Regulatory T cells in cancer

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Increasing evidence supports the existence of elevated numbers of regulatory T cells (Treg cells) in solid tumors and hematologic malignancies. Whereas the biology of CD4+CD25+FOXP3+ Treg cells in murine models seems to be rather straightforward, studies in human diseases are more difficult to interpret due to expression of CD25 on activated effector T cells as well as Treg cells.

More importantly, early studies in human tumors were mainly focused on CD4+CD25+ Treg cells lacking interrogation of more specific markers such as FOXP3 expression. Although the increase of Treg cells seems to be a characteristic feature in most tumors, little is known about the molecular and cellular mechanisms responsible for the increase and maintenance of elevated levels of Treg cells in cancer. We will discuss earlier data in the context of recent findings in Treg-cell biology with a particular emphasis on CD4+CD25+FOXP3+ Treg cells in human malignancies. (Blood. 2006;108:804-811)

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Introduction

In 1971, Gershon and Kondo identified so-called “suppressor” cells when they transferred antigen-specific tolerance to naive animals by transferring antigen-experienced T cells. Due to conflicting results, the concept of T-cell suppression vanished into relative obscurity in the late 1980s. However, reports describing murine T cells responsible for suppression of antitumor immune responses2 by transferring antigen-experienced memory T cells, although lately some reports have described naive CD4+CD25+FOXP3+ T cells in mice as well as humans.23,24 Among the cell-surface markers associated with Treg-cell phenotype and function, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR) are the most prominent molecules.25-28 Additionally, IL-10 and TGF-β, although rarely expressed in vitro, might have functional importance for Treg cells in vivo, particularly in the context of disease.29,30 Major topics of current research are the characterization of Treg-cell defects in autoimmune diseases and their role in infectious diseases and transplantation tolerance, particularly after allogeneic bone marrow transplantation.5,6,31-34 Although research in Treg-cell biology is intensifying, it is still unclear whether Treg cells in human tumors are primed mainly in the thymus or emerge in the periphery due to antigen-specific stimulation. The lack of more specific cell-surface markers is a major reason that many functionally relevant aspects of Treg cells are still unknown. In this review, we focus mainly on naturally occurring CD4+CD25+FOXP3+ Treg cells with particular emphasis on novel aspects in malignant disease as well as potentials for improving antitumor immunity by targeting Treg cells.

Increase of Treg cells in cancer-bearing mice

Treg cells protect the host from autoimmune disease by suppressing self-reactive cells. As such, Treg cells may also block antitumor immune responses. Particularly in the context of cancer, Treg-cell frequencies and function are important because...
increased numbers might favor tumor development or growth and influence the course of the disease. Currently, a number of important questions are under intense investigation. Is the increase of T\textsuperscript{reg}-cell frequencies an early event at the onset of disease or more likely a response of the immune system during tumor progression? Do organ-specific and more importantly, do tumor-specific T\textsuperscript{reg} cells, play a role? How does therapy influence T\textsuperscript{reg}-cell numbers, particularly in already established tumors? Is there a possibility of long-term depletion of T\textsuperscript{reg} cells and is this connected to induction of autointoimmunity?

The development of T\textsuperscript{reg} cells during tumor progression has been addressed in a fibrosarcoma model in C57BL/6N mice as well as in a colon adenocarcinoma model in BALB/c mice. Transfer of unfractionated tumor-draining lymph node (LN) cells isolated on day 9 after tumor challenge achieved complete rejection of established tumors, whereas even 4-fold higher numbers of cells harvested on day 12 seldom prevented lethal tumor progression. This treatment failure was due to cotransfer of tumor-induced T\textsuperscript{reg} cells, indicating that during this relatively short time span of tumor development the induction of a highly suppressive T\textsuperscript{reg}-cell population occurred.\textsuperscript{35} Relatively early induction of T\textsuperscript{reg} cells during tumor development has significant impact in human disease as the time point of T\textsuperscript{reg}-cell induction in cancer patients would certainly precede the time of diagnosis in the majority of patients.

The suppressive effect of naturally occurring T\textsuperscript{reg} cells against tumor-specific CD8\textsuperscript{+} T cells was established in a poorly immunogenic B16 melanoma model. T\textsuperscript{reg} cells efficiently suppressed cytotoxic T lymphocyte (CTL)–mediated concomitant immunity against a rechallenge with the same tumor, demonstrating that precursor T\textsuperscript{reg} cells in naive hosts gave rise to effective suppressors during tumor development. These findings clearly suggest that T\textsuperscript{reg} cells are major regulators of concomitant tumor immunity.\textsuperscript{36} Further evidence for the interference of T\textsuperscript{reg} cells with CD8\textsuperscript{+} T cell-mediated antitumor immune responses in vivo was established in a transgenic murine colon carcinoma model where T\textsuperscript{reg} cells abrogated CD8\textsuperscript{+} T cell–mediated tumor rejection by specifically suppressing cytotoxicity of CTLs.\textsuperscript{37}

Selective accumulation of T\textsuperscript{reg} cells in the tumor environment was studied in a murine fibrosarcoma model where the majority of tumor-infiltrating lymphocytes (TILs) at late stage of tumor progression were T\textsuperscript{reg} cells. Their depletion during the effector rather than priming phase successfully enhanced antitumor immunity. Blockade of IL-10 and TGF-\beta partially reversed the suppression imposed by CD4\textsuperscript{+} T cells. Furthermore, local depletion of CD4\textsuperscript{+} T cells inside the tumor led to eradication of well-established tumors and development of long-term antitumor memory. This study suggested that suppression of antitumor immunity by T\textsuperscript{reg} cells occurs predominantly at the tumor site and that local reversal of suppression, even late during tumor development, can be an effective treatment.\textsuperscript{38} This has been confirmed in a murine pancreatic cancer model, suggesting that the tumor actively promotes the accrual of T\textsuperscript{reg} cells through several mechanisms involving activation of naturally occurring T\textsuperscript{reg} cells as well as conversion of non-T\textsuperscript{reg} cells into T\textsuperscript{reg} cells.\textsuperscript{39}

Analysis of tumor-draining LNs demonstrated that both antitumor effector T cells and FOXP3\textsuperscript{+} T\textsuperscript{reg} cells are primed in the same LNs during tumor progression. These tumor antigen-specific T\textsuperscript{reg} cells possessed the same functional properties as T\textsuperscript{reg} cells that arise naturally in the thymus.\textsuperscript{40} Whether there is a systemic increase of T\textsuperscript{reg} cells in cancer-bearing mice is not yet clearly defined because conflicting data have been reported. In a colon carcinoma model, an expansion of T\textsuperscript{reg} cells was observed only in the spleen but not in peripheral blood (PB).\textsuperscript{41} In contrast, in a murine transgenic model of prostate dysplasia, increased frequencies of T\textsuperscript{reg} cells and enhanced production of inhibitory cytokines were observed in PB, resulting in impaired T-cell function, which also correlated with tumor progression.\textsuperscript{42}

Although these experiments focused on accumulation and function of T\textsuperscript{reg} cells, the trafficking behavior of T\textsuperscript{reg} cells and their cellular interactions and localization to and within the tumor microenvironment and in tumor-draining LNs were not studied in these models. Immunochemistry revealed FOXP3\textsuperscript{+} T\textsuperscript{reg} cells in close proximity to CD11c\textsuperscript{+} DCs, FOXP3\textsuperscript{+} CD4\textsuperscript{+} T cells, and CD8\textsuperscript{+} T cells in the T-cell regions of lymphoid tissues in normal and tumor-bearing mice.\textsuperscript{43} Further insights into the signals involved in T\textsuperscript{reg}-cell attraction to tumor sites came from studies in a lung cancer model. Tumor-derived prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) resulted in an increase of T\textsuperscript{reg}-cell activity and Foxp3 expression (Figure 1). Assessment of E-prostanoid (EP) receptor requirements revealed that absence of EP4 receptor led to reduced induction of Foxp3, whereas absence of EP2 ablated expression. In vivo, COX2 inhibition reduced T\textsuperscript{reg}-cell frequency and activity, attenuated Foxp3 expression, and decreased tumor burden. Transfer of T\textsuperscript{reg} cells or administration of PGE\textsubscript{2} to mice receiving COX2 inhibitors reversed these effects, indicating that COX2 inhibition suppressed T\textsuperscript{reg}-cell activity and might be useful to enhance antitumor responses.\textsuperscript{44} Interestingly, PGE\textsubscript{2} also enhanced the in vitro inhibitory function of human T\textsuperscript{reg} cells and induced a regulatory phenotype in CD4\textsuperscript{+}CD25\textsuperscript{+} T cells. Furthermore, PGE\textsubscript{2} exposure induced FOXP3 expression in CD4\textsuperscript{+}CD25\textsuperscript{+} T cells and up-regulated its expression in T\textsuperscript{reg} cells. Similarly, incubation with supernatants from COX2-overexpressing lung cancer cells secreting PGE\textsubscript{2} significantly induced FOXP3, indicating that PGE\textsubscript{2} can indeed modulate FOXP3 expression and T\textsuperscript{reg} function.\textsuperscript{45}

\textbf{T\textsuperscript{reg}-cell depletion leads to restoration of antitumor immunity}

Even before the identification of CD4\textsuperscript{+}CD25\textsuperscript{+} T\textsuperscript{reg} cells, early data indicated that nonspecific depletion of CD4\textsuperscript{+} T cells can lead to the...
induction of efficient antitumor immunity (Figure 2). More specifically targeting Treg cells by administration of CD25 monoclona-
nal antibody (mAb) abrogated immunologic unresponsiveness to
tumors and induced spontaneous development of tumor-specific
CD8+ effector T cells and NK cells. Interestingly, depletion of
Treg cells led to cross-reactive tumor immunity against tumors of
diverse origins. Timing of Treg-cell elimination also seems to be
an important aspect. Administration of CD25 mAb later than 2 days
after inoculation of myeloma cells caused no tumor regression,
irrespective of Treg-cell depletion. As already outlined, this might
due to the induction of antitumor tolerance at a relatively early
time point of tumor development, resulting in inefficient activation
of effector cells. Furthermore, the number of Treg cells after CD25
depletion is restored over time and the capacity to mount an
antitumor response progressively diminishes.

Depletion of Treg cells together with other immunostimulatory
approaches, for example, CTLA-4 blockade, has also been tested.
Combination of Treg-cell depletion and CTLA-4 blockade was
synergistic and resulted in maximum tumor rejection. The observed
synergism indicates that both pathways represent 2 alternatives for
suppression of autoreactive T cells so that simultaneous interven-
tion might be a promising concept for the induction of therapeutic
antitumor immunity. Because immune responses to malignant
tumors often are weak and ineffective, solely depleting Treg cells
might not always result in tumor regression. Approaches combing-
ing Treg-cell depletion with other immunologic interventions, for
example, transfer of activated T cells or DC-based vaccinations,
therefore might be more beneficial.

Reduction of Treg cells is associated with
the immunostimulatory effect of
cyclophosphamide

It has long been recognized that cyclophosphamide exerts an
immunostimulatory effect. Early data indicated that cyclophospha-
mide preferentially destroys CD4+ suppressor T cells causing
immunologically mediated regression of immunogenic lymphomas
in mice. In a rat colon cancer model, administration of cyclophos-
phamide depleted Treg cells and delayed the outgrowth of tumors.

Combining cyclophosphamide and immunotherapy even cured the
mice, whereas both strategies applied alone had no curative
effect. Low-dose cyclophosphamide not only decreases num-
bers of Treg cells but also leads to decreased function, enhanced
apoptosis, and decreased homeostatic proliferation. This suggests
that cyclophosphamide might be successfully integrated into
chemoimmunotherapy, as recently shown by Dudley et al. The
combination of adoptive transfer of ex vivo activated tumor-
specific T cells to patients with lymphopenic melanoma after
chemotherapy with cyclophosphamide and fludarabine induced
tumor regression in up to 50% of patients treated.

Collateral damage needs to be accounted for
after Treg-cell depletion

Because Treg cells are an important cellular mechanism suppressing
autoantigen-specific conventional T cells from attacking self tis-

ue-tumor cells are not known. In a series of elegant experiments, Nishikawa et al

demonstrated that immunization with tumor-associated self-

antigens and tumor-specific CTL epitopes heightened CD8+ T-cell

responses and increased resistance to tumor challenge in a CD4+ T
cell-dependent manner. In contrast, immunization with self-

antigens alone increased the susceptibility to tumor challenge,

leading to the development of highly active Treg cells with

enhanced expression of Foxp3. Induction of Treg cells was also

associated with acceleration of tumor development, which was

abolished by depletion of CD4+ T cells or CD25+ T cells. Acceleration of tumorigenesis

was not only observed in self-

antigen vaccinated mice but could also be adoptively transferred

with Treg cells derived from immunized mice.

Broadly expressed self-antigens are
recognized by tumor-associated Treg cells

For most current tumor models the antigens recognized by Treg cells

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Human Treg cells in cancer: current
knowledge and open questions

Even in the early 1990s, T cells with regulatory function were
reported in patients with cancer; however, these reports were not
followed up until the identification of CD4+CD25+ Treg cells in the mid-1990s. Since then, an increase of Treg cells in cancer patients has been reported by numerous investigators. In contrast to the murine system, definition of human Treg cells has been more difficult, and assessment of the most specific marker—namely, FOXP3—has not been performed in many of the early studies. Although human CD4+CD25high T cells are most enriched for FOXP3+ T cells, there are still significant numbers of FOXP3+ cells within the CD4+CD25low T-cell population. In the absence of more specific cell-surface markers, it is not yet possible to study human FOXP3+ Treg cells irrespective of their CD25 expression. These limitations also explain why Treg cells in humans currently need to be characterized by a combination of FOXP3 and CD25 expression as well as analysis of inhibitory function of T-cell populations enriched for FOXP3+ cells, mainly by sorting CD25high T cells.

Comparability of previous reports is further challenged by use of different antibodies to detect CD25 or different gating strategies when assessing CD25+CD25high cells. Similarly, function of Treg cells has been assessed with numerous in vitro approaches, making it rather difficult to compare results of different studies. To reconcile our recent findings about Treg cells, it is most important to identify specific cell-surface markers for Treg cells that allow us to isolate these cells and functionally test them in the context of malignant disease.

**Increased frequencies of Treg cells in solid cancers**

Woo et al were the first to report increased percentages of CD4+CD25+ Treg cells in TILs in non–small-cell lung cancer and ovarian cancer. These Treg cells were shown to secret TGF-β, providing first evidence that Treg cells contribute to immune dysfunction in patients with cancer. Further characterization of these cells showed constitutive high-level expression of CTLA-4. More importantly, Treg cells mediated potent inhibition of T-cell proliferation. Supporting this initial report, a larger study concluded that prevalence of CD4+CD25+ Treg cells is increased not only in the tumor microenvironment of patients with invasive breast or pancreas cancers but also in PB, suggesting that the increase of Treg cells is a generalized phenomenon.

In malignant melanoma, an increase of functional CD4+CD25+ Treg cells was observed, which was further linked to increases in the serum level of H-ferritin. Release of H-ferritin by melanoma cells led to activation of IL-10–producing functional Treg cells as a potential mechanism of Treg-cell induction in cancer patients. A more recent study demonstrated FOXP3 mRNA expression in the increased subset of CD4+CD25high Treg cells in patients with melanoma, confirming the previous reports.

Patients with gastrointestinal malignancies, the relative increase of Treg cells might actually be explained by a significant reduction of CD4+CD25+ T cells. Interestingly, in patients with gastric carcinoma, poor prognosis and decreased survival rates were closely correlated with higher Treg-cell frequencies. After curative resections, previously elevated Treg cells numbers were significantly reduced. In contrast, prevalence of Treg cells increased again in patients having a relapse after tumor resection. These findings underline the close correlation of tumor growth and Treg-cell frequencies.

Curiel et al demonstrated that CD4+CD25+FOXP3+ Treg cells suppress tumor-specific T-cell immunity in ovarian cancer, contribute to tumor growth, and accumulate during progression. Furthermore, increased frequencies of Treg cells were associated with a high death hazard ratio and reduced survival. Treg cells preferentially moved to and accumulated in tumors and ascites, but rarely entered draining LNs in later cancer stages. Tumor cells and surrounding macrophages produced the chemokine CCL22, which mediated trafficking of Treg cells to the tumor via CCR4 (Figure 1). This specific recruitment of Treg cells might represent a mechanism by which tumors may foster immune privilege.

For patients with squamous-cell carcinoma of the head and neck, a significantly elevated frequency of FOXP3+GITR+ Treg cells was shown. These Treg cells were significantly more sensitive to apoptosis than non-Treg cells, which might hint at a rapid turnover in the peripheral circulation. How the higher sensitivity to apoptosis influences Treg-cell frequencies, however, has not been addressed yet.

Increased numbers of Treg cells have also been reported in PB and TILs of patients with hematologic malignancies. Although the increase of Treg cells seems to be a common theme in solid tumors, there are clear but yet unexplained differences between individual tumor entities. In a comparative study, differences in Treg-cell frequencies were shown for malignant pleural effusions from patients with mesothelioma compared with carcinomatous pleural effusions from patients with non–small-cell lung cancer or breast cancer.

Overall, previous work has clearly established that Treg cells are increased in most human solid tumors. Furthermore, there seems to be a stage-dependent increase of Treg cells with frequencies of Treg cells probably correlated to overall survival. However, little is known about the mechanisms leading to this increase. A first study by Wolf et al might help us to understand the underlying molecular mechanisms. This study showed that increased frequencies of Treg cells in PB of cancer patients are due to active proliferation rather than redistribution from other compartments (ie, secondary lymphoid organs or bone marrow). This finding, in combination with the proposed attraction of Treg cells to the tumor via CCL22/CCR4 and induction of Treg cells by PGE2 or H-ferritin, might be one possible mechanism responsible for expansion of Treg cells in cancer patients (Figure 1).

**Treg cells in hematologic malignancies**

Whereas the question of Treg cells in solid tumors sparked interest relatively early, studies addressing Treg cells in hematologic malignancies have been conducted only recently.

The first study by Marshall et al demonstrated large populations of both IL-10–secreting Tr1 and CD4+CD25+ Treg cells in Hodgkin lymphoma (HL) infiltrating lymphocytes and peripheral-blood mononuclear cells (PBMCs). Suppressive function was mediated by IL-10 secretion, cell-to-cell contact, and CTLA-4 expression. The difficulty of applying FOXP3 as a Treg cell–specific marker in human diseases is exemplified by a second study in HL. The frequency of FOXP3+ cells was determined in lymphoma-affected LNs. Low frequencies of FOXP3+ cells and high frequencies of CTLs in the reactive background of LNs were correlated with poor overall survival. However, costaining for FOXP3 and CD4 or CD8 was not performed, limiting the significance of this finding because FOXP3 expression in humans might not be confined to Treg cells only. Alternatively, Treg cells might actually play a beneficial role in HL, which is characterized by a chronic inflammatory response, similar to Helicobacter-associated lymphoma.

In patients with B-cell chronic lymphocytic leukemia (CLL) we recently established a stage-dependent increase of CD4+CD25highFOXP3+CTLA4+GITR+ Treg cells with full suppressive capacity. However, when patients with CLL were treated with fludarabine, frequencies of Treg cells decreased and
Treg cells showed impaired function. Ongoing studies are addressing the question of how fludarabine mediated this effect.83 The increase of CTLA-4+ Treg cells in untreated CLL patients, which correlated with advanced disease stage and unfavorable cytogenetics, was recently confirmed by others.84 Similarly, in patients with B-cell non-Hodgkin lymphomas (B-NHLs) increased frequencies of FOXP3+CTLA4+ Treg cells have been observed. PD1 expression was partly responsible for the suppressive activity of these LN-infiltrating Treg cells. Furthermore, as reported for ovarian cancer, the tumor cells released CCL22 and thereby attracted CCR4+ Treg cells into the area of the lymphoma (Figure 1).85 For patients with acute myeloid leukemia (AML) higher frequencies of CD4+CD25high Treg have been observed. Similar to observations in solid tumors, Treg cells of patients with AML were less resistant to apoptosis but showed higher proliferation compared with healthy individuals.86

Comparable to other hematologic malignancies, we were also able to demonstrate increased frequencies of CD4+CD25highFOXP3+ Treg cells in patients with monoclonal gammopathy of undetermined significance (MGUS) or multiple myeloma (MM).24 Independent of prior therapy or stage of disease Treg cells exhibited a strong inhibitory capacity. Moreover, the increase of Treg cells was also dependent on stage and resulted from peripheral expansion. We also established for the first time that naive CD4+CD25highFOXP3+ Treg cells coexpressing CD45RA and CCR7 are expanded in MM patients, further supporting the concept of peripheral expansion of this T-cell compartment. The importance of identifying more specific markers as well as more standardized functional assays is supported by a recent report on FOXP3+ cells in PB from patients with MM.87 Due to an alternative experimental approach only assessing FOXP3 in context of CD4+ T cells but not CD25+ cells, these data are difficult to compare with other studies on naturally occurring CD4+CD25high Treg cells. Although this report came to the conclusion that Treg cells are dysfunctional in MM patients, the assays chosen to assess Treg-cell function allowed for alternative explanations of the observed results including already described defects in conventional autologous T cells in MM patients.88

Taken together, these data established the concept of increased Treg cells in solid tumors as well as hematologic malignancies. However, some of the early studies need to be validated by using more specific markers such as FOXP3 as well as more sophisticated and standardized functional assays.

Specificity of Treg cells in human tumors

So far, little is known about the antigen-specificity of human Treg cells. Wang et al reported the identification of LAGE1-specific CD4+CD25+GITR+ fully functional Treg-cell clones in cancer patients.89 Ligand-specific activation and cell-to-cell contact were required for Treg cells to exert suppressive activity, suggesting that the presence of tumor-specific Treg cells at tumor sites may have profound effects on the inhibition of T-cell responses against cancer. In a second study, a CD4+CD25+ Treg-cell line was established from a patient with colorectal carcinoma. This T-cell line was tumor-cell dependent in its growth but did not lyse autologous tumor cells and suppressed proliferative responses of allogeneic lymphocytes and autologous CTLs as well as the induction of CTLs from autologous PBMCs. These effects were mediated by TGF-β and did not require cell-to-cell contact, which would be in line with induced regulatory capacity.90 Clearly, further work is needed to understand how the enrichment of Treg cells in cancer patients occurs and if accumulation or preferential induction of clonal, oligoclonal, or polyclonal tumor-specific Treg cells plays a role during tumor progression.

The question of whether induction of Treg cells might be inversely correlated with the induction of tumor-antigen specific immunity during cancer development in vivo was addressed by Nishikawa et al.91 Using NY-ESO-1 as a model, the authors demonstrated that the in vitro generation of NY-ESO1-specific TH1 cells in NY-ESO-1–seropositive cancer patients was independent of Treg-cell depletion, indicating that tumor-antigen-associated Treg cells were not enriched in patients naturally mounting an immune response against this antigen. In contrast, Treg-cell depletion in NY-ESO1–seronegative patients was always required for induction of NY-ESO-1–specific TH1 cells, which suggests the existence of Treg cells specifically suppressing the expansion of tumor-antigen–specific T cells. Moreover, NY-ESO-1–specific TH1 cells were derived from naive precursors in seronegative patients, whereas preexisting memory populations were detectable exclusively in NY-ESO-1–seropositive patients. The memory populations were also less sensitive than naive populations toward Treg-cell–mediated suppression. These results strongly support the hypothesis that tumor-specific Treg cells exist in patients with tumors and actively suppress antigen-specific antitumor immunity.

Influence of treatment on Treg-cell frequency and function

As already outlined, a correlation of increased Treg cells with greater disease burden and poorer overall survival has been reported. In CLL, we have observed reduced frequencies of functionally impaired Treg cells after fludarabine treatment; however, not every chemotherapeutic agent seems to induce this effect, because in CLL or MM no other treatment including autologous stem cell transplantation induced similar effects. In line with this observation, frequency and suppressive function of Treg cells in tumor-draining LNs derived from patients with cervical cancer were not influenced by chemotherapy or combined chemoradiation.92

Recent work has demonstrated that IL-2 signaling is required for thymic development, peripheral expansion, and suppressive activity of Treg cells.93 During immune reconstitution after chemotherapy, IL-2 therapy led to a homeostatic peripheral expansion of Treg cells and to a markedly increased Treg-cell compartment. IL-2 therapy induced expansion of existent Treg cells in healthy hosts and this expansion was further augmented by lymphopenia. Treg cells generated by IL-2 therapy expressed FOXP3 at levels observed in healthy individuals and these Treg cells also were of similar potency, suggesting that IL-2 and lymphopenia are modulators of Treg-cell homeostasis.94 Similarly, in patients with melanoma or renal-cell carcinoma (RCC), the frequency of fully functional Treg cells was significantly increased after IL-2 treatment, which was also accompanied by an increase of FOXP3, demonstrating that administration of high-dose IL-2 increases the frequency of circulating FOXP3+ Treg cells.95 This might also explain why therapy with IL-2 in patients with RCC has not yet fully lived up to expectations because significant induction of Treg cells might counteract potential antitumor effects of IL-2.

Surprisingly, vaccination of melanoma patients with DCs either loaded with synthetic peptides or tumor lysates was also shown to induce increased frequencies of Treg cells, concomitant with the expansion of tumor-specific CTLs. Whether this enhances antitumor tolerance and negatively influences the induction of clinically efficient antitumor immune responses needs further attention, because the mechanisms of this phenomenon are not yet understood.96
First clinical studies toward selective elimination of T<sub>reg</sub> cells

Murine models have established that selective elimination of T<sub>reg</sub> cells alone or in combination with other treatment options might induce regression of already established tumors. First pilot studies have been initiated in cancer patients to selectively eliminate T<sub>reg</sub> cells. A promising and specific approach might be targetting of CD25 on the surface of T<sub>reg</sub> cells. Danull et al used IL-2 diphtheria toxin conjugate DAB(389)IL-2 (denileukin diftitox) to selectively eliminate CD25-expressing T<sub>reg</sub> cells from the PBMCs of cancer patients without inducing toxicity on other cells that expressed CD25 at only intermediate to low levels.97 DAB(389)IL-2 significantly reduced the number of T<sub>reg</sub> cells present in the PB of patients with metastatic RCC and abrogated T<sub>reg</sub> cell-mediated immunosuppressive activity in vivo. Moreover, elimination of T<sub>reg</sub> cells followed by vaccination with RNA-transfected DCs significantly improved stimulation of tumor-specific T-cell responses when compared with vaccination alone.

In summary, this first clinical study specifically eliminating T<sub>reg</sub> cells has shown promising results that need to be further evaluated. An important aspect of future studies will be to clearly describe the therapeutic window of deleting T<sub>reg</sub> cells as a major gatekeeper of self-antigen recognition.

**Future directions**

In patients with tumors, characterization of T<sub>reg</sub> cells has focused mainly on coexpression of CD4 and CD25, whereas differentiation status, frequencies of T<sub>reg</sub>-cell subtypes, eg, natural or induced T<sub>reg</sub> cells, Tr1, or TH3 cells are less well characterized. To better understand and study T<sub>reg</sub>-cell biology in relation to tumor development and progression, it will be most critical to identify more specific cell-surface markers. Assessment of FOXP3, lacking in many of the early studies, is certainly a minimum requirement for future studies; however, it does not allow for subsequent functional testing of FOXP3<sup>+</sup> cells.

One of the most burning questions concerning T<sub>reg</sub> cells in cancer remains the specificity of these cells. Although tumor specificity was demonstrated for single T<sub>reg</sub>-cell clones, so far our understanding is still limited. The lack of specific cell-surface markers allowing us to follow such cells in vivo is again the major hurdle.

The recent finding demonstrating recruitment of CCR4-expressing naive T<sub>reg</sub> cells by tumor cells secreting CCL22 was intriguing. Similarly, the role of PGE<sub>2</sub> expressed by tumor cells and tumor-associated macrophages on development of T<sub>reg</sub> cells demonstrated that there is a rather coordinated cross-talk between tumor environment and T<sub>reg</sub> cells. It is very likely that additional signals and interactions with other cells, such as tolerogenic DCs, are involved in recruitment of T<sub>reg</sub> cells to the tumor site. Whether this is a uniform process in all cancer types or whether organ-specific mechanisms might also play a role is only one of the many still unanswered questions of T<sub>reg</sub>-cell biology in human cancer.

**References**

28. Chen W. Dendritic cells and CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells: crosstalk between two professional cells in immunity versus tolerance. Front Biosci. 2006;11:1360-1370.


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