REVIEW

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The significance of Treg cells in defective tumor immunity

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Abstract

Regulatory T cells (Treg) enriched in FoxP3$^+$, glucocorticoid-induced TNF receptor$^+$, and cytotoxic T-lymphocyte-associated antigen-4$^+$ exert a potential to suppress effector T cells in the periphery. These cells exist in markedly higher proportions within tumor-infiltrating lymphocytes, peripheral blood lymphocytes, and/or regional lymph node lymphocytes of patients with cancer and their frequencies are suggested to be strongly related to tumor progression and inversely correlated with the efficacy of treatment. Tumor-specific Treg cells require ligand-specific activation and cell-to-cell contact to exert their suppressive activity on tumor-specific effector cells (CD8$^+$ cytotoxic T lymphocytes and CD4$^+$ Th cells), which includes decreased cytotoxity, proliferation, and Th1 cytokine secrection. Depletion or blockade of Treg cells can enhance immune protection from tumor-associated antigens that are expressed as self antigens. Recent studies revealed that lymphoma T cells might adopt a Treg profile as well. Studies assessing the influence of chemotherapy on Treg cells have also been included in this review.

Key words: Treg cells, FoxP3, immunosuppression, tumor immunity.

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INTRODUCTION

The adaptive immune response is a dynamic system that can protect a host from alloantigens and tumors based on the ability of the immune system to discriminate between self and non-self. Immune tolerance plays a key role in this process and its dysregulation may induce a breakdown of immune homeostasis. A number of recent studies have provided firm evidence for the presence of certain subsets of CD4$^+$ T cells in the periphery which are generally termed regulatory T cells (Treg) and which have the potential to suppress "conventional" T cells in the periphery [77, 82].

Treg cells include at least four populations that differ in phenotype, cytokine secretion profile, and suppressive mechanism [69]. Three subsets of Treg cells (Tr1, Th3, and Tr1-like cells) require repetitive antigen stimulation *in vivo* and *in vitro* for their generation. Adaptive T regulatory 1 (Tr1) cells arise in the periphery upon encountering antigen in a tolerogenic environment, produce interleukin (IL)-10 at high levels, and mediate suppression via IL-10 [26, 35, 36]. Th3 cells originate from oral tolerant animals, display an inhibitory role through a transforming growth factor (TGF)-β-dependent mechanism, and secrete, besides TGF-β, IL-4 and IL-10 [22, 113]. Tr1-like cells develop by co-culture with allogeneic immature dendritic cells (DCs) [42]. All the Treg cells mentioned above inhibit the antigen-specific immune response in a cytokine- or cell contact-dependent manner and play a role in preventing autoimmunity [15, 17, 35, 77]. In this review we will focus our attention on naturally occurring Treg cells and their role in tumor immunity.

PHENOTYPIC CHARACTERISTICS OF Treg CELLS

The induction of autoimmunity was prevented by the transfer of a T-cell population from syngeneic healthy donors to lymphopenic recipients, which suggested the presence of a population of professional Treg cells in the normal immune system that were capable of suppressing autoreactivity [7, 14]. Subsequent studies confirmed the existence in human peripheral blood of these Treg cells with the same phenotypic and functional characteristics as those in mice [27]. In healthy individuals (both mice and humans), CD25$^+$ T cells constitute

5–10% of peripheral $CD4^+$ T cells [77, 100] and less than 1% of $CD8^+$ T cells [51, 77]. Neonatal thymectomy results in autoimmunity due to dramatically decreased numbers of Treg cells in the periphery [77], indicating that Treg cells originate in the thymus [41, 82]. Indeed, these cells naturally arise in the thymus, where they represent 2–5% of $CD4^+$ single-positive thymocytes and are functionally competent, which was documented by the protection of mice from autoimmunity upon adoptive transfer of this population of thymocytes [41, 44]. After differentiation in the thymus, these naive $CD4^+CD25^+CD45RA^+$ T cells are exported to the periphery, where they suppress the activation of T cells potentially reactive to the self [7, 77].

A pure population of human Treg cells contains both $CD45RO^+$ and $CD45RO^-$ cells, but the suppressive effects are enriched in the $CD45RO^+$ memory fraction [43]. Highly differentiated $CD4^+$ T cells can exhibit higher suppressor activity when stimulated repeatedly by antigens presented by non-professional antigen-presenting cells (APCs) *in vivo* and by TGF-β *in vitro*, which enhances the expression of CD25 on $CD4^+$ T cells and accelerates their differentiation to the $CD45RA^-RO^+$ activated/memory phenotype [90, 110]. These findings strongly suggest that Treg cells, besides being generated in the thymus, may also be generated in the periphery. This would provide a mechanism for the generation of a population of adaptive Treg cells that induce tolerance to a wide array of antigens that may not be encountered in the thymus [35, 36, 90].

Analysis of the CD95 expression on the cell surface showed that freshly isolated Treg cells acquired the apoptotic phenotype. In fact, after isolation from peripheral blood, human Treg cells were highly sensitive toward CD95-mediated apoptosis [29, 79]. The finding of apoptotic properties of Treg cells makes possible the use of the CD95/CDC95L system to specifically modulate Treg survival/apoptosis [62].

MARKERS OF Treg CELLS

The naturally activated $CD4^+CD45RB^{low}$ T-cell pool contains most of the $CD25^+$ T cells, which contribute to controlling the size of the peripheral T-cell population [3]. CD45RB expression is up-regulated during thymic development and decreased upon T-cell activation [52, 103]. Although both the $CD25^+$ and the $CD25^-CD4^+CD45RB^{low}$ T-cell pools contain Treg cells, only the $CD25^+$ population can efficiently regulate the size of the memory $CD4^+$ T-cell compartment via a mechanism involving the production of IL-10 [4]. In contrast, $CD4^+CD25^-CD45RB^{low}$ T cells do not significantly regulate T-cell homeostasis [4]. Early studies suggested that the expression of IL-2Rα (CD25) could be a useful marker of Treg cells [77], as the frequency of $CD25^+$ T cells is constant throughout the adult life of normal mice, in contrast to the transient CD25 expression on activated T cells in general [85, 88]. Its constitutive expression on Treg cells implies that these cells are continuously activated *in vivo* [51, 93]. Existing evidence indicates that IL-2-induced signals in Treg cells are required for their maintenance, expansion, and/or survival rather than for the generation of Treg cells in the thymus [31]. However, as CD25 is unable to distinguish Treg cells from activated T cells precisely, its expression seems to be insufficient to identify Treg cells.

The tumor necrosis factor (TNF) receptor family member glucocorticoid-induced TNF receptor (GITR) [48, 61, 84] was also previously proposed to define these naturally arising Treg cells more accurately than CD25, since GITR also identifies a small population of $CD4^+CD25^-$ T cells which are also capable of suppressing autoimmunity [97]. GITR-depleted $CD4^+$ T cells are more autoaggressive than CD25-depleted T cells. However, the percentage of $GITR^+$ T cells in the Treg cell subpopulation was found to be significantly higher than within the $CD4^+CD25^-$ T-cell subset, suggesting a preferential co-expression of GITR with CD25 [57]. Augmented expression of GITR and of other closely related members of the TNF receptor (TNFR) family, such as OX40, TNFRII, and 4-1BB, in Treg cells contributes to the survival of these cells [32]. The agonistic antibodies to GITR, natural GITR ligand (GITRL) (expressed on DCs, macrophages, and B cells) and soluble GITRL, neutralize the suppressive activity of Treg cells [55, 84, 96, 111]. Contrary to the proposed negative regulation of suppressive activity by GITR, T cells in $GITR^{-/-}$ mice displayed increased IL-2 production in addition to increased sensitivity to apoptosis [74], suggesting a need for further elucidation of its role in the development and function of Treg cells. Based on the study performed by Li et al. [57], GITR seems to be an activation marker for $CD4^+$ T cells (both $CD25^+$ and $CD25^-$) in human peripheral blood. Since both CD25 and GITR can be induced by T cell receptor (TCR) activation, they have been excluded as highly specific markers for Treg cells.

Resting murine and human Treg cells intracellularly express cytotoxic T-lymphocyte-associated antigen (CTLA)-4, which also becomes detectable on the surface after activation by allogeneic DCs as well as anti-CD3 or phytohemagglutinin [54, 91]. This constitutive and long-lasting (over three weeks) membrane expression of CTLA-4 after activation is a characteristic property of human and murine Treg cells [54, 73, 91]. There have been contradictory results regarding the function of CTLA-4 in the immunoregulatory activity of Treg cells *in vitro* [73, 91, 93, 114]. The study performed by Read et al. [73] revealed that the immune-suppressive function of these Treg cells was dependent on signaling via CTLA-4 and allowed the control of intestinal inflammation. Another group confirmed complete removal of the inhibitory capacity of Treg cells by blockade of the murine CTLA-4 molecule on these cells [89]. A different pathway for their suppressive function was recently suggested because delivery of anti-CTLA-4 monoclonal antibody did not abrogate the regulatory activity of

human Treg cells *in vitro* [93]. This contradiction could also be a result of the fact that anti-human CTLA-4 monoclonal antibody has a comparatively low neutralizing activity [43]. Thus the relevance of this marker to the function of Treg cells is not defined in detail.

The currently used cell-surface molecules are unable to discriminate completely between Treg cells and activated/memory effector T cells. Furthermore, both CTLA-4 and GITR are also expressed at certain levels by activated $CD4^+CD25^-$ T cells; therefore the utility of these molecules for unequivocal identification of Treg cells seems to be limited. Recent studies revealed that the most specific marker to identify Treg cells is Foxp3, a forkhead transcription factor family member encoded on the X chromosome [28, 38, 47]. Foxp3 is an essential factor for the developmental differentiation of Treg cells in the thymus and the periphery [38]. Foxp3 mutations in mice and humans lead to the development of fatal lymphoproliferative disorders associated with multiorgan pathology in males, but not in heterozygote female carriers. These disorders result from inactivation of Foxp3 leading to Treg-cell deficiency [95]. The phenotypes of mice and patients with immune dysregulation polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), or X-linked autoimmunity-allergic dysregulation syndrome (XLAAD) indicate a critical role for the Foxp3 gene product, scurfin, in the negative regulation of T-cell homeostasis [11, 56]. Scurfin presumably regulates $CD4^+$ T-cell activation by direct repression of nuclear factor of activated T cells-mediated cytokine gene transcription [80]. The population of Treg cells controls disease development and preferentially expands when transferred into neonatal $Foxp3^{-/-}$ mice [28]. The forced expression of Foxp3 also delays disease in $CTLA\text{-}4^{-/-}$ mice, indicating that the Foxp3 and CTLA-4 pathways may intersect and providing further insight into the Treg cell lineage [47]. Furthermore, ectopic or TGF-β-induced Foxp3 expression in $CD4^+CD25^-$ or in $CD8^+$ T cells confers suppressive activity to these cells [20, 28, 47], and IL-2 plays an essential role in this process [115]. Thus Foxp3 gene specifically controls the development and suppressor function of the Treg cell population.

THE MECHANISM OF IMMUNOSUPPRESSION MEDIATED BY Treg CELLS

Functional analysis of murine Treg cells revealed that these cells were naturally anergic to activation via the TCR [81, 101]. Interestingly, this stimulation is required to exert the suppressive function and to inhibit the activation and expansion of the conventionally responsive $CD4^+$ and $CD8^+$ T cells when they are co-cultured [4, 63, 72, 92]. Furthermore, TCR restimulation of Treg cells *in vitro* revealed a reduced sensitivity toward activation-induced cell death compared with effector T cells [29]. The suppressive activity of Treg cells is related to their ability to inhibit both IL-2 production and cell cycle progression in responders [93]. The exact mechanism of their action remains to be defined, but once activated, their suppressive function is antigen-unspecific, requires cell-to-cell contact, and probably involves signals through CTLA-4 and/or GITR [61, 73, 84, 91].

The role of cytokines in the immunoregulatory effect mediated by Treg cells remains unsettled. While cell-to-cell contact is important for Treg-mediated suppression in *in vitro* assays, the physiological significance of direct cell-cell contact *in vivo* has not been established and cytokines may be more critical. Some data showed secretion of IL-10 and/or TGF-β in response to a variety of stimuli [27, 42, 54, 55], whereas others failed to detect the production of these cytokines by human Treg cells [10, 66]. Of note, when Treg cells were activated in response to alloantigens, they produced IL-10, TGF-β, low amounts of interferon (IFN)-γ, but no IL-2 or IL-4 [54]. However, neither IL-10 nor TGF-β appeared to be required for their *in vitro* suppressive function, since anti-IL-10 and anti-TGF-β could not abrogate the suppressive capacity of Treg cells [42, 43, 55, 66, 90]. In addition, Treg cells isolated from mice genetically deficient in IL-10 and/or TGF-β were fully suppressive *in vitro* and *in vivo* [71, 91, 93]. It was also shown that targeted cells which were genetically altered to be unresponsiveness to TGF-β were susceptible to suppression mediated by Treg cells [71].

In contrast to these findings, a control role of cytokines in the function of Treg cells was postulated in recent studies [8, 73]. A major role in the regulatory T cell-mediated mechanism of immunosuppression may be played by TGF-β bound to their surface and presented to its receptors on target cells [5, 21, 65]. The expression of surface-bound TGF-β reaches a peak on day 3 after stimulation, and Treg cells maintain its high level for quite a long time (over 6 days). This finding is in agreement with the previous observation that after stimulation, Treg cells exhibit a more potent suppressor function without an additional stimulation through the TCR in an antigen-nonspecific manner, probably due to surface-bound or secreted TGF-β [94].

In light of the amount of evidence discounting the role of cytokines in immune regulation mediated by Treg cells, a new model of suppression mediated by these cells was recently proposed which postulated a role for reverse signaling through the co-stimulatory molecules B7 expressed on activated T cells [32]. It has been suggested that the high expression of CTLA-4 on Treg cells may not only deliver a suppressive or anti-proliferative signal to Treg cells, but may also act as a ligand for B7 on the surface of activated T cells (effector T cells), sending a reverse signal to responding T cells that suppresses their activation [31]. In this system, CTLA-4 seems to be the key suppressor molecule by activating the reverse signal through B7, since blockade of CTLA-4/B7 interactions completely abrogates regulatory Treg cell-mediated suppression *in vitro*, whereas cells from $CD28^{-/-}$ mice are still competent suppressors [91].

Recently it was demonstrated that Treg cells could exert dual effects on the course of an immune response. Besides the production of immunosuppressive TGF-β when activated, they could induce Th17 cells, which are capable of IL-17 production [109]. Treg cells producing high amounts of soluble TGF-β could function as inducers of Th17 cells from the population of $CD4^+CD25^-Foxp3^-$ cells when co-cultured in the presence of IL-6. Another source of Th17 cells could be activated Treg cells themselves, differentiating into IL-17-producing cells in the presence of IL-6 and in the absence of exogenous TGF-β. This property of Treg cells could be particularly useful in settings where IL-17 mediates certain homeostatic functions rather than a proinflammatory effect [86].

Treg CELLS AND TUMOR IMMUNITY

There are accumulating data that many tumor-associated antigens recognized by autologous cytotoxic T lymphocytes (CTLs) are antigenically normal constituents of the self (host) [39, rev. in 83]. The possibility of antigenic cross-reactions between tumor antigens and normal tissue antigens may lead to autoimmunity resulting from immunotherapy of cancer by augmentation of T-cell function and/or vaccination with tumor antigens [76]. Based on the fact that Treg cells function *in vivo* to prevent the host from mounting an immune response to self antigens, including tumor antigens [68], it was suggested that a preponderance of these Treg cells could lead to enhanced tumor growth. In an animal model, a rapid growth of tumors with local invasion and metastatic spread following initially slow growth seems to coincide with the development of suppressor $CD4^+$ T cells [rev. in 18, 30]. Further experiments performed in humans confirmed the above observations. It has been found that human Treg cells preferentially move to and accumulate at tumor sites [2, 24]. Tumor-infiltrating lymphocytes (TILs) were demonstrated to be enriched in $FoxP3^+$, $GITR^+$, and $CTLA\text{-}4^+$ Treg cells [2]. Within tumor-involved and -uninvolved lymph node lymphocytes (LNLs) and autologous peripheral blood mononuclear cells (PBMCs), the frequencies of Treg cells were lower than those of TILs, although higher compared with T cells from healthy individuals.

Treg cells exist in markedly higher proportions within TILs, PBLs, and/or regional LNLs of patients with ovarian cancer [106, 107], non-small-cell lung cancer [106, 107, 108], esophageal cancers [40, 106], gastrointestinal malignancies [46, 78, 106], hepatocellular cancers [70], pancreas or breast cancers [58, 106], head and neck cancers [79], other epithelial malignancies [106], and B-cell chronic lymphocytic leukemia (B-CLL) [13] compared with healthy individuals. Increased frequencies of Treg cells were also recently found in malignant pleural effusions [25]. Furthermore, the increase in the proportion of human Treg cells is suggested to be strongly related to tumor progression [46, 49, 78] and inversely correlated with the efficacy of treatment [49].

Experimental models in mice showed that the effector T-cell population within TILs or PBMCs was unresponsive to activation in the tumor microenvironment enriched in tumor-specific Treg cells [104] (see Fig. 1). In fact, Treg cells seem to be potent inhibitors of an anti-tumor response, including decreased cytotoxic activity of tumor-specific $CD8^+$ effector T cells and both NK and NK T cells [2, 9, 37, 67]. Further analysis revealed the impairment of tumor-infiltrating $CD8^+$ and $CD4^+CD25^-$ T-cell function after *ex vivo* stimulation compared with autologous PBMCs or PBMCs from healthy controls resulting from the interference of Treg cells with these tumor-specific T cells [2, 46, 99]. However, it was shown that $CD8^+$ T cells might even expand to the same extent and produce similar levels of IFN-γ in the presence or absence of specific Treg cells, but these Treg cells abrogated $CD8^+$ T cell-mediated tumor rejection due to TGF-β signals [16, 19].

As the suppression of anti-tumor immunity by Treg cells occurs predominantly at the tumor site, the local reversal of suppression, even at a late stage of tumor development, was shown to be an effective treatment for well-established cancers [6, 50, 112]. Depletion or blockade of Treg cells can enhance immune protection from tumor-associated antigens that are expressed as self antigens [64, 85, 89]. Removal of Treg cells evokes effective immune responses to tumor cells, resulting from more vigorous activation of the tumor-specific effector cells $CD8^+$ CTLs and $CD4^+$ Th cells and also tumor-unspecific $CD4^-CD8^-$ NK-like effector cells [85, 89]. The other effect of Treg-cell elimination was vigorous proliferation of $CD4^+$ T cells reactive to the self, presumably responding to self peptides or class II MHC molecules expressed on APCs [33, 85]. These observations imply a common regulatory basis between tumor immunity and autoimmunity [85].

Recent studies have strongly suggested a role for CTLA-4 expressed on regulatory $CD4^+CD25^+$ T cells in the down-regulation of tumor immunity [106, 108]. In contrast to previous observations, recent studies showed that Treg cells in tumors had marked surface expression of CTLA-4 (in addition to a cytoplasmic store) and membrane-bound TGF-β and that they directly inhibited the proliferation of autologous peripheral blood T cells [58, 106, 108]. As CTLA-4 is thought to be an activation marker, its augmented baseline surface expression on Treg cells most likely reflects vigorous ongoing activation of these cells in the tumor microenvironment, probably by tumor antigens [107]. On the other hand, the engagement of CTLA-4 strongly expressed on Treg cells may be a source of increased levels of TGF-β produced at baseline by these cells resident in tumors [106, 108]. Treg cell-derived TGF-β has been postulated to play a major role in tumor-induced immunosuppression, in particular leading to the inhibition of CTL proliferation, which contributes to escape of the tumor from immunosurveillance by CTLs [87]. Although the significance of CTLA-4 in defective T cell-mediated tumor immunity arose in the light of findings

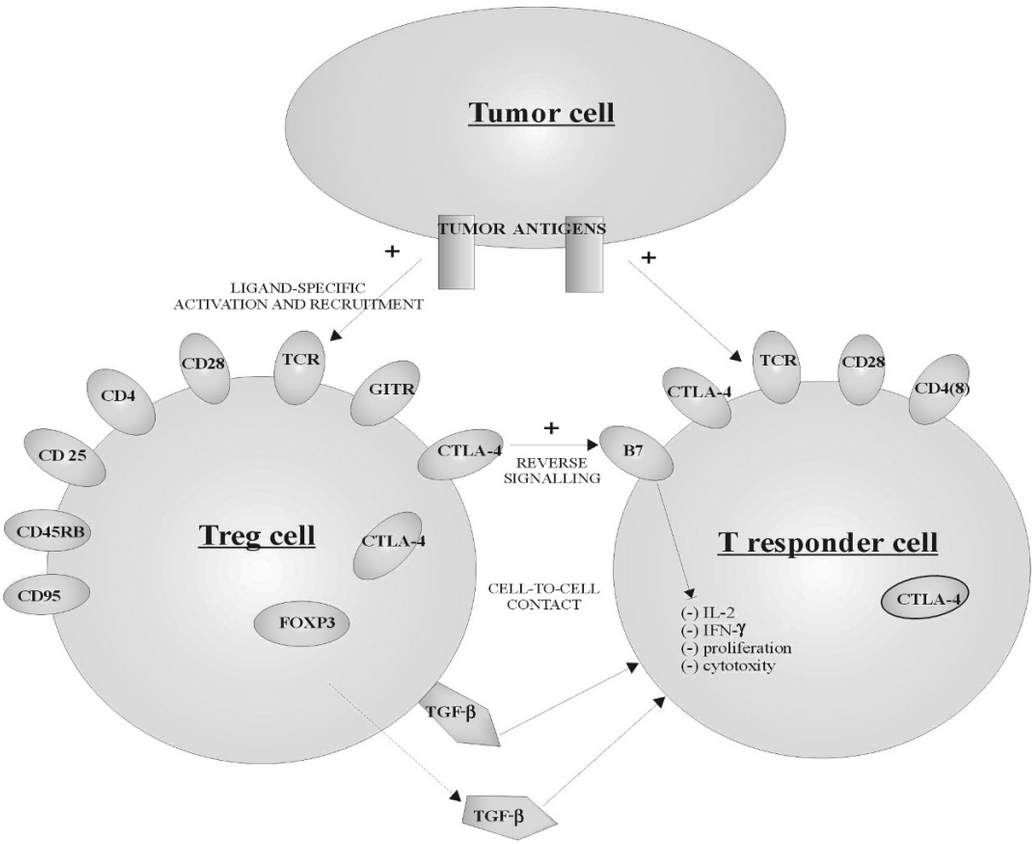

Fig. 1. The model of anti-tumor immunity suppression by Treg cells. The tumor-specific Treg cells require ligand-specific activation and cell--to-cell contact to exert suppressive activity on tumor-specific effector cells: CD8 CTLs and $CD4^+$ Th cells (T responder cells). Treg cells have striking surface expression of CTLA-4 (in addition to cytoplasmic store) and membrane-bound TGF-β. Engagement of surface CTLA-4 molecule may be a source of increased levels of TGF-β produced at baseline by these cells resident in tumors. Treg cell-derived TGF-β plays a major role in tumor-induced immunosuppression, leading to inhibition of Th1 cytokine production, proliferation, and decreased cytotoxic activity of CTLs, which contributes to escape of the tumor cells from immunosurveillance by CTLs. This model includes a role for reverse signaling through the costimulatory B7 molecules expressed on activated T responder cells, which has been recently postulated [32]. High expression of CTLA-4 on Treg cells may send a reverse inhibitory signal to responding T cells in addition to its role in the suppression of Treg cell proliferation.

on tumor regression after a blockade of CTLA-4 on Treg cells [89], the exact nature of its role in Treg cell function in tumors requires further studies. Nevertheless, it is becoming clear that the modulation of Treg cells, including a blockade of the CTLA-4 signaling, appears to be a powerful and promising strategy in cancer therapy, even though it potentates autoimmune complications [89, 105].

THE LIGANDS FOR THE TUMOR-SPECIFIC HUMAN Treg CELLS

It has been postulated that progressively growing tumors present or shed/secrete self antigens that subsequently activate naturally occurring Treg cells [23, 101]. Wang et al. [104] established for the first time tumor--specific Treg cell clones from the TILs of patients with cancer, which was the first step in the identification of the target ligands for Treg cells. Little is known about the nature of the physiological target antigens for tumor-specific Treg cells. Since tumor-specific Treg cells require ligand-specific activation and cell-to-cell contact to exert suppressive activity on both $CD4^+$ and $CD8^+$ effector T cells, tumor-specific self antigens may stimulate and preferentially expand Treg cells at the tumor sites, thus inducing immune tolerance in patients with cancer [2, 104].

LAGE-1 protein was recently identified as a possible ligand for tumor-specific Treg cells from TILs of cancer patients [53, 104]. This protein is an antigen expressed in tumor cells (such as non-small-cell lung carcinomas, melanomas, bladder, prostate, and head and neck cancers) and normal testis, but not in other normal tissues [53]. Although LAGE1 is also recognized by effector $CD4^+$ T cells, most of the $CD4^+$ T-cell clones generated in the study performed by Wang et al. [104] were exclusively $CD4^+$ Treg cells. Another group identified

NY-ESO-1, a germ-cell protein that is expressed by cancer cells but not by normal somatic cells as a ligand for NY-ESO-1-specific Treg cells [67]. Of note, the expression of NY-ESO-1 is strongly correlated with that of LAGE-1 at both the mRNA and protein levels [53]. The majority of human cancers overexpress p53, and the involvement of wild-type p53 epitopes in the specific recruitment of tumor-associated Treg cells was also demonstrated [2].

Beside the tumor-associated antigens, an influence of tumor-mediated soluble factors on tumor-specific Treg cell expansion in the tumor microenvironment has also been postulated. Recently, Curiel et al. [24] revealed in a study performed on $CD4^+$ T cells from ovarian cancer patients that tumor cells and microenvironment macrophages produce the chemokine CCL22, which mediates trafficking of specific Treg cells to tumor sites and ascites. Other groups demonstrated TGF-β secretion by melanoma cells [98], thus inducing FoxP3 expression in $CD4^+CD25^-$ T cells. This observation points to the possibility that tumor cells themselves participate locally in the induction of such $CD4^+CD25^-FoxP3^+$ suppressor T cells. In addition, TGF-β has been shown to promote the expansion of Treg cells in the tumor microenvironment [110], and blockade of TGF-β signaling has been recently implicated in the immune-mediated eradication of tumors [34]. These observations suggest that tumor cells could use many factors to induce a local suppressive network and that distinct subtypes of suppressive T cells may be involved [99].

MALIGNANT PROLIFERATION OF Treg CELLS

Recent studies revealed that Treg cells might also be generated from T cell-derived tumor cells [12, 45]. Adult T-cell leukemia/lymphoma (ATLL) and cutaneous T-cell lymphoma (CTCL) were suggested to be a source of Treg cells, since these highly neoplastic diseases usually exhibit the $CD4^+CD25^+$ T-cell phenotype [12, 45]. A study performed by Karube et al. [45] on ATLL cells from 17 patients demonstrated for the first time the expression of FoxP3, which has been shown to be expressed exclusively in Treg cells. FoxP3 expression in ATLL cells strongly suggests a potential for immunosuppressive activity of these neoplastic cells. Although $CD4^+CD25^-$ T cells were induced to express FoxP3 and achieved regulatory function by special stimuli through the TCR in humans [102] and by TGF-β in mice [20], the mechanism of the adoption of a Treg profile by ATLL cells is still unknown.

CTCL is a clonal malignancy of $CD4^+$ helper T cells expressing a memory phenotype and accumulating in the epidermis in the proximity to Langerhans cells, which are immature DCs [99]. The mechanism of adopting a Treg phenotype by CTCL cells is based on the direct contact with immature DCs presenting antigens, which consequently stimulates CTCL cell proliferation and promotes CTCL progression. Of note, Berger et al. [12] demonstrated that only DCs loaded with autologous apoptotic tumor cells were able to convert $CD4^+$ CTCL cells to Treg cells. After exposure to DCs, CTCL cells respond by proliferating, up-regulating cytoplasmic and surface CTLA-4, FoxP3, and CD25, and secreting the immunosuppressive cytokines IL-10 and TGF-β. Thus the secretion of these cytokines by Treg cells may provide a continual source of immature DCs, which in turn ensures the continued presentation of antigens derived from apoptotic cells, inducing further CTCL proliferation and up-regulation of the Treg profile. In fact, the growth dependency of CTCL cells on immature DCs is a well-known feature of CTCL. The conversion of CTCL cells to Treg cells may explain the anergic, immunosuppressive nature of the malignancy that accompanies the evolution of the disease. In advanced CTCL, the normal T-cell compartment is depleted, suggesting that CTCL cells have an inhibitory influence on normal T-cell poiesis [75], which may be explained, at least in part, by their adoption of Treg functions. Adoption of a Treg profile by ATLL and CTCL cells identifies new avenues for therapeutic intervention in these hematological malignancies.

THE INFLUENCE OF THERAPY ON Treg FREQUENCIES/FUNCTION

Studies assessing a potential influence of chemotherapy on Treg cells have been initiated [13, 59, 60]. In an animal model, low-dose cyclophosphamide was shown to augment the efficacy of anti-tumor immune responses by decreasing the frequencies of Treg cells and increasing both lymphocyte proliferation and memory T cells [59, 60]. Similar findings on the down-regulatory effect of fludarabine on both the number and, most surprisingly, inhibitory function of Treg cells was found in B-CLL patients [13]. Moreover, patients treated with regimens containing fludarabine had significantly reduced Treg cells, independent of stage of disease. Although the molecular basis for the action of fludarabine on Treg cells has not yet been elucidated, *in vitro* experiments revealed a preferential induction of apoptosis in Treg cells after incubation with fludarabine.

It has been shown that COX-2 inhibitors might also negatively control Treg cell activity and, consequently, enhance anti-tumor responses [1]. This assumption resulted from the observation that tumor-derived COX-2 and its product, prostaglandin E (PGE)-2, were shown to be involved in the pathogenesis of non-small-cell lung cancer by inducing suppressor activities of Treg cells [1]. In fact, the PGE-2-mediated induction of Treg-cell FoxP3 gene expression was significantly reduced in the absence of the E-prostanoid-4-receptor (EP-4R) and completely ablated in the absence of EP-2R expression. Thus mice receiving COX-2 inhibitors showed reduced Treg cell frequency and activity within TILs and decreased tumor burden [1].

In summary, defining the pathways controlling Treg cell activities will enhance our understanding of the limitation of the host anti-tumor immune responses. All the presented observations suggest that the success of peptide-based immunotherapy for cancer may depend on the balance between Treg and effector T cells. New strategies involving simultaneous blocking of the migration/function or depletion of Treg cells and the stimulation of $CD4^+$ effector T cells are needed to shift this dynamic balance toward effector T cells in the treatment of cancer patients.

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