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The Role of T_{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 $CD4^+CD25^+$ regulatory T (T_{reg}) cells represents a milestone in the filed of immunology and provides an explanation for T-cell-mediated immunosuppression. Although T_{reg} cells were originally identified for their ability to prevent organ-specific autoimmune disease in mice, emerging evidence suggests that T_{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_{reg} cells affect anti-tumor immunity. **Results:** T_{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_{reg} cells and creating a suppressive tumor microenvironment. Strategies to deplete T_{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_{reg} cells not only increased our knowledge in a variety of aspects of immunology but also potentially benefited cancer patients.

Key words: $CD4^+CD25^+$, T_{reg} cells, Foxp3, cancer, tumor immunity, immune response

INTRODUCTION

T cell-mediated immuno-suppression has been observed for decades. In 1970, Gershon *et al*.[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*.[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 *in vitro* and *in vivo* studies. A series of studies by North *et al*.[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 characterization in this subset. This hurdle persisted until a subset of $CD4^+$ T cells expressing IL-2 receptor α-chain (CD25) were identified in 1995 and found to be critical in the control of self-tolerance[4]. In this study, Sakaguchi *et al*.[4] found that depletion of $CD25^+$ T cells resulted in spontaneous development of autoimmune diseases and reconstitution of $CD4^+CD25^+$ cells prevented these autoimmune diseases in a dose-dependent fashion. This finding was subsequently confirmed by a study showing that $CD4^+CD25^+$ T cells inhibited both the induction and effector function of autoreactive T cells and suggested that $CD4^+CD25^+$ T cells represent a unique lineage of immunoregulatory cells[5]. Since then, tremendous effort has been put into investigating $CD4^+CD25^+$ T cells in a variety of settings. In this article, we will review recent advances regarding the role of $CD4^+CD25^+$ regulatory T cells in the cancer immunological response.

Characterization of regulatory T cells: Regulatory T (T_{reg}) cells were originally identified as a small subset of $CD4^+$ T cells expressing IL-2 receptor α-chain (CD25) and represent approximately 5-10% of peripheral $CD4^+$ 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_{reg}

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cells[6-8]. To date, it is generally believed that $CD4^+$ T_{reg} subsets include naturally occurring T_{reg} cells and peripherally induced T_{reg} cells. Naturally occurring T_{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_{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_{reg} cells, T_{reg} cells can be induced in the periphery under particular conditions of antigenic stimulation[9-11]. The presence of inducible T_{reg} cells in the periphery is supported by the observation in adult mice that depletion of T_{reg} cells by means of an anti-CD25 monoclonal antibody and thymectomy is followed by complete reconstitution within 48 days[12]. Studies have revealed that several molecules and signaling pathways are involved in inducing the development of T_{reg} cells in the periphery. These include glucosteroids[13], estrogen[14], TGF-β[9,10] and IL-2[10], as well as co-stimulatory molecules such as CD80/CD86[15] and CD70[16]. Along with naturally occurring T_{reg} cells, peripherally induced T_{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[17-19] have independently shown that Foxp3 is specifically expressed in T_{reg} cells and is necessary for T_{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_{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_{reg} cells and is now used as a specific marker for T_{reg} cells.

Regulatory property of T_{reg} cells: $CD4^+CD25^+$ T_{reg} cells have been demonstrated to suppress various types of immune responses, including autoimmune, antimicrobial and antitumor immune responses by inhibiting T cell, B cells and NK cells. T_{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_{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[20,21], inhibition of $CD8^+$ T cell-mediated skin inflammation[22], maintenance of persistent hepatitis C virus infection[23] as well as elimination of tumor cytotoxicity by $CD8^+$ T cells[24].

In addition to the suppression of T cells, T_{reg} cells can also suppress proliferation and immunoglobulin production of $CD19^+$ B cells. Firstly, T_{reg} cells can indirectly inhibit the B cell immunoglobulin response by suppressing $CD57^+$ GC-T_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_H cell-stimulated B cell immunoglobulin production[25]. Secondly, T_{reg} cells can also directly suppress the B cell immunoglobulin response without having to suppress T_H cells. Under this circumstance, T_{reg} cells directly suppress B cell class switch recombination and thereby regulate B cell immunoglobulin production[26].

In addition to suppressing adaptive immune cells, T_{reg} cells also have an impact on innate immune cells. It has been reported that T_{reg} cells inhibit the cytotoxicity of $CD3^-CD56^+$ NK cells[27-29] and steer monocyte differentiation toward alternatively activated macrophages (AAM), a subset of cells with immune regulatory properties that contribute to tumor promotion[30].

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_{reg} cell-mediated suppression cannot be abrogated by neutralizing soluble inhibitory cytokines and T_{reg} cells cultured with $CD4^+CD25^-$ T cells in a transwell system are unable to suppress the proliferation of responder cells[31,32]. In this regard, membrane-bound TGF-β has

been shown to play an important role in T_{reg} cell-mediated, cell contact-dependent suppression of T and B cells given that T_{reg} cells express high levels of TGF-β on the cell surface[33] and T_{reg} cells mediate immunosuppression via cell surface presentation of TGF-β to TGF-βR on target cells. In contrast, soluble factors are involved in T_{reg} 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 $CD4^+CD25^+$ T_{reg} cells, or direct lysis of T cells via perforin and granzymes are involved in suppressive effects of T_{reg} cells. For example, Grossman *et al*.[34,35] showed that human peripherally induced and naturally occurring T_{reg} cells express granzyme-B upon activation and that these T_{reg} cells display perforin-dependent cytotoxicity against autologous target cells, including activated $CD4^+$ and $CD8^+$ 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_{reg} cells in the murine system[36,37].

Reciprocal regulation of T_{reg} and T_H17: T_{reg} cells and T-helper (T_H) cells constitute two opposing immune responses. Newly-identified IL-17-secreting $CD4^+$ helper T cells expand the family of T_H cells into 3 major lineages, T_H1, T_H2 and T_H17 cells[38,39]. $CD4^+CD25^+$ T_{reg} cells form the other major lineage of $CD4^+$ T cells[40]. T_H17 and T_{reg} cells are critically involved in the modulation of inflammation induced by either autoimmunity or bacterial infection. T_H17 and T_{reg} cells develop from precursor naïve $CD4^+$ T cells. The selective differentiation of precursor $CD4^+$ T cells into T_H17 or T_{reg} 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 $CD4^+$ T cells into T_H17 cells in mice, whereas the presence of TGF-β alone promotes differentiation of T_{reg} cells. Unlike mice, IL-1β (but not TGF-β) plus IL-6 have been demonstrated to drive the differentiation of T_H17 cells in humans. The differentiation of precursor $CD4^+$ T cells into T_{reg} or T_H17 cells is mutually exclusive. Tumor cells commonly participate in the generation of T_{reg} cells, which provides an explanation for the observation that elevated numbers of T_{reg} 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_{reg} cells by converting naïve T cells into T_{reg} cells.

The decision of naive $CD4^+$ T cells to become T_H17 or T_{reg} cell has important consequences in the success of an immune response and the progression of disease. $CD4^+$ T cell infiltration into tissue occurs whenever pathological changes are initiated. These pathological changes include infection, autoimmunity and malignant cell transformation. Interestingly, infiltrating $CD4^+$ T cells take distinct differentiation directions in different pathological scenarios. T_H17 cells and T_{reg} cells are prototypical subsets of $CD4^+$ T cells whose infiltration in tissues with each of those pathological changes represents the result of $CD4^+$ T cell differentiation affected by different pathological changes. $CD4^+$ T cells migrating into tissue with autoimmune disease adopt a pro-inflammatory phenotype while $CD4^+$ T cells invading into the tissues with malignant disease adopt an inhibitory phenotype. The mechanism responsible for the distinct differentiation direction of $CD4^+$ T cells is largely unknown.

T_{reg} cells in the tumor microenvironment: Although infiltration by CTL and T_H 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_{reg} 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_{reg} cells are involved in the regulation of antitumor immunity. Consistent with this concept, experimental depletion of T_{reg} cells in mice with tumors improves immune-mediated tumor clearance and enhances the response to immune-based therapy. T_{reg} cells have been shown to suppress tumor-specific T-cell immunity and therefore may contribute to the progression of human tumors. Furthermore, tumor T_{reg} cells are associated with a reduced survival in patients with various malignancies.

The number of T_{reg} cells in tumor microenvironment: Since Woo *et al*.[41] reported in 2001 that $CD4^+CD25^+$ 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_{reg} cells. Elevated numbers of T_{reg} cells correlate with disease stage, histologic subtypes or overall survival of cancer patients. For example, it has been found that T_{reg} cells are increased in patients with advanced-stage breast cancer and that HER^+, but not HER^-, tumors account for this increase[42]. Although it has been shown that the number of T_{reg} cells is associated with overall survival in most studies, there is no agreement regarding whether elevated number of T_{reg} cells predicts a poor or favorable outcome for all cancer patients. It appears that high numbers of T_{reg} cells are associated with a poor prognosis in patients with most types of solid tumors. In contrast, highly-representative T_{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_{reg} cells. Because the malignant cells are immune cells, T_{reg} cells may interact differently with these cells than with malignant cells in solid tumors. In fact, it has been shown that T_{reg} cells directly suppress B cell-dependent immunoglobulin production and class switch recombination, without having to suppress T_H cells[26] and can induce apoptosis of activated B cells via the upregulation of perforin and granzymes[37]. T_{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_{reg} cells predicts a better overall survival in patients with hematological malignancies.

Recruitment and generation of intratumoral T_{reg} cells: Several mechanisms that may explain the elevated number of T_{reg} cells in the tumor microenvironment have been proposed. Firstly, T_{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_{reg} cells into the tumor site. A study by Curiel *et al*.[49] showed that ovarian tumor T_{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_{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_{reg} cells into tumors. In pancreatic cancer patients, intratumoral T_{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_{reg} cells by abolishing CCL5 expression in pancreatic tumor cells or blockade CCR5 expression on intratumoral T_{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_{reg} cells and enables T_{reg} cells to migrate toward CXCL12 in the tumor microenvironment thereby increasing T_{reg} cell accumulation[54].

A second mechanism for the increased number of intratumoral T_{reg} cells is the expansion and *de novo* generation of T_{reg} cells within tumors. As discussed above, naturally occurring T_{reg} cells are anergic and do not proliferate in response to TCR stimulation unless in the presence of IL-2. However, naturally occurring T_{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_{reg} cells[55], which may explain the elevated number of T_{reg} cells in Hodgkin lymphoma patients[56]. In myeloma, monocyte-derived DCs maintained and expanded $CD4^+Foxp3^+$ T_{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_{reg} cells seen within 1 week after DC injection[57]. These observations suggest that naturally occurring T_{reg} cells can be expanded within the tumor microenvironment. In addition to expansion of T_{reg} cells, *de novo* generation of T_{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_{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_{reg} cells in thymectomized mice with depletion of $CD25^+$ T cells using an anti-CD25 antibody, suggesting tumor development in mice led to a *de novo* generation of T_{reg} cells. Another study[59] described a subset of tumor-induced CD25- regulatory T cells (TMT_{reg}) in mice that arise after the mice are inoculated with lymphoma B cells. These TMT_{reg} have increased expression of Foxp3 and IL-10, develop independently of pre-existing natural T_{reg} cells and maintain suppressive properties long term in the absence of antigen stimulation. In conjunction with naturally occurring T_{reg} cells, TMT_{reg} induced tumor-specific $CD4^+$ T cell tolerance. In patients with B-cell NHL, several studies[16,60,61] have shown that lymphoma B cells induce Foxp3 expression in intratumoral $CD4^+CD25^-$ T cells and participate in the generation of T_{reg} cells, which may account for elevated number of T_{reg} cells seen in B-cell NHL.

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

Specificity of intratumoral T_{reg} cells: Most $CD4^+$ T cells persist as an antigen-specific subset, but it is not clear whether antigen-specific T_{reg} cells exist. The observation that tumor cells are able to induce the development of T_{reg} cells suggests that T_{reg} 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 $CD4^+$ T cells into cells capable of suppressing naïve and T_H1 effector cells. These $CD4^+$ T cells have increased expression of Foxp3 and IL-10 with suppressive activity and were described as tumor-induced regulatory T cells[59]. Further study showed that this *de novo* generation of T_{reg} cells contributed to tumor-specific T cell tolerance[59,64]. Wang *et al*.[65,66] generated a panel of $CD4^+$ T-cell clones isolated from a melanoma. One of the clones had a phenotype similar to T_{reg} 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 $CD4^+$ T cells. This result demonstrated that T_{reg} cells recognizing tumor antigens can be generated *in vitro*. In ovarian cancer, it has been shown that tumor T_{reg} cells disabled tumor antigen-specific T cell immunity *in vivo* and in turn allow tumor growth[49].

Reversal and enhancement of function of T_{reg} cells: The suppressive effect of T_{reg} cells is a major obstacle to developing effective cancer immunotherapy. Although it has been shown that depletion of T_{reg} 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_{reg} 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_{reg} cell depletion promptly induces conversion of peripheral precursors into T_{reg} cells and the number of T_{reg} cells will be restored over a period of time. Second is that some $CD4^+CD25^-$ T cells in the tumor microenvironment also express Foxp3 and possess similar regulatory function to naturally occurring T_{reg} cells. Therefore, while targeting $CD4^+CD25^+$ T_{reg} cells may augment tumor-specific immune responses, residual $CD4^+CD25^-Foxp3^+$ 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_{reg} cells in the tumor microenvironment has led to a second strategy to reverse T_{reg} cell function. Several groups have reported that the function of T_{reg} cells can be reversed by Toll-Like Receptor (TLRs) ligation by CpG[67,68], OX40 costimulation[69], or functional blockade of galactin-1[70] or -10[71]. Toll-like receptors control activation of adaptive immune responses by Antigen-Presenting Cells (APCs) such as DCs. Ligation of TLRs on DCs overcomes $CD4^+CD25^+$ T cell-mediated suppression[67]. Further study identified that it is TLR8 that is responsible for TLR-mediated reversal of $CD4^+$ regulatory T cell function[68]. 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 $Foxp3^+$ T_{reg} cells to suppress T effector cells without affecting their

proliferation or survival[69]. Importantly, OX40 costimulation of T effector cells prevented the induction of new inducible Foxp3$^+$ T_{reg} cells[69] and facilitated tumor rejection[72]. In contrast to reversal of T_{reg} cell function, the function of T_{reg} cells can also be enhanced. It has been shown that tumor-derived prostaglandin E2 induced Foxp3 expression and enhanced the suppressive activity of CD4$^+$CD25$^+$ regulatory cells. Furthermore, inhibition of cyclooxygenase-2 reduced T_{reg} cell activity and tumor burden *in vivo*[73]. The ability of these strategies to enhance or suppress T_{reg} cell function may provide future options for modulating the antitumor immune response.

T_{reg} cells, tumor immunity and tumor growth: Before the recent expansion of interest and publications in T_{reg} 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[74-78]. 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$^+$ T_{reg} cells, the role of this subset in tumor-immunity has drawn great interest. Although T_{reg} cells were originally identified for their ability to prevent organ-specific auto-immune disease in mice, emerging evidence suggests that T_{reg} cells are able to suppress tumor-specific T-cell immunity thereby contributing to the progression of tumors. *In vitro* studies consistently showed that T_{reg} 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[79-81]. These T_{reg} cells abrogate CD8$^+$ T cell-mediated tumor rejection by specifically suppressing the cytotoxicity of expanded CD8$^+$ T cells[82]. In addition, release of suppression of NK cell function by depletion of T_{reg} cells is another mechanism accounting for tumor regression. A study by Smyth *et al*.[27] showed that NKG2D-mediated NK cell cytotoxicity is suppressed by T_{reg} cells and depletion of T_{reg} cells and IL-12 therapy synergize to promote NK cell-mediated tumor suppression in mice. The IL-2 immunotoxin, denileukin diftitox, depleted and prevented accumulation of T_{reg} 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[83].

The role of CD4$^+$CD25$^+$ T_{reg} 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 T_{reg} cells have been consistently found in tissues and peripheral blood from patients with a wide variety of types of cancers. These tumor T_{reg} cells are functional and inhibit tumor-specific T cell immunity and contribute to growth of human tumors *in vivo*[24,49,84]. Using biopsy specimens from B-cell NHL, we have found that T_{reg} 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 T_{reg} cell-mediated inhibition of the immune response by recruiting natural occurring T_{reg} cells and also generating inducible T_{reg} cells in the tumor site[16,50].

T_{reg} cells and therapeutic approaches in cancer patients: Studies in animal models have convincingly shown that depletion of T_{reg} 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 T_{reg} cells can be clinically beneficial. Several studies observed that administration of dinileukin diftitox (Ontak) in cancer patients (melanoma, renal, ovarian, breast, squamous-cell lung carcinoma) effectively depletes T_{reg} cells and leads to an increased tumor-specific CD4$^+$ and CD8$^+$ responses[85-88]. Studies showing that administration of denileukin diftitox depletes CD4$^+$CD25^{high}Foxp3$^+$ T_{reg} cells and enhances T-cell proliferation in normal donors[87-89] have significant implications for cancer vaccine strategies. Based on these observations, Morse *et al*.[88] 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 T_{reg} cells by denileukin diftitox specifically enhanced the T-cell response to carcinoembryonic antigen CEA[88]. The importance of T_{reg} cells in vaccine therapy was further shown in a pilot study[90] 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. In patients with a partial response, decreased T_{reg} cells recovered 12 months after vaccination. 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 $CD4^+CD25^{high}$ 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 $IL\text{-}17^+$ 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 T_H17 cells. Along with elevated number of T_{reg} cells,

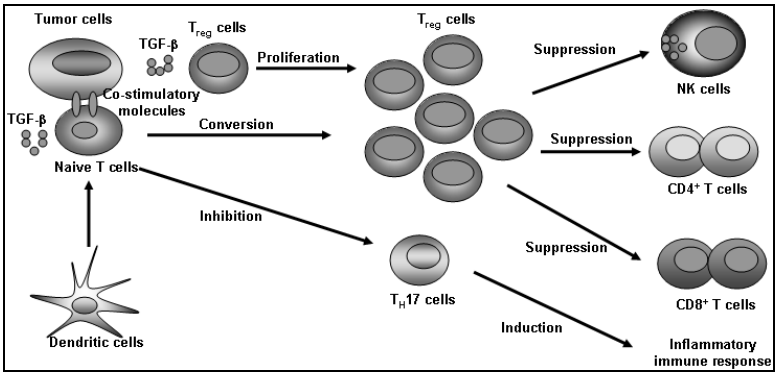

Fig. 1: Tumor-mediated generation of T_{reg} cells and the impact on the tumor microenvironment

an insufficient number of T_H17 cells contribute to the inadequate immune response and the limited anti-tumor immunity. Strategies that deplete or inhibit T_{reg} cells and thereby promote a competent immune response in the tumor microenvironment should be the goal in future immunotherapeutic studies in cancer patients.

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