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Depletion of Tregs for adoptive T-cell therapy using CD44 and CD137 as selection markers

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Abstract

Several types of cancer have been shown to be susceptible to cellular immune responses, leading to investigations using various forms of T cell-based, tumor-directed immunotherapy. One potential obstacle for the successful application of these therapies is the suppressive function of Tregs. Goldstein and colleagues evaluate a strategy to identify and remove Tregs from an adoptive T-cell therapy product generated by *in vivo* vaccination. They demonstrate that the depletion of Tregs characterized by CD44 and CD137 expression enhances antitumor immunity in their mouse model.

Keywords

adoptive T-cell therapy; CD137; lymphoma; Tregs; tumor immunotherapy; tumor vaccines

Adoptive T-cell transfer has emerged as a promising form of immunotherapy for several malignancies, including lymphoma, leukemia and melanoma. A recent clinical trial testing CD19-directed adoptive T-cell therapy for chronic lymphocytic leukemia demonstrated that this strategy has the potential to induce complete remissions in human patients [1]. However, to date, most other studies have yielded disappointing response rates [2,3], suggesting that there are still barriers to overcome before this approach is reliably effective. One of these potential impediments is the suppression of therapeutic immune responses by Tregs. Theoretically, Treg-mediated inhibition could occur in several ways: inhibition of transferred T cells at the tumor site or secondary lymphoid tissues by resident Tregs; conversion of therapeutic T cells to induced Tregs in the tumor microenvironment under the influence of TGF- β and IL-2, if naive CD4⁺ cells are present; or cotransfer of natural Tregs activated in *ex vivo* cultures along with the therapeutic T cells.

Goldstein *et al.* address the last of these possibilities by examining the effects of adoptive transfer of different T-cell subsets, demonstrating that the removal of Tregs characterized by

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CD44 and CD137 (4-1BB) expression significantly enhances antitumor immune responses [4].

Summary of methods & results

The authors employed a model in which donor C57/BL6 mice were vaccinated with congenic B-cell lymphoma cells pulsed with a CpG-enriched oligodeoxynucleotide, a TLR9 agonist that enhances the immunogenicity of vaccines by activating APCs. A stem cell transplant was then performed, in which bone marrow and splenocytes from vaccinated donor mice were injected into irradiated congenic C57/BL6 recipient mice, along with additional irradiated CpG-enriched oligodeoxynucleotide-pulsed tumor cells as a post-transplant 'booster' vaccine. By transferring sorted subsets of *in vivo*-activated donor lymphocytes, the authors found that CD4⁺ but not CD8⁺ cells protected against tumor challenges, and that this required recipient CD8⁺ cells, presumably activated after reconstitution post-transplant. In the process of attempting to define more precisely the active donor CD4⁺ subset, the authors observed that a higher percentage of T cells from vaccinated mice expressed CD137 compared with those from unvaccinated mice, and that the majority of these cells coexpressed FoxP3, suggesting that they were Tregs. A comparison of FoxP3⁺ cells with or without CD137 expression revealed that CD137⁺ Tregs had a more activated phenotype, with higher levels of CD25, CD44 and CD69, as well as slightly higher levels of CD103 and GITR. The investigators hypothesized that sorting cells for CD44 expression (a marker of antigen experience) would enrich for vaccine-reactive cells, and that these cells could be subdivided on the basis of CD137 expression to separate the effector cell (CD137⁻) and Treg (CD137⁺) subsets.

This strategy initially seems counterintuitive, since CD137 is best known as a costimulatory molecule expressed on the surface of activated effector T cells [5]. However, it is tightly regulated on effector CD4⁺ and CD8⁺ cells, and is expressed only transiently (<72 h), at least in the setting of transient antigen exposure [6,7]. By contrast, CD137 is expressed constitutively on Tregs [5]. Thus, depletion of CD44⁺CD137⁺ cells is a reasonable approach to examine for removing Tregs from a cell product generated by *in vivo* vaccination. By sorting cells in this way, the authors demonstrated that CD44⁺CD137⁺ cells derived from vaccinated donors have higher FoxP3 expression and suppress *in vitro* proliferation of CD44⁺CD137⁻ effector cells in a contact-dependent manner. *In vivo*, cotransfer of CD44⁺CD137⁺ cells blocked tumor killing and IFN- γ production by the CD44⁺CD137⁻ effectors. The authors also presented data demonstrating that CD4⁺CD137⁺ cells are expanded in tumor biopsies of patients with follicular and mantle cell lymphoma, compared with peripheral blood samples (although normal lymph node controls were not included for comparison), and that the majority of these cells express FoxP3, suggesting that this immunophenotype likely identifies an active Treg subset in human tumors.

Discussion & significance

The observations in this study lead to several insights and potential applications. While CD137 is known to be expressed on Tregs, this is the first demonstration of CD137 as a selection marker for clinically relevant Tregs, and the authors provide a potentially useful algorithm for isolating and depleting this Treg population. The transcription factor FoxP3 is the most biologically important marker of Treg differentiation and function discovered to date, but it cannot itself be used for cell sorting, since it is intracellular and therefore requires fixation and permeabilization prior to staining with antibody. Cell-surface markers must therefore be used for either magnetic or flow cytometric cell sorting. Several candidate proteins on CD4⁺ cells have been proposed as Treg selection markers, most commonly CD25, but other surface antigens may be more specific, including CD147, anti-FR4 and

CTLA-4 [8–10], and it is now appropriate to add CD137 to this list. Further studies are needed to determine which combination of markers is the most clinically useful, recognizing that the answer may vary depending on the particular application.

The observation by Goldstein *et al.* that CD137 appears to correlate with a more potent suppressive function also raises questions about the biological role of CD137 in Tregs [4]; is it primarily a marker of activation for Tregs, or does it somehow play a direct role in suppressing T cells? If the latter, by what mechanism? Does its constitutive expression indicate an essential function in these cells, or is it merely associated with other important proteins? Further studies evaluating the effect of CD137 on Treg function are warranted.

Although the methods outlined in this study are designed to enhance the immune potency of T cells for adoptive transfer, the same methods might reasonably be employed in an inverse manner to generate a Treg product for treatment of graft-versus-host disease, organ transplant rejection or autoimmune disease.

There are several inherent limitations to this study. The utility of the findings are primarily restricted to the transfer of CD4⁺ cells; there is presumably no benefit in sorting CD8⁺ cell products in this way. In addition, the selection strategy is most relevant to the situation of *in vivo* vaccination of autologous or syngeneic donors. Although the authors' CpG-enriched oligodeoxynucleotide-pulsed tumor vaccine could conceivably be used in the allogeneic transplant setting, removal of Tregs would likely increase the risk of graft-versus-host disease. This type of Treg depletion could potentially be used with *in vitro*- or *ex vivo*-generated T cells, although in this setting, the desired T-cell subsets could perhaps be more easily selected prior to activation and expansion.

An important consideration regarding the approach outlined by Goldstein *et al.* is the timing of CD137 depletion [4]. The beneficial effect could be lost if carried out too early after vaccination, since activated effectors expressing CD137 might be removed. Indeed, the selection of CD137⁺ cells 24-h post-stimulation has been proposed as a method for selecting reactive T cells [7].

As with all mouse studies, another potential limitation is that it may not accurately reflect human biology. For example, there may be differences in CD137 expression following vaccination in humans, such as relevant CD137⁺ Treg populations that may fail to be eliminated with the proposed cell sorting approach or, conversely, desirable CD137⁺ effector cells might be removed, particularly if there was a delay in response to the vaccine, leading to activation of effector cells within 72 h of T-cell harvest.

Future perspective

As the field of adoptive cell therapy evolves, our understanding of the biological behavior and immunophenotypic characteristics of the various T-cell subsets continues to grow. We continue to appreciate the importance of the intrinsic qualities of these subsets in terms of their interaction with each other and other immune cells, and their capacity to proliferate and survive, secrete cytokines, home to lymph nodes and tumor sites, and generate or suppress tumor immune responses. Ultimately, the success of adoptive cell therapy for cancer will depend on our ability to achieve potent but controlled immune responses. It is likely that, in order to achieve this, future therapeutic products will need to consist of carefully selected T-cell subsets with predictable *in vivo* behavior, tailored to the specific clinical application. Work such as that of Goldstein *et al.* [4] will continue to move us towards that goal.

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Executive summary

- Tregs can be functionally separated from effector T cells on the basis of CD44 and CD137 expression in a mouse model of *in vivo* vaccination and adoptive transfer.
- The CpG tumor vaccination and adoptive transfer model employed by the authors eradicates tumors in mice and is a promising strategy for lymphoma patients.
- The presence of increased numbers of CD4⁺CD137⁺ T cells expressing FoxP3 in human lymphoma specimens indicates that CD137 may be a useful marker to characterize and isolate Tregs in humans.
- Additional studies are needed to determine the biological role of CD137 in Tregs, as well as to identify the ideal combination of surface markers to identify human Tregs.