The Role of T\textsubscript{reg} Cells in the Cancer Immunological Response

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Abstract: Problem statement: T cell-mediated immunosuppression has been observed for decades without clarification as to which factor was responsible for this observation. The identification of CD4\textsuperscript{+}CD25\textsuperscript{+} regulatory T (T\textsubscript{reg}) cells represents a milestone in the field of immunology and provides an explanation for T-cell-mediated immunosuppression. Although T\textsubscript{reg} cells were originally identified for their ability to prevent organ-specific autoimmune disease in mice, emerging evidence suggests that T\textsubscript{reg} cells play a pivotal role in tumor immunity and contribute to tumor growth and progression, thereby having an important impact on the outcome of cancer patients. Approach: This article reviewed the medical literature to describe how T\textsubscript{reg} cells affect anti-tumor immunity. Results: T\textsubscript{reg} cells suppressed anti-tumor immunity by inhibiting the effector functions of tumor-specific T cells and NK cells. Importantly, tumor cells played an active role in recruiting and generating T\textsubscript{reg} cells and creating a suppressive tumor microenvironment. Strategies to deplete T\textsubscript{reg} cells or inhibit their function had yielded promising results by enhancing anti-tumor immunity in experimental studies as well as clinical practice. Conclusion: A better understanding of the pathophysiology of T\textsubscript{reg} cells not only increased our knowledge in a variety of aspects of immunology but also potentially benefited cancer patients.

Key words: CD4\textsuperscript{+}CD25\textsuperscript{+}, T\textsubscript{reg} cells, Foxp3, cancer, tumor immunity, immune response

INTRODUCTION

T cell-mediated immuno-suppression has been observed for decades. In 1970, Gershon et al.\textsuperscript{[1]} found that there were populations of bone marrow-derived precursors of antibody-making cells (B cells) which could not be rendered tolerant to Sheep Red Blood Cells (SRBC) unless thymus-derived lymphocytes (T cells) were present. In 1972, Gershon et al.\textsuperscript{[2]} further found that thymocytes were capable of suppressing the antigen-induced response of other thymocytes without the mediation of B cells and defined these thymocytes as suppressor T cells. Since then, T-cell-mediated suppression of immune response has been investigated under a variety of pathophysiological conditions including malignant transformation in animal model by \textit{in vitro} and \textit{in vivo} studies. A series of studies by North et al.\textsuperscript{[3]} has shown that the acquisition of suppressor T cells by a tumor-bearing host is responsible for the failure of passively transferred, tumor-sensitized T cells to cause regression of the tumor. The attempt to isolate suppressor T cells using different methods was unsuccessful simply due to a lack of phenotypic characterisation in this subset. This hurdle persisted until a subset of CD4\textsuperscript{+} T cells expressing IL-2 receptor \textalpha-chain (CD25) were identified in 1995 and found to be critical in the control of self-tolerance\textsuperscript{[4]}. In this study, Sakaguchi et al.\textsuperscript{[5]} found that depletion of CD25\textsuperscript{+} T cells resulted in spontaneous development of autoimmune diseases and reconstitution of CD4\textsuperscript{+}CD25\textsuperscript{+} cells prevented these autoimmune diseases in a dose-dependent fashion. This finding was subsequently confirmed by a study showing that CD4\textsuperscript{+}CD25\textsuperscript{+} T cells inhibited both the induction and effector function of autoreactive T cells and suggested that CD4\textsuperscript{+}CD25\textsuperscript{+} T cells represent a unique lineage of immunoregulatory cells\textsuperscript{[5]}. Since then, tremendous effort has been put into investigating CD4\textsuperscript{+}CD25\textsuperscript{+} T cells in a variety of settings. In this article, we will review recent advances regarding the role of CD4\textsuperscript{+}CD25\textsuperscript{+} regulatory T cells in the cancer immunological response.

Characterization of regulatory T cells: Regulatory T (T\textsubscript{reg}) cells were originally identified as a small subset of CD4\textsuperscript{+} T cells expressing IL-2 receptor \textalpha-chain (CD25) and represented approximately 5-10\% of peripheral CD4\textsuperscript{+} T cells in both mice and humans. In addition to sustained high surface expression of CD25, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR) expression are features of suppressive T\textsubscript{reg}
cells. To date, it is generally believed that CD4\(^+\) T\(_{\text{reg}}\) subsets include naturally occurring T\(_{\text{reg}}\) cells and peripherally induced T\(_{\text{reg}}\) cells. Naturally occurring T\(_{\text{reg}}\) cells have a phenotype as originally identified, arise as a distinct lineage from the thymus and migrate into blood and peripheral tissues. These T\(_{\text{reg}}\) cells are anergic in vitro and do not proliferate in response to T-cell receptor (TCR) stimulation. This anergy can be overcome by the addition of high doses of exogenous IL-2 or the use of mature Dendritic Cells (DCs) as antigen-presenting cells. In addition to naturally occurring T\(_{\text{reg}}\) cells, T\(_{\text{reg}}\) cells can be induced in the periphery under particular conditions of antigenic stimulation. The presence of inducible T\(_{\text{reg}}\) cells in the periphery is supported by the observation in adult mice that deletion of T\(_{\text{reg}}\) cells by means of an anti-CD25 monoclonal antibody and thymectomy is followed by complete reconstitution within 48 days.

Studies have revealed that several molecules and signaling pathways are involved in inducing the development of T\(_{\text{reg}}\) cells in the periphery. These include glucocorticoids, estrogen, TGF-\(\beta\), IL-2, and IL-20, as well as co-stimulatory molecules such as CD80/CD86 and CD70. Along with naturally occurring T\(_{\text{reg}}\) cells, peripherally induced T\(_{\text{reg}}\) cells play an important role in suppressing the immune response, especially the anti-tumor immune response.

**Foxp3 identification:** The forkhead/winged helix transcription factor family member Foxp3 (forkhead box P3) plays a critical role in suppression of immune system responses and inhibition of Foxp3 function results in significant immune dysregulation as illustrated by the following findings. A mutation in the gene Foxp3 carried by the mutant mouse strain scurfy results in a CD4\(^+\) T-cell–mediated lymphoproliferative disease. Mutations in the human homolog of Foxp3 lead to onset of a human genetic disease called immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) characterized by global immune dysregulation with autoimmunity. From these clinical observations, three studies have independently shown that Foxp3 is specifically expressed in T\(_{\text{reg}}\) cells and is necessary for T\(_{\text{reg}}\) cell development and function. It has been convincingly shown that ectopic expression of Foxp3 in CD4\(^+\)CD25\(^-\) naive T cells by retroviral gene transfer can convert them to natural T\(_{\text{reg}}\)-like cells functionally and phenotypically. Transgenic mice lacking Foxp3 lack T cells with regulatory function and have dysregulated T cell proliferation resulting in a severe autoimmune disease. These results indicated that Foxp3 is a master transcriptional factor for development and function of T\(_{\text{reg}}\) cells and is now used as a specific marker for T\(_{\text{reg}}\) cells.

**Regulatory property of T\(_{\text{reg}}\) cells:** CD4\(^+\)CD25\(^+\) T\(_{\text{reg}}\) cells have been demonstrated to suppress various types of immune responses, including autoimmunity, antimicrobial and antitumor immune responses by inhibiting T cell, B cells and NK cells. T\(_{\text{reg}}\) cells were originally identified as a subset of CD4\(^+\) T cells suppressing the proliferation and cytokine production of conventional CD4\(^+\)CD25\(^+\) T cells. Further studies found that T\(_{\text{reg}}\) cells are also able to suppress the proliferation, cytokine production and granule secretion of CD8\(^+\) T cells. This suppression results in the prevention of CD8\(^+\) T cell-mediated graft rejection, inhibition of CD8\(^+\) T cell-mediated skin inflammation, maintenance of persistent hepatitis C virus infection, as well as elimination of tumor cytotoxicity by CD8\(^+\) T cells.

In addition to the suppression of T cells, T\(_{\text{reg}}\) cells can also suppress proliferation and immunoglobulin production of CD19\(^+\) B cells. Firstly, T\(_{\text{reg}}\) cells can indirectly inhibit the B cell immunoglobulin response by suppressing CD57\(^+\) GC-T\(_{\text{H}}\) cells, a subset of cells specifically present within GCs with highly efficient T helper function to stimulate B cells to produce immunoglobulin, thereby interfering with GC-T\(_{\text{H}}\) cells-stimulated B cell immunoglobulin production. Secondly, T\(_{\text{reg}}\) cells can also directly suppress the B cell immunoglobulin response without having to suppress T\(_{\text{H}}\) cells. Under this circumstance, T\(_{\text{reg}}\) cells directly suppress B cell class switch recombination and thereby regulate B cell immunoglobulin production.

In addition to suppressing adaptive immune cells, T\(_{\text{reg}}\) cells also have an impact on innate immune cells. It has been reported that T\(_{\text{reg}}\) cells inhibit the cytotoxicity of CD3\(^+\)CD56\(^+\) NK cells and steer monocyte differentiation toward alternatively activated macrophages (AAM), a subset of cells with immune regulatory properties that contribute to tumor promotion.

The mechanisms mediating these immunosuppressive effects still remain to be fully understood. Several studies suggest that the immunosuppression is cell contact-dependent, while other studies demonstrate that suppression can also be cell contact-independent. Cell contact-dependent mechanisms represent circumstances in which T\(_{\text{reg}}\) cell-mediated suppression cannot be abrogated by neutralizing soluble inhibitory cytokines and T\(_{\text{reg}}\) cells cultured with CD4\(^+\)CD25\(^+\) T cells in a transwell system are unable to suppress the proliferation of responder cells. In this regard, membrane-bound TGF-\(\beta\) has...
been shown to play an important role in T<sub>reg</sub> cell-mediated, cell contact-dependent suppression of T and B cells given that T<sub>reg</sub> cells express high levels of TGF-β on the cell surface and T<sub>reg</sub> cells mediate immunosuppression via cell surface presentation of TGF-β to TGF-βR on target cells. In contrast, soluble factors are involved in T<sub>reg</sub> cell-mediated cell contact-independent mechanism. In this regard, the production of the immunosuppressive cytokines IL-10 and TGF-β, preferential IL-2 consumption by CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells, or direct lysis of T cells via perforin and granzymes are involved in suppressive effects of T<sub>reg</sub> cells. For example, Grossman et al. showed that human peripherally induced and naturally occurring T<sub>reg</sub> cells express granzyme-B upon activation and that these T<sub>reg</sub> cells display perforin-dependent cytotoxicity against autologous target cells, including activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells. This finding has been confirmed by other studies showing perforin-granzyme B pathway can also be served as a suppressive mechanism for T<sub>reg</sub> cells in the murine system.

Reciprocal regulation of T<sub>reg</sub> and T<sub>h17</sub>: T<sub>reg</sub> cells and T-helper (T<sub>h</sub>) cells constitute two opposing immune responses. Newly-identified IL-17-secreting CD4<sup>+</sup> helper T cells expand the family of T<sub>h</sub> cells into 3 major lineages, T<sub>h</sub>1, T<sub>h</sub>2 and T<sub>h</sub>17 cells. CD4<sup>+</sup>CD25<sup>+</sup> T<sub>reg</sub> cells form the other major lineage of CD4<sup>+</sup> T cells. T<sub>h</sub>17 and T<sub>reg</sub> cells are critically involved in the modulation of inflammation induced by either autoimmunity or bacterial infection. T<sub>h</sub>17 and T<sub>reg</sub> cells develop from precursor naïve CD4<sup>+</sup> T cells. The selective differentiation of precursor CD4<sup>+</sup> T cells into T<sub>h</sub>17 or T<sub>reg</sub> cells is established during the initial priming of these cells and is influenced by a variety of extracellular factors, such as the cytokine environment, the dose of antigen and the source of costimulation. Among these, the most effective polarizing factor is the cytokine environment. The presence of TGF-β plus IL-6 during activation drives the differentiation of precursor CD4<sup>+</sup> T cells into T<sub>h</sub>17 cells in mice, whereas the presence of TGF-β alone promotes differentiation of T<sub>reg</sub> cells. Unlike mice, IL-1β (but not TGF-β) plus IL-6 have been demonstrated to drive the differentiation of T<sub>h</sub>17 cells in humans. The differentiation of precursor CD4<sup>+</sup> T cells into T<sub>reg</sub> or T<sub>h</sub>17 cells is mutually exclusive. Tumor cells commonly participate in the generation of T<sub>reg</sub> cells, which provides an explanation for the observation that elevated numbers of T<sub>reg</sub> cells have been found in many types of cancers. It appears that TGF-β, secreted by the tumor itself or tumor-stimulated myeloid cells, plays a central role in tumor-mediated development of T<sub>reg</sub> cells by converting naïve T cells into T<sub>reg</sub> cells.

The decision of naïve CD4<sup>+</sup> T cells to become T<sub>h</sub>17 or T<sub>reg</sub> cell has important consequences in the success of an immune response and the progression of disease. CD4<sup>+</sup> T cell infiltration into tissue occurs whenever pathological changes are initiated. These pathological changes include infection, autoimmunity and malignant cell transformation. Interestingly, infiltrating CD4<sup>+</sup> T cells take distinct differentiation directions in different pathological scenarios. T<sub>h</sub>17 cells and T<sub>reg</sub> cells are prototypical subsets of CD4<sup>+</sup> T cells whose infiltration in tissues with each of those pathological changes represents the result of CD4<sup>+</sup> T cell differentiation affected by different pathological changes. CD4<sup>+</sup> T cells migrating into tissue with autoimmune disease adopt a pro-inflammatory phenotype while CD4<sup>+</sup> T cells invading into the tissues with malignant disease adopt an inhibitory phenotype. The mechanism responsible for the distinct differentiation direction of CD4<sup>+</sup> T cells is largely unknown.

T<sub>reg</sub> cells in the tumor microenvironment: Although infiltration by CTL and T<sub>h</sub> cells as well as other immune cells in tumor microenvironment is commonly seen, spontaneous clearance of established tumors by endogenous immune mechanisms is rare. The attempts at using immunotherapy to supplement essential immunogenic elements to boost tumor-specific immunity have shown limited clinical benefit. The generally accepted reason is that tumor cells develop diverse strategies that escape tumor-specific immunity. It has been shown that immunosuppression exists in the tumor microenvironment and contributes to the progression of cancer. T<sub>reg</sub> cells have profound inhibitory properties to suppress the function of effector T cells and account for a significant proportion of the immunosuppression in the tumor microenvironment. Indeed, emerging evidence suggests that T<sub>reg</sub> cells are involved in the regulation of antitumor immunity. Consistent with this concept, experimental depletion of T<sub>reg</sub> cells in mice with tumors improves immune-mediated tumor clearance and enhances the response to immune-based therapy. T<sub>reg</sub> cells have been shown to suppress tumor-specific T-cell immunity and therefore may contribute to the progression of human tumors. Furthermore, tumor T<sub>reg</sub> cells are associated with a reduced survival in patients with various malignancies.

The number of T<sub>reg</sub> cells in tumor microenvironment: Since Woo et al. reported in 2001 that CD4<sup>+</sup>CD25<sup>+</sup> T cells exist in significant
numbers in tumor tissue from patients with early-stage non-small cell lung cancer or later-stage ovarian cancer, a number of studies have consistently found that CD4\(^+\)CD25\(^+\) T cells as well as CD4\(^+\)Foxp3\(^+\) T cells are highly represented in tumor tissue (tumor masses, ascites, draining lymph nodes and spleen) and peripheral blood from patients with a wide variety of cancers. CD4\(^+\)CD25\(^+\) T cells from tumor-bearing mice and cancer patients show similar Foxp3 expression and suppressive activity in vitro when compared to naturally occurring T\(_{\text{reg}}\) cells. Elevated numbers of T\(_{\text{reg}}\) cells correlate with disease stage, histologic subtypes or overall survival of cancer patients. For example, it has been found that T\(_{\text{reg}}\) cells are increased in patients with advanced-stage breast cancer and that HER\(^{2}\), but not HER\(^{1}\), tumors account for this increase\(^{[45]}\). Although it has been shown that the number of T\(_{\text{reg}}\) cells is associated with overall survival in most studies, there is no agreement regarding whether elevated number of T\(_{\text{reg}}\) cells predicts a poor or favorable outcome for all cancer patients. It appears that high numbers of T\(_{\text{reg}}\) cells are associated with a poor prognosis in patients with most types of solid tumors. In contrast, highly-representative T\(_{\text{reg}}\) cells correlate with a favorable outcome in some patients with hematological malignancies\(^{[43-47]}\). The reason for this discrepancy is unknown. In hematological malignancies, malignant T, or B, or myeloid cells are the target of T\(_{\text{reg}}\) cells. Because the malignant cells are immune cells, T\(_{\text{reg}}\) cells may interact differently with these cells than with malignant cells in solid tumors. In fact, it has been shown that T\(_{\text{reg}}\) cells directly suppress B cell-dependent immunoglobulin production and class switch recombination, without having to suppress T\(_{\beta}\) cells\(^{[26]}\) and can induce apoptosis of activated B cells via the upregulation of perforin and granzymes\(^{[35]}\). T\(_{\text{reg}}\) cells may therefore directly suppress malignant cells in hematologic malignancies and this may explain, in part, why the increased percentage of tumor infiltrating T\(_{\text{reg}}\) cells predicts a better overall survival in patients with hematological malignancies.

**Recruitment and generation of intratumoral T\(_{\text{reg}}\) cells:** Several mechanisms that may explain the elevated number of T\(_{\text{reg}}\) cells in the tumor microenvironment have been proposed. Firstly, T\(_{\text{reg}}\) cells express a number of chemokine receptors such as CCR2, CCR4, CCR5, CCR7, CCR8 and CXCR4 and are able to migrate in response to a variety of chemokines such as CCL22, CCL17, CCL1 and CCL4\(^{[48]}\). Among those chemokines and chemokine receptors, CCR4 and CCL22 are particularly important in terms of their role in attracting T\(_{\text{reg}}\) cells into the tumor site. A study by Curiel et al.\(^{[49]}\) showed that ovarian tumor T\(_{\text{reg}}\) cells express functional CCR4 and migrate toward CCL22 in the tumor microenvironment. They showed that cancer cells and tumor-associated macrophages are the source of CCL22. These ovarian tumor T\(_{\text{reg}}\) cells are functionally suppressive and able to block tumor-specific immunity, foster tumor growth and predict poor patient survival\(^{[49]}\). This finding has been also observed in other malignancies such as B-cell NHL\(^{[50]}\), Hodgkin lymphoma\(^{[51]}\) and gastric cancer\(^{[52]}\). In addition to the CCR4-CCL22 pair, other chemokines and receptors have been also found to play an important role in recruiting T\(_{\text{reg}}\) cells into tumors. In pancreatic cancer patients, intratumoral T\(_{\text{reg}}\) cells expressed high-level of CCR5 and respond to CCL5 produced by pancreatic cancer cells\(^{[53]}\). Interestingly, disruption of CCR5-dependent homing of T\(_{\text{reg}}\) cells by abolishing CCL5 expression in pancreatic tumor cells or blockade CCR5 expression on intratumoral T\(_{\text{reg}}\) cells by CCR5 antagonists inhibits tumor growth in a murine model of pancreatic cancer\(^{[53]}\). Furthermore, another study found that IL-2 stimulates CXCR4 expression on T\(_{\text{reg}}\) cells and enables T\(_{\text{reg}}\) cells to migrate toward CXCL12 in the tumor microenvironment thereby increasing T\(_{\text{reg}}\) cell accumulation\(^{[54]}\).

A second mechanism for the increased number of intratumoral T\(_{\text{reg}}\) cells is the expansion and *de novo* generation of T\(_{\text{reg}}\) cells within tumors. As discussed above, naturally occurring T\(_{\text{reg}}\) cells are anergic and do not proliferate in response to TCR stimulation unless in the presence of IL-2. However, naturally occurring T\(_{\text{reg}}\) expansion has been reported in Hodgkin lymphoma and myeloma. In Hodgkin lymphoma, *in vitro* pre-exposure of PBMCs to a Hodgkin lymphoma cell line (HRS) supernatant significantly increased the expansion of T\(_{\text{reg}}\) cells\(^{[55]}\), which may explain the elevated number of T\(_{\text{reg}}\) cells in Hodgkin lymphoma patients\(^{[56]}\). In myeloma, monocyte-derived DCs maintained and expanded CD4\(^+\)Foxp3\(^+\) T\(_{\text{reg}}\) cells under *in vitro* culture conditions. Furthermore, it has been found that injection of DCs matured by inflammatory cytokines into patients with myeloma in a clinical trial results in a rapid expansion of T\(_{\text{reg}}\) cells seen within 1 week after DC injection\(^{[57]}\). These observations suggest that naturally occurring T\(_{\text{reg}}\) cells can be expanded within the tumor microenvironment. In addition to expansion of T\(_{\text{reg}}\) cells, *de novo* generation of T\(_{\text{reg}}\) cells is another important mechanism and has been reported in several types of tumors. The tumor microenvironment is able to induce the development of T\(_{\text{reg}}\) cells through converting CD4\(^+\)CD25\(^+\) T cells into CD4\(^+\)CD25\(^+\) T cells. Valzasina et al.\(^{[58]}\) observed increased numbers of CD4\(^+\)CD25\(^+\) cells in spleen and draining lymph nodes
of tumor-bearing mice and significant recovery of T<sub>reg</sub> cells in thymectomized mice with depletion of CD25<sup>+</sup> T cells using an anti-CD25 antibody, suggesting tumor development in mice led to a de novo generation of T<sub>reg</sub> cells. Another study<sup>[59]</sup> described a subset of tumor-induced CD25<sup>-</sup> regulatory T cells (TMT<sub>reg</sub>) in mice that arise after the mice are inoculated with lymphoma B cells. These TMT<sub>reg</sub> have increased expression of Foxp3 and IL-10, develop independently of pre-existing natural T<sub>reg</sub> cells and maintain suppressive properties long term in the absence of antigen stimulation. In conjunction with naturally occurring T<sub>reg</sub> cells, TMT<sub>reg</sub> induced tumor-specific CD4<sup>+</sup> T cell tolerance. In patients with B-cell NHL, several studies<sup>[60,61,16]</sup> have shown that lymphoma B cells induce Foxp3 expression in intratumoral CD4<sup>+</sup>CD25<sup>+</sup> T cells and participate in the generation of T<sub>reg</sub> cells, which may account for elevated number of T<sub>reg</sub> cells seen in B-cell NHL.

A number of additional mechanisms have been proposed to explain how T<sub>reg</sub> cells are generated in the tumor microenvironment. Given that TGF-β is able to convert CD4<sup>+</sup>CD25<sup>-</sup> T cells into T<sub>reg</sub> cells and tumor cells are a rich source of TGF-β, TGF-β can be the key factor contributing to tumor-mediated conversion of normal CD4<sup>+</sup> T cells into T<sub>reg</sub> cells. Indeed, several studies have shown that tumor-derived TGF-β played an important role in the generation of T<sub>reg</sub> cells in the tumor microenvironment.<sup>[62,63]</sup> In addition, our group has found that CD70-expressing lymphoma B cells induced Foxp3 expression in intratumoral CD4<sup>+</sup>CD25<sup>-</sup> T cells and interaction between CD27<sup>-</sup>-CD70 was involved in lymphoma B cell-mediated generation of T<sub>reg</sub> cells<sup>[16]</sup>. Although conversion of CD4<sup>+</sup>CD25<sup>-</sup> T cells to T<sub>reg</sub> cells has been described as a physiological process that maintains the peripheral T<sub>reg</sub> population, the data would suggest that this process is used by tumor cells to evade immune surveillance.

**Specificity of intratumoral T<sub>reg</sub> cells:** Most CD4<sup>+</sup> T cells persist as an antigen-specific subset, but it is not clear whether antigen-specific T<sub>reg</sub> cells exist. The observation that tumor cells are able to induce the development of T<sub>reg</sub> cells suggests that T<sub>reg</sub> cells may recognize tumor antigens and may be tumor-specific. It has been shown that specific recognition of tumor antigen led to differentiation of a subset of CD4<sup>+</sup> T cells into cells capable of suppressing naïve and T<sub>eff</sub> effector cells. These CD4<sup>+</sup> T cells have increased expression of Foxp3 and IL-10 with suppressive activity and were described as tumor-induced regulatory T cells.<sup>[59]</sup>

Further study showed that this de novo generation of T<sub>reg</sub> cells contributed to tumor-specific T cell tolerance<sup>[59,64]</sup>. Wang et al.<sup>[65,66]</sup> generated a panel of CD4<sup>+</sup> T-cell clones isolated from a melanoma. One of the clones had a phenotype similar to T<sub>reg</sub> cells in that the cells expressed CD25, GITR and Foxp3 and recognized a tumor-specific antigen and this clone was shown to inhibit the proliferation of conventional CD4<sup>+</sup> T cells. This result demonstrated that T<sub>reg</sub> cells recognizing tumor antigens can be generated in vitro. In ovarian cancer, it has been shown that tumor T<sub>reg</sub> cells disabled tumor antigen-specific T cell immunity in vivo and in turn allow tumor growth.<sup>[69]</sup>

**Reversal and enhancement of function of T<sub>reg</sub> cells:** The suppressive effect of T<sub>reg</sub> cells is a major obstacle to developing effective cancer immunotherapy. Although it has been shown that depletion of T<sub>reg</sub> cells led to inhibition and rejection of tumor growth in animal models and an increased anti-tumor immunity in cancer patients in some studies, T<sub>reg</sub> depletion with therapies targeting CD25 has not consistently improved the clinical outcome and overall survival of cancer patients. At least two reasons have been proposed to explain this. One explanation is that T<sub>reg</sub> cell depletion promptly induces conversion of peripheral precursors into T<sub>reg</sub> cells and the number of T<sub>reg</sub> cells will be restored over a period of time. Second is that some CD4<sup>+</sup>CD25<sup>-</sup> T cells in the tumor microenvironment also express Foxp3 and possess similar regulatory function to naturally occurring T<sub>reg</sub> cells. Therefore, while targeting CD4<sup>+</sup>CD25<sup>-</sup> T<sub>reg</sub> cells may augment tumor-specific immune responses, residual CD4<sup>+</sup>CD25<sup>-</sup>Foxp3<sup>+</sup> cells capable of mediating immune suppression would still remain and would continue to inhibit the host’s anti-tumor response.

Inability of CD25-depletion to eliminate the T<sub>reg</sub> cells in the tumor microenvironment has led to a second strategy to reverse T<sub>reg</sub> cell function. Several groups have reported that the function of T<sub>reg</sub> cells can be reversed by Toll-Like Receptor (TLRs) ligation by CpG<sup>[67,68]</sup>, OX40 costimulation<sup>[60]</sup>, or functional blockade of galactin-1<sup>[70,71]</sup>. Toll-like receptors control activation of adaptive immune responses by Antigen-Presenting Cells (APCs) such as DCs. Ligation of TLRs on DCs overcomes CD4<sup>+</sup>CD25<sup>+</sup> T cell-mediated suppression<sup>[67]</sup>. Further study identified that it is TLR8 that is responsible for TLR-mediated reversal of CD4<sup>+</sup> regulatory T cell function<sup>[68]</sup>. OX40 belongs to the TNF receptor family and co-stimulation of OX40 in vivo has been shown to prevent tolerance induction and to reverse lymphocyte hyporesponsiveness in experimental tolerogenic systems. Triggering OX40 profoundly inhibited Foxp3 gene expression and abrogated the ability of naturally arising Foxp3<sup>+</sup> T<sub>reg</sub> cells to suppress T effector cells without affecting their
showed that Treg cells reduced Tregulatory cells. Furthermore, inhibition of the role of this subset in tumor-immunity has drawn great interest. Although Treg cells were originally identified for their ability to prevent organ-specific autoimmune disease in mice, emerging evidence identified for their ability to prevent organ-specific and facilitated immune responses to poorly specific immune T cells in tumor-draining lymph nodes. NK cell function by depletion of Treg cells is functional and enhances T-cell-mediated tumor suppression in mice. The IL-2 immunotoxin, denileukin diftitox, depleted and prevented accumulation of Treg cells. This depletion was accompanied by increased Ag-specific immunity against the neu protein, a self Ag and markedly inhibited tumor growth of breast cancers in neu-transgenic mice.

The role of CD4+CD25+ Treg cells in human tumor growth is more difficult to address simply because human studies are more restricted and are largely observational in nature. Highly-representative Treg cells have been consistently found in tissues and peripheral blood from patients with a wide variety of types of cancers. These tumor Treg cells are functional and inhibit tumor-specific T cell immunity and contribute to growth of human tumors in vivo. Using biopsy specimens from B-cell NHL, we have found that Treg cells are highly-represented in biopsy specimens and strongly inhibit the functions of CD4+ and CD8+ effector T cells, resulting in decreased lysis of human NHL B cells. Our previous studies have shown that NHL B cells play an active role in Treg cell-mediated inhibition of the immune response by recruiting natural occurring Treg cells and also generating inducible Treg cells in the tumor site.

**Treg cells, tumor immunity and tumor growth:** Before the recent expansion of interest and publications in Treg cells, there was already published evidence that suppressor T cells play a role in tumor growth. During 1970s and 1980s, a number of studies revealed that tumor growth was influenced by suppressor T cells. These studies observed that depletion of suppressor T cells led to an inhibition of tumor growth and that activation of suppressor T cells resulted in enhanced tumor growth in mouse models. Importantly, tumor growth favored the generation of suppressor T cells. These results indicated that T-cell-mediated immunosuppression had an impact on tumor growth.

Since the identification of CD4+CD25+ Treg cells, the role of this subset in tumor-immunity has drawn great interest. Although Treg cells were originally identified for their ability to prevent organ-specific autoimmune disease in mice, emerging evidence suggests that Treg cells are able to suppress tumor-specific T-cell immunity thereby contributing to the progression of tumors. In vitro studies consistently showed that Treg cells isolated from tumor tissues exhibited profound inhibition of autologous intratumoral CD4+ and CD8+ T cells as well as NK cells. In vivo studies showed that depletion of CD4+CD25+ T cells augmented the generation of specific immune T cells in tumor-draining lymph nodes and facilitated immune responses to poorly immunogenic murine tumors. These Treg cells abrogate CD8+ T cell-mediated tumor rejection by specifically suppressing the cytotoxicity of expanded CD8+ T cells. In addition, release of suppression of NK cell function by depletion of Treg cells is another mechanism accounting for tumor regression. A study by Smyth et al. showed that NKG2D-mediated NK cell cytotoxicity is suppressed by Treg cells and depletion of Treg cells and IL-12 therapy synergize to promote NK cell-mediated tumor suppression in mice. The IL-2 immunotoxin, denileukin diftitox, depleted and

**Treg cells and therapeutic approaches in cancer patients:** Studies in animal models have convincingly shown that depletion of Treg cells alone or combined with other therapeutical reagents results in elevated levels of anti-tumor immunity and longer survival of inoculated mice. Recent human cancer trials suggest that depletion of Treg cells can be clinically beneficial. Several studies observed that administration of denileukin diftitox (Ontak) in cancer patients (melanoma, renal, ovarian, breast, squamous-cell lung carcinoma) effectively depletes Treg cells and leads to an increased tumor-specific CD4+ and CD8+ responses. Studies showing that administration of denileukin diftitox depletes CD4+CD25+Foxp3+ Treg cells and enhances T-cell proliferation in normal donor have significant implications for cancer vaccine strategies. Based on these observations, Morse et al. performed a phase 1 clinical trial of a DC vaccine modified to express carcinoembryonic antigen (CEA), which was administered to patients with advanced CEA-expressing malignancies (colorectal cancer or breast cancer) after denileukin diftitox administration in 2 different schedules (before the first dose of vaccine and before all 4 doses of the vaccine). They found that depletion of Treg cells by denileukin diftitox specifically enhanced the T-cell response to carcinoembryonic antigen CEA. The importance of Treg cells in vaccine therapy was further shown in a pilot study of 18 previously treated patients with
measurable indolent NHL. Patients were injected subcutaneously with DCs loaded with autologous heat-shocked and UVC–treated tumor cells. The vaccination was well tolerated without autoimmune reactions and resulted in significant objective clinical responses. Interestingly, in patients with complete response, the number of CD4⁺CD25⁺Foxp3⁺ T_reg cells significantly decreased 6 months after vaccination, while the number of CD4⁺CD25⁺Foxp3⁺ T_reg cells did not change in patients with no response to the vaccine. The finding that clinical responses were associated with a reduction in CD4⁺CD25⁺Foxp3⁺ T_reg cells suggests that the decreased number of T_reg cells contributed to favorable clinical responses to the vaccine.

A number of anti-cancer drugs have been shown to regulate T_reg cells. Low dose administration of cyclophosphamide, a chemotherapy agent with tumoricidal activity, has been shown to selectively deplete T_reg cells thereby enhancing antitumor immunity\[91,92\]. In contrast, rapamycin, a small molecule that inhibits signal transduction, has been shown to expand T_reg cells thereby suppressing the immune response. Recombinant IL-2 induces clinical responses in malignant melanoma and renal cell carcinoma, suggesting that IL-2 therapy predominantly induces immune activation. But response rates to IL-2 are low and some studies have shown reduced vaccine responses with IL-2 therapy. Studies that monitored T_reg cells during immune reconstitution in individuals with cancer who did or did not receive IL-2 therapy found that CD4⁺CD25high cells underwent homeostatic peripheral expansion during immune reconstitution and in lymphopenic individuals receiving IL-2, the T_reg cell compartment was markedly increased\[93,94\]. These studies suggest that IL-2 and lymphopenia are primary modulators of CD4⁺CD25⁺ T_reg cell homeostasis. In addition to IL-2, IFN-α2b up-regulates STAT5 and down-regulates STAT3, resulting in up-regulation of T_reg cells and inhibition of IL-17 expressing lymphocytes in melanoma\[95\]. These observations suggest that selective inhibition of IFN-α and IL-2-mediated enhancement of T_reg cells might be of therapeutic benefit.

**CONCLUSION**

Experimental and clinical findings have demonstrated that profound immunosuppression is present in the tumor microenvironment and that T_reg cells are a major factor contributing to this immunosuppressive tumor microenvironment. Significant interest has recently focused on the premise that tumors may subvert tumor immunity by promoting the expansion, recruitment and activation of T_reg cells. Figure 1 provides a schematic diagram of tumor-mediated generation of T_reg cells and the consequence of elevated T_reg cells in tumor microenvironment. Basically, tumor cells induce the generation of T_reg cells through both cell contact-dependent and cell contact-independent mechanisms. Soluble proteins such as TGF-β produced by tumor cells promote the proliferation of T_reg cells and induce the conversion of naïve CD4⁺CD25⁻ T cells into T_reg cells. Tumor cells also express surface proteins such as CD80/CD86 or CD70 and interact with naïve cells in a cell contact-dependent manner to convert these naïve T cells into T_reg cells. In addition to tumor cells, dendritic cells are also able to convert naïve T cells into T_reg cells and contribute to the elevated numbers of T_reg cells seen in the tumor microenvironment. Elevated numbers of T_reg cells participate in creating an immunosuppressive tumor microenvironment by suppressing the innate and adaptive immune responses thereby contributing to the progression of tumors. In contrast to inducing the generation of T_reg cells, tumor cells may also inhibit the development of inflammatory immune cells such as Th17 cells. Along with elevated number of T_reg cells,
an insufficient number of T<sub>H</sub>17 cells contribute to the inadequate immune response and the limited anti-tumor immunity. Strategies that deplete or inhibit T<sub>reg</sub> cells and thereby promote a competent immune response in the tumor microenvironment should be the goal in future immunotherapeutic studies in cancer patients.

REFERENCES


