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Roles of regulatory T cells in cancer immunity

Yoshiko Takeuchi[1] and **Hiroyoshi Nishikawa**[1,2]

[1]Division of Cancer Immunology, EPOC, National Cancer Center, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan and
[2]Department of Immunology, Nagoya University Graduate School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466-8550, Japan

Correspondence to: H. Nishikawa; E-mail: hnishika@east.ncc.go.jp

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REVIEW

Abstract

CD4$^+$ regulatory T cells (T_{regs}) expressing the transcription factor FoxP3 are highly immune suppressive and play central roles in the maintenance of self-tolerance and immune homeostasis, yet in malignant tumors they promote tumor progression by suppressing effective antitumor immunity. Indeed, higher infiltration by T_{regs} is observed in tumor tissues, and their depletion augments antitumor immune responses in animal models. Additionally, increased numbers of T_{regs} and, in particular, decreased ratios of CD8$^+$ T cells to T_{regs} among tumor-infiltrating lymphocytes are correlated with poor prognosis in various types of human cancers. The recent success of cancer immunotherapy represented by immune checkpoint blockade has provided a new insight in cancer treatment, yet more than half of the treated patients did not experience clinical benefits. Identifying biomarkers that predict clinical responses and developing novel immunotherapies are therefore urgently required. Cancer patients whose tumors contain a large number of neoantigens stemming from gene mutations, which have not been previously recognized by the immune system, provoke strong antitumor T-cell responses associated with clinical responses following immune checkpoint blockade, depending on the resistance to T_{reg}-mediated suppression. Thus, integration of a strategy restricting T_{reg}-mediated immune suppression may expand the therapeutic spectrum of cancer immunotherapy towards patients with a lower number of neoantigens. In this review, we address the current understanding of T_{reg}-mediated immune suppressive mechanisms in cancer, the involvement of T_{regs} in cancer immunotherapy, and strategies for effective and tolerable T_{reg}-targeted therapy.

Keywords: immune suppression, immune checkpoint inhibitors, T_{reg}-targeted therapy

Introduction

CD4$^+$ regulatory T cells (T_{regs}) are a highly immune suppressive subset of CD4$^+$ T cells, characterized by expression of the master regulatory transcription factor FoxP3 (1–3). T_{regs} were originally identified as CD4$^+$CD25$^+$ T cells by Sakaguchi *et al.* (4) and are proven to play central roles in the maintenance of self-tolerance in healthy individuals (5–9). T_{reg} deficiency due to mutations in the *FOXP3* gene results in fatal autoimmune disorders and allergy in both mice and humans (5–7). T_{regs} are therefore involved in maintaining immune homeostasis: they protect hosts from developing autoimmune diseases and allergy, whereas in malignancies, they promote tumor progression by suppressing effective antitumor immunity (8, 9).

Cancer cells harboring inherent genetic instability form new antigens (so-called neoantigens), which have not been previously recognized by the immune system. To avoid immune surveillance targeting immunogenic cancer antigens including neoantigens, cancers acquire resistance and escape machineries against the immune system by selecting less-immunogenic cells, and establishing an immunosuppressive environment using immunosuppressive elements to become clinically apparent 'cancers'. In cancer tissues, immune suppressive cytokines, molecules and cells including T_{regs} constitute the immunosuppressive network to inhibit effective antitumor immunity, thereby promoting cancer progression (10, 11).

Cancer immunotherapy represented by blockade of immune checkpoint molecules such as CTLA-4 and PD-1 has provided remarkable clinical efficacy across multiple cancer types even in patients with advanced cancers (12–27). Long-term follow-up in a pooled meta-analysis of 1861 melanoma patients receiving the anti-CTLA-4 antibody, ipilimumab, in phase II or III trials revealed prolonged survival in approximately 20 percent, in some cases extending to 10 years (28). The cohort of the phase I clinical trial for the anti-PD-1 antibody, nivolumab, in heavily pretreated solid cancers showed overall survival of 9.9, 22.4 and 16.8 months in melanoma, non-small cell lung cancer and renal cell carcinoma, respectively (14).

However, accumulating data have uncovered that these durable responses are only observed in approximately 20–30% of the treated patients (28), indicating the importance

of identifying biomarkers to predict clinical responses in addition to developing novel cancer immunotherapies. Clinical efficacy after immune checkpoint blockade is reportedly associated with the somatic mutational burden in the tumor cells (29–32); that is, clinical benefit is limited to those whose cancer cells harbor mutation-derived neoantigens (not present in normal cells) being recognized as 'non-self' by the immune system (33, 34). T_{regs} engaged in self-tolerance favorably control the activation of T cell responses to cancer antigens that are derived from self-constituents (so-called shared antigens), but are less suppressive to T cells recognizing foreign antigens (35). Therefore, it is anticipated that integration of approaches reducing the suppressive activity and/or number of T_{regs} with approaches blocking immune checkpoint molecules, can broaden the therapeutic spectrum of cancer immunotherapy to cancer patients who have a lower number of neoantigens.

Here, we will review the current understanding of T_{reg}-mediated immune suppressive mechanisms in cancer, the involvement of T_{regs} in cancer immune therapy, and future therapeutic strategies targeting T_{regs}.

Natural and induced T_{regs}

T_{regs} are separated into natural/thymic and peripherally induced T_{regs} on the basis of the sites in which they are generated (8, 36). Although not fully clarified in humans, natural/thymic T_{regs} stem from self-reactive thymocytes present in the thymus (8). A fraction of $CD4^+CD8^-$ thymocytes receive TCR stimulation by complexes of MHC plus self-peptide and acquire expression of CD25, through which IL-2 transmits signals via STAT5 to express FoxP3, resulting in differentiation into T_{regs} (37–39). Natural/thymic T_{regs} reportedly express high levels of Helios (a member of the Ikaros transcription factor family) and Neuropilin-1(a type-1 transmembrane protein). In contrast, T_{regs} that develop in the periphery often lack or have a low level expression of these molecules.

According to data from animal models, these peripherally induced T_{regs} are readily converted from conventional T cells by *in vitro* stimulation with TGF-β or retinoic acid (40). However, in humans, $FoxP3^+$ T cells induced from conventional T cells by *in vitro* TCR stimulation with TGF-β fail to gain suppressive function and rather produce pro-inflammatory cytokines (41, 42). At present, the function of peripherally induced T_{regs} such as TGF-β-induced ones in humans is obscure though there are some reports showing that several cytokines or a specific microbiota environment can induce T_{regs} with a suppressive function from $CD4^+CD25^-$ T cells (43, 44). Yet it remains to be determined whether these peripherally induced $FoxP3^+$ T_{regs} are functionally stable *in vivo*. Therefore, in this review, the T_{regs} we will refer to are natural/thymic T_{regs} unless otherwise specified.

Identification and functional classification of human T_{regs}

FoxP3 is the master regulatory molecule in T_{regs}, and expression of FoxP3 represents the T_{reg} population in mice. In contrast, to define T_{regs} definitely in humans causes difficulty due to the upregulation of FoxP3 following activation of naive T cells (42). As CD25 is an activation marker and its expression is not confined to T_{regs}, additional markers are needed. Although $CD4^+CD25^+$ T cells with additional low level expression of CD127 (the α-chain of the IL-7 receptor) were reported to possess FoxP3 expression and suppressive function (45, 46), CD127 is also down-regulated following recent activation of naive T cells that also express a low level of FoxP3 (47), suggesting possible contamination of non-T_{regs} in the $CD127^{low}CD4^+CD25^+$ T-cell fraction.

We have therefore proposed a classification of human T_{regs} based on expression levels of CD45RA and FoxP3 (Fig. 1; Table 1) (8, 11, 48). $FoxP3^+CD4^+$ T cells can thus be divided into three fractions: naive T_{regs} (nT_{regs}: $CD45RA^+FoxP3^{low}CD4^+$);

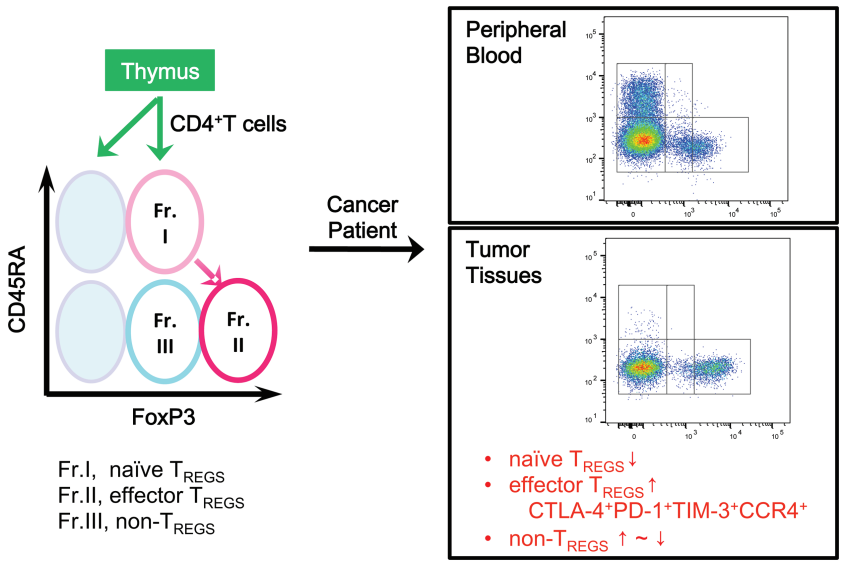

Fig. 1. Identification of human T_{regs}. Human T_{regs} are classified into naive and effector T_{regs} by the expression levels of a naive marker CD45RA and of FoxP3. In TMEs compared with blood, naive T_{reg} (fraction I, Fr. I) numbers are reduced and highly suppressive effector T_{reg} (fraction II) numbers are increased, expressing CTLA-4, PD-1, TIM-3 and CCR4. The frequency of $FoxP3^+$ non-T_{reg} cells (fraction III) is variable depending on cancer types.

effector T_{regs} (eT_{regs}: $CD45RA^-FoxP3^{high}CD4^+$); and non-$T_{regs}$ ($CD45RA^-FoxP3^{low}CD4^+$). The nT_{regs} have recently egressed from the thymus, have not yet been activated in the periphery and possess weak suppressive activity. Upon activation with TCR stimulation, nT_{regs} vigorously proliferate and differentiate into highly suppressive eT_{regs}. In contrast, non-T_{regs} are not immune suppressive but are rather immune stimulatory T cells, producing inflammatory cytokines including IFN-γ and IL-17 (48).

This classification, based on T_{reg} function, reflects the pathophysiology of autoimmune and inflammatory diseases. Both sarcoidosis patients lacking tuberculin reaction because of an immune suppressive state and systemic lupus erythematosus (SLE) patients with systemic auto-immunity have increased $FoxP3^+CD4^+$ T cells in the peripheral blood (48). In our classification with CD45RA and FoxP3 expression, highly suppressive eT_{regs} ($CD45RA^-FoxP3^{high}CD4^+$) are the dominant component of $FoxP3^+CD4^+$ T cells in the former, whereas $FoxP3^+$ non-T_{regs} ($CD45RA^-FoxP3^{low}CD4^+$) are increased in the latter (48), clearly demonstrating the clinical state of these patients—an immune suppressive state and a dysregulation of self-tolerance in sarcoidosis and SLE, respectively.

Suppressive mechanisms of T_{regs}

T_{regs} exhibit their suppressive activity by numerous cellular and humoral mechanisms (summarized in Table 2) such as suppression of antigen-presenting cells via CTLA-4, secretion of inhibitory cytokines (IL-10, TGF-β and IL-35), expression of granzyme/perforin, consumption of IL-2, and degradation of ATP (reviewed in [8]).

Among these mechanisms, suppression via CTLA-4 (a co-inhibitory receptor constitutively expressed by T_{regs}) and IL-2 consumption via CD25 (the IL-2 receptor α-chain, also constitutively expressed by T_{regs}) appear to play key roles for the following reasons: T_{reg}-specific CTLA-4 deficiency impairs *in vitro* and *in vivo* T_{reg}-mediated suppression (49); FoxP3 directly suppresses IL-2 gene transcription and up-regulates *CTLA4* and *IL2RA* (which encodes CD25) gene transcription (2); and high-dose IL-2 neutralizes *in vitro* T_{reg}-mediated suppression (50, 51). CTLA-4 engages with B7 molecules (i.e. B7-1 and B7-2; CD80 and CD86) on antigen-presenting cells with greater avidity compared with CD28 (52) and provides inhibitory reverse signaling to antigen-presenting cells. In addition, B7 molecules are physically transferred to the surface or the inside of T_{regs} together with CTLA-4 (52). Then, maturation of antigen-presenting cells (via the co-stimulatory signal from B7 to CD28 on effector cells) is strongly blocked.

T_{regs} suppress effective antitumor immune responses

In animal models

The involvement of T_{regs} in tumor immunity was originally reported in 1999 (10, 53). Mice treated with anti-CD25 antibody (which depleted the $CD4^+CD25^+$ T_{regs}) and nude (T cell deficient) mice that were given splenocytes that had been treated with anti-CD25, exhibited tumor rejection and retardation of tumor growth, and interestingly the latter mice simultaneously

Table 1. Classification of $FoxP3^+CD4^+$ T cells

Cell subset	Phenotype/cytokines	Characteristics
Naive T_{regs} (nT_{regs}): fraction I, resting T_{regs} $CD45RA^+FoxP3^{low}CD4^+$	$CTLA\text{-}4^{low}CD25^{high}$ $CD127^{low/-}Ki\text{-}67^-$	Weak suppressive activity Differentiate to effector T_{regs} upon TCR stimulation
Effector T_{regs} (eT_{regs}): fraction II, activated T_{regs} $CD45RA^-FoxP3^{high}CD4^+$	$CTLA\text{-}4^{high}CD25^{high}$ $Ki\text{-}67^+$, $PD\text{-}1^+$, $TIM\text{-}3^+$, $GITR^+$, Fas^+, $IL\text{-}10^+$, $TGF\text{-}\beta^+$	Strong suppressive and proliferative activity Prone to apoptosis Tend to increase in peripheral blood with aging
Non-T_{regs}: fraction III $CD45RA^-FoxP3^{low}CD4^+$	$IL\text{-}2^+$, $IFN\text{-}\gamma^+$, $IL\text{-}17^+$	Heterogeneous population No suppressive activity

Table 2. T_{reg}-mediated suppressive mechanisms

Molecules[a]	Ligands	Function
Contact-dependent suppression		
CTLA-4	B7-1/B7-2	Blockade of B7–CD28 costimulatory signals by binding to B7 with greater avidity Inhibition of maturation of antigen-presenting cells (APCs) by physical transfer of B7 on/in T_{regs} or transmitting reverse signals to induce IDO in APCs
?	?	Rendering self-antigen-specific $CD8^+$ T cells to a stable anergic state expressing CCR7 and CTLA-4
CD39, CD73	A_{2A} receptor	Conversion of ATP, an inflammatory molecule and a danger signal, to inhibitory adenosine by CD39/CD73
Granzyme, perforin	Not applicable	Direct cytotoxicity against effector cells
Cytokine-mediated suppression		
CD25 (IL-2 receptor α-chain)	IL-2	Inhibition of differentiation to effector cells by consuming IL-2
TGF-β, IL-10,IL-35	Not applicable	Inhibition of effector T cells, macrophages, cancer-associated fibroblasts, etc.

[a]The major mechanisms are mediated by CTLA-4 and by CD25.

exhibited autoimmunity in the stomach and the thyroid (10). Another study showed that intra-tumoral injection of anti-CD4 antibody in tumor-bearing mice caused rejection of late-stage tumors by depleting T_{regs} and altering the cytokine milieu in the tumor microenvironment (TME) (54). In addition, concomitant tumor immunity, which is a phenomenon that tumor-bearing mice can reject the same tumor cells when inoculated at a distant site, is also suppressed by T_{regs}; mice bearing a poorly immunogenic B16 melanoma, in which concomitant tumor immunity is not evoked, rejected a secondary B16 melanoma challenge when T_{regs} were depleted by anti-CD4 antibody (55). Taken together, T_{regs} suppress anti-tumor immunity and promote tumor progression.

In humans

In the TME in melanoma, non-small cell lung, gastric and ovarian cancers, eT_{regs} heavily infiltrate and account for 20–50% of $CD4^+$ T cells, as compared with 5 to 10 percent in the peripheral blood of healthy individuals (8, 11). High infiltration of T_{regs} in tumors is associated with a poor prognosis in various types of cancers including melanoma, non-small cell lung, gastric, hepatocellular, pancreatic, renal cell, breast and cervical cancers (11, 56). In ovarian cancer, a decreased ratio of $CD8^+$ T cells to T_{regs} in tumors is related to poor prognosis (57), indicating suppression of effector $CD8^+$ T cells by T_{regs}. Yet in some cancers such as colorectal, head and neck, and bladder cancers, a higher infiltration of $FoxP3^+$ T cells is reportedly correlated with better prognosis (56).

In fact, in colorectal cancer we have recently shown that $FoxP3^+$ non-T_{regs} heavily infiltrated a fraction of colorectal cancers containing high levels of inflammatory cytokines such as TGF-β and IL-12 and were associated with a better prognosis (58). The difficulty of distinguishing $FoxP3^+$ non-T_{regs} from $FoxP3^{high}$ eT_{regs} in tumor tissues would have been a major confounding factor in previous studies evaluating the clinical significance of $FOXP3^+CD4^+$ T cells in colorectal cancers using immunohistochemistry. Therefore, although in some cancers controversies do exist regarding the significance of T_{regs}, T_{reg}-infiltration into a tumor suppresses anti-tumor immunity and generally corresponds to poor prognosis.

Trafficking and characteristics of T_{regs} in cancer

How and why are activated T_{regs} present in high numbers in tumor sites? T_{regs} appear to chemo-attracted to the TME (summarized in Table 3). Although the combination of chemokines and their receptors differs in each cancer—i.e. CCR4 with CCL22 in breast cancer (59); CCR4 with undefined chemokines in colorectal (60) and oral squamous cancers (61) and in Hodgkin lymphoma (62); CCR4 with CCL22, CCR10 with CCL28 and CXCR4 with CXCL12 in ovarian cancer (63–65); and CCR5 with CCL5 in pancreatic cancer (66)—blockade of chemotaxis by antibodies or small molecules may result in a reduction in T_{reg} numbers in tumors (66, 67).

These T_{reg}-recruiting chemokines are generated in TMEs by macrophages and/or tumor cells. Hypoxia is also reported to induce CCL28 production by ovarian cancer cells and to recruit T_{regs} (64). Additionally, activated $CD8^+$ T cells infiltrating into the tumor stimulate production of the T_{reg}-recruiting chemokine CCL22 by tumor cells (67). Moreover, in a mouse model with a xenograft of human melanoma, infiltration by T_{regs} was decreased in the tumor if T_{regs} were transferred alone compared with tumors where T_{regs} and $CD8^+$ T cells were co-transferred, suggesting that initial $CD8^+$ T-cell infiltration stimulates CCL22 production by tumors as an escape mechanism (67).

In the TME, highly immune suppressive eT_{regs} with high-level expression of suppression-related molecules such as CTLA-4 and TIGIT are detected with reduced number of nT_{regs}, indicating a highly activated status of tumor-infiltrating T_{regs} (11, 72). In breast cancer, RANKL-expressing T_{regs} are reported to promote metastasis of RANK-expressing cancer cells (73) (Fig. 2). One possible mechanism of T_{reg} activation in tumors is that proliferating and dying tumor cells provide a large amount of self-antigens, which T_{regs} might recognize and be activated by as tumors contains a subset of immature

Table 3. Chemokines and chemokine receptors related to T_{reg} trafficking

Cancer	Chemokine receptor on T_{regs}	Chemokine	Origin of chemokines	Ref
Human				
Breast	CCR4	CCL22	Tumor cells	(59)
Cervical	ND	CXCL12	Tumor cells	(68)
Colorectal	CCR4	ND	ND	(60)
Oral squamous	CCR4	ND	ND	(61)
Ovarian	CCR4	CCL22	TAMs	(63)
	CCR10	CCL28	Tumor cells	(64)
	CXCR3	ND	Tumor	(69)
	CXCR4	CXCL12	Tumor	(65)
Pancreatic	CCR5	CCL5	Tumor cells	(66)
Hodgkin lymphoma	CCR4	ND	ND	(62)
Mouse				
Colorectal	CCR6	CCL20	TAMs	(70)
Melanoma	CCR4	CCL22	Tumor	(67)
	CCR5	CCL3,4,5	MDSCs	(71)
Pancreatic	CCR5	CCL5	Tumor cells	(66)

MDSCs, myeloid-derived suppressor cells; ND, not described; TAMs, tumor-associated macrophages.

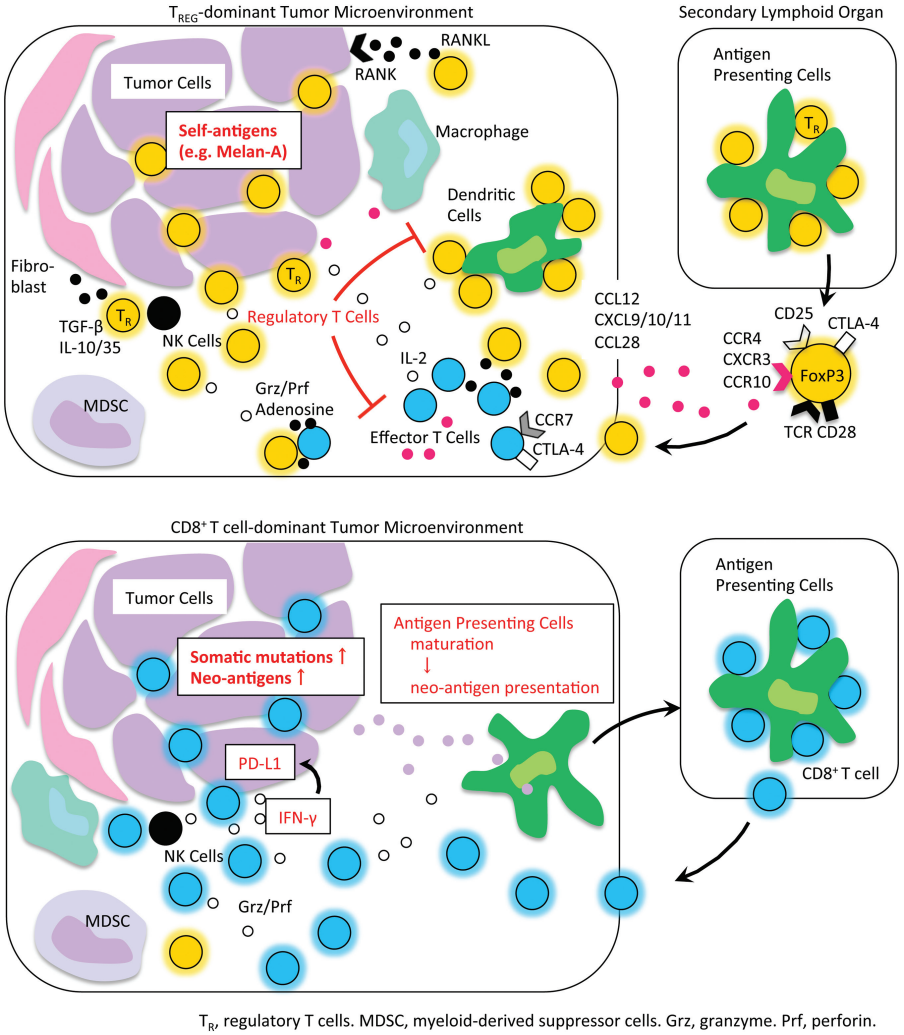

Fig. 2. T_{regs} in cancer immunity. In cancer patients with minimal neoantigens (top part of the figure), T_{regs} appear to be primed at the secondary lymphoid organs and traffic to the TME by chemotaxis. T_{regs} suppress effective antitumor immunity and/or contribute to tumor progression and metastasis. In contrast, in cancer patients with abundant neoantigens (bottom part of the figure), effector cells including $CD8^+$ T cells are primed and expanded; while they are suppressed in local tumor sites by the immune suppressive network and chronic exposure to cancer antigens in tumors, they are yet on stand-by for tumor killing upon re-stimulation with inhibition of the immune suppressive network, particularly PD-1 signaling. Grz, granzyme; MDSC, myeloid-derived suppressor cell; Prf, perforin; T_R, regulatory T cell.

dendritic cells that promote the proliferation/stimulation of T_{regs} in a TGF-β-dependent manner (74, 75).

In accordance with this, the TCR repertoire of tumor-infiltrating T_{regs} is skewed and largely distinct from that of tumor-infiltrating conventional T cells, suggesting that T_{regs} recognize certain skewed antigens and clonally expand in the TME (76, 77). Indeed, T_{reg} clones established from human melanoma recognize cancer-testis antigens including NY-ESO-1 (78, 79), TRAG-3 (78), LAGE-1 (80) and ARTC1 (antigen recognized by T_{reg} cells) (81), and differentiation/overexpression self-antigens including gp100, TRP1, and survivin (79). T_{regs} in human colorectal cancer are known to be reactive to Mucin-1, HER2/neu, CEA, telomerase, survivin and EGFR (82). WT1 is also reported to be recognized by leukemia-derived T_{regs} (83). Yet whether these antigens are exclusively recognized by T_{regs} or recognition is shared by helper $CD4^+$ T cells is unclear; however, T_{regs} usually harbor higher affinity TCRs compared with conventional T cells and are predominantly activated in tumors.

Strategies for T_{reg}-targeted therapy

As discussed above, activated eT_{regs} are present at a high frequency in tumors and need to be controlled for the generation/activation of antitumor immunity. Some clinical studies indicated the potential of depleting CD25-expressing lymphocytes to augment anti-tumor immune responses; yet, other similar studies failed to support this. As activated effector T cells in addition to T_{regs} also express CD25, CD25-based cell depletion may reduce activated effector T cells as well, cancelling the effect of T_{reg} depletion to augment anti-tumor immunity. Additionally, one plausible concern is increased autoimmunity-related toxicities following T_{reg} depletion. In order to secure safety of T_{reg}-targeted therapy, selective

depletion of eT_{regs} in tumors rather than the entire T_{reg} population can be exploited to augment anti-tumor immunity without eliciting deleterious autoimmunity (72). Targeting molecules and signals specific for eT_{regs} is being tested in clinical trials as an effective strategy for eT_{reg} depletion.

Humanized IgG1 monoclonal antibody targeting CCR4: mogamulizumab

We showed that CCR4 was specifically expressed by a subset of suppressive eT_{regs} abundant in melanoma, and treatment using anti-CCR4 antibody depleted the melanoma-infiltrating T_{regs} that expressed CCR4 and efficiently induced/augmented both $CD4^+$ and $CD8^+$ T cells that were specific for cancer-testis antigen (72). Mogamulizumab has been approved in Japan for the treatment of CCR4-expressing adult T-cell leukemia/lymphoma (ATLL). Anti-CCR4 antibody markedly reduced eT_{regs} as well as ATLL cells and augmented ATLL antigen (cancer-testis antigen)-specific $CD8^+$ T-cell responses in an ATLL patient, possibly in association with the prolonged survival of this patient (72).

Based on these preclinical data, multiple early phase clinical trials with mogamulizumab as an eT_{reg} depletion reagent are being conducted as monotherapy (trial numbers NCT02281409 and NCT01929486 (84)) and in combination with anti-PD-1 antibody (NCT02476123 and NCT02705105), anti-PD-L1 (PD-1 ligand 1) antibody or anti-CTLA-4 antibody (NCT02301130) and anti-4-1BB agonistic antibody (NCT02444793) in advanced solid tumors, and in combination with docetaxel in non-small cell lung cancer (NCT02358473).

Anti-OX-40 antibody and anti-GITR antibody

OX-40 and GITR are members of the TNF receptor superfamily and are both co-stimulatory receptors expressed by activated T cells. On T_{regs}, OX-40 is induced after activation and GITR is constitutively expressed (85–90). These signals reduce the suppressive activity of T_{regs} as well as enhancing activation of effector T cells.

In mouse models, an anti-OX-40 agonistic antibody augmented anti-tumor immunity in melanoma, colon cancer, glioma, breast cancer, sarcoma, renal cancer and prostate cancer (91). Its effect was mainly dependent on the reduction of T_{regs} in tumor tissues. A phase I trial of an OX-40 agonist demonstrated anti-tumor activity in melanoma and renal cell cancer (92). Early phase clinical trials evaluating OX-40 agonists in head and neck, breast and prostate cancer and in B cell lymphoma are also being investigated (NCT01862900, NCT02274155, NCT02318394 and NCT02205333). Additionally, combination of an OX-40 fusion protein (MEDI6383) and an anti-PD-L1 antibody, durvalumab, is also being investigated (NCT02221960). In mouse models, an anti-GITR agonistic antibody stimulated strong anti-tumor immunity in fibrosarcoma, colorectal carcinoma and melanoma models by decreasing T_{reg} numbers and converting T_{reg}-mediated resistance to effector T cell activation (93–95). Phase I clinical trials evaluating GITR agonists in solid tumors are being tested (NCT 02583165 and NCT02628574).

Small molecules targeting T_{reg}-specific signals

T_{regs} are highly dependent on PI3K signals for their maintenance and function. Inactivation of PI3K signals in T_{regs} activates $CD8^+$ T cells and induces tumor regression (96). Therefore, not only molecules specifically expressed by T_{regs}, but also signals on which T_{regs} specifically depend could become targets to control T_{regs}.

T_{reg} depletion with vaccination

T_{reg} depletion alone may not be sufficient to establish effective antitumor immunity. We have shown that self-antigen (Melan-A, a differentiation antigen of melanocytes)-reactive $CD8^+$ T cells fall into an irreversible anergic state (i.e. hyporoliferative and with low cytokine production) with a unique phenotype ($CCR7^+CTLA\text{-}4^+$) after T_{reg}-mediated suppression and they cannot be re-activated even in the absence of T_{regs} (35). Thus, in addition to overcoming T_{reg}-mediated suppression, subsequent re-priming of effector T cells from the naive T-cell population would be necessary. At least two strategies to augment anticancer immunity by depleting T_{regs} prior to administering cancer vaccines have been evaluated: daclizumab or cyclophosphamide (CPA).

Humanized IgG1 monoclonal antibody targeting CD25: daclizumab. Since T_{regs} are enriched in the $CD4^+CD25^+$ T cell fraction, T_{reg}-depletion by the CD25-depleting antibody daclizumab has been evaluated in clinical trials. When daclizumab was administered following dendritic cell vaccination in metastatic melanoma (n = 15), not only T_{regs} but also activated effector cells were depleted and neither antitumor immune responses nor antibody production was observed (97). In contrast, in breast cancer patients, administration of daclizumab followed by vaccination consisting of multiple tumor-associated peptides succeeded in T_{reg}-depletion and demonstrated favorable clinical responses (98). Stable disease was obtained in 6 out of 10 patients. Progression-free survival was 4.8 months (95% Confidence Interval, 3.0–6.5 months). The overall survival (OS) was 27.8 months (19.5–36.1). The 2-year survival was 65.5 ± 17.3% (rate ± SD). No immune related adverse reaction was observed.

Cyclophosphamide. CPA is an alkylating agent that reportedly depletes T_{regs} when used in low doses. In a phase II clinical trial, patients with advanced renal cell cancer received therapeutic vaccination of IMA901 consisting of multiple tumor-associated peptides and GM-CSF with or without preceding CPA administration (99). Patients treated with IMA901/GM-CSF/CPA showed T_{reg} reduction with augmented antitumor immune responses. The OS tended to be extended in the IMA901/GM-CSF/CPA-treated group (n = 33) compared with the IMA901/GM-CSF-treated group (n = 35) (23.5 months versus 14.8 months). A phase III trial investigating the addition of IMA901/GM-CSF/CPA to the standard care of sunitinib was completed in 2015 and the results are awaited.

Involvement of T_{regs} in immune checkpoint inhibitors

Immune checkpoint blockade—inhibiting the immunosuppressive signals from co-inhibitory molecules—allows a resurgence in the effector function of tumor-infiltrating T cells and provides clinical success in various types of cancers including malignant melanomas and lung cancers. As

immune checkpoint molecules such as CTLA-4 and PD-1 are expressed by both tumor-infiltrating effector T cells and T_{regs}, current immune checkpoint blocking agents could target T_{regs} as well. Analyses of anti-CTLA-4 antibodies in mouse models revealed that the antitumor efficacy was dependent on depletion of CTLA-4-expressing T_{regs} in tumors through the antibody-dependent cellular cytotoxic (ADCC) activity of the anti-CTLA-4 antibody; depletion of Fc function totally abrogated the anti-tumor effect of the anti-CTLA-4 antibody (94, 100–102). Additionally, PD-1-expressing T_{regs} reportedly possess higher immune suppressive function than T_{regs} without PD-1 expression in a mouse model (103). Therefore, PD-1-blocking antibodies might act on T_{regs} to augment anti-tumor immunity as well as reversing the effector function of dysfunctional effector cells.

Yet, more than half of the treated patients did not respond to immune checkpoint blockade therapy, even if combinations of blocking antibodies were used. Immuno-monitoring of biomarkers to properly evaluate immune responses in cancer patients is critical for detecting responders. There are two types of tumor antigens: tumor-specific antigens (TSAs), which are either oncogenic viral proteins or abnormal proteins that stem from somatic mutations (neoantigens); and tumor-associated antigens (TAAs), which are highly or aberrantly expressed normal proteins. It is not yet determined how $CD8^+$ T cells specific for each antigen contribute to clinical tumor regression and whether activation of these $CD8^+$ T cells specific for self-antigens versus non-self-antigens are controlled differently.

In vitro experiments comparing T_{reg}-mediated suppression of self-antigen (Melan-A)-specific $CD8^+$ T cells versus non-self (cytomegalovirus)-specific $CD8^+$ T cells showed that cytomegalovirus-specific $CD8^+$ T cells were resistant to suppression by T_{regs} (35), indicating that T_{reg}-mediated suppression is more prominent on self-antigen-expressing tumor cells rather than those expressing neoantigens. It is therefore noteworthy that cancers in patients susceptible to immune checkpoint blockade monotherapy contain a large number of neoantigens and that $CD8^+$ T cells specific for the antigens are resistant to T_{reg}-mediated immune suppression. In contrast, cancers with a lower number of neoantigens did not respond to immune checkpoint blockade and $CD8^+$ T cells are under the control of T_{reg}-mediated immune suppression. Thus, integration of T_{reg}-targeting therapies that reduce T_{reg} function and/or number may expand the therapeutic spectrum of cancer immunotherapy.

Conclusion

T_{regs}, initially found as a key player of self-tolerance, have been revealed to play a critical role in tumor immunity and become a promising therapeutic target of cancer immunology. Yet their contribution in current cancer immunotherapy has not been fully determined and further detailed studies are essential for developing novel effective cancer immunotherapies.

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