune surveillance and immune therapy of cancer due to suppressing anti-tumor immune responses. However, studies using $Apc^{Min/+}$ mice revealed a protective role of IL-10-producing T_{Reg} cells in bacterial-induced chronic inflammation and cancer [47] and even hereditary colon cancer [48], suggesting that T_{Reg} cells might play a protective role in cancer by suppressing inflammation (Figure 2). Mast cells are essential hematopoietic components promoting the development of adenomatous polyps [49]. Adoptive transfer of T_{Reg} cells protect against colon cancer by suppressing focal mastocytosis in adenomatous polyps due to IL-10 production [7]. Conversely, in polyp-

ridden mice, T_{Reg} cells are aberrant, fail to produce IL-10 and instead produce IL-17 thereby promoting rather than suppressing mastocytosis [7, 50]. These observations suggest that the role of T_{Reg} cells in the development of cancer depends on their unique functional properties as identified by their ability to produce specific cytokines.

IL-17, produced locally in the tumor microenvironment, plays important roles in both angiogenesis and tumor immunity. Inhibition of IL-17A at tumor sites by intratumoral injection of an adenovirus vector expressing siRNA against the mouse IL-17A gene (Ad-si-IL-17) significantly inhibited tumor growth. It was found that inhibition of IL-17 at tumor sites significantly suppressed CD31, MMP9 and VEGF expression in tumor tissue. Furthermore, the cytotoxic activity of tumor infiltrating $CD8^+$ lymphocytes in mice treated with ad-si-IL17A was significantly higher than in control mice [51]. Further experimental evidence supports the idea that IL-17 promotes tumor growth. Specifically, ablation of IL-17 significantly reduced tumor development in mice bearing a heterozygote mutation in the adenomatous polyposis coli (APC) gene ($Apc^{Min/+}$ mice). There was also a decrease in inflammatory cytokines and proinflammatory mediators and reduced lymphocytic infiltration, suggesting that IL-17 promotes spontaneous intestinal tumorigenesis [52]. Consistently with a positive role of IL-17 in promoting tumor development, tumor tissues have a higher frequency of $IL\text{-}17^+$ cells T cells compared with untransformed bowel tissues [53]. The role of IL-17 in promoting tumor growth provides additional support for the already well-established connection between inflammation and tumorigenesis. However, this study did not identify whether Ad-si-IL-17 induced IL-17 ablation only in T effector cells or whether its effects were also extended to T_{Reg} cells, a population that is also capable of producing IL-17 and is intimately linked to the development of inflammation and cancer in the bowel.

T_{Reg} cells can affect tumor progression by mediating suppression of the effector T-cell responses and by inducing production of angiogenetic factors by T_H17 cells. Tumor infiltrating T_H17 cells themselves have also been postulated to offset the anti-tumor response of IFN-γ^+ effector cells [54]. Thus, both T_{Reg} cells and T_H17 cells can affect cancer progression through independent and interconnected effects although the precise mechanisms of such counter-regulation remain poorly understood. Understanding the regulation of the T_{Reg}/T_H17 axis may result in novel approaches for the control of tumor progression. Studies by Baba et al. suggested that human $CD45RA^+$ T_{Reg} cells promote the differentiation of human T_H17 cells, implying that $CD45RA^+$ T_{Reg} cells positively govern T_H17 development. These findings might provide an explanation why T_H17 cells are often detected alongside T_{Reg} cells within tumor tissues or systemically in cancer patients [7, 55, 56] and further suggest that the T_{Reg} population might be an appropriate target for cancer immunotherapy.

5. Paradoxical roles of IL-17-producing $Foxp3^+CD4^+$ cells in anti-inflammatory response and anti-tumor immunity

Inflammatory and tumor tissues often have lower frequency of $CD69^+$ T effector cells but a higher frequency of T_H17 cells and T_{Reg} cells compared to healthy or untransformed tissues [53]. The mechanisms by which T_H17 cells and T_{Reg} cells accumulate in such inflammatory and tumor tissues and their roles in the pathogenesis of these diseases remain unsolved. The identification of $IL\text{-}17^+Foxp3^+$ T cells in inflamed intestinal mucosa of patients with IBD and cancer but not in healthy control might provide some clues to the above questions.

$IL\text{-}17^+Foxp3^+$ cells accumulated in colorectal cancer (CRC) tissue express CCR6, TGF-β and IL-6, and significantly suppress $CD8^+$ cell-mediated immune responses during *in vitro* culture [57, 58]. Interestingly, the suppressive activity of $IL\text{-}17^+Foxp3^+$ cells is attenuated

by pretreatment with an anti-IL-17 antibody, suggesting that this subset of T cells may be a novel therapeutic target in the treatment of CRC [58]. In a similar study, abundant IL-17$^+$Foxp3$^+$ cells expressing high levels of TGF-β, CXCR3, CCR6, and RORγt were also detected in CRC. In addition, co-culture of IL-17$^+$Foxp3$^+$ cells isolated from transformed tissues with sphere cells generated from bone marrow derived mononuclear cells under hypoxia, could induce sphere cells to express CRC markers such as CD133, CD44s, CD166, EpCAM, and ALDH1 [59, 60]. Addition of neutralizing anti-IL-17 antibody in the co-culture abolished the expression of CRC markers in sphere cells, suggesting that IL-17$^+$Foxp3$^+$ T_{Reg} cells drive cancer-initiating mechanisms and that IL-17 itself plays a critical role in this process [60].

A different study showed that T_{Reg} cells become pathogenic in the context of CRC when they acquire expression of RORγt [61]. In this model, expression of RORγt marks a distinct subset of activated T_{Reg} cells, which have the potential to express IL-17 and have been identified in human CRC tumor. These RORγt-expressing T_{Reg} cells expand in CRC in a manner dependent on cancer-stage. Moreover, enriched RORγt$^+$ T_{Reg} cells obtained from CRC display potent T-cell suppressive function. Conversely, RORγt deficiency restored the anti-inflammatory properties of T_{Reg} cells, protected against polyposis and improved anti-tumor immunity as determined by increased IFN-γ production [61]. These findings suggest that RORγt expressed in Foxp3$^+$ T_{Reg} cells might serve as a primary therapeutic target for improvement of the anti-inflammatory effects of T_{Reg} cells, thereby inhibiting tumor development. Furthermore, these findings suggest that RORγt$^+$ Treg rather than IL-17-producung cells might have a causative role in the pathogenesis of CRC development in the context of bowel inflammation (Figure 1).

6. Conclusions

The immunobiology of T_{Reg} cells has been intensively investigated since the discovery of T_{Reg} cells and their master transcription factor Foxp3, more than a decade ago. Such efforts have led to significant advances in our understanding of the development of T_{Reg} cells and the molecular mechanisms underlying their suppression function. Recent studies have focused on understanding the reciprocal relationship between T_{Reg} and other immune components surrounding T_{Reg} cells. Through these studies it has been recognized that T_{Reg} cells are subjected to regulation mediated by other immune cell populations, and as a result, the functional and phenotypic properties of T_{Reg} cells can be altered. Currently developing evidence supports the idea that the role of T_{Reg} cells in various disease settings varies considerably depending on disease stage, inflammatory conditions and activation of effector cells. Identification of the IL-17$^+$Foxp3$^+$$T_{Reg}$ and RORγt$^+$Foxp3$^+$ T_{Reg} subsets in human IBD and CRC confirm that Foxp3$^+$ T_{Reg} cells are capable of expressing transcription factors that control the differentiation of effector cells and can potentially produce proinflammatory cytokines. Although the exact mechanisms underlying the generation of IL-17$^+$ T_{Reg} cells remain unclear, soluble mediators such as IL-1β, TGF-β and IL-23 might be involved in this process. The precise role of these T_{Reg} subsets in inflammation and tumor immunity is still debatable but most studies agree that IL-17$^+$$T_{Reg}$ and RORγt$^+$$T_{Reg}$ suppress anti-tumor immunity and promote tumor growth. Thus, targeting RORγt and IL-17 in T_{Reg} cells might be required in order to improve the properties of T_{Reg} cells employed for cell-based therapies in the context of inflammation and cancer.

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References

1. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008; 133:775–787. [PubMed: 18510923]
2. Bienenstock J, McDermott M, Befus D, O'Neill M. A common mucosal immunologic system involving the bronchus, breast and bowel. Adv Exp Med Biol. 1978; 107:53–59. [PubMed: 742502]
3. Jorde R, Burhol PG. Diurnal profiles of gastrointestinal regulatory peptides. Scand J Gastroenterol. 1985; 20:1–4. [PubMed: 2859649]
4. Smart CJ, Trejdosiewicz LK, Badrel-Din S, Heatley RV. T lymphocytes of the human colonic mucosa: functional and phenotypic analysis. Clin Exp Immunol. 1988; 73:63–69. [PubMed: 2971488]
5. Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Buschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD). Clin Exp Immunol. 1995; 102:448–455. [PubMed: 8536356]
6. Bos PD, Rudensky AY. Treg cells in cancer: a case of multiple personality disorder. Sci Transl Med. 2012; 4:164fs144.
7. Gounaris E, Blatner NR, Dennis K, Magnusson F, Gurish MF, Strom TB, Beckhove P, Gounari F, Khazaie K. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. Cancer Re. 2009; 69:5490–5497.
8. Badoual C, Hans S, Rodriguez J, Peyrard S, Klein C, Agueznay Nel H, Mosseri V, Laccourreye O, Bruneval P, Fridman WH, Brasnu DF, Tartour E. Prognostic value of tumor-infiltrating CD4+ T-cell subpopulations in head and neck cancers. Clin Cancer Res. 2006; 12:465–472. [PubMed: 16428488]
9. Haas M, Dimmler A, Hohenberger W, Grabenbauer GG, Niedobitek G, Distel LV. Stromal regulatory T-cells are associated with a favourable prognosis in gastric cancer of the cardia. BMC Gastroenterol. 2009; 9:65. [PubMed: 19732435]
10. Ladoire S, Arnould L, Mignot G, Coudert B, Rebe C, Chalmin F, Vincent J, Bruchard M, Chauffert B, Martin F, Fumoleau P, Ghiringhelli F. Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2011; 125:65–72. [PubMed: 20229175]
11. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003; 4:330–336. [PubMed: 12612578]
12. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003; 299:1057–1061. [PubMed: 12522256]
13. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol. 2003; 4:337–342. [PubMed: 12612581]
14. Roncador G, Brown PJ, Maestre L, Hue S, Martinez-Torrecuadrada JL, Ling KL, Pratap S, Toms C, Fox BC, Cerundolo V, Powrie F, Banham AH. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. Eur J Immunol. 2005; 35:1681–1691. [PubMed: 15902688]
15. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol. 2010; 10:490–500. [PubMed: 20559327]
16. Seddiki N, Santner-Nanan B, Tangye SG, Alexander SI, Solomon M, Lee S, Nanan R, Fazekas de Saint Groth B. Persistence of naive CD45RA+ regulatory T cells in adult life. Blood. 2006; 107:2830–2838. [PubMed: 16332974]
17. Valmori D, Merlo A, Souleimanian NE, Hesdorffer CS, Ayyoub M. A peripheral circulating compartment of natural naive CD4 Tregs. J Clin Invest. 2005; 115:1953–1962. [PubMed: 16007258]
18. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity. 2009; 30:899–911. [PubMed: 19464196]
19. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells

via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med. 2007; 204:1757–1764. [PubMed: 17620361]

20. Hill JA, Feuerer M, Tash K, Haxhinasto S, Perez J, Melamed R, Mathis D, Benoist C. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity. 2007; 27:786–800. [PubMed: 18024188]
21. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity. 2009; 30:636–645. [PubMed: 19464986]
22. Zhou L, Lopes JE, Chong MM, Ivanov, Min R, Victora GD, Shen Y, Du J, Rubtsov YP, Rudensky AY, Ziegler SF, Littman DR. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008; 453:236–240. [PubMed: 18368049]
23. Voo KS, Wang YH, Santori FR, Boggiano C, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR, Liu YJ. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A. 2009; 106:4793–4798. [PubMed: 19273860]
24. Li L, Kim J, Boussiotis VA. IL-1beta-mediated signals preferentially drive conversion of regulatory T cells but not conventional T cells into IL-17-producing cells. J Immunol. 2010; 185:4148–4153. [PubMed: 20817874]
25. Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C. DC activated via dectin-1 convert Treg into IL-17 producers. Eur J Immunol. 2008; 38:3274–3281. [PubMed: 19039774]
26. Radhakrishnan S, Cabrera R, Schenk EL, Nava-Parada P, Bell MP, Van Keulen VP, Marler RJ, Felts SJ, Pease LR. Reprogrammed FoxP3+ T regulatory cells become IL-17+ antigen-specific autoimmune effectors in vitro and in vivo. J Immunol. 2008; 181:3137–3147. [PubMed: 18713984]
27. Xu L, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. J Immunol. 2007; 178:6725–6729. [PubMed: 17513718]
28. Li L, Patsoukis N, Petkova V, Boussiotis VA. Runx1 and Runx3 are involved in the generation and function of highly suppressive IL-17-producing T regulatory cells. PLoS One. 2012; 7:e45115. [PubMed: 22984619]
29. Komatsu N, Mariotti-Ferrandiz ME, Wang Y, Malissen B, Waldmann H, Hori S. Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proc Natl Acad Sci U S A. 2009; 106:1903–1908. [PubMed: 19174509]
30. Schmidl C, Hansmann L, Andreesen R, Edinger M, Hoffmann P, Rehli M. Epigenetic reprogramming of the RORC locus during in vitro expansion is a distinctive feature of human memory but not naive Treg. Eur J Immunol. 2011; 41:1491–1498. [PubMed: 21469109]
31. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, Sallusto F, Napolitani G. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol. 2007; 8:639–646. [PubMed: 17486092]
32. Kleinewietfeld M, Puentes F, Borsellino G, Battistini L, Rotzschke O, Falk K. CCR6 expression defines regulatory effector/memory-like cells within the CD25(+)CD4+ T-cell subset. Blood. 2005; 105:2877–2886. [PubMed: 15613550]
33. Raffin C, Raimbaud I, Valmori D, Ayyoub M. Ex vivo IL-1 receptor type I expression in human CD4+ T cells identifies an early intermediate in the differentiation of Th17 from FOXP3+ naive regulatory T cells. J Immunol. 2011; 187:5196–5202. [PubMed: 21998454]
34. Izcue A, Coombes JL, Powrie F. Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol Rev. 2006; 212:256–271. [PubMed: 16903919]
35. Newberry RD, Lorenz RG. Organizing a mucosal defense. Immunol Rev. 2005; 206:6–21. [PubMed: 16048539]
36. Round JL, Mazmanian SK. 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. [PubMed: 20566854]

37. Schmitt EG, Haribhai D, Williams JB, Aggarwal P, Jia S, Charbonnier LM, Yan K, Lorier R, Turner A, Ziegelbauer J, Georgiev P, Simpson P, Salzman NH, Hessner MJ, Broeckel U, Chatila TA, Williams CB. IL-10 produced by induced regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012; 189:5638–5648. [PubMed: 23125413]
38. Aujla SJ, Dubin PJ, Kolls JK. Th17 cells and mucosal host defense. Semin Immunol. 2007; 19:377–382. [PubMed: 18054248]
39. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. Gastroenterology. 2011; 140:957–965. [PubMed: 21147109]
40. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. Immunity. 2008; 28:454–467. [PubMed: 18400188]
41. Wan YY, Flavell RA. TGF-beta and regulatory T cell in immunity and autoimmunity. J Clin Immunol. 2008; 28:647–659. [PubMed: 18792765]
42. Kanai T, Nemoto Y, Kamada N, Totsuka T, Hisamatsu T, Watanabe M, Hibi T. Homeostatic (IL-7) and effector (IL-17) cytokines as distinct but complementary target for an optimal therapeutic strategy in inflammatory bowel disease. Curr Opin Gastroenterol. 2009; 25:306–313. [PubMed: 19448533]
43. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, Bamba T, Fujiyama Y. Increased expression of interleukin 17 in inflammatory bowel disease. Gut. 2003; 52:65–70. [PubMed: 12477762]
44. Raza A, Shata MT. Letter: pathogenicity of Th17 cells may differ in ulcerative colitis compared with Crohn's disease. Aliment Pharmacol Ther. 2012; 36:204. author reply 205. [PubMed: 22703461]
45. Eastaff-Leung N, Mabarrack N, Barbour A, Cummins A, Barry S. Foxp3+ regulatory T cells, Th17 effector cells, and cytokine environment in inflammatory bowel disease. J Clin Immunol. 2010; 30:80–89. [PubMed: 19936899]
46. Maul J, Loddenkemper C, Mundt P, Berg E, Giese T, Stallmach A, Zeitz M, Duchmann R. Peripheral and intestinal regulatory CD4+ CD25(high) T cells in inflammatory bowel disease. Gastroenterology. 2005; 128:1868–1878. [PubMed: 15940622]
47. Erdman SE, Rao VP, Poutahidis T, Ihrig MM, Ge Z, Feng Y, Tomczak M, Rogers AB, Horwitz BH, Fox JG. CD4(+)CD25(+) regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. Cancer Res. 2003; 63:6042–6050. [PubMed: 14522933]
48. Erdman SE, Sohn JJ, Rao VP, Nambiar PR, Ge Z, Fox JG, Schauer DB. CD4+CD25+ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice. Cancer Res. 2005; 65:3998–4004. [PubMed: 15899788]
49. Gounaris E, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, Lee DM, Zhang G, Glickman JN, Shin K, Rao VP, Poutahidis T, Weissleder R, McNagny KM, Khazaie K. Mast cells are an essential hematopoietic component for polyp development. Proc Natl Acad Sci U S A. 2007; 104:19977–19982. [PubMed: 18077429]
50. Huber S, Gagliani N, Esplugues E, O'Connor W Jr, Huber FJ, Chaudhry A, Kamanaka M, Kobayashi Y, Booth CJ, Rudensky AY, Roncarolo MG, Battaglia M, Flavell RA. 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. [PubMed: 21511184]
51. Hayata K, Iwahashi M, Ojima T, Katsuda M, Iida T, Nakamori M, Ueda K, Nakamura M, Miyazawa M, Tsuji T, Yamaue H. Inhibition of IL-17A in tumor microenvironment augments cytotoxicity of tumor-infiltrating lymphocytes in tumor-bearing mice. PLoS One. 2013; 8:e53131. [PubMed: 23372655]
52. Chae WJ, Gibson TF, Zelterman D, Hao L, Henegariu O, Bothwell AL. Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. Proc Natl Acad Sci U S A. 2010; 107:5540–5544. [PubMed: 20212110]
53. Girardin A, McCall J, Black MA, Edwards F, Phillips V, Taylor ES, Reeve AE, Kemp RA. Inflammatory and regulatory T cells contribute to a unique immune microenvironment in tumor tissue of colorectal cancer patients. Int J Cancer. 2013; 132:1842–1850. [PubMed: 23002055]

54. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. Cell. 2010; 140:845–858. [PubMed: 20303875]

55. Le Gouvello S, Bastuji-Garin S, Aloulou N, Mansour H, Chaumette MT, Berrehar F, Seikour A, Charachon A, Karoui M, Leroy K, Farcet JP, Sobhani I. High prevalence of Foxp3 and IL17 in MMR-proficient colorectal carcinomas. Gut. 2008; 57:772–779. [PubMed: 17965063]

56. Maruyama T, Kono K, Mizukami Y, Kawaguchi Y, Mimura K, Watanabe M, Izawa S, Fujii H. Distribution of Th17 cells and FoxP3(+) regulatory T cells in tumor-infiltrating lymphocytes, tumor-draining lymph nodes and peripheral blood lymphocytes in patients with gastric cancer. Cancer Sci. 2010; 101:1947–1954. [PubMed: 20550524]

57. Kryczek I, Wu K, Zhao E, Wei S, Vatan L, Szeliga W, Huang E, Greenson J, Chang A, Rolinski J, Radwan P, Fang J, Wang G, Zou W. IL-17+ regulatory T cells in the microenvironments of chronic inflammation and cancer. J Immunol. 2011; 186:4388–4395. [PubMed: 21357259]

58. Ma C, Dong X. Colorectal cancer-derived Foxp3(+) IL-17(+) T cells suppress tumour-specific CD8+ T cells. Scand J Immunol. 2011; 74:47–51. [PubMed: 21352252]

59. Lugli A, Iezzi G, Hostettler I, Muraro MG, Mele V, Tornillo L, Carafa V, Spagnoli G, Terracciano L, Zlobec I. Prognostic impact of the expression of putative cancer stem cell markers CD133, CD166, CD44s, EpCAM, and ALDH1 in colorectal cancer. Br J Cancer. 2010; 103:382–390. [PubMed: 20606680]

60. Yang S, Wang B, Guan C, Wu B, Cai C, Wang M, Zhang B, Liu T, Yang P. Foxp3+IL-17+ T cells promote development of cancer-initiating cells in colorectal cancer. J Leukoc Biol. 2011; 89:85–91. [PubMed: 20952660]

61. Blatner NR, Mulcahy MF, Dennis KL, Scholtens D, Bentrem DJ, Phillips JD, Ham S, Sandall BP, Khan MW, Mahvi DM, Halverson AL, Stryker SJ, Boller AM, Singal A, Sneed RK, Sarraj B, Ansari MJ, Oft M, Iwakura Y, Zhou L, Bonertz A, Beckhove P, Gounari F, Khazaie K. Expression of RORgammat marks a pathogenic regulatory T cell subset in human colon cancer. Sci Transl Med. 2012; 4:164ra159.

Highlights

- Foxp3^{+} T_{Reg} cells are able to express RORγt and produce IL-17
- IL-17^{+} T_{Reg} cells are accumulated in the inflamed intestinal mucosa.
- IL-17^{+} T_{Reg} cells have potent T-cell suppressive activity.
- RORγt $^{+}$ T_{Reg} cells are unable to suppress inflammation.
- IL-17^{+}/RORγt^{+} T_{Reg} cells are pathogenic T_{Reg} cells with proinflammatory properties.

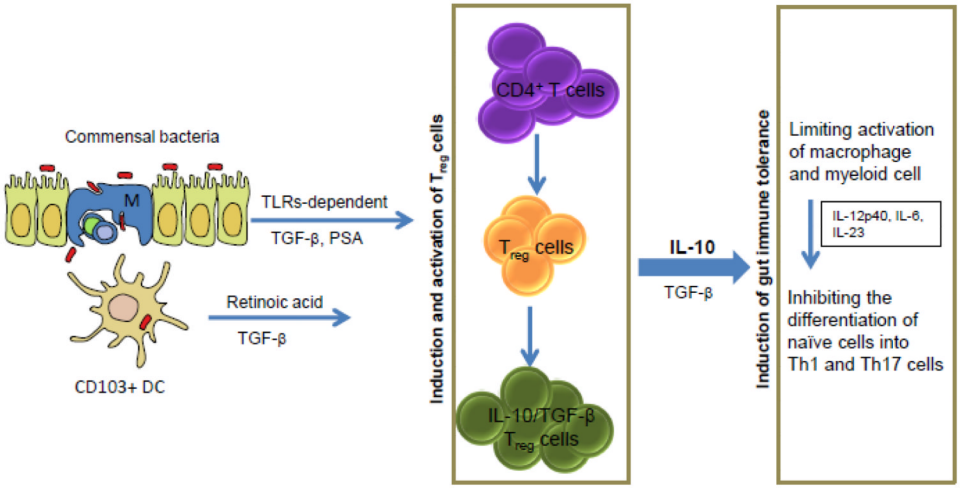

Figure 1. Induction of T_{Reg} cell mediated gut immune tolerance

In the steady state, gut immune tolerance is induced and maintained by a variety of mechanisms, which regulate the differentiation and activation of T_{Reg} cells. Some commensal bacteria can induce the differentiation of $CD4^+$ T cells into T_{Reg} cells in a TLRs dependent manner. *Clostridium spp*. can induce generation of IL-10 producing T_{Reg} cells via epithelial cell-derived TGF-β, whereas *Bacteroides fraglilis* can induce IL-10 producing T_{Reg} cells via polysaccharide A (PSA). $CD103^+$DC can also induce T_{Reg} cells through their ability to produce retinoic acid and TGF-β. T_{Reg} cells act on macrophage and myeloid cells to inhibit their ability to produce colitogenic cytokines including IL-12p40, Il-6 and IL-23, which are required for the differentiation of $CD4^+$ T cells into Th1 and Th17 cells. Both Th1 and Th17 cells are associated with initiation and progression of IBD and colon cancer. T_{Reg} cells suppress inflammatory responses mainly through production of anti-inflammatory cytokines TGF-β and more importantly IL-10.

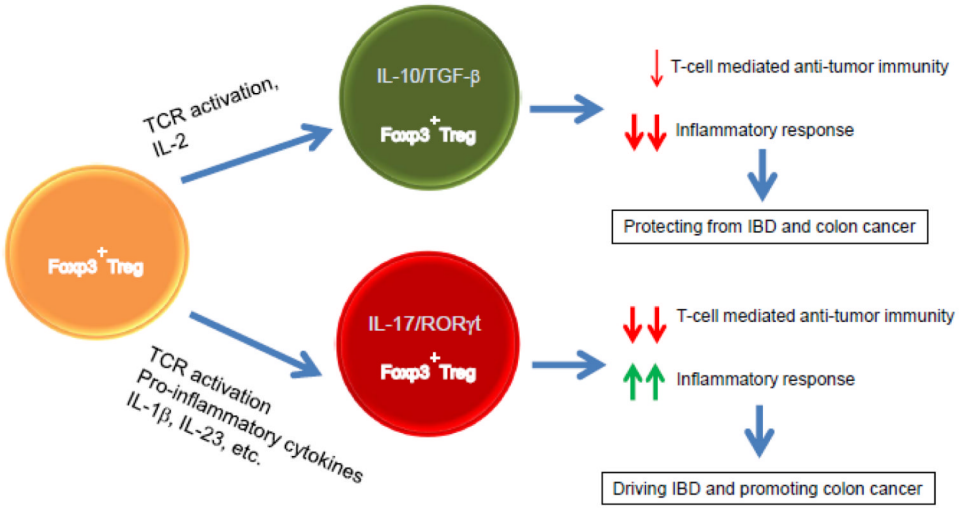

Figure 2. Opposing effects of Foxp3$^+$T$_{Reg}$ and RORγt$^+$Foxp3$^+$T$_{Reg}$ cells in the development of IBD and colon cancer

Foxp3$^+$ T$_{Reg}$ cells suppress both T-cell mediated anti-tumor immune response and inflammatory response at least in part by secretion of IL-10 and TGF-β in response to TCR-mediated stimulation. Given their ability to suppress inflammatory processes, Foxp3$^+$ T$_{Reg}$ cells play an important role in controlling and suppressing the pathogenesis of IBD and subsequently the development of colon cancer. However, in the context of an inflammatory milieu, Foxp3$^+$ T$_{Reg}$ cells are able to express RORγt and have the potential to express and secrete IL-17. The resultant RORγt$^+$Foxp3$^+$T$_{Reg}$ cells display potent suppressive activity on T-cell mediated immune responses, whereas their antiinflammatory function is compromised. As a consequence, RORγt$^+$Foxp3$^+$ T$_{Reg}$ cells are unable to control inflammation and rather promote instead of suppressing the development of colon cancer.