Roles of regulatory T cells in cancer immunity

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Abstract

CD4+ regulatory T cells (Treg) 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 Treg is observed in tumor tissues, and their depletion augments antitumor immune responses in animal models. Additionally, increased numbers of Treg and, in particular, decreased ratios of CD8+ T cells to Treg 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 Treg-mediated suppression. Thus, integration of a strategy restricting Treg-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 Treg-mediated immune suppressive mechanisms in cancer, the involvement of Treg in cancer immunotherapy, and strategies for effective and tolerable Treg-targeted therapy.

Keywords: immune suppression, immune checkpoint inhibitors, Treg-targeted therapy

Introduction

CD4+ regulatory T cells (Treg) are a highly immune suppressive subset of CD4+ T cells, characterized by expression of the master regulatory transcription factor FoxP3 (1–3). Treg 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). Treg deficiency due to mutations in the FOXP3 gene results in fatal autoimmune disorders and allergy in both mice and humans (5–7). Treg 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 Treg 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). Tregs 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 Tregs 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 Tregs-mediated immune suppressive mechanisms in cancer, the involvement of Tregs in cancer immune therapy, and future therapeutic strategies targeting Tregs.

Natural and induced Tregs

Tregs are separated into natural/thymic and peripherally induced Tregs on the basis of the sites in which they are generated (8, 36). Although not fully clarified in humans, natural/thymic Tregs 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 Tregs (37–39). Natural/thymic Tregs reportedly express high levels of Helios (a member of the Ikaros transcription factor family) and Neuropilin-1 (a type-1 transmembrane protein). In contrast, Tregs 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 Tregs 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 Tregs 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 Tregs with a suppressive function from CD4+CD25− T cells (43, 44). Yet it remains to be determined whether these peripherally induced FoxP3+ Tregs are functionally stable in vivo. Therefore, in this review, the Tregs we will refer to are natural/thymic Tregs unless otherwise specified.

Identification and functional classification of human Tregs

FoxP3 is the master regulatory molecule in Tregs, and expression of FoxP3 represents the Treg population in mice. In contrast, to define Tregs 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 Tregs, 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-Tregs in the CD127+CD4+CD25− T-cell fraction.

We have therefore proposed a classification of human Tregs 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 Tregs (nTregs; CD45RA+FoxP3lowCD4+);

![Thymus](Thymus.png)

**Thymus**

- CD4+T cells
- Fr. I
- Fr. II
- Fr. III

**Cancer Patient**

- CD45RA
- Fr. I: naive Tregs
- Fr. II: effector Tregs
- Fr. III: non-Tregs

**Peripheral Blood**

- Fr. I: naive Tregs
- Fr. II: effector Tregs
- Fr. III: non-Tregs

**Tumor Tissues**

- naive Tregs
- effector Tregs
- CTLA-4+PD-1+TIM-3+CCR4+
- non-Tregs

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

Suppressive mechanisms of Tregs

Tregs 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 Tregs) and IL-2 consumption via CD25 (the IL-2 receptor α-chain), also constitutively expressed by Tregs, appear to play key roles for the following reasons: Treg-specific CTLA-4 deficiency impairs in vitro and in vivo Treg-mediated suppression ([49]); FoxP3 directly suppresses IL-2 gene transcription and up-regulates CD4 and IL2RA (which encodes CD25) gene transcription ([2]); and high-dose IL-2 neutralizes in vitro Treg-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 Tregs 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.

Table 1. Classification of FoxP3+CD4+ T cells

<table>
<thead>
<tr>
<th>Cell subset</th>
<th>Phenotype/cytokines</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive Treg (nTreg):</td>
<td>CD127low/Ki-67</td>
<td>Weak suppressive activity</td>
</tr>
<tr>
<td>fraction I, resting</td>
<td></td>
<td>Differentiate to effector Treg upon TCR stimulation</td>
</tr>
<tr>
<td>CD45RA-FoxP3+CD4+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effector Treg (eTreg):</td>
<td>CD25high</td>
<td>Strong suppressive and proliferative activity</td>
</tr>
<tr>
<td>fraction II, activated</td>
<td></td>
<td>Prone to apoptosis</td>
</tr>
<tr>
<td>CD45RA-FoxP3+CD4+</td>
<td></td>
<td>Tend to increase in peripheral blood with aging</td>
</tr>
<tr>
<td>Non-Treg:</td>
<td></td>
<td>Heterogeneous population</td>
</tr>
<tr>
<td>fraction III</td>
<td>IL-2+, IFN-γ+, IL-17+</td>
<td>No suppressive activity</td>
</tr>
<tr>
<td>CD45RA-FoxP3+CD4+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Treg-mediated suppressive mechanisms

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

The major mechanisms are mediated by CTLA-4 and by CD25.
exhibited autoreactivity 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 Tregs 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 Tregs; mice bearing a poorly immunogenic B16 melanoma, in which concomitant tumor immunity is not evoked, rejected a secondary B16 melanoma challenge when Tregs were depleted by anti-CD4 antibody (55). Taken together, Tregs suppress anti-tumor immunity and promote tumor progression.

In humans
In the TME in melanoma, non-small cell lung, gastric and ovarian cancers, eTregs 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 Tregs 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 Tregs in tumors is related to poor prognosis (57), indicating suppression of effector CD8+ T cells by Tregs. 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-Tregs 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-Tregs from FoxP3+ Tregs in tumors 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 Tregs, Treg-infiltration into a tumor suppresses anti-tumor immunity and generally corresponds to poor prognosis.

### Trafficking and characteristics of Tregs in cancer

How and why are activated Tregs present in high numbers in tumor sites? Tregs 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 Treg numbers in tumors (66, 67).

These Treg-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 Tregs (64). Additionally, activated CD8+ T cells infiltrating into the tumor stimulate production of the Treg-recruiting chemokine CCL22 by tumor cells (67). Moreover, in a mouse model with a xenograft of human melanoma, infiltration by Treg was decreased in the tumor if Tregs were transferred alone compared with tumors where Tregs 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 eTregs with high-level expression of suppression-related molecules such as CTLA-4 and TIGIT are detected with reduced number of nTregs indicating a highly activated status of tumor-infiltrating Tregs (11, 72). In breast cancer, RANKL-expressing Tregs are reported to promote metastasis of RANK-expressing cancer cells (73) (Fig. 2). One possible mechanism of Treg activation in tumors is that proliferating and dying tumor cells provide fine tuned chemokines in colorectal (67); CCR4 with CCL22, CCR10 with CCL28 and CXCR4 with CXCL12 in breast 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 Treg numbers in tumors (66, 67).

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Table 3. Chemokines and chemokine receptors related to Treg trafficking

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Chemokine receptor on Tregs</th>
<th>Chemokine</th>
<th>Origin of chemokines</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Breast</td>
<td>CCR4</td>
<td>CCL22</td>
<td>Tumor cells</td>
<td>(59)</td>
</tr>
<tr>
<td>Cervical</td>
<td>ND</td>
<td>CXCL12</td>
<td>Tumor cells</td>
<td>(68)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>CCR4</td>
<td>ND</td>
<td>ND</td>
<td>(60)</td>
</tr>
<tr>
<td>Oral squamous</td>
<td>CCR4</td>
<td>ND</td>
<td>ND</td>
<td>(61)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>CCR4</td>
<td>CCL22</td>
<td>TAMs</td>
<td>(63)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>CCR4</td>
<td>CCL28</td>
<td>Tumor cells</td>
<td>(64)</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>CCR4</td>
<td>CCL12</td>
<td>Tumor cells</td>
<td>(65)</td>
</tr>
<tr>
<td>Mouse Colorectal</td>
<td>CCR6</td>
<td>CCL20</td>
<td>TAMs</td>
<td>(70)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>CCR4</td>
<td>CCL22</td>
<td>Tumor</td>
<td>(67)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>CCR5</td>
<td>CCL3,4,5</td>
<td>MDSCs</td>
<td>(71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCL5</td>
<td>Tumor cells</td>
<td>(66)</td>
</tr>
</tbody>
</table>

MDSCs, myeloid-derived suppressor cells; ND, not described; TAMs, tumor-associated macrophages.
dendritic cells that promote the proliferation/stimulation of T\textsubscript{reg} in a TGF-β-dependent manner (74, 75).

In accordance with this, the TCR repertoire of tumor-infiltrating T\textsubscript{reg} is skewed and largely distinct from that of tumor-infiltrating conventional T cells, suggesting that T\textsubscript{reg} recognize certain skewed antigens and clonally expand in the TME (76, 77). Indeed, T\textsubscript{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\textsubscript{reg} cells) (81). and differentiation/overexpression self-antigens including gp100, TRP1, and survivin (79). T\textsubscript{reg} 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\textsubscript{reg} (83). Yet whether these antigens are exclusively recognized by T\textsubscript{reg}, or recognition is shared by helper CD4\textsuperscript{+} T cells is unclear; however, T\textsubscript{reg} usually harbor higher affinity TCRs compared with conventional T cells and are predominantly activated in tumors.

**Strategies for T\textsubscript{reg} targeted therapy**

As discussed above, activated eT\textsubscript{reg} 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\textsubscript{reg} also express CD25, CD25-based cell depletion may reduce activated effector T cells as well, cancelling the effect of T\textsubscript{reg} depletion to augment anti-tumor immunity. Additionally, one plausible concern is increased autoimmunity-related toxicities following T\textsubscript{reg} depletion. In order to secure safety of T\textsubscript{reg} targeted therapy, selective...
depletion of eTregs in tumors rather than the entire Treg population can be exploited to augment anti-tumor immunity without eliciting deleterious autoimmunity (72). Targeting molecules and signals specific for eTregs is being tested in clinical trials as an effective strategy for eTreg depletion.

**Humanized IgG1 monoclonal antibody targeting CCR4: mogamulizumab**

We showed that CCR4 was specifically expressed by a subset of suppressive eTregs abundant in melanoma, and treatment using anti-CCR4 antibody depleted the melanoma-infiltrating Treg 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 eTregs 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 eTreg-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 Treg, OX-40 is induced after activation and GITR is constitutively expressed (85–90). These signals reduce the suppressive activity of Tregs 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 Tregs 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. 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. hypoproliferative and with low cytokine production) with a unique phenotype (CCR7-CTLA-4+) after Treg-mediated suppression and they cannot be re-activated even in the absence of Treg (35). Thus, in addition to overcoming Treg-mediated suppression, subsequent re-priming of effector T cells from the naive T-cell population would be necessary. At least two strategies to augment antitumor immunity by depleting Tregs prior to administering cancer vaccines have been evaluated: daclizumab or cyclophosphamide (CPA).

**Humanized IgG1 monoclonal antibody targeting CD25: daclizumab.** Since Tregs are enriched in the CD4+CD25+ T cell fraction, Treg-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 Tregs 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 Treg-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 Tregs 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 Treg 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 Tregs 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 Tregs. Current immune checkpoint blocking agents could target Tregs as well. Analyses of anti-CTLA-4 antibodies in mouse models revealed that the anti-tumor efficacy was dependent on depletion of CTLA-4-expressing Tregs in tumors through the antibody-dependent cellular cytotoxicity (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 Tregs reportedly possess higher immune suppressive function than Tregs without PD-1 expression in a mouse model (103). Therefore, PD-1-blocking antibodies might act on Tregs 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 Tregs-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 Tregs (35), indicating that Tregs-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 Tregs-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 Tregs-mediated immune suppression. Thus, integration of Tregs-targeting therapies that reduce Tregs function and/or number may expand the therapeutic spectrum of cancer immunotherapy.

Conclusion

Tregs, 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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