Targeting the right regulatory T-cell population for tumor immunotherapy

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Abbreviations: Treg, regulatory T cells; Cy, cyclophosphamide; neu-vaccine, neu-targeted whole cell GM-CSF-secreting vaccine; neu-N, HER-2/neu transgenic

Regulatory T cells (Treg) that suppress tumor-specific T-cell-mediated immune responses are the subject of an intense wave of investigation. We recently reported that a subset of Treg, namely effector/memory CD25low cells, are responsible for suppressing high avidity tumor-specific T cells in mouse mammary tumors. Here, we discuss additional findings that clarify this mechanism of Treg-mediated immunosuppression.

Introduction

Regulatory T cells (Treg) pose a significant barrier to the success of immunotherapeutic strategies aimed at activating high-avidity tumor-specific T-cells. Initial strategies to downmodulate the effect of Treg targeted the interleukin 2α (IL-2α) receptor CD25 with little success.1,2 We have previously shown that low-dose cyclophosphamide (Cy) given one day prior to a neu-targeted, whole cell, granulocyte macrophage colony-stimulating factor (GM-CSF)-secreting vaccine (neu-vaccine) leads to the depletion of CD4+FOXP3+CD25+ Tregs.3 More recently, we have demonstrated that a subpopulation of CD25low effector/memory Treg were particularly sensitive to Cy and preferentially suppressed high- vs. low-avidity HER-2/neu-specific T cells in the HER-2/neu transgenic (neu-N) mouse model of breast carcinoma.4 Here, we present companion data showing that anti-CD25 therapy preferentially affects CD25high Treg, leaving CD25low effector/memory Treg unaffected and capable of suppressing high-avidity T cells. These findings provide a possible mechanism for the lack of enhanced antitumor activity when CD25-targeted approaches are used to inhibit Treg.

Results

To elucidate differences in the activation of tumor-specific CD8+ T cells with distinct avidities, we developed high- and low-avidity CD8+ T-cell receptor transgenic mice specific for the immunodominant epitope of HER-2/neu (RNEU420–429), which is expressed by mammary tumors in the neu-N mouse model of breast carcinoma. We specifically addressed the role played by Treg in suppressing high- and low-avidity T-cells. Using adoptive transfer studies, we showed that in non-tolerant (parental FVB/N) mice, high- and low-avidity RNEU420–429-specific T cells given with neu-vaccine accumulate in the tumor bed, but only high-avidity T cells cause tumor rejection. In neu-N mice, high-avidity T cells failed to accumulate (and function) in tumors, unless mice were pretreated with Cy and the neu-vaccine. On the contrary, low-avidity RNEU420–429-specific T cells do not invade the tumor microenvironment even in the presence of Cy plus the neu-vaccine. High avidity RNEU420–429-specific T cells transferred with Cy and neu-vaccine upregulate integrins, which are required for trafficking to the tumor site, and secrete high levels interferonγ. Importantly, we found that Cy selectively depletes a CD4+FOXP3+CD25low subpopulation of Treg that displays an effector/memory phenotype similar to a Treg population that has been recently described in reference 4 and 5.

Companion data from simultaneous studies not published in reference 4, evaluated the anti-CD25 antibody PC61 as another method for depleting Treg. We previously showed that Cy given with PC61 and the neu-vaccine allowed for the systemic activation of endogenous high-avidity T cells in the neu-N model. However, in other models, PC61 given with vaccine was unable to elicit a significant anti-tumor response.2,6,7 We therefore adoptively transferred high avidity RNEU420–429-specific T cells into tumor bearing neu-N mice receiving the neu-vaccine and either PC61, Cy or Cy the PC61. When treated with PC61 plus the neu-vaccine, neu-N mice had significantly fewer CD4+FOXP3+ Treg in their tumor draining lymph nodes than mice given Cy plus the neu-vaccine. However, this Treg depletion did not translate into tumor clearance as tumors in the mice treated with...
PC61 plus the neu-vaccine grew as quickly as tumors in mice given the vaccine and high-avidity T cells (a tumor permissive environment, Fig. 1A and B). When we compared the function of adoptively transferred high-avidity RNEU_{420-429}-specific T cells in tumor draining nodes from mice treated with neu-vaccine plus either Cy or PC61, we found that mice given Cy plus the neu-vaccine plus high-avidity T cells had a significant population of T cells that secreted interferon γ, whereas mice given...
PC61 plus the neu-vaccine plus high avidity T cells had few, if any, such T cells (Fig. 1C). In addition, high-avidity Tcells given with PC61 plus the neu-vaccine did not upregulate integrins or accumulate into the tumor bed (Fig. 1D). These data led us to suspect that there might be specific Treg subpopulations that are responsible for suppressing activated T cells at the tumor site that differ from those that operate in draining lymph nodes. Based on our data with PC61 depletion, we suspected that these subpopulations differed in CD25 expression. As suspected, Cy depleted mainly CD4+FOXP3+CD25high Tregs, whereas PC61 treatment depleted only CD4+FOXP3+CD25high Tregs (Fig. 1E and F).

Discussion

Here, we describe companion data to our recent publication “Trafﬁcking of high avidity HER-2/neu-speciﬁc T cells into HER-2/neu-expressing tumors after depletion of effector/memory-like Treg in which we showed that the Treg-depleting antibody PC61 is unable to cause the same antitumor effects as the Cy-mediated depletion of Treg. Even though PC61 treatment led to a greater decrease in CD4+FOXP3+CD25+ Tregs, high-avidity CD8+ T cells did not upregulate integrins, secrete interferon-γ or effect tumor killing after PC61-mediated Treg depletion. Though the possibility exists that PC61 might have depleted adoptively transferred T cells or activated CD4+ T cells, this is unlikely because the administration of Cy plus PC61 plus the neu-vaccine allowed for similar extents of tumor killing as the administration of Cy plus the neu-vaccine alone, and we previously reported that the combination of Cy plus PC61 plus the neu-vaccine is able to elicit endogenous high-avidity T-cell responses against HER-2/neu.7

Similar results showing the ineffectiveness of PC61 in melanoma and pancreas cancer models may be explained by the fact that PC61-like therapies fail to attack effector Tregs that traffic with regular effector CD8+ T cells into the tumor microenvironment. Recent reports have demonstrated that PC61-mediated Treg depletion effectively reduces tumor growth but only when given with lymphodepleting doses of temozolomide.8 Altogether, these data highlight the complexity of Treg phenotypes, but also the fact that they can be effectively nullified to enhance tumor immunotherapy. Further elucidation of Treg subpopulations and their functions should lead to new ways to more efﬁciently depleting the Treg populations that de facto suppress the tumor-specific effector T cells while leaving in place populations that suppress non-specific T cell populations that are also activated by cancer immunotherapies.

Materials and Methods

All assays, adoptive transfer studies, and data analyses were performed as in reference 4, except that the anti-CD25 antibody PC61 was given in place of cyclophosphamide at a dose of 150 μg/mouse one day prior to vaccine. PC61 was puriﬁed from culture supernatants of the PC61.5.5 cell line (American Type Culture Collection) grown in Protein Free Hybridoma Medium II.3

Disclosure of Potential Conﬂicts of Interest

This work involves a GM-CSF-secreting vaccine. Through a licensing agreement with BioSante, Johns Hopkins has the potential to receive royalties in the future. None of the authors have ﬁnancial interest in this work.

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