Roles of regulatory T cells in cancer immunity

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Received 18 April 2016, accepted 6 May 2016

Abstract

CD4+ regulatory T cells (T_{reg}) 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_{reg} is observed in tumor tissues, and their depletion augments antitumor immune responses in animal models. Additionally, increased numbers of T_{reg} and, in particular, decreased ratios of CD8+ T cells to T_{reg} 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_{reg} 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_{reg}) are a highly immune suppressive subset of CD4+ T cells, characterized by expression of the master regulatory transcription factor FoxP3 (1–3). T_{reg} 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_{reg} 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_{reg} 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+);

![Fig. 1. Identification of human Tregs.](image-url)

**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.
The involvement of Treg in tumor immunity was originally reported in 1999 (10, 53). Mice treated with anti-CD25 antibody (which depleted the CD4+CD25+ Treg) 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>
<thead>
<tr>
<th>Molecules</th>
<th>Ligands</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact-dependent suppression CD25</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 Treg or transmitting reverse signals to induce IDO in APCs</td>
</tr>
<tr>
<td>CD39, CD73</td>
<td>Aα receptor</td>
<td>Rendering self-antigen-specific CD8+ T cells to a stable anergic state expressing CCR7 and CTLA-4</td>
</tr>
<tr>
<td>Granzyme, perforin</td>
<td>Not applicable</td>
<td>Conversion of ATP, an inflammatory molecule and a danger signal, to inhibitory adenosine by CD39/CD73</td>
</tr>
<tr>
<td>Cytokine-mediated suppression CD25 (IL-2 receptor α-chain) TGF-β, IL-10, IL-35</td>
<td>IL-2</td>
<td>Inhibition of differentiation to effector cells by consuming IL-2</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td>Inhibition of effector T cells, macrophages, cancer-associated fibroblasts, etc.</td>
</tr>
</tbody>
</table>

aThe 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 T<sub>reg</sub> 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<sub>reg</sub>; mice bearing a poorly immunogenic B16 melanoma, in which concomitant tumor immunity is not evoked, rejected a secondary B16 melanoma challenge when T<sub>reg</sub> were depleted by anti-CD4 antibody (55). Taken together, T<sub>reg</sub> suppress anti-tumor immunity and promote tumor progression.

In humans

In the TME in melanoma, non-small cell lung, gastric and ovarian cancers, eT<sub>reg</sub> heavily infiltrate and account for 20–50% of CD4<sup>+</sup> T cells, as compared with 5 to 10 percent in the peripheral blood of healthy individuals (8, 11). High infiltration of T<sub>reg</sub> 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<sup>+</sup> T cells to T<sub>reg</sub> in tumors is related to poor prognosis (57), indicating suppression of effector CD8<sup>+</sup> T cells by T<sub>reg</sub>. Yet in some cancers such as colorectal, head and neck, and bladder cancers, a higher infiltration of FoxP3<sup>+</sup> T cells is reportedly correlated with better prognosis (56).

In fact, in colorectal cancer we have recently shown that FoxP3<sup>+</sup> non-T<sub>reg</sub> 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<sup>+</sup> non-T<sub>reg</sub> from FoxP3<sup>+</sup>eT<sub>reg</sub> in tumor tissues would have been a major confounding factor in previous studies evaluating the clinical significance of FOXP3<sup>+</sup>CD4<sup>+</sup> T cells in colorectal cancers using immunohistochemistry. Therefore, although in some cancers controversies do exist regarding the significance of T<sub>reg</sub>, T<sub>reg</sub>-infiltration into a tumor suppresses anti-tumor immunity and generally corresponds to poor prognosis.

### Table 3. Chemokines and chemokine receptors related to T<sub>reg</sub> trafficking

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Chemokine receptor on T&lt;sub&gt;reg&lt;/sub&gt;</th>
<th>Chemokine</th>
<th>Origin of chemokines</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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></td>
<td>CCR10</td>
<td>CCL28</td>
<td>Tumor cells</td>
<td>(64)</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>CCL5</td>
<td>Tumor cells</td>
<td>(66)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>CCR5</td>
<td>CCL5</td>
<td>Tumor cells</td>
<td>(66)</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>CCR4</td>
<td>ND</td>
<td>ND</td>
<td>(62)</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>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></td>
<td>CCR5</td>
<td>CCL3,4,5</td>
<td>MDSCs</td>
<td>(71)</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>CCR5</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\(_{\text{reg}}\) in a TGF-\(\beta\)-dependent manner (74, 75).

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

**Strategies for T\(_{\text{reg}}\)-targeted therapy**

As discussed above, activated eT\(_{\text{reg}}\) 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\(_{\text{reg}}\) also express CD25, CD25-based cell depletion may reduce activated effector T cells as well, cancelling the effect of T\(_{\text{reg}}\) depletion to augment anti-tumor immunity. Additionally, one plausible concern is increased autoimmunity-related toxicities following T\(_{\text{reg}}\) depletion. In order to secure safety of T\(_{\text{reg}}\)-targeted therapy, selective

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**Fig. 2.** T\(_{\text{reg}}\) in cancer immunity. In cancer patients with minimal neoantigens (top part of the figure), T\(_{\text{reg}}\) appear to be primed at the secondary lymphoid organs and traffic to the TME by chemotaxis. T\(_{\text{reg}}\) 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\(_{\text{reg}}\), regulatory T cell.
depletion of $T_{reg}$ 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 $eT_{reg}$ 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 $eT_{reg}$ abundant in melanoma, and treatment using anti-CCR4 antibody depleted the melanoma-infiltrating $T_{reg}$ 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 $eT_{reg}$ as well as ATL 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 $T_{reg}$, OX-40 is induced after activation and GITR is constitutively expressed (85-90). These signals reduce the suppressive activity of $T_{reg}$, 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 $T_{reg}$ 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, NCT0218394 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**

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

**$T_{reg}$ 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. hypoproliferative and with low cytokine production) with a unique phenotype (CCR7+CTLA-4-) after $T_{reg}$-mediated suppression and they cannot be re-activated even in the absence of $T_{reg}$ (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 antitumor immunity by depleting $T_{reg}$ prior to administering cancer vaccines have been evaluated: daclizumab or cyclophosphamide (CPA).

**Humanized IgG1 monoclonal antibody targeting CD25: daclizumab.** Since $T_{reg}$ 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 $T_{reg}$ 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 report-edly depletes $T_{reg}$ 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 $T_{reg}$ in immune checkpoint inhibitors**

Immune checkpoint blockade—inhibiting the immuno-suppressive 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_{reg}^{*}, current immune checkpoint blocking agents could target T_{reg} 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_{reg} 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 T_{reg} reportedly possess higher immune suppressive function than T_{reg} without PD-1 expression in a mouse model (103). Therefore, PD-1-blocking antibodies might act on T_{reg} 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_{reg} (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_{reg}, 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.

**Funding**

This study was supported by Grants-in-Aid for Scientific Research (B) (H.N., No. 26290054) and for challenging Exploratory Research (H.N., No. 16K15551) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by The National Cancer Center Research and Development Fund (H.N., No. 28-A-7).

**Conflict of interest:** The authors have no conflict of interest on this manuscript.

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