Failure at the Effector Phase: Immune Barriers at the Level of the Melanoma Tumor Microenvironment

Thomas F. Gajewski

Abstract
The clinical investigation of numerous therapeutic cancer vaccine strategies has resulted in relative disappointment. Whereas a minority of patients have indeed experienced clinical benefit, the majority of patients show disease progression even in cases in which induction of functional tumor antigen–specific T-cell responses as measured in the blood is easily detected. This observation has led to interrogation of the tumor microenvironment for potential mechanisms of tumor resistance to the effector phase of the antitumor T-cell response. Poor chemokine-mediated trafficking of effector cells and the action of negative regulatory pathways that inhibit T-cell function have been identified as key limiting factors. Important negative regulatory pathways include T-cell anergy from insufficient B7 costimulation, extrinsic suppression by regulatory T-cell populations, direct inhibition through inhibitory ligands such as PD-L1, and metabolic dysregulation such as through the activity of indoleamine 2,3-dioxygenase. Recognition of these evasion mechanisms has pointed toward new therapeutic approaches for cancer immunotherapy.

Over the past decade, it has clearly been shown that most cancers express antigens that can be recognized by T cells of the host immune system. However, despite expression of antigens, spontaneous immune-mediated rejection of cancer, including of melanoma, seems to be a rare event. Based on the presumption that the priming phase of the antitumor adaptive immune response is inadequate, many investigators have pursued tumor antigen–based vaccine strategies in clinical trials. In several instances, increased frequencies of circulating tumor antigen–specific T cells result from such vaccination approaches, and some clinical responses, have been seen. However, the clinical response rate is in the range of only 5% to 10%. Although it could be argued that the magnitude or quality of these immune responses might be insufficient, other lines of evidence suggest that this is not the entire explanation. Tumor regressions when they are seen can occur with relatively low overall frequencies of tumor antigen–specific T cells being generated (1). In addition, very high preexisting frequencies of specific T cells even without immunization have been seen under conditions when tumors are obviously growing (2, 3). Together, these notions have prompted investigation into downstream barriers to T-cell–mediated tumor rejection, largely at the level of the tumor microenvironment. This brief review will focus on two major candidate categories of such resistance: deficient recruitment of T cells into tumor sites and immunosuppressive mechanisms that restrain the function of T cells that have successfully been recruited.

Chemokine-Mediated Migration into Target Tumor Sites

Following active immunization or adoptive T-cell therapy, effector T cells must migrate to target tissue sites to mediate ultimate tumor destruction. Homing of effector T cells to inflamed tissues is thought to depend on adhesion molecules, such as LFA-1 and VLA-4, and also on the activity of specific chemokines (4, 5). Chemokines are chemotactic cytokines that support the migration of different categories of inflammatory cells into tissues. It has been reported that primary melanoma lesions that contain a T-cell infiltrate express higher levels of the chemokines CXCL9/Mig and CXCL10/IP10 (6). This observation makes sense as these factors have been shown to recruit Th1-type T cells in vitro (7). Preclinical experiments have given conflicting information about chemokines produced directly by melanoma cells that could be relevant. Whereas some in vitro studies have suggested that CXCL12/stromal cell–derived factor-1 produced by melanoma tumor cells might mediate recruitment of CD8+ CTL in vitro, other studies have suggested that CXCL12/stromal cell–derived factor-1 might actually repel T cells (8, 9). In our own laboratory, we have gained additional insights by doing Affymetrix gene expression profiling on metastatic melanoma lesions, which revealed interesting categories based on chemokine patterns. We observed that approximately one third of tumors contained high levels of T-cell–specific transcripts, whereas the rest did not. The presence of T cells was associated with high expression of a wide range of chemokine genes. To narrow the list of candidates to those relevant for recruiting activated T cells, we examined the...
expression of chemokine receptors on effector versus naive human CD8+ T cells and found that CCR1, CCR2, CCR5, and CXCR3 were up-regulated in the effector state. Based on known chemokine/chemokine receptor interactions, these combined data narrowed the candidates to CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 as potentially important factors. Each of these was confirmed to induce migration of human CD8+ effector T cells using a Transwell assay in vitro, suggesting that this set may be most relevant for recruiting activated CD8+ effector T cells into metastatic melanoma tumor sites. Of note, the majority of melanoma tumors tested expressed CXCL12/stromal cell–derived factor-1 and CXCL8/interleukin-8 even if they lacked T cells, suggesting that these two chemokines are not sufficient for T-cell recruitment in vivo either directly or indirectly. These factors could be involved in recruiting other cell types, such as neutrophils, and also may contribute to carcinogenesis (e.g., through promoting angiogenesis; refs. 10, 11). Together, these data also argue that the failed production of specific chemokines represents one important barrier at the level of the effector phase of the antitumor immune response in that it limits recruitment of activated T cells into the location required for direct tumor cell killing. A hypothetical model by which T-cell–attracting chemokines might be produced within the tumor microenvironment is depicted in Fig. 1.

Fig. 1. Hypothetical chemokine network that may support recruitment of CD8+ T cells into tumor sites. Depicted are cellular components of the tumor microenvironment that may produce chemokines that recruit CD8+ effector T cells. The attribution of these chemokines to specific cell subsets is speculative based on the literature. MIP-1β, macrophage inflammatory protein-1β; MIP-1α, macrophage inflammatory protein-1α; IL-8, interleukin-8; SDF-1, stromal cell–derived factor-1; MCP-1, monocyte chemotactic protein-1; SLC, secondary lymphoid tissue.

Negative Regulatory Factors within the Tumor Microenvironment

It seems paradoxical that some tumors do indeed contain T cells, as one might expect that if activated effector T cells specific for tumor antigens became successfully primed and homed to tumor sites, then the tumor should not have progressed to the point of becoming clinically important. This apparent paradox seems to be explained by the presence of multiple negative regulatory mechanisms that inhibit T-cell function within the tumor microenvironment. Several studies have confirmed that T cells in tumor sites have an activated phenotype and include cells that react with tumor antigen peptide/class I MHC tetramers. However, in multiple cases, these CD8+ T cells have been found to be dysfunctional when analyzed ex vivo (12–14). Defective production of cytokines as well as deficient lytic activity associated with poor expression of granule proteins have been observed. Because functional antigen-specific T cells can be found in the blood, it seems that
this dysfunctional state is associated with their presence in the tumor microenvironment.

Several important candidate mechanisms of negative regulation have been identified to be relevant in human melanoma metastases and validated in animal models to limit antitumor immunity in vivo (15). These include T-cell anergy, extrinsic suppression by regulatory T cells (Treg), inhibition by ligation of negative regulatory receptors, metabolic perturbations through amino acid catabolism, and other mechanisms (15).

Metastatic melanoma tumors seem to be deficient in expression of B7-1 and B7-2 (16), which are important costimulatory factors for full T-cell activation (17). Stimulation through the T-cell antigen receptor without B7 costimulation has been shown to induce a hyporesponsive state termed anergy (18, 19). In support of this possibility, we recently have observed in a mouse model that a T-cell hyporesponsive state consistent with anergy occurs in mice bearing B7-negative tumors (20). In this situation, tumor rejection indeed can occur when B7-1 is transfected into the tumor cells, an observation that has been reported by multiple laboratories in other model systems (21, 22).

Several T-cell–extrinsic suppressive mechanisms also have been implicated at the tumor site. The circulating number of CD4+CD25+FoxP3+ Tregs has been reported to be increased in patients with several types of advanced cancer (15, 23). Tregs are a subpopulation of T cells that express high levels of the transcriptional regulator FoxP3 and inhibit the activation of conventional T cells (24, 25). Tregs also have been found at increased numbers within the tumor microenvironment, as detected using FoxP3 reverse transcription-PCR or analysis of FoxP3+ cells by flow cytometry or immunohistochemistry (26–28). Preclinical models depleting Tregs using anti-CD25 monoclonal antibodies cells have shown improved tumor control in vivo (29), although this intervention alone usually does not promote complete tumor rejection. Using FoxP3-green fluorescent protein knock-in mice generated by the Rudensky laboratory, we have recently examined complete depletion of Tregs by sorting for green fluorescent protein–negative cells and using these for adoptive transfer in vivo. Like CD25 depletion, this does result in improved tumor control but fails to lead to complete rejection.2 Still, these results clearly implicate the FoxP3+ T-cell population as having negative regulatory properties in the tumor context.

A third important negative regulatory pathway seems to be engagement of the inhibitory receptor PD-1 on T cells by PD-L1 expressed by tumor cells. PD-1 is a receptor expressed by activated T cells, which mediates inhibition of T-cell activation (30) likely through the recruitment of tyrosine phosphatases (31). The vast majority of melanomas and other tumors express PD-L1 either constitutively or in response to IFN-γ exposure (32). Interfering with PD-L1/PD-1 interactions has been shown to yield improved T-cell effector function in vitro and augmented tumor control in vivo (33, 34).

Metabolic dysregulation represents a fourth potential escape mechanism. The tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) is expressed in a subset of metastatic melanoma tumor sites. Although expression by tumor cells themselves has been reported (35), in some settings IDO seems to be expressed by infiltrating dendritic-like cells and endothelial cells (36). IDO has been implicated in immunologic tolerance at the maternal/fetal interface, and its activity has been shown to lead to T-cell dysfunction and also apoptosis (37, 38). Other model systems have implicated another amino acid–catabolizing enzyme, arginase, which can be produced by myeloid lineage cells and lead to T-cell dysfunction (15, 39).

Additional negative regulatory mechanisms that may be involved at the level of the tumor microenvironment include the soluble factors transforming growth factor-β (40) and interleukin-10 (41), the inhibitory ligand B7-H4 (42), the activity of inducible nitric oxide synthase (43, 44), glucose deprivation (45), and inhibition by other regulatory cell populations in addition to thymically derived Tregs. An illustration of key negative regulatory mechanisms within the tumor microenvironment is represented in Fig. 2. It is important to note that most melanoma metastases seem to have multiple negative regulatory factors in play simultaneously, implying that uncoupling two or more inhibitory mechanisms may be required for maximal therapeutic benefit. The relative contribution of each of these inhibitory processes in human metastatic melanoma will require careful systematic analysis in future studies.

FEATURES OF TUMOR CELL BIOLOGY THAT SHAPE THE MICROENVIRONMENT

The heterogeneity observed with respect to features of the melanoma tumor microenvironment suggests that there may be differences in the tumor cells themselves that dictate the nature of the microenvironment vis-à-vis the immune system. Several signal transduction pathways become active in melanoma either through mutation, epigenetic regulation, or overexpression. Activating mutations in Ras and B-Raf, loss of PTEN expression, and constitutive activation of Notch or signal transducer and activator of transcription (STAT) 3 are among the biochemical alterations seen in melanoma (46–49). As the patterns of these molecular alterations are variable between tumors, it seems likely that variation in the tumor microenvironment is at least partially dictated by differences in the expression of downstream genes that are regulated by differentially activated oncogenic pathways. Among the first possible links in this regard are the association between active B-Raf signaling in melanoma cells with defects in dendritic cell function (50). In addition, STAT3 activation in tumor cells has been associated with production of vascular endothelial growth factor that inhibits antigen presentation and also with deficient production for specific chemokines (51). Understanding further this interface between cancer biology and immunology represents a ripe area for future research.

HOW ARE HOST T CELLS EVER SPONTANEously PRIMED TO TUMOR ANTIGENS?

Although negative regulatory influences seem to be present that suppress the effector phase of the antitumor immune

2 J. Kline and T. Gajewski, unpublished data.
response, it is striking that tumor antigen–specific T cells can be found in tumor sites at all. This observation suggests that the priming phase that drives the differentiation of tumor-specific T cells from a naive into an effector phenotype can at times occur spontaneously. Many patients have spontaneous antibody responses that can be detected in the serum that recognize proteins on autologous tumor cells, supporting the notion that spontaneous priming of immune responses against cancers frequently occurs (52, 53). Murine models have shown a similar phenomenon, with the group of Spiotto et al. (54) reporting that the level of antigen expression by the tumor cells determines the efficiency of cross-presentation and tumor elimination. Interestingly, the dominant APC type that seems to be presenting tumor-derived antigens in mice may be a macrophage lineage cell (55). However, although spontaneous induction of adaptive immune responses does indeed occur, it is not at all clear what are the endogenous innate immune factors that enable productive tumor antigen cross-presentation to T cells in the absence of exogenous Toll-like receptor ligands. Inasmuch as tumor antigen cross-presentation may be important not only for the early priming phase of antitumor T cells but also for reinforcement of the effector phase of the immune response in the tumor microenvironment (56), gaining a better understanding of the innate factors involved in bridging to an effective adaptive immune response when it does occur spontaneously should provide important insights with therapeutic potential.

Opportunities for Therapeutic Intervention

Identification of these numerous limiting factors at the level of the effector phase of the antitumor T-cell response has pointed to several opportunities for the development of new therapeutics. The overall notion is that strategies to increase the frequency of tumor antigen–specific effector T cells ultimately may need to be combined with strategies to improve the effector phase to achieve optimal clinical benefit.

To support optimal migration of effector CD8+ T cells into the tumor microenvironment, strategies to introduce chemokines into tumor sites should be developed. Preclinical experiments have suggested the value of transfecting tumor cells to express specific chemokines to promote T-cell recruitment and improve tumor control in vivo (57–59). An alternative consideration is to use the tumor necrosis factor family member LIGHT. This ligand has been shown to promote generation of secondary lymphoid-like structures through interaction with the lymphotixin B receptor (60). Introduction of LIGHT into
tumors has been shown to promote recruitment of both naïve and activated T cells, which is associated with the production of CCL21 and other chemokines (61) and improved tumor rejection. Thus, this single factor may give a broader chemokine pattern that could be advantageous.

The potential to interfere with specific negative regulatory pathways also is becoming clinically feasible. T-cell anergy may be uncoupled by proliferation driven by homeostatic cytokines, such as interleukin-15 (62). Homeostatic proliferation in a lymphopenic host also seems to prevent or reverse T-cell anergy (20). Introduction of B7-1 into melanoma tumor sites using a recombinant vaccinia virus vector has been explored and has been shown to promote tumor regression in a subset of patients (63). Identification of molecular mediators of the anergic state also should yield pharmacologic targets that, when inhibited, might support reversal of anergy. We recently have identified diacylglycerol kinase as a potential anergy mediator that should be amenable to pharmacologic manipulation (64).

Extrinsic suppression by CD4+CD25+FoxP3+ Tregs also is being pursued. Dannull et al. were the first to explore the use of Ontak, an interleukin-2-diphtheria toxin fusion protein, to deplete CD25+ Tregs in patients. They observed partial and transient decreases in the number of circulating Tregs that was associated with augmented T-cell responses following active immunization (65). However, not all investigators have obtained similar results (66), so the optimal strategy to decrease the number of Tregs while increasing the number of effector CTL in patients is not yet clear. Preparation of a CD25-depleted T-cell product for adoptive T-cell therapy also is quite feasible for clinical translation.

Interference with the action of specific inhibitory molecules also is being evaluated. PD-L1/PD-1 interactions can be targeted using specific blocking monoclonal antibodies. Medarex and collaborators are currently evaluating a neutralizing antibody against PD-1 in early-phase clinical trials. IDO can be targeted using specific inhibitors as well. The furthest along in development is 1-methyltryptophan, which has been confirmed to be effective in preclinical models (35). Interestingly, 1-methyltryptophan may act synergistically with homeostatic proliferation to support improved tumor rejection (67), suggesting that anergy reversal and IDO inhibition may work cooperatively. We also have observed that homeostatic proliferation and Treg depletion synergistically promote tumor rejection in mice.3 These early observations are giving the first hints that the combinatorial approaches to eliminate several negative regulatory influences in concert may be necessary for an optimal therapeutic effect.

Conclusions

Evidence is accumulating that features of the tumor microenvironment could influence the outcome of T-cell–based immunotherapeutic interventions in the treatment of cancer. Two identified categories of barrier include deficient chemokine-mediated migration into tumors and presence of negative regulatory mechanisms that suppress T-cell function even if recruitment is successful. Manipulation of these mechanisms in mouse models has shown encouraging results at improving tumor control. Importantly, strategies amenable to clinical translation of these concepts are in development. Other investigators writing in this issue of Clinical Cancer Research have developed parallel strategies to increase the expression of costimulatory molecules (68), reduce the expression of inhibitory factors such as transforming growth factor-beta (69), and increase the activity of the innate immune system (70). It will be exciting to discover whether facilitating the effector phase of the antitumor immune response through such interventions, either alone or in combination with vaccines or T-cell adoptive transfer, will improve the therapeutic benefit of cancer immunotherapy as these studies unfold.

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References


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