Tumor-Specific T-Cell Memory: Clearing the Regulatory T-Cell Hurdle

Anik L. Côté, Edward J. Usherwood, and Mary Jo Turk

Department of Microbiology and Immunology, and the Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, New Hampshire

Abstract

Antitumor immune responses can be stimulated by interfering with regulatory T-cell (T_{reg}) function. However, this effect is short lived unless T-cell memory to tumor antigens can be generated. Our recent studies show that T_{reg} cells not only limit primary responses to tumor/self-antigens in tumor-bearing hosts but also prevent the natural generation of T-cell memory to such antigens. Here, we discuss the role of T_{reg} cells in suppressing T-cell memory after surgical excision of tumors and the potential clinical benefits of overcoming this suppression. [Cancer Res 2008;68(6):1614–7]

Background

The generation of CD8 T-cell memory is one of the major goals of tumor immunotherapy. For years, studies in infectious disease models have shown that memory T cells are required for pathogen clearance and protection against reinfection. More recently, these lessons are being applied to cancer models. Although surgery currently remains the leading cure for solid tumors, memory T-cell responses may be required for the durable prevention of tumor recurrence and metastasis following surgery. In vitro–generated memory CD8 T cells have also been shown to be highly effective at treating large established melanomas (1, 2). However, in stark contrast with infectious disease models, most human tumors are poorly immunogenic, and most tumor antigens are unaltered self-proteins. This presents a significant challenge, as mechanisms of central and peripheral tolerance prevent the priming of T-cell responses against self-antigens. Even if tolerance is broken, T cells remain exposed to self-antigens in the periphery, which may lead to the development of functionally impaired memory, as observed with chronic viral infections (3, 4).

Despite these challenges, some vaccination strategies have been capable of inducing long-lived protective T-cell responses against poorly immunogenic tumors. In one of the earliest examples, Mullins et al. (5) showed that a CD40L-matured dendritic cell vaccine could generate CD8 T-cell recall responses against the melanocyte differentiation antigen tyrosinase, as well as long-term protection against melanoma. Cytokines can also drive the development of memory in vivo, as shown by the development of a durable and protective central and effector memory CD8 T-cell response following administration of a DNA vaccine encoding the tumor antigen Fra-1 and the cytokine interleukin-18 (IL-18; ref. 6). Costimulatory molecules may also play an important role. A xenogeneic DNA vaccine encoding gp100, which typically induces only short-term immunity, induced long-lived T-cell responses and tumor protection when coupled with a stimulatory antibody to GITR (7). However, aside from these studies, there are few examples of active immunotherapy inducing durable T-cell memory to tumor/self-antigens. More frequently, memory is shown following the immune-mediated rejection of primary tumors. For example, regression of B16 melanoma, induced by a GM-CSF–producing tumor cell vaccine and CTLA-4 blockade, protected mice from tumor challenge as long as 100 days after vaccination (8). Such studies illustrate that some aspect of active tumor rejection may lead to the development of immunologic memory.

Interestingly, historical data show that progressive tumors themselves can induce functional T-cell memory. In the 1980s, Bursuker and North (9) found that protective T-cell memory resulted after surgical excision of a highly immunogenic methyl-cholanthrene-induced tumor. This memory arose naturally in response to tumor growth and without a need for vaccination. However, it was crucial to excise primary tumors when they were small to prevent the generation of "suppressor" cells that would attenuate the response. Notably, postsurgical immunity was only a phenomenon of highly immunogenic tumors and was not observed in hosts bearing poorly immunogenic cancers (10).

In hindsight, the suppressor cells identified by Bursuker and North may likely have been tumor-induced regulatory T cells (T_{reg}). T_{reg} cells are crucial mediators of peripheral tolerance (11). They possess a CD4+CD25+Foxp3+ phenotype and arise both in the thymus and through the conversion of Foxp3−CD4+ T cells in the periphery (11, 12). T_{reg} cells suppress the development of CD8 T-cell memory in infectious disease models (13), but until recently, their role in preventing memory against poorly immunogenic tumors had not been shown.

Many studies have illustrated that T_{reg} cells prevent primary T-cell responses against poorly immunogenic cancers (11, 12). We previously showed that CD4+CD25+ T_{reg} cells suppress the de novo priming of CD8 T cells in response to growth of the poorly immunogenic B16 melanoma (14). If T_{reg} cells were deleted during growth of the melanoma, mice primed CD8 T cells against differentiation antigens expressed by both the tumor cells and normal melanocytes. Melanoma tumor-bearing mice that lacked T_{reg} cells also developed concomitant immunity, evidenced by the rejection of a secondary melanoma inoculated at a different site. Thus, T_{reg} cells functioned early to suppress the de novo priming of immunity against this poorly immunogenic tumor. However, whether such tumor/self-antigen–specific T cells could develop into functional T-cell memory remained unknown.

Removing T_{reg} Cells during Tumor Growth Drives the Natural Development of T-Cell Memory

We recently asked whether tumor growth and T_{reg} depletion could induce functional T-cell memory by studying immunity following curative surgery in mice bearing B16 melanoma (15).
As in our previous work (14), mice were inoculated with B16 melanoma, and then T<sub>reg</sub> cells were eliminated with a CD4-depleting antibody. This strategy eliminates CD4<sup>+</sup>CD25<sup>+</sup> T cells as well as any CD4<sup>+</sup> precursors of induced T<sub>reg</sub> cells. Following T<sub>reg</sub> depletion, i.d. primary tumors were surgically excised to attenuate T-cell priming and to extend the life span of the mice. Defining T-cell memory was challenging because classic memory T cells are defined based on their ability to persist following the clearance of antigen (4). Because tumor/self-antigens are never cleared, we chose to use an operational definition of memory as a functional T-cell response present at least 1 month following surgery.

To assess the development of T-cell memory, mice were challenged with B16 tumor cells in the flank 1 month after surgery. Not surprisingly, mice that had received surgical treatment alone were overtaken by secondary tumors. Mice that had received CD4 depletion alone, but no primary tumor, also succumbed to the second tumors. However, 40% to 60% of mice that had been depleted of CD4 T cells during growth of their primary tumors were protected against secondary tumors given as long as 2 months after surgery. Moreover, these mice developed systemic immunity, evidenced by their rejection of lung tumors inoculated i.v. Lung tumors that were already established at the time of surgery were also rejected, indicating a potential control of metastatic disease. Importantly, the depletion of CD8 T cells abrogated this long-lived tumor protection, providing evidence of CD8 T-cell memory.

These data established that growth of a poorly immunogenic tumor could induce functional T-cell memory, although the specificity of these memory T cells was not known. Our previous work had shown that short-term CD8 T-cell responses in T<sub>reg</sub>-depleted, B16 melanoma-bearing mice were specific for tumor/self-antigens (14). Among these antigens were the melanosomal membrane proteins TRP-2/DCT and gp100 (14). However, due to immunologic tolerance and antigen persistence, it seemed unlikely that T cells against melanosomal proteins would be sustained following surgery. Because of this, we were surprised to find memory CD8 T cells specific for both TRP-2/DCT and gp100 in mice with postsurgical immunity. TRP-2/DCT–specific T cells were present at least 30 days after surgery, and IFN-γ–producing and IL-2–producing transgenic T cells specific for gp100 were found as long as 150 days following surgery. Thus, tumor growth in the absence of T<sub>reg</sub> cells induced durable T-cell memory against self-antigens expressed by the tumor.

**Figure 1.** Model for the generation of postsurgical tumor protection and T-cell memory in hosts bearing poorly immunogenic tumors. Left, surgery alone is insufficient for providing immunity against poorly immunogenic tumors that do not naturally prime functional CD8 T-cell responses and may instead induce T<sub>reg</sub> development. Right, however, the depletion of CD4 T cells eliminates natural and induced populations of T<sub>reg</sub> cells, thereby enabling the priming of protective antitumor immunity during growth of a poorly immunogenic tumor. This tumor-primed immune response develops into functional CD8 T-cell memory against tumor/self-antigens following surgical excision of the primary tumor. CD4 depletion in tumor-bearing mice also leads to concurrent autoimmunity against the normal tissue counterpart of the tumor. This model shows that T<sub>reg</sub> depletion in hosts bearing poorly immunogenic tumors is sufficient for the generation of CD8 T-cell memory following surgical tumor excision.
Because little was known about T-cell memory against tumor/self-antigens, it was also important to characterize these T cells with regard to their phenotype and localization. Based on infectious disease models, the memory CD8 T-cell compartment can be divided into two phenotypically and functionally distinct subpopulations: central (TCM) and effector (TEM) memory (16). In vitro–generated TCM are more potent than TEM for mediating tumor rejection (2). However, it was unknown whether tumor antigen–specific TEM could be generated in hosts with persistent peripheral self-antigen. Interestingly, we found that mice with postsurgical immunity developed a mixed population of antigen-specific TEM and TCM. TEM dominated the population and were found in lung as well as lymphoid tissues, whereas TCM represented a smaller population that was only found in lymphoid tissues. These data illustrated that T cells recognizing tumor/self-antigens can develop into long-lived populations of TEM and TCM even in the face of persistent antigen.

Finally, we observed that a high proportion of Treg-depleted tumor-excised mice also developed an autoimmune response against normal melanocytes. This was evidenced by the outgrowth of white hair (on black mice) beginning at the surgery site and progressing to other locations with time. This showed that melanoma growth can induce an immune response against normal host melanocytes, and that such autoimmunity is normally prevented when tumor-excised mice also developed an autoimmune response against normal melanocytes. This was evidenced by the outgrowth of white hair (on black mice) beginning at the surgery site and progressing to other locations with time. This showed that melanoma growth can induce an immune response against normal host melanocytes, and that such autoimmunity is normally prevented when tumor-excised mice also developed an autoimmune response against normal melanocytes.

T reg cells that reemerge in tumor-excised hosts are less suppressive than those that arise in tumor-bearing hosts. For example, we have previously observed in murine gammaherpesvirus (MHV-68) infection that most antiviral effector functions are intact (20). In fact, CD8 T cells from MHV-68 persistently infected mice mediate more efficient control of a challenge infection compared with cells from mice that have cleared the virus (20). Therefore, some memory cells generated in the face of persistent antigen may actually be better adapted for long-term immune surveillance. Memory T cells in mice with postsurgical immunity might also possess this capability.

In summary, there is now convincing evidence that functional CD8 T-cell memory can be generated against tumor/self-antigens. In contrast to studies that use active immunization, our study shows that poorly immunogenic tumors themselves can induce tumor-specific T-cell memory after the hurdle of Treg suppression is overcome. This work stresses the importance of exploring immunotherapies in conjunction with Treg depletion and the surgical treatment of cancer to provide long-lived and meaningful control of recurrent and metastatic disease.

Acknowledgments

Grant support: NIH grant R01 CA120777, Melanoma Research Foundation New Investigator Award (M.J. Turka), and NIH grant CA103642 (E.J. Ushewood).

References

8. van Elias A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of


Tumor-Specific T-Cell Memory: Clearing the Regulatory T-Cell Hurdle

Anik L. Côté, Edward J. Usherwood and Mary Jo Turk


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/6/1614

Cited articles
This article cites 20 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/6/1614.full.html#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/6/1614.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.