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Regulatory T cells and treatment of cancer

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Summary of recent advances

CD4⁺CD25⁺Foxp3⁺ regulatory T cells are elevated in cancers and can thwart protective anti-tumor immunity. Recent human cancer trials suggest that depleting regulatory T cells can be clinically beneficial. Additional types of deleterious regulatory cells are also increased in cancer. Regulatory T cells also play unanticipated roles in cancer therapy in that some drugs unexpectedly increase (cancer vaccines or IL-2 treatment, for example) or decrease (anti-neoangiogenesis agents or receptor tyrosine kinase inhibitors, for example) their numbers or function. Managing deleterious effects of regulatory cells represents a novel and potentially effective way to effect immunotherapy for cancer. New insights into molecular mechanisms governing trafficking, differentiation and function of these cells suggest novel approaches to manipulating them as treatment strategies.

Introduction

This commentary examines the enormous increases in knowledge regarding dysfunctional regulatory cells in cancer, primarily citing work appearing in the past three years. Older works are acknowledged in existing comprehensive reviews. This commentary focuses on CD4⁺CD25⁺FOXP3⁺ regulatory T cells (Tregs). It is not meant to be an exhaustive review of tumor Tregs, which are treated in excellent reviews such as [1]. Tregs inhibit otherwise protective anti-tumor immunity and predict reduced survival in cancer [1]. Important developments in the understanding of Treg differentiation and accumulation in tumors will be discussed with respect to using such knowledge therapeutically.

Current cancer immunotherapies capitalize on infectious disease concepts that might not apply to malignancies [2]. Recent work demonstrates that reversing deleterious immune dysfunction makes active interventions such as vaccines more effective in cancer [3]. Much experimental, and emerging clinical data suggest that managing deleterious Treg effects are important components of these novel anti-cancer immune strategies [4].

Regulatory cells other than CD4⁺CD25⁺FOXP3⁺ Tregs have also been recently described in cancer [1–4]. Their relative contributions to immune dysfunction, and the clinical consequences of interfering with their function remain to be defined, but offer many additional approaches to treatment.

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Main text of review

Background

Malignant tumors pose special challenges to immunity. On one hand, their abnormalities should target them for immune destruction. On the other hand, they are self. Thus, protective mechanisms against autoimmunity could impede anti-tumor immunity.

Peripheral self-tolerance is mediated by CD4+CD25+ Tregs. However, activated effector T cells express the identical CD4+CD25+ phenotype, and thus Tregs cannot be distinguished by surface phenotype alone. FOXP3 was recently identified as essential to Treg differentiation, offering a relatively selective Treg marker. Nonetheless, confirming Treg identity with a functional assay is essential because not all FOXP3+ cells are functional Tregs [5].

Tumor Tregs are highly heterogeneous populations that arise through disparate pathways and mediate immunologic effects through various means including soluble factors and contact-dependent mechanisms [1,6,7]. They are elevated in peripheral blood and tumors of cancer patients. Three principal mechanisms explaining their increase include: i) tumor-specific factors (enhanced *de novo* differentiation, proliferation, or recruitment; or reduced death), ii) reaction to autoimmunity, and iii) control of inflammation. The following paragraphs expand on current information regarding these three principal mechanisms, although relative contributions from each in specific tumors remains poorly studied.

Natural Tregs mediate peripheral self tolerance, whereas induced Tregs dampen inflammation. Natural Tregs could thus increase in tumor to impede anti-tumor immunity sensed as anti-self (as surveillance against autoimmunity is their primary function), and induced Tregs could increase to dampen ongoing inflammation. Both natural and induced Tregs contribute to tumor tolerance [8] although contributions of each to tumor-related immune dysfunction remain little understood.

Tumor Treg properties

Tumor Tregs are phenotypically indistinguishable from other Tregs. Nonetheless, they likely possess specific characteristics bestowed by the tumor microenvironment. Npr-1 expression identifies a functional Treg population in mice. A similar population was recently described in human cervical cancer [9]. Lack of CD127 expression predicts functional Tregs in normal humans [10], but is relatively unstudied in tumor Tregs. CD4+CD25+FOXP3+ Tregs in blood of patients with prostate cancer are more suppressive than those in blood from normals, even if total numbers do not differ [11].

Tumor-derived factors

Recent work demonstrates that tumor cells secrete many factors that induce Tregs, either by direct action on T cells, or indirectly through effects on accessory cells, especially dendritic cells, or produce factors that attract Tregs locally [1]. *In vitro* experiments suggest several soluble or contact-dependent tumor factors contributing to Treg generation including cyclooxygenase-2 [12], CD70 [13], Gal1 [14] TGF- β [15,16], indoleamine 2,3-dioxygenase [17] and yet-to-be-identified factors [18,19]. Enhanced local Treg proliferation or reduced apoptosis are additional mechanisms that could augment tumor Treg numbers.

Tumor microenvironmental factors

Cells rendered dysfunctional in the tumor microenvironment can induce or attract Tregs or enhance their function. For example, plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate Tregs via indoleamine 2,3-dioxygenase. The B7-H1/

PD-1 suppressive mechanism of these Tregs differs from Tregs activated without indoleamine 2,3-dioxygenase [20]. Tregs induce macrophages to express inhibitory B7-H4 and secrete IL-6 and IL-10, demonstrating dysfunctional immune cell cross-talk [21]. Impeding tumor-specific factors boosting Treg numbers or function is a potential means to reverse immune dysfunction in cancer. Specific strategies have been discussed [4].

Tumor Treg content and prognosis

Many studies report that increased tumor Tregs predict reduced survival or treatment response. Some studies show no predictive value, such as the recent demonstration that tumor-infiltrating FOXP3+CD4+CD25+ T cells did not predict survival in renal cell carcinoma [22]. Several studies, particularly in hematologic malignancies, suggest that increased Tregs portend a favorable prognosis, as in a recent study of specific lymphomas [23]. These conflicting results likely relate to issues discussed below.

Proving Treg function is difficult, especially in human tissues where sample size is limiting. Many (but not all) prognostic studies rely on FOXP3 expression to identify Tregs without a confirmatory functional test. However, not all FOXP3+ cells are functional Tregs [5]. Further, patient populations and potential confounders of response or survival data were not always identified. Nonetheless, a mouse model for melanoma demonstrated a beneficial role for Tregs. The authors speculated that CD4+CD25+Foxp3+ Tregs blocked complete tumor eradication by maintaining tumor(antigen) that stimulated anti-tumor immunity [24].

Treg prognostic potential may depend not only on numbers or function, but also on relative abundance, specific anatomic compartmentalization and even distribution within a compartment. For example, the localization pattern but not total numbers of FOXP3+ cells predicted survival in stomach cancer [25]. The FOXP3+/granzyme B+ cell ratio predicted prognosis in Hodgkin lymphoma [26].

Tregs in cancer prevention

Most work focuses on the role of Tregs in existing cancers. However, Tregs may also modulate immunoediting and premalignant progression. For example, Tregs prevented colorectal cancer in a mouse model for chronic microbial inflammation by dampening inflammation [27]. Resident Tregs inhibited tumor immune surveillance in a mouse model for carcinogen-induced sarcoma [28]. Ultraviolet radiation induces skin Tregs that could contribute to skin cancer [29]. In cervical cancer, Tregs may foster malignant progression from non-malignant cells, also suggesting that Tregs in viral-associated cancers might play unique roles [30]. Managing Tregs in cancer prevention remains a little explored area worthy of more attention.

Therapeutic interventions induce Tregs

Vaccines—It is well-known that vaccines induce antigen-specific effector cells. Tregs are also antigen-specific cells, but until recently their possible generation in active immunization was unstudied. We now know that vaccines induce tumor-specific Tregs in mice [31]. A cervical cancer vaccine induced CD4+CD25+FOXP3+ cells in humans [32], but function and specificity were not assessed. Means to generate antigen-specific effector cells while limiting generation of antigen-specific Tregs is critical to optimal immunotherapy strategies. Several groups are now testing such strategies, including CD40 agonists plus toll-like receptor activation [33], a liposomal vaccine [34], CD137 sFv-expressing tumor cells [35], and specific dendritic cells [36].

Therapeutic cytokines—In a mouse sarcoma model, GM-CSF and Flt3-L induced dysfunctional Foxp3+ cells [37]. Tregs reduced benefits of IL-12 gene therapy in a mouse

model for hepatocellular carcinoma [38]. Systemic IL-2 alters Treg trafficking molecules and may enhance their accumulation in ovarian malignancies in humans [39].

Other approaches—Histone deacetylase inhibitors augment Treg suppressive functions [40], which effect might be ameliorated with IL-2 [41]. Retinoids, including all-trans retinoic acid induce gut-homing Tregs [42]. Numerous additional unexpected effects on Tregs of drugs used for various indications have been discussed [4]. It will be important to reassess the mechanism of action of many anti-cancer agents, including tumor vaccines, anti-angiogenesis agents and tyrosine kinase inhibitors, in addition to those mentioned here in light of their potential to modulate Treg activity. Such studies may provide insights into why certain drugs (such as IL-2) work or fail in specific patients, or provide clues as to how to alter use for better effect.

Strategies to manage Tregs

Reducing Treg function is a logical therapeutic strategy given the large body of evidence suggesting that elevated Tregs are detrimental in cancer. Based on current data regarding how Tregs arise and function in cancer [1], and as many tumor immune dysfunctional pathways lead to Treg induction [2], I suggested several principal means to thwart Treg function: depletion; blocking differentiation, trafficking or effector functions; or raising effector cell suppression threshold [4]. Although *depleting* Tregs is most studied, it is more useful to consider *managing* Tregs, as strategies aside from elimination are also effective, and depletion might foster rapid Treg regeneration [43,44]. The following represent newer concepts in managing Tregs to treat cancer.

Non-specific Treg depletion—Anti-CD25 antibody remains the prototypical reagent for depleting Tregs in mouse models, although denileukin diftotox (Ontak, a protein fusing IL-2 and diphtheria toxin), is also effective [45]. Cyclophosphamide depletes Tregs and boosts efficacy of a dendritic cell vaccine in mouse models for melanoma or colon carcinoma [46]. Vaccination against Foxp3 improved tumor immunity in a model for renal cell carcinoma [47]. Denileukin diftotox [48] or LMB-2, a CD25-directed *Pseudomonas* immunotoxin [49], reduces Tregs in cancer patients. *In vivo* transfer of autologous CD25-depleted mononuclear cells plus high-dose IL-2 did not mediate prolonged Treg reduction [50]. CpG treatment lowers FOXP3+ T cells in lymph nodes of melanoma patients [51].

Antigen-specific Treg elimination—Targeting antigen-specific Tregs might prevent pathologic autoimmunity. Tumor antigen-specific Tregs occur naturally [52,53] and are induced by vaccination [31]. As yet, there are no known means to deplete these specifically, although folate receptor 4-expressing tumor Tregs may be enriched for antigen-specific cells, and their depletion in tumor-bearing mice improved immune-mediated tumor rejection [54].

Raising effector cell threshold—Whether anti-CTLA-4 antibody depletes Tregs, or works through augmenting effector cell suppression threshold remains controversial. In a recent human trial, anti-CTLA-4 antibody reduced phenotypic Tregs in blood, but CD8+ cytotoxic T cells did not increase, and phenotypic Tregs rebounded quickly, although their function was not specifically determined [55]. Tumor infiltrating T cells activated by dendritic cells in which the zinc finger protein A20 was genetically silenced were resistant to Treg-mediated suppression [56].

Block Treg trafficking—It took years to realize that clinical activity of adoptively transfused effector T cells was compromised, among other reasons, because they did not traffic well to tumor, an insight appreciated in Treg studies. IL-2 alters Treg trafficking, and

expands Treg numbers [39,57] although *in vitro* functionality may be reduced [57]. Preventing Treg ingress into areas where anti-tumor immunity is primed may be beneficial [58].

Block Treg effector functions—Tumor Tregs work through myriad mechanisms [1], which can be countered by previously discussed means [4]. *Escherichia coli* expressing Listeriolysin-O induces specific cytotoxic T lymphocytes, but Tregs become nonfunctional [59]. Inhibiting STAT3 reduces Treg suppressive function [60]. Triggering OX40 on tumor Tregs abrogates their suppressive function and augments tumor rejection [44]. Sendai virus in CT26 colon cancer inhibited Treg function in mice, possibly through induced IL-6 [61]. Blocking effector cell IL-10 receptor or other strategies might also be useful.

Block Treg differentiation—FOXP3 mediates Treg differentiation through increasingly understood mechanisms that could be exploited therapeutically [62,63]. Alternatively, agents inducing Treg differentiation, such as IL-2, could be avoided.

Subvert Treg differentiation—Many tumor-associated Tregs are tumor antigen-specific. If their differentiation could be redirected into a useful pathway, Th1 for example, it might be possible to change a tumor-specific Treg into a tumor-specific effector T cell. This concept is appealing as depleting Tregs could produce a void that refills with Tregs [43], and because altering differentiation increases the number of potentially beneficial effector cells. The recent discovery that a common aryl hydrocarbon receptor controls Th17 versus Treg differentiation [64] may be usefully exploited in subverting Treg differentiation, provided that the Th17 cells so produced are not detrimental.

Other regulatory cells

CD8+ T cells, various myeloid cells and NKT cells have previously been described as dysfunctional regulatory cells in cancer [3]. A recent study demonstrates a population of suppressive CD8+FOXP3+ cells in prostate cancer that function through a contact-dependent mechanism, and whose effects are reduced by TLR8 agonists [65]. Relative contributions to tumor immunopathology, and therapeutic effects of management of each of these various types of regulatory cells remain to be defined, but represent interesting areas for future study.

Combining Treg management with other treatment modalities

Timing of Treg depletion with other interventions critically affects the efficacy of the combination [45]. Little is known regarding how to combine Treg management strategies outside of depletion, with other modalities. Depleting T cell pools to allow homeostatic expansion is an interesting concept, and may partly underlie the mechanism of action of denileukin diftitox. Combining certain cytotoxic agents that provoke significant tumor immunogenicity [66] with Treg management may be useful as a sort of endogenous vaccination.

Is there a malignancy of Tregs?

Cutaneous T-cell lymphomas can express FOXP3 and adopt an *in vitro* Treg phenotype [67]. FOXP3 expression may contribute to aggressive behavior of mycosis fungoides after large cell transformation [68].

Conclusions

Tumors employ myriad mechanisms to defeat host immunity. Many such mechanisms ultimately induce functional tumor CD4+CD25+FOXP3+ Treg accumulation. Reducing

Treg function in patients with cancer should allow more effective immune-based therapies, alone or in combination with other therapies. Recent insights into mechanisms of Treg differentiation, trafficking and suppressive function suggest novel means to manage deleterious Treg effects. Treg management is a better general term than Treg depletion, encompassing the many means by which their dysfunctional attributes can be overcome. Additional regulatory cells aside from CD4+CD25+FOXP3+ Tregs also contribute to cancer immune dysfunction, the relative contributions of which, and effects of management of which remain to be fully understood, but which afford additional therapeutic targets. Aside from their roles in ongoing cancers, Tregs now also appear to influence immunoediting and malignant progression in certain animal models. The relevance to human disease or prevention strategies remains to be defined.

Denileukin diftitox and cyclophosphamide are two agents that deplete Tregs in human clinical trials. Additional agents are under per-clinical and clinical investigation. Issues related to dosing, schedules and combining Treg management with other therapies remain incompletely resolved. Not all types of cancers, or at all stages, or in all hosts will benefit from specific strategies. Further, appropriate, tractable monitoring tests must be developed to follow the immunopharmacodynamics of interventions to manage Tregs.

Appropriate proof-of-concept trials are required to address the unique demands of these strategies. For example, it will be important to show not only that Treg function is reduced in a general suppression assay, but more specifically, to show that tumor antigen-specific immunity is improved, itself another challenge. In a vaccine trial for example, response to the vaccine antigen can be studied in detail. On the other hand, when Treg-mediated suppression is reduced, general measures of anti-tumor immunity (such as interferon- γ production) are augmented, although specifics, such as effects on a given antigen-specific CD8+ cytotoxic T lymphocyte, are usually not known. Further complicating matters, what specifically mediates tumor protection likewise is generally unknown, making it difficult to know what precisely should be monitored to assess immunologic efficacy.

Tregs for prognosis may ultimately be useful in selected settings after functionality, effects of anatomic distribution, and relationships to other important cells, among other factors, are better understood. Despite our current state of ignorance and incomplete understanding, we can be guardedly optimistic that Treg management will find a useful place in the therapeutic arsenal in our war against cancer. As we understand more about additional regulatory cell populations, their management might also be added to treatment strategies.

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