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REVIEW

# Regulatory T cells in inflammatory bowel diseases and colorectal cancer

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## Abstract

Regulatory T cells ($T_{regs}$) are key elements in immunological self-tolerance. The number of $T_{regs}$ may alter in both peripheral blood and in colonic mucosa during pathological circumstances. The local cellular, microbiological and cytokine milieu affect immunophenotype and function of $T_{regs}$. Forkhead box P3+ $T_{regs}$ function shows altered properties in inflammatory bowel diseases (IBDs). This alteration of $T_{regs}$ function can furthermore be observed between Crohn's disease and ulcerative colitis, which may have both clinical and therapeutical consequences. Chronic mucosal inflammation may also influence $T_{regs}$ function, which together with the intestinal bacterial flora seem to have a supporting role in colitis-associated colorectal carcinogenesis. $T_{regs}$ have a crucial role in the immunoevasion of cancer cells in sporadic colorectal cancer. Furthermore, their number and phenotype correlate closely with the clinical outcome of the disease, even if their contribution to carcinogenesis has previously been controversial. Despite knowledge of the clinical relationship between IBD and colitis-associated colon cancer, and the growing number of immunological aspects encompassing sporadic colorectal carcinogenesis, the molecular and cellular links amongst $T_{regs}$, regulation of the inflammation, and cancer development are still not well understood. In this paper, we aimed to review the current data surrounding the role of $T_{regs}$ in the pathogenesis of IBD, colitis-associated colon cancer and sporadic colorectal cancer.



**Key words:** Regulatory T cells; Forkhead box P3; Inflammatory bowel diseases; Colitis-associated colon cancer; Colorectal cancer

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## INTRODUCTION

Immunological self-tolerance is crucial for preventing autoimmune diseases and maintaining immune homeostasis[1]. Regulatory T cells ($T_{regs}$) are a subpopulation of T cells including CD4+CD25+ forkhead box P3 (Foxp3)+ T cells, Tr1, Th3, and CD8+ $T_{regs}$. They serve to suppress the immune system and maintain self-tolerance. Inappropriate T cell response to intestinal microflora has a role in the pathological process of inflammatory bowel diseases (IBD)[2]. Accumulating evidence indicates that the imbalance of immunity, including a discrepancy between the number of $T_{regs}$ in peripheral blood and in inflamed colonic mucosa, an excess of pro-inflammatory stimuli and an altered function of immunoregulatory cells has a central role in the pathogenesis of IBD[3].

Risk for developing cancer rises substantially as a result of poorly regulated inflammatory responses to pathogenic bacteria. $T_{regs}$ function to restore immune homeostasis during chronic inflammation, and therefore it seems logical that $T_{regs}$ would reduce the risk of inflammation-associated colon cancer by down-regulating inflammation. Alteration of T cell phenotype could also have an effect not only on the regulation of the chronic mucosal inflammation[3], but on colitis-associated carcinogenesis as well[4,5].

On the other hand, it is also widely believed, that $T_{regs}$ in cancer primarily suppress protective anticancer immune responses. This alteration of $T_{regs}$ function, together with the tumor-helping chemokine milieu produced by the cancer tissue could support cancer progression[4,5]. $T_{regs}$ role in the carcinogenesis of inflammation-associated colon cancer and sporadic colorectal cancer is paradoxical due to conflicting results.

The bone marrow progenitors of all T cells become committed to their lineage in the thymus. The double negative T cells (CD4-CD8-) will rearrange their T cell receptor (*TCR*) genes to form a unique, functional molecule, which they test against cells in the thymic cortex for a minimal level of interaction with self-major histocompatibility complex (MHC). Upon receiving these signals, they begin to proliferate and express both CD4 and CD8. Then, the double-positive cells are selected by their interaction with thymic cells, begin the transcription of Foxp3, becoming $T_{regs}$. Foxp3 expression begins at the single-positive stage, at which point they are functional $T_{regs}$[6].

Foxp3 is a member of the forkhead and winged helix family of transcriptional regulators, and plays a critical role in the development and function of CD4+ $T_{regs}$[6]. In previous studies, CD4 and CD25 were selected as the markers for $T_{regs}$, but in recent studies Foxp3 has been proposed as a more reliable and superior biomarker[7,8].

The exact process of $T_{reg}$ cell selection is not well known. It appears to be a process determined by the affinity of interaction with the self-peptide MHC complex[9]. Characteristic features of CD4+CD25+Foxp3+ $T_{regs}$ are their anergic state, and their ability to actively inhibit CD4+CD25- T cells, CD8+ T cells, dendritic cells, natural killer cells, natural killer T cells, and B cells in a cell-to-cell contact and dose-dependent manner[10].

Recent results[3-5] suggest that $T_{regs}$ could have an important, but controversial regulatory role in both the pathogenesis of chronic colonic inflammation and colorectal carcinogenesis. Despite the clinical relationship between IBD and IBD-associated colon cancer, and the growing number of immunological aspects encompassing sporadic colon cancer, the molecular and cellular links between infiltrating immune cells, regulation of the inflammation, and cancer development are not well understood. Therefore, we aimed to review the current information available on the role of $T_{regs}$ in the pathogenesis of IBD, colitis-associated colon cancer and sporadic colorectal cancer.

## $T_{REGS}$ IN IBD

### *Discrepancy in the distribution of $T_{regs}$ in peripheral blood and mucosa*

$T_{regs}$ are able to suppress the expansion of effector T cells by interleukin (IL)-10 and transforming growth factor (TGF)-β secretion and cytotoxic T lymphocyte-associated antigen expression[11]. The frequency of circulating $T_{regs}$ was shown to be lower in patients with relapsing than in remitting autoimmune diseases[12,13], therefore leading to the speculation that insufficient levels of $T_{regs}$ in peripheral blood are critical in inducing a relapse of autoimmune disease.

Maul *et al*[14] reported that CD4+CD25+ cells were decreased in the peripheral blood of IBD patients. Moreover, Wang *et al*[3] found the percentage of CD4+Foxp3+ $T_{regs}$ in peripheral blood to be significantly lower in IBD (both ulcerative colitis and Crohn's disease) patients compared with healthy controls. However, at the mucosal level, Maul *et al*[14] and Wang *et al*[3] found the frequency of $T_{regs}$ higher in active and inactive IBD samples compared to healthy controls. Their results and the results of Holmén *et al*[15] indicated that the increased frequency of CD25+ T cells in inflamed mucosa might be associated with the decreased number of circulating CD4+CD25+ T cells. During active inflammation, circulating $T_{regs}$ migrate into the lamina propria to maintain homeostasis. As direct cell-to-cell contact is critical for $T_{regs}$ to function, a low number of $T_{regs}$ may result in a disturbance in the inhibition of the inflammatory reaction[15,16]. The results of Wang *et al*[3] suggest that an insufficient number of $T_{regs}$ in the peripheral blood may be associated with the recurrence of IBD.

While Wang *et al*[3] did not find any alteration in the frequency of mucosal CD4+Foxp3+ $T_{regs}$ in ulcerative colitis and Crohn's disease, Hovhannisyan *et al*[17] showed that the inflammatory environment in the colonic mucosa of Crohn's disease patients (but not in that of ulcerative colitis patients) contributes to the generation of a distinct population of $T_{regs}$ that are Foxp3+ and produce IL-17. These Foxp3+ IL-17-producing cells also produced large amounts of interferon (IFN)-γ, another effector cytokine. On the other hand, Kryczek *et al*[18] observed mucosal Foxp3+IL17+CD4+ $T_{regs}$ in ulcerative colitis that induce proinflammatory cytokines and suppress effector T cell function, exhibiting their dual inflammatory and regulatory function.

The balance between these subpopulations of mucosal $T_{reg}$ cells, which may be regulated by the inflammatory milieu, seems to be crucial in the pathogenesis of IBD.

### *Phenotypic plasticity of $T_{regs}$ in IBDs*

The IL-17-producing CD4+ T (Th17) cells constitute a subset of helper T cells that has been linked to the pathogenesis of IBD[19]. Mucosal Th17 cells are thought to protect the host from infection.

The development of Th17 cells and $T_{regs}$ requires TGF-β which suggests that both cell types may be related

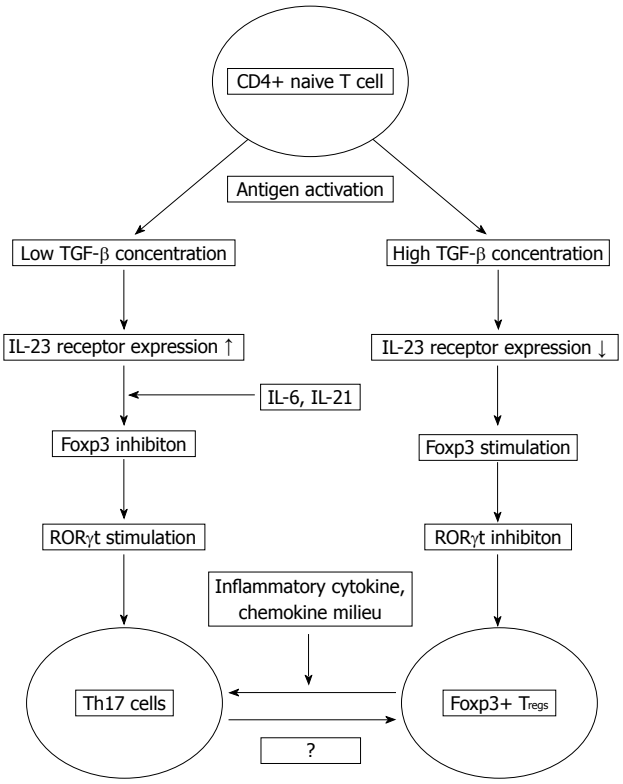


**Figure 1 Phenotypic plasticity of T cells in inflammatory bowel disease.** The mucosal concentration of transforming growth factor (TGF)-β influences the interleukin (IL)-23 receptor expression of antigen activated CD4+ T cells, which results imbalance in forkhead box P3 (Foxp3) and retinoic acid-related orphan receptor (RORγt) transcription factor activity. The Foxp3 dependent inhibition or stimulation of RORγt may regulate helper T cell (Th) 17 or Foxp3+ regulatory T cell ($T_{reg}$) differentiation. The inflammatory cytokine/chemokine milieu may also influence the phenotypic changes of T cells.

and may arise from the same precursor based on the distinct cytokine milieus[20-23]. TGF-β exposure of antigen-activated naive CD4+ T cells results in upregulation of Foxp3 and retinoic acid-related orphan receptor (RORγt), two transcription factors that regulate $T_{reg}$ and Th17 cell differentiation[24]. Zhou *et al*[25] showed that at low concentrations, TGF-β synergizes with IL-6 and IL-21 to promote IL-23 receptor (IL23R) expression, favouring Th17 cell differentiation, while in high concentrations it represses IL23R expression and favours Foxp3+ $T_{regs}$ differentiation. *In vitro*, TGF-β-induced Foxp3 inhibits RORγt function, at least in part through their interaction. Consequently, lamina propria Foxp3+RORγt+T cells produce less IL-17 than those that express RORγt alone. IL-6, IL-21 and IL-23 inhibit Foxp3-mediated inhibition of RORγt, therefore promoting Th17 cell differentiation. Based on these data it seems, that the decision of antigen-stimulated cells to differentiate into either Th17 or $T_{reg}$ cell depends on the cytokine-regulated balance of Foxp3 and RORγt[25].

Hovhannisyan *et al*[17] identified such a Foxp3+ IL-17-producing CD4+ $T_{reg}$ cell population in the lamina propria of Crohn's disease patients that shared phenotypic characteristics of both Th17 and $T_{reg}$ cells, and showed potent suppressor activity *in vitro*. Their analysis of the TCR repertoire revealed a similar TCR β-chain variable region usage by Foxp3+IL-17+ and Foxp3+CD4+ T cells, suggesting that Foxp3+ IL-17-producing cells may come from Foxp3+ $T_{regs}$ when exposed to unique inflammatory signals present in Crohn's disease (Figure 1).

Increased mucosal TGF-β and IL-6 expression have been detected in Crohn's disease[26,27]. Similar results were not found in ulcerative colitis associated mucosa[26,27]. These cytokines are supposed to promote the conversion of $T_{regs}$ into effector T cells, while retaining their suppressive properties[17,20]. Despite the presence of cytokines involved in Th17 and $T_{reg}$ cell differentiation, the generation of Foxp3+IL-17+ $T_{regs}$ from normal lamina proprial or peripheral blood CD4+ T cells failed[17]. Therefore it is speculated that additional, presumably local, factors are also required for the induction of these cells *in vivo*. Kryczek *et al*[18] found that these inflammatory $T_{regs}$ are induced from memory chemokine (CC motif) receptor 6+ T cells and $T_{regs}$. Furthermore, TGF-β, IL-2 and the presence of antigen presenting cells are also essential for their induction.

The better understanding of the origin and function of $T_{regs}$, and the interaction between effector and regulatory sites of mucosal immune cells in IBD may lead to new therapeutic possibilities.

## $T_{REGS}$ IN IBD-ASSOCIATED COLON CANCER

In mice, Faubion *et al*[28] showed that chronic inflammation arising from the bowel may induce thymic involution and $T_{reg}$ cell suppression. This was suggested to lead to the enhancement of inflammation-mediated events that worsen IBD. Restoration of homeostasis through suppression of tumor necrosis factor (TNF)-α production and fortification of $T_{regs}$ were proposed for human IBD patients[4]. These data on IBD may support to the concept that uncontrolled inflammation weakens the $T_{reg}$-mediated inhibition and increases the risk for inflammation-associated carcinogenesis.

In colitis-associated colon cancer, a high frequency of Foxp3+IL-17+CD4+ $T_{regs}$ was found in the tumor, but not in the adjacent mucosa[18]. Though human tumor-infiltrating $T_{regs}$ express limited effector cytokines[29], colitic Foxp3+IL-17+CD4+ $T_{regs}$ express a moderate amount of IFN-γ, TNF-α and IL-2, and shares similar cytokine profiles to blood Foxp3+IL-17+ $T_{regs}$[18]. This cytokine profile reveals a phenotype for polyfunctional effector T cells similar to that observed in infectious diseases[30,31] and tumor-infiltrating Th17 cells in multiple human cancers[32]. These data indicate that Foxp3+IL-17+ $T_{regs}$ express effector cytokines and are distinct from $T_{regs}$ within the same inflammatory milieu.

Moreover, in suppressive assays, Foxp3+IL-17+ T cells from colitis-associated colon cancer were able to suppress T cell proliferation and IFN-γ production in a dose-dependent manner[18], which suggests that Foxp3+IL-17+ T cells belong to a functional $T_{reg}$ cell population.

Kryczek *et al*[18] also found that $T_{regs}$ induce the production of IL-1 and IL-6 in ulcerative colitis. IL-17 derived from Foxp3+IL-17+CD4+ T cells may also induce IL-6 expression[33,34]. As IL-1 and IL-6 play a crucial role in ulcerative colitis[35,36], it is possible that IL-17-producing Foxp3+CD4+ T cells together with Th17 cells may potentially contribute to the early tumorigenesis seen in chronic ulcerative colitis.

The role of pathogenic microbial infection in colitis-associated carcinogenesis is also known[4]. After pathogenic microbial challenges, a pro-inflammatory response mediated by effector T cells serves to eliminate pathogenic bacteria. Educated CD4+CD45RB$^{low}$ $T_{regs}$ then subsequently suppress inflammation, offering protection from immunological self-destruction. It was shown that IL-10-dependent $T_{regs}$ protect from malignancy[37,38]. Deficiency in IL-10 increases susceptibility to IBD-associated colorectal cancer (CRC) with poor prognosis[39]. Mice lacking IL-10 were shown to be susceptible to colitis-associated CRC after *Helicobacter hepaticus* (*H. hepaticus*) infection, while their wild type counterparts infected with *H. hepaticus* had only minimal bowel alterations[37,38,40,41]. The lack of IL-10 leads to elevated levels of TNF-α, IL-6 and IL-17, therefore allowing chronic inflammation to persist[42]. After *H. hepaticus* infection, IL-10 insufficient CD4+ T cells preferentially recruit to a Th-17 phenotype in the case of a pro-inflammatory challenge[4-45]. It was also shown in mice that bacteria-triggered inflammation mobilizes neutrophils bearing TGF-β1 and IL-6, which may inhibit $T_{regs}$ and promote Th-17 response, a phenomenon that has been linked to carcinogenesis[40]. Based on these results, one may conclude that colonic bacterial infections supposed to trigger protective IL-10-dependent $T_{regs}$, while, under hygienic conditions, individuals with insufficiently educated $T_{regs}$ are predisposed to sustained inflammatory reactions and may subsequently developed cancers[4].

Acting downstream of TGF-β1 signaling, Runx proteins are the interacting and functional partners of R-Smad proteins[46]. Among Runx proteins, Runx3 is involved in the differentiation of immune cells[47], and acts as a homeostasis maintainer and tumor suppressor in the gastrointestinal epithelium[48,49]. In mice, Sugai *et al*[47] found that the loss of Runx3 in T cells resulted in suppression of $T_{reg}$ cell function which lead to the development of colitis and colonic tumors (associated with luminal microorganisms) in Runx3$^{-/-}$ animals.

Further studies are needed to reveal the interaction between the commensal intestinal bacterial flora and $T_{regs}$ for the better understanding of colitis-associated colorectal carcinogenesis.

## $T_{REGS}$ IN SPORADIC COLORECTAL CANCER

### *Presence of $T_{regs}$ and disease outcome*

Cancer cells have evolved to develop strategies of immunoevasion to escape control by the immune system[50]. There are quite numerous possible immunoevasion mechanisms that appear to affect diverse steps in cancer-specific lymphocyte priming, activation, and effector function. These mechanisms may also operate simultaneously[51,52]. Evidence is accumulating that CD4+CD25+Foxp3+ $T_{regs}$ are crucially involved in cancer immunobiology[10]. The number of Foxp3+ $T_{regs}$ was found to be significantly increased in the peripheral blood, lymph nodes and surrounding tumors of patients with colorectal cancer compared to that of healthy controls[20,53]. Several data show that increased frequencies of $T_{regs}$ prognosticate a worse outcome for colon cancer patients, as this may reflect the inability of the immune system to adequately respond to cancer[54-56]. Moreover, studies aimed at reducing $T_{reg}$ cell frequencies in experimental models have resulted in successful control of cancer[57].

On the contrary, others showed that an increased intratumoral Foxp3+ $T_{reg}$ cell number correlates with a favourable clinical outcome[58]. This can be the result of mast cell-$T_{reg}$ cell interaction, which induces $T_{regs}$ to switch function and escalate inflammation in CRC without losing T-cell-suppressive properties in an IL6 and IL17 independent manner[59].

Szczepanik *et al*[60] found that the absolute number of $T_{regs}$ in the peripheral blood of gastric cancer patients, especially in those with lymph node metastases was significantly decreased in comparison to that of healthy controls. This phenomenon was not observed in CRC patients. Their results suggest that the population of $T_{regs}$ in peripheral blood does not simply mimic intratumoral $T_{regs}$.

Based on this data, one can conclude that when inflammatory cells that promote tumor progression dominate the immune response, $T_{regs}$ may be beneficial in suppressing carcinogenesis. However, when T cells dominate the immune response, $T_{regs}$ may promote disease progression by suppressing their anti-tumoral immunological effects. The discrepancy between the peripheral blood and local, intratumoral number phenotype of $T_{regs}$ in sporadic CRC may have an effect on this theory, and should be further investigated.

### *Contribution of $T_{regs}$ to carcinogenesis and cancer cell survival*

The colonic adenoma-dysplasia-carcinoma sequence is associated less clearly with inflammation then it is with colitis-associated colon cancer. C57BL/6 mice heterozygous for a mutation in the *Apc* gene (Apc$^{Min/+}$) are genetically at risk for intestinal polyps and mimic early stages of sporadic CRC in humans[61]. It was observed, that Apc$^{Min/+}$ mice have higher serum and intestinal levels of TNF-α, IL-6 and IL-17 than matched wild type animals, which may indicate a subclinical inflammatory condition[4]. Elevated inflammatory cytokine levels were found in human colorectal cancer as well[62].

Using $T_{reg}$ cell titration assay it was also shown, that $T_{regs}$ from *H. hepaticus* infected donor Apc$^{Min/+}$ mice con-

sistently provided complete protection from cancer and more effectively suppressed TNF-α, IL-6 and IL-17 cytokine levels in tumor-prone tissues than $T_{regs}$ from uninfected donor mice[4,63]. It is thought that the protection from cancer is attributable to $T_{reg}$ cell functions, and is more dependent upon the former conditions of $T_{regs}$ than that of the recipient mice.

In humans, Ma *et al*[64] revealed that CD4+CD25+Foxp3+IL-17+ T cells in CRC tissue express TGF-β and IL-10, the functional molecules of $T_{regs}$. This subset of T cells suppresses anti-tumoral peripheral CD8+ T cells in a tumor antigen-specific manner, therefore contributing to cancer development. If the effector CD8+ T cells could disengage from the suppression of CD4+CD25+Foxp3+IL-17+ $T_{regs}$, they might again proliferate and fight against tumor development.

It has been shown that chemokine (CC motif) ligand (CCL) 5 is highly expressed in colon tumor tissues from early-stage CRC compared with paired normal tissues[65]. CCL5 promotes tumor growth and metastasis by inducing tumor cell proliferation, angiogenesis, and expression of matrix metalloproteinases[66,67]. It can also diminish anti-tumoral immune responses by increasing the presence of tumor-infiltrating macrophages and $T_{regs}$[67]. Chang *et al*[68] have recently found that CCL5 expression could not only promote migration of $T_{regs}$ to tumors, but also enhance their killing ability on CD8+ T cells. Furthermore, this enhanced killing ability was associated with an increase in production of TGF-β by $T_{regs}$. Based on these results, it seems that the production of CCL5 by cancer cells is a part of CRC's immunoevasion repertoire.

Further studies may help us to better understand the interaction between tumor cells and $T_{regs}$ and to identify an unrevealed mechanism by which tumor cells induce immune tolerance in CRC.

## CONCLUSION

Targeting $T_{regs}$ may offer an important therapeutic strategy as an adjunct to treatment of patients.

A better understanding of the microenvironmental cues that influence $T_{reg}$ cell commitment toward a given lineage and their balance between regulation and inflammation may result in new therapeutic targets in IBD and colitis-associated cancer.

$T_{regs}$ and IL-10 producing Tr1 cells have the potential to prevent or cure colitis in graft-versus-host disease, a finding supported by a favourable safety profile was demonstrated in phase I clinical trials[69]. Although it must be confirmed in further clinical trials, these results emphasize that $T_{regs}$ may also be promising tools for therapeutic applications in IBD[70]. Changing the distribution of peripheral and mucosal $T_{regs}$ may also have therapeutic potential in colonic inflammations.

We presently lack sufficient data to determine the clinical effect of an immunotherapy targeting $T_{regs}$ in patients with sporadic CRC. The unexpected finding[58] that tumor-infiltrating Foxp3+ T cells could be associated with a favourable prognosis in CRC, suggests the potential in targeting these cells in immune-based anti-cancer therapies. On the other hand, eliminating CD4+CD25+Foxp3+IL-17+ $T_{regs}$ may help the effector CD8+ T cells to fight against cancer cells. Therefore this subset of T cells may offer a novel therapeutic target in the treatment of CRC[64]. In conclusion, these conflicting results should be appreciated with some discretion, as the mechanisms linking tumor-infiltrating Foxp3+ $T_{reg}$ cell density with a favourable outcome remain equivocal.

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