

# Review Article: Regulatory T Cells in Colorectal Cancer



Morteza Jafarinia<sup>1</sup> , Noushin Lotfi<sup>1</sup> , Mazdak Ganjalikhani-hakmi<sup>1</sup> , Abbas Rezaei<sup>1\*</sup>

1. Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.



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## ABSTRACT

One of the essential protection mechanisms of immune system is Treg cells which play an important role in maintenance of immune homeostasis. However, they may also inhibit immune functions against tumor cells. It has been reported that Treg cells are increased in patients suffering from different types of cancer. Increased number of Treg cells has been shown in tumor lymph nodes, peripheral blood, and tumor sites of patient with Colorectal Cancer (CRC). However, it is clear that Treg cells are increased in CRC patients, but the prognostic impact of Treg cells in CRC patients is a matter of debate. It seems that the function of these cells depend on the stage of CRC development. The aim of the present review article is to make an attempt to provide vision on the role of Treg in CRC. Finally, the potential approaches for the treatment of CRC are discussed.

## Introduction

Colorectal Cancer (CRC) develops in the colon or rectum and is the third most common cancer and the third leading cause of death due to cancer in the US [1]. This cancer is usually asymptomatic, so screening is essential in detecting it. The main risk factors for this cancer include alcohol, red meat, obesity, smoking, lack of physical exercise, and genetic predisposition [2].

Treg cells are specific population of T cells with suppression/regulatory properties and play a major role in maintenance of self-tolerance. These cells comprise 5-10% of the circulating CD4<sup>+</sup> population. The initial characteristic

phenotype of Tregs is CD4<sup>+</sup>CD25<sup>+</sup> [3]. However, CD25 expression cannot be used in human studies. Previous studies have suggested that peripheral blood isolated from an outbred human population contains up to 30% CD4<sup>+</sup>CD25<sup>+</sup> T cells; only 1-2% of cells with the highest CD25 expression have been shown to be functionally suppressive and can be considered as Tregs [4]. Besides CD4 and CD25, transcription factor FoxP3 is also required for isolation of Treg cells. In fact, Foxp3 is specifically required for Treg cell development [5]. Recent studies have shown that human Tregs are functionally and phenotypically diverse.

Treg cells can be classified in two main subtypes: naturally occurring Treg cells, which are CD4<sup>+</sup>CD25<sup>hi</sup>CD127<sup>low</sup>FoxP3<sup>+</sup> Helios<sup>+</sup> and induced regulatory T

## \* Corresponding Author:

Abbas Rezaei, PhD.

**Address:** Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

**Phone:** +98 (31) 37929060

**E-mail:** rezaei@mui.ac.ir

cells (iTreg) which are generated in the periphery. These iTreg can exert their suppressive effects through production of IL-10 and TGF- $\beta$  [4, 6]. Moreover, there are Foxp3 negative suppressor T cells, including Tr1, Th3 cells, CD8+CD28+/-, and Qa1-restricted T cells [7, 8]. Treg cells also could be divided in to three categories based on CD45RA expression and intensity of FoxP3 expression: 1) CD45RA+FoxP3low resting Treg cells, 2) CD45RA-FoxP3hi activated Treg cells, and 3) cytokine-secreting CD45RA-FoxP3low non-Treg cells [9]. In addition to CD25 and FoxP3, there are other markers that have been shown to express on Treg cells, including CD127 [10], CTLA-4 [11], GITR [12] and HLA-DR [13]. There is also another classification of Treg cells based on the expression of HLA-DR and CD45RA: Naïve Tregs (CD45RA+, HLA-DR-), memory Tregs (CD45RA-, HLA-DR-), and memory/activated Tregs (CD45RA-, HLA-DR+) [14].

Treg cells play a critical role in inflammation resolution and restoration of immune homeostasis. They can functionally suppress immune responses by influencing the activity of another cell type [15], including CD4+ and CD8+ T cells, Natural Killer and NKT cells, B cells, and Antigen-Presenting Cells (APCs) [16]. In spite of this activity, sometimes Treg cells play negative roles in immune system and could protect virus or carcinoma from immune clearance and promote diseases development [17].

## Treg Cells in Cancer

Regulatory T cells play an important role in cancer, although partially controversial role. In many human cancers, the prevalence of Treg and their suppressor actions are increased as compared to those reported for healthy participants [18-20]. Despite the general concept that Treg aggregation in cancer is related with poor prognosis [21-23], several reports have demonstrated that Treg numbers and activity are associated with improved prognosis [24-28]. While the role of Treg in tumor growth, progression to metastasis, and the disease outcome are still controversial, there is considerable experimental and clinical evidence that Treg plays a crucial role in suppression of antitumor immune responses and so contributes to tumor escape from the host immune system [28, 29]. Different studies showed an increase of Treg cells in circulation, draining lymph nodes, and the tumor site of patients with malignancies [30, 31].

The potential of Treg application either for protection from tissue destruction by activated T cells or for invasion against antitumor effector immune cells has led to a more extensive attention of mechanisms that support Treg recruitment to tissue sites. It is accepted, for example, that Treg express Toll-Like Receptors (TLRs), and that TLR li-

gands can control functions of Treg, presumably including their migration [32]. Employment of Treg to tumor sites is regulated by chemokines formed in the Tumor Micro-environment (TME) such as, CCL22, a ligand for CCR4 [33]. Activated Treg express several chemokine receptors (i.e. CCR4, CCR5, CCR6, CCR7, and CCR10) that can mediate Treg trafficking to tumor micro environment [34].

In the presence of tumor-derived chemokines, Treg gather in the tumor site, and, before taking place, progress to prevent antitumor responses of immune cells penetrating the TME. Hence, Treg which accumulate in situ and in the peripheral circulation of cancer patients can be noticed as one of multiple attempts by the tumor to develop its own escape from the host immune system by dampening anti-tumor immune effector cells. On the other hand, it looks likely that in tumors characterized by vast inflammatory infiltrates, such as colon or breast cancers, Treg are vital for controlling chronic inflammation, preventing tissue damage, and reducing tumor development associated with inflammation [35, 36].

Several studies have suggested that many cancers can induce proliferation of Treg cells from naïve T cells. It is also shown that the presence and number of Treg in the tumor are associated with improved prognosis in patients with colon or breast carcinomas [28, 35, 37, 38]. Recently, the origin and phenotypic features of Treg infiltrating human tumors have been a matter of debate [39, 40] and although rapid progress have been made in our understanding of how Treg work, many conditions of their interactions with the tumor and other immune or non-immune cells remain ambiguous [41, 42].

## Regulatory T Cells in CRC and Controversial Function of Treg Cells in CRC

CRC is one of the most common cancer and cause of the death in the world [1]. Treg cells could have an important role in CRC, and have potential roles in therapeutic strategies [43]. Increased number of Treg cells has been shown in tumor lymph nodes, peripheral blood, and tumor site in patients with CRC [15, 44, 45]. It is also shown that Treg cells could infiltrate lymph nodes in patients with CRC. These cells have critical roles in the colon and are necessary for homeostasis and maintaining intestinal immune tolerance in colon. The abundance of Treg cells in the colon is due to commensal microbiota and colonic DCs which are tolerogenic in this part of body. Reduction of Treg cells have been shown in the germ free mice or vancomycin-treated mice [46].

Recently, Treg cells have received special attention because of their inhibitory role on effector T cells and high number of these cells that enable the cancer cells to evade the host immune responses [47]. Previous studies were shown that Treg cells in patients are specific for a set of Tumor Associated Antigens (TAAs), suggesting that Treg suppress other cells in an antigen selective manner [48].

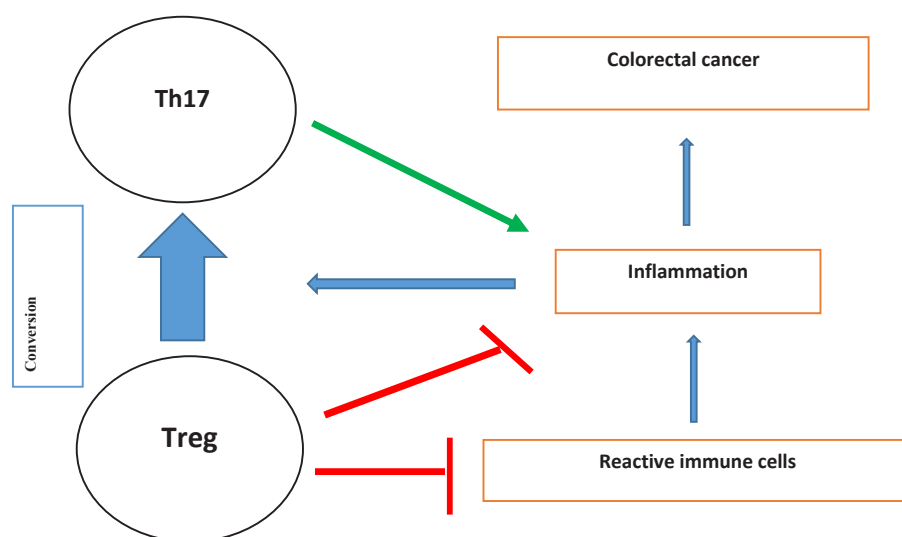
Several studies have shown that anti-tumor T cell immune responses were enhanced in mice after depletion of Treg cells subpopulation by anti-CD25 monoclonal antibodies. Also, high number of tumor infiltrating FoxP3 cells has been associated with poor overall survival of patients with solid tumors [49-51]. So, it is reasonable to consider that high level of Treg cells in CRC patients can inhibit anti-tumor response and cause immune escape of tumor cells and disease progression [51]. One study showed that after Treg cells depletion, there will be an increase in effector T cells responses against TAAs in CRC patients [48].

Although Treg cells are increased in CRC patients, the prognostic impact of Treg cells in these patients is a matter of debate. Different studies support the different roles of Treg cells in CRC patients. In one study, it was shown that the density of FoxP3 cells infiltrating CRCs was significantly higher in parallel with enhanced number of CD8+ T cells in CRC with level microsatellites instability [52]. Also, several other studies have reported that high density of Treg cells in Sentinel Lymph Node could suppress the antitumor immune response, whereas in CRC patients, Treg cells correlate with increased tumor protection and survival [53].

Different studies suggested that in early stages of CRC, Treg cells may have a protective role by suppressing cancer-associate inflammation, but after conversion of the Treg cells into a pro-inflammatory phenotype, this benefit is lost [54]. On the other hand, in early carcinogenesis, Treg cells are able to limit the inflammation that ultimately leads to cancer [29]. One report showed that high number of intra-tumoral FoxP3 cells is associated with better survival of CRC patients [45]. In fact, the role of Treg cells in CRC patients depend on the type of immune responses in the tumor microenvironment and the stage of the disease. Treg cells may be beneficial when inflammatory cells that promote tumor progression are dominate; however, Treg cells may promote tumor progression and suppress the anti-tumor immunity when the immune response is dominated by T cells [45]. Treg cells can also convert into pro-inflammatory Th17 cells under strong inflammatory stimuli and lead to cancer initiation [55].

### Potential Approaches for Treatment of CRC

Targeting Tregs may suggest an important therapeutic plan as an adjunct to the treatment of patients besides surgery and neoadjuvant chemotherapy [45]. In fact, it is determined that immunosuppression by T-reg cell is one of the vital tumor immune-evasion mechanisms and the main restriction of successful tumor immunotherapy [56]. Treg cells are necessary to tumor-induced peripheral tolerance and are an obstacle to tumor immunotherapy. Some cytotoxic agents systemically deplete Treg cells, and Treg modulation in patients with CRCs might progress antitumor immunity or the response to immunotherapy [50, 57]. It is also reported that depletion of Tregs in the peripheral blood



**Figure 1.** Role of Treg in colorectal cancer  
Conversion of Treg cells into Th17 cells can initiate the colorectal cancer.

of patients with CRC enhances CD4<sup>+</sup> T cell responses to Tumor-Associated Antigens (TAAs) [48].

However, the profit or risk of Tregs increase in CRC is currently controversial. Some reports concluded that using Tregs for immunotherapy has a strong preclinical animal database, which support the safety and efficacy of Treg immunotherapy protocols in patients who need induction of clinical tolerance, like allograft tolerance, atopic and autoimmune disease, and acute inflammatory disorders [58]. However, because there are differences between Tregs and Effector T cells in the supply of the known TAAs, alternative choice is testing selected TAAs for tumor vaccinations that motivates suitable effector T-cell responses but reduces Treg activity, which may boost the efficacy of vaccination programs without the need for depletion of Tregs [54].

### Concluding Remarks

CRC is one of the most common cancers in the world having a significant impact on the health care outcomes. Already it is known that Treg cells increase and enrich in CRC patients. But the action of these cells is still a matter of debate. It seems that the function of these cells depend on the stage of CRC development. In the early stages, Treg cells play a positive role by suppressing the inflammatory cells and inflammation. However, when effector cells dominate in the tumor microenvironment, they play a negative role by suppressing the effector cells. So, learning more about the exact role of these cells in CRC patients can help us to adopt different strategies to manipulate Treg cells and eventually improve the patient survival.

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