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Antigen loss and tumor-mediated immunosuppression facilitate tumor recurrence

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Abstract

While tumor immunotherapy has seen notable success in recent years, mechanisms that tumors utilize to escape immune responses have provided significant hurdles to maximal clinical benefit. Escape mechanisms such as antigen loss, decreased MHC expression, as well as tumor-mediated suppressive effects on antitumor immune responses, can cause the most potent antitumor immune response to be rendered powerless at the tumor site. In this study, the authors show that the adoptive transfer of tumor antigen-specific $CD4^+$ and $CD8^+$ T cells combined with tumor cell immunization can elicit regression of established subcutaneous tumors in lymphopenic, but not lymphoreplete, animals. However, using a suboptimal dose of transferred cells followed by vaccination, the authors identify the development of recurrent tumors with reduced antigen expression. These tumors could still be eradicated in similarly treated animals; however, they found that transferred $CD4^+$ T cells from animals with recurrent tumors acquired a suppressive phenotype. This work highlights the importance of understanding mechanisms of tumor escape, particularly underscoring the role of the tumor in modulating antigen-specific immune responses, and the critical importance of finding mechanisms to avoid the development of viable escape variants.

Keywords

adoptive transfer; antigen-loss variant; immunotherapy; tumor escape variant; tumor immunosuppression

This article reviews the study of Jensen *et al.*, summarizes the key findings and discusses the impact this will have on our understanding of tumor escape following immunotherapy [1]. It is becoming more evident that the escape mechanisms that tumors utilize to avoid antitumor immune responses are significant hurdles to maximizing clinical benefit from immunotherapies. These mechanisms include tumor variants that decrease the recognition of the tumor itself, as well as tumor-mediated immunosuppressive functions that can dampen the antitumor immune effector function [2,3].

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The importance of regulatory responses in hampering antitumor vaccine responses has led several groups to be interested in immunizing in settings where these regulatory responses have been eliminated. In this report, Jensen *et al.* utilize lymphopenic $RAG^{-/-}$ mice and reconstitute these animals with adoptively transferred tumor antigen-specific $CD4^+$ and $CD8^+$ T cells, along with a tumor cell vaccine, to evaluate the ability of this immunotherapeutic approach to eliminate established tumors.

Methods

In this study, the authors utilize tumor antigen-specific pmel $CD8^+$ T cells targeting gp100 and TRP1 $CD4^+$ T cells targeting tyrosinase-related protein 1. Transgenic mice expressing pmel or TRP1 T-cell receptors were backcrossed onto the $RAG^{-/-}$ background to eliminate endogenous T cells. These cells were used in combination with the B16BL6-D5 (D5) murine melanoma tumor cell line, which is a poorly immunogenic subclone of the B16 cell line [4]. This cell line was used for both subcutaneous tumor challenges, as well as an immunizing vector (transfected to express GM-CSF, D5-G6).

Lymphoreplete C57BL/6 or lymphopenic $RAG^{-/-}$ mice were challenged with D5 tumors and then received 10^6 pmel and 5×10^5 TRP1 splenocytes as well as 10^7 irradiated D5-G6 cells. Subsequent studies utilized suboptimal doses of transferred cells to study the contribution of $CD8^+$ T cells in tumor rejection, as well as mechanisms of tumor escape in recurrent tumors. Recurrent tumors were isolated and used to grow subclonal cell lines, which were examined for tyrp1, gp100 and MHC class I expression, as well as its immunogenicity and tumorigenicity in naive tumor-bearing $RAG^{-/-}$ mice similarly treated with adoptive transfer and vaccination.

Results

The adoptive transfer of naive pmel-specific $CD8^+$ and TRP1 $CD4^+$ T cells did not alter tumor growth in wild-type animals, even when combined with immunization using a GM-CSF-secreting irradiated tumor cell line (D5-G6) and IL-2 supplementation. However, in lymphopenic $RAG^{-/-}$ mice the transfer of tumor antigen-specific $CD4^+$ and $CD8^+$ T cells in combination with D5-G6 immunization was sufficient to elicit tumor regression, even in the absence of supplemental IL-2. Furthermore, a suboptimal dose of TRP1 $CD4^+$ T cells, when combined with a suboptimal dose of pmel $CD8^+$ T cells and D5-G6 immunization, led to tumor regression and an increase in overall survival. However, approximately 25% of the animals eventually developed recurrent, depigmented tumors, presumably an indicator of immune editing and antigen loss [5]. Splenocytes from tumor-free animals mediated tumor regression in naive tumor-bearing recipients, however splenocytes from animals with recurrent tumors had no effect on tumor growth. TRP1 $CD4^+$ T cells from animals with recurrent tumors were found to have increased expression of Foxp3 suggesting that these transferred tumor-specific $CD4^+$ T cells were converted to exhibit a regulatory phenotype.

Discussion

In this article, Jensen *et al.* show that lymphopenic hosts are more amenable to adoptive transfer strategies, potentially due to the lack of intrinsic regulatory T-cell populations. This alone is an interesting observation, as these animals should have tumor-associated myeloid-derived suppressor cells, yet these appear to not play a central role in mediating tolerance in this model system. An additional unexplored possibility is that these lymphopenic hosts have altered systemic levels of cytokines (e.g., IL-7) that may affect the function, proliferation or persistence of transferred T-cell populations.

Isolating recurrent tumors to generate cell lines, the authors show that recurrent tumors no longer express detectable levels of gp100, in line with data showing that antigen loss is a primary means of tumor escape [6]. However, the ability of these recurrent 'gp100-negative' tumors to activate pmel $CD8^+$ T cells *in vitro*, as well remain susceptible to their immunotherapeutic regimen, suggests that decreases in antigen expression in itself is not sufficient to protect tumors from antitumor immune responses. It has been suggested that low levels of residual tumor antigen expression can lead to T-cell tolerance and the loss of effector immune responses, which could potentially contribute to the generation of immunosuppressive splenocytes in animals with recurrent tumors [7]. Strikingly, in this system it was the presence of transferred $CD4^+$ T cells of a regulatory T-cell phenotype ($FoxP3^+$) and suppressive function that was most associated with the development of recurrent tumors.

Expert commentary

As new immunotherapeutic approaches enter clinical testing, identifying mechanisms that tumors can utilize to escape these antitumor responses is increasingly important. In this report, Jansen and colleagues provide further evidence that antigen loss, or at least decrease in target expression, can occur, which is an example of immunoediting. However, their studies suggest that even with reduced expression these tumors are still amenable to antigen-specific immunotherapy, and as such these studies underscore the importance of antigen selection for antitumor vaccines. Targeting antigens critical to the function or oncogenicity of tumor cells may make these cells less susceptible to antigen loss. At this point, it is unknown whether the changes in antigen expression in the tumors led to the development of regulatory responses, although this is an interesting possibility, as previously suggested [7].

While antigen loss must certainly play a role in tumor escape, the authors highlight that this process of antigen immunoediting alone is insufficient in leading to escape, and that tumor-mediated changes in the $CD4^+$ T cell population (from an effector to regulatory phenotype) also play a central role in avoiding antitumor immune responses. As these studies were performed in lymphopenic $RAG^{-/-}$ mice, and regulatory $CD4^+$ T cells were detected only in animals with recurrent tumors, it suggests that the tumor was directly mediating a suppressive effect on the transferred T cells. Whether this is through an inhibitory ligand such as PD-L1 or rather through a secreted factor remains to be evaluated. Furthermore, the lack of antitumor response from the same adoptive transfer and vaccine regimen in lymphoreplete animals suggest this suppression likely occurs, at least initially, independent of the adoptively transferred cells, whether by tumor-mediated suppression of T cells, altered cytokine levels or other methods.

Many recent studies have suggested that effective immunotherapy should include conditioning regimens to deplete host leukocytes [8]. At this point it is unclear whether these strategies 'make space' for adoptively transferred cells, or provide a cytokine environment most conducive to adoptive transfer and/or vaccination. The studies here, demonstrating an effect from combined immunotherapy with adoptive transfer followed by vaccine activation only in $RAG^{-/-}$ mice, suggests an alternative that the depletion of intrinsic lymphocytes, potentially regulatory T cells induced by the tumor, could enhance the efficacy of immunotherapy. It remains to be demonstrated if similar results could be obtained by selectively removing regulatory populations, or skewing the cytokine balance to optimize conditions for T-cell activation or maintaining effector function.

Five-year view

The ability of tumor cells to suppress effector responses, as well as cause effector cells to gain suppressive function themselves, highlights several areas of future research that will be

crucial to enhance the clinical efficacy of immunotherapeutic approaches. This tumor-mediated conversion needs to be better understood to identify therapeutic modalities (potentially antibodies blocking checkpoint inhibitors such as CTLA-4 or PD-1) to maintain effector activity or even reverse regulatory reprogramming. If tumors continue to convert antigen-specific T cells to a suppressive phenotype, future strategies may need to block this conversion rather than simply deplete regulatory cells prior to beginning immunotherapy.

The generation of antigen-loss variants that escape immune targeting also raises important questions regarding antigen-specific immunization approaches. Not only is the selection of the appropriate target a central component of vaccine design, but further suggests that it may be advantageous to target multiple antigens simultaneously. However, there may be differences in terms of pre-existing regulatory responses as well as regulation following immunization with respect to individual antigens. In the study by Jensen *et al.*, regulatory cells were only identified for the $CD4^+$ T cells, and did not occur in all animals, suggesting that there may be differences among antigens or a means to control the conversion of antigen-specific responses to a suppressive phenotype. Taken together, these findings demonstrate that future studies with antitumor vaccines should monitor for the presence of antigen-specific regulatory cells as well as effector cells.

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Key issues

- Adoptive transfer of tumor antigen-specific $CD4^+$ and $CD8^+$ T cells combined with tumor vaccine immunization elicited tumor regression in lymphopenic $RAG^{-/-}$ mice, but not lymphoreplete mice.
- A suboptimal dose of transferred cells resulted in recurrence of tumors, which displayed decreased antigen expression.
- $CD4^+$ T cells from animals with recurrent tumors expressed a suppressive phenotype.
- This study highlights the importance of understanding mechanisms of tumor escape, suggesting that tumor-mediated T-cell regulation is critical to tumor immune escape, and that finding mechanisms to avoid the development tumor-mediated regulatory T cells is important to the success of future immunotherapy approaches.