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Absence of CD4 T-cell help provides a robust CD8 T-cell response while inducing effective memory in a preclinical model of melanoma

Maria Muccioli¹, Caitlin Longstaff², and Fabian Benencia^{1,2,3,*}

¹Molecular & Cell Biology Program, Ohio University, OH, USA

²Department of Biomedical Sciences, College of Osteopathic Medicine, Ohio University, OH, USA

³Biomedical Engineering Program, Russ College of Engineering & Technology, Ohio University, OH, USA

Abstract

Immunotherapy strategies for cancer are focused on inducing effective and specific cytotoxic responses mediated by CD8 T cells. On the other hand, immunosuppressive mechanisms induced by the tumor, such as the generation of tumor-specific CD4⁺CD25⁺FoxP3⁺ Tregs, conspire against the efficacy of immunotherapies. It has been considered that, similar to what has been observed in the context of immunological responses towards microbes, CD4 help is indispensable for the development of a successful and long-lasting (memory) CD8 immune response. In the recent article, Côté *et al.* reported that, in a mouse model of melanoma, total ablation of CD4 help does not hamper the development of a specific antitumor memory CD8 response. In addition, ablation of CD4 was more successful than strategies to deplete CD25 Tregs in generating memory CD8 T cells. These data opens the door for therapies destined to induce effective antitumor immune responses by ablation of whole CD4 T-cell populations.

Keywords

helpless CD8 cells; immunotherapy; melanoma; Tregs

The protective role of the immune system against tumors has been widely studied; for example, tumor-infiltrating lymphocytes (TILs) have been associated with improved survival of patients with melanoma, prostate, breast, colorectal and ovarian carcinomas, among others [1–5]. In particular, a high number of melanomas harbor TILs with protective properties, making this tumor a susceptible target for immunotherapy [5,6]. In this context, foundational work has been performed by Rosenberg *et al.* [7]. As early as 1985, his group reported that coadministration of autologous cytokine-activated killer cells and IL-2, a cytokine that induced proliferation of T cells, a reduction in tumor size in several patients

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*Author for correspondence: benencia@ohio.edu.

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with advanced cancer and a complete tumor regression in one patient with metastatic melanoma. Further studies demonstrated that IL-2 treatment has been successful in approximately 10–20% of patients with melanoma [8–11]. In theory, the use of T cells for the treatment of cancers would provide specific tumor attack without the side effects of chemotherapeutic drugs. Adoptive T-cell transfer is an immunotherapeutic strategy that has been developed to fight tumors. Here, the patients' own T cells are activated and induced to proliferate *in vitro*, and are then readministered to the same patient. T cells can be recovered from the circulation, and tumor-specific T cells induced to proliferate upon incubation with APCs pulsed with tumor antigens [12]. Then, cells that have been specifically activated by tumor antigens are reintroduced to the patient. In addition, TILs that might be specific for tumor antigens can be recovered from tumor samples and induced to proliferate for their use in adoptive transfer protocols [13,14]. This can circumvent the fact that some tumor antigens are unknown. Some protocols involve the use of CD8 cytotoxic T cells or a mixture of CD4 and CD8 T cells [13]. It is considered that the presence of CD4 helper cells may be relevant for the generation of effective CD8 cytotoxic cells while they are expanded *in vitro*, and also *in vivo* during tumor regression [13].

Although the immune system provides protection against cancer, in some cases it might collaborate with tumor development. Leukocyte infiltration can precede the development of a neoplasm, which is an important risk factor for the development of cancer [15–17]. Indeed, one of the hurdles for cancer immunotherapy is the presence of immunosuppressive mechanisms triggered by tumors [18]. In particular, great importance has been given to the role of Tregs in tumor development. Naturally occurring Tregs are key mediators of peripheral tolerance and protect the organism against autoimmunity [19–21], but in a tumor setting, they may help to suppress the specific antitumor immune response [22,23]. In addition, during cancer progression, Tregs that abrogate specific anticancer immune responses are originated *de novo* in the periphery [24]. It has been shown in several experimental animal models that Treg depletion improves immune-mediated tumor clearance [23,25]. Furthermore, Prasad et al. showed that depletion of Tregs before vaccination and in the absence of tumor, created a 'blank slate' condition, which led to complete tumor rejection and establishment of long-lasting tumor immunity in a considerable proportion of tumor-inoculated mice [26]. Finally, in a mouse model of melanoma, Turk et al. demonstrated that Tregs were the major regulators of the capability of animals harboring a progressive tumor to reject the same tumor at a remote site [27].

In humans, therapies destined to ablate immunosuppressive lymphocyte populations have been used as part of immunotherapy protocols during clinical trials against different tumors [28–31]. Indeed, as reviewed by Muranski *et al.*, a 50% response rate (according to Response Evaluation Criteria In Solid Tumors [RECIST] criteria) was observed in metastatic melanoma upon combinatorial treatment with *in vitro*-expanded TILs and IL-2 following a lymphodepleting protocol [11]. This clearly highlights the relevance of the suppressive mechanisms that conspire against the success of T-cell therapies in cancer.

Finally, it has been proposed that, in order to maintain an effective immune response upon tumor vaccination or T-cell therapy, there is a need to generate a robust memory CD8 T-cell response that will be able to cope with tumor recurrence [32]. Taking into account all these data, an effective antitumor T-cell therapy must circumvent the challenges imposed by the mechanisms of immunosuppression orchestrated by the tumor, while generating a long-lasting CD8 T-cell memory.

Summary of methods & results

In a recent manuscript published in *PLoS ONE*, Côté *et al.* investigated the capability of inducing memory CD8 T-cell responses in the absence of CD4 T-cell help in a mouse model of melanoma [33]. The goal of their studies was to determine the relevance of CD4 T-cell help in the generation of protective CD8 T-cell memory to melanoma. One of the highlights of these studies is that they investigated the immune parameters following surgical removal of the primary tumor. This is very relevant because it depicts a scenario that more closely follows the treatment of the disease in humans than other animal models where prophylactic or therapeutic treatment of a primary tumor is investigated.

In the first series of experiments, C57BL/6 mice were inoculated with the poorly immunogenic B16-F10 mouse melanoma tumor (day 0). On days 4 and 10 or 2, 4 and 10, tumor-bearing mice were treated with 250 µg of an anti-CD4 antibody (clone GK 1.5) intraperitoneally. The growing tumors were measured using calipers three-times a week, and primary tumors were removed from the skin on day 12. Subsequently, CD8 T cells were purified from spleen and inguinal lymph node samples and cocultured with EL-4 thymoma cell targets pulsed with MHC-I-restricted peptide epitopes (including TRP₂₁₈₀₋₁₈₈, gp100₂₅₋₃₃ or irrelevant OVA₂₅₇₋₂₆₄). The activation of T cells was determined by measuring IFN-γ by enzyme-linked immunosorbent spot. The authors determined that no IFN-γ was produced in the absence of anti-CD4 treatment, following stimulation of CD8 T cells with melanoma antigen. By contrast, CD8 T cells recovered from mice treated with the anti-CD4 antibody displayed an increase in IFN-γ production. In fact, the highest IFN-γ levels were produced by cells recovered from mice treated three times with anti-CD4, suggesting that CD4 T-cell depletion results in a greater CD8 T-cell priming response.

In a series of complementary studies, the authors measured antimelanoma CD8 T-cell priming in mice treated with 250 µg of an anti-CD25 antibody (clone PC61) intraperitoneally in a dose scheme similar to that described for anti-CD4 antibodies. It has been previously shown that this antibody is able to deplete Tregs *in vivo* [26]. It is particularly effective in depleting Tregs as these cells express high levels of CD25 on their surface.

Surprisingly, the authors found that CD25 T-cell depletion did not result in an antigen priming response in the tumor-bearing mice. These could be due to the fact that CD25 depletion treatment was not able to delete all Tregs, while anti-CD4 did.

Côté and colleagues went on to examine the details of CD4 T-cell repopulation following depletion using flow cytometry. They found that 10 days after the last anti-CD4 antibody treatment, the CD4 T cells became detectable again, reaching normal levels approximately 1.5 months after depletion. In addition, the repopulation of T helper cells (FoxP3⁻) and Tregs (FoxP3⁺) was investigated. It was determined that the proportion of Tregs among all CD4 T cells increased to higher-than-normal levels by day 10, and then decreased gradually. Also, it was found that both FoxP3⁻ and FoxP3⁺ T cells displayed a CD44^{hi} memory phenotype. In addition, following anti-CD4 treatment, and before CD4 T-cell recovery, the levels of CD8 T cells markedly increased, with 25% of those cells showing the CD44^{hi} phenotype. This was probably due to CD4 T-cell depletion, as the CD8 T cell levels decreased to normal levels approximately 2 weeks after the anti-CD4 treatment.

In addition to their studies on CD4 T-cell depletion effects on CD8 T-cell priming, the authors also investigated whether CD4 T cells were necessary for the formation of the protective memory response against the melanoma. The B16 mice were treated on varying days with the anti-CD4 antibody, pre- and post-tumor induction, and the anti-CD4 treatment continued weekly for 60 days after tumor excision. First, the authors assessed the mice for

vitiligo, the autoimmune destruction of pigment cells associated with antimelanoma immunity [34], and found that no significant differences in occurrence or severity of the vitiligo between mice that received transient CD4 T-cell help, and those that received no help. Choosing only vitiligo-affected mice for analysis, as only these mice develop an antimelanoma memory immune response, Côté and colleagues determined that the population of Thy1.1⁺/CD44^{hi} memory T cells was slightly decreased in the lymph nodes when there was no help provided by CD4 T cells. This difference was not observed in the spleen, and no significant difference could be observed between mice receiving transient help and no help 30 days after discontinuation of the anti-CD4 treatment. The authors also confirmed that CD4 depletion did not have an effect on CD8 T-cell function, as indicated by normal levels of IFN- γ , TNF- α and IL-2 in a flow cytometry analysis. However, enzyme-linked immunosorbent spot analysis revealed less IFN- γ production in mice receiving no T-cell help in the lymph nodes, although there was no significant difference in IFN- γ levels found between the two in the spleen.

Conclusion

In summary, Côté and colleagues found that CD8 T cells were able to undergo priming and protective memory responses in the absence of CD4 helper T cells. Significantly, complete and temporary depletion of CD4 T cells boosted the efficacy of the treatment. The researchers showed that this treatment was able to eliminate Tregs from tumor-bearing mice. Focusing on the CD4 T-cell population has the following advantages: this strategy is able to completely eliminate Tregs compared with CD25 ablation treatments and, since CD25 is also expressed by activated CD8 T cells, this treatment can also eliminate specific CD8 T cells that might be activated against the tumor. Thus, strategies focused on CD4 T-cell depletion opposed to CD25 depletion might help preserve naturally occurring CD8 cytotoxic populations that can help control the tumor. Furthermore, this CD4 T-cell ablation, together with a therapeutic CD8 T-cell treatment, can hypothetically be boosted by combination with IL-2 therapy without risking Treg activation during the first few days of treatment. As discussed by the authors, these results contradict previous reports indicating that CD4 help is relevant for CD8 therapeutic efficacy in tumor settings [22]. This could be due to the particular characteristics of the model used by Côté *et al.*, in which primary tumors were surgically resected possibly modifying the strong immunosuppressive milieu imposed by large established tumors, as previously described [18,35].

The relevance of these studies lies in the discovery that a robust CD8 memory response against the tumor can be obtained in the absence of CD4 help. As the authors discuss, the present work demonstrates that CD8–CD4 interactions documented in microbial infections are not easily translatable to other settings such as cancer; for example, it has been shown that helpless CD8 T cells are immunologically impaired during viral infections or intracellular bacteria [36–38], but the current work shows that this is not the case in an experimental model of melanoma. This clearly indicates that the mechanisms regulating immune responses against microbes are different to those involved in tumor recognition attack and, thus, not easy to extrapolate.

Future perspective

The capability of generating a robust memory CD8 response in the absence of CD4 help, in contradiction to what is observed in the context of infectious disease, generates a paradigm shift regarding the nature of the immune response in the context of tumor immunity. This might be relevant in designing future antitumor immune therapies. In particular, new strategies destined to temporary ablate total CD4 T cells in the context of immunotherapy,

rather than CD25 T cells, could provide a more effective and longstanding antitumor CD8 T-cell response.

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Executive summary

- In the context of infection, no effective memory CD8 T-cell response is obtained in the absence of CD4 T-cell help.
- The paper under evaluation revealed that effector and memory cytotoxic CD8 antimeelanoma T cells can be generated in the absence of helper CD4 T cells. The authors describe that full CD4 T-cell ablation was more effective than therapies destined to specifically ablate Tregs.
- These results open the door for novel antitumor therapies involving transient ablation of CD4 T cells in combination with other immunotherapeutic approaches, such as tumor vaccination or adoptive cell therapy.