Cranking the Immunologic Engine with Chemotherapy: Using Context to Drive Tumor Antigen Cross-Presentation towards Useful Antitumor Immunity

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Abstract

This review shows how tumor antigen cross-presentation is affected by the major therapeutic modalities including chemotherapy, radiotherapy, and surgery. We argue that this process could affect the way that a tumor works as its own cellular vaccine, and that it is differentially modulated by the choice of treatment. (Cancer Res 2006; 66(2): 601-4)

It is estimated that tumors typically accumulate $10^{12}$ or more mutations (1). Even though many of these were already present in the tissue cells from which the tumor originated and only a small proportion occur in the open reading frames of genes expressed by tumor cells, it is inevitable that any given cancer will express at least a few new antigenic determinants that could be recognized by the immune system. Despite this, the immune system rarely stops a tumor growth. It is likely that the same tolerance mechanisms that prevent immune responses against normal tissue cells expressing random mutations also block antitumor responses. Similar to self-antigens, tumor neo-antigens are constitutively and efficiently offered to the immune system through a process known as cross-presentation (2, 3). The fact that tumor neo-antigens are likely to be highly individuated suggests that the most promising approaches to immunotherapy will depend on our ability to turn a tumor into its own cellular vaccine (4).

Which Antigens Are Cross-Presented?

The process of antigen cross-presentation was discovered in 1976, when it was shown that immunization with lymphoid cells congenic for minor histocompatibility antigens (7) or with allogeneic or semisyngeneic SV40-transformed cells (8) resulted in the generation of specific CTLs that were restricted by host MHC class I molecules. Thus, antigen must have been transferred from the donor cells to the hosts APCs. Now, almost 30 years later, it is evident that cross-presentation of tumor and self-antigens is a very efficient process, but it is still not entirely clear where the antigen comes from. Controversy persists over the question of whether live, apoptotic, or necrotic cells are the best source of cross-presented antigen. Also it is not clear how antigen transfer takes place and what form of antigen is required. Answering these questions will be important for the design of new immunotherapeutic strategies.

Particulate and cellular antigens are cross-presented much more efficiently than soluble antigens (3), suggesting that antigen is captured by phagocytosis. Several different mechanisms have been proposed to explain antigen transfer from donor cells to APC. These mechanisms include phagocytosis of apoptotic bodies, nibbling from live cells and transfer of antigen by molecular chaperones such as heat-shock proteins (Hsp). A related issue is whether the transferred antigen consists of mature proteins, peptides, or defective ribosomal products, which are known to be the main source of antigenic peptides for the cells’ endogenous MHC class I processing pathway (9). Current evidence implicates mature proteins as the primary source of cross-presented antigen. First, treating donor cells with irreversible proteasome inhibitors blocks direct antigen presentation but enhances cross-presentation (10). Second, epitopes located within cleaved leader sequences are not cross-presented, suggesting that short-lived peptides cannot enter the cross-presentation pathway (11). On the other hand, data from Binder and Srivastava indicate that when cell lysates are used as a source of antigen, transfer depends on Hsps (12). This suggests that Hsp-chaperoned peptides can enter the cross-presentation pathway, presumably via receptor-mediated endocytosis (12). The issue is further complicated by another study implicating mature proteins as the source of cross-presented antigen in cell lysates (13). Irrespective of the mechanism, it is evident that tumor antigens are efficiently cross-presented to the immune system (Fig. 1). The question, therefore, is why the immune system fails to respond in an adequate fashion because tumors usually induce only relatively weak immune responses.
What Does the Immune System Do with Cross-Presented Antigens?

Cross-presented antigens do not necessarily induce an immune response (3). Cross-presentation is involved in the maintenance of tolerance to self-antigens (cross-tolerance) as well as in the induction of immune responses (cross-priming). In both situations, a subset of DCs that, at least in the mouse, expresses CD8 is most likely responsible for cross-presentation (3). We will now deal with the question of how such APC “decide” whether the outcome of cross-presentation is tolerogenic or immunogenic.

Recent data indicate that the difference between tolerance and immunity depends on the presence or absence of inflammatory signals associated with viral or bacterial pathogens, as well as on costimulatory signals. Pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide, double-stranded RNA, and unmethylated CpG motifs, are recognized by specific receptors, including the Toll-like receptors. Through their receptors, PAMPs activate DCs and promote immune responses (14). In addition, soluble mediators, including the type I IFNs stimulate immune responses to cross-presented antigens (15).

The necessity to activate DCs by external stimuli such as PAMPs, costimulatory signals, and IFN-α/β suggests that the default action of such DCs is to maintain tolerance to cross-presented antigens. This is important for tumor immunity because tumors often masquerade as normal tissue. In an elegant series of experiments, Sherman and coworkers showed that when the influenza virus HA gene is transgenically expressed in the islets of Langerhans, it is constitutively cross-presented in the draining lymph nodes. In the absence of any other signals, HA-specific CD8 T cells proliferate but fail to acquire effector functions and are eventually deleted (16). In other words, tolerance is maintained. Adding the proinflammatory cytokine IL-12 and a costimulatory signal in the form of an agonistic anti-CD40 antibody, triggered acquisition of effector functions by HA-specific CD8 T cells and led to islet infiltration of these T cells (16).

Taken together, the data suggest that cross-presented antigens are likely to promote tolerance, or partial activation, in the absence of inflammatory signals or costimulation. In contrast, cross-priming occurs only when antigen is cross-presented in the context of microbial infection or costimulation, or in the presence of cognate CD4 T cell help.

Why Does the Immune System Fail to Attack Tumors?

The two key features that differentiate tumors from somatic self in this regard are (a) antigenicity and (b) rate of growth. So it

Figure 1. Tumors constitutively present antigens via DCs (1). Chemotherapy increases the rate at which these antigens are cross-presented, but in the absence of any further signals, there is only weak T cell priming. APC-directed immunotherapy (2) promotes efficient cross-priming and can drive the activation and expansion of antigen-specific T cells (3).
is reasonable to ask how important is the process of antigen cross-presentation in the development of an immune response to tumors? The observation by Trinchieri et al. that SV40-transformed tumor cells induce SV40-specific CTL that are restricted by the host’s MHC class I molecules showed for the first time that not only are tumor antigens cross-presented, but also that they can induce specific responses (8). It is now clear that in the same way as normal self-antigens, tumor-derived antigens are constitutively cross-presented in draining lymph nodes (17–20). Cross-presentation of tumor antigens has also been shown in cancer patients who received a vaccine comprised of irradiated allogeneic tumor cells (21). The rest of this section will deal with the issue of how cross-presented tumor antigens are perceived by the immune system and what regulates whether cross-presentation results in tolerance or immune activation.

The evidence indicates that the immunogenicity of cross-presented tumor antigens ranges from tolerogenic (20) to weakly immunogenic (19). Using HA-transfected mesothelioma cells, Marzo et al. showed that the HA protein is cross-presented in the tumor-draining lymph nodes and triggers a weak, transient cytotoxic T cell response (19). Subsequent experiments revealed that weak in vivo CTL activity was sustained over 4 weeks of tumor growth (22). Thus, cross-presentation in this tumor model seems to be a cross-priming rather than a cross-tolerizing event. Nevertheless, cross-priming does not prevent tumor progression. Sherman and coworkers reported slightly different results using a tumor model in which transgenic mice expressed both the HA protein and the SV40 large T antigen under the control of the rat insulin promoter (20). These mice spontaneously develop pancreatic β-cell tumors. The authors then adoptively transferred HA-specific CD8 T cells into tumor-bearing mice and found that the acquisition of effector functions by these T cells depended on the number of cells transferred. Adoptive transfer of low numbers of HA-specific T cells resulted in proliferation without effector function. Armed CD8 effector T cells were only observed when very high numbers of HA-specific precursor T cells were injected. Because only small numbers of tumor-specific CD8 T cells would be present under normal conditions, it can be argued that tumor antigen cross-presentation is nominally tolerogenic in that the tumor does not provide the inflammatory signals needed to support differentiation of CD8 T cell precursors into effector cells (20).

In summary, tumor antigens are efficiently cross-presented but it is less predictable how CD8 T cells will respond to these antigens. The examples that we have discussed illustrate this (19, 20, 22). The variable levels of cross-priming, in the face of quite robust cross-presentation, may also provide an explanation for Zinkernagel’s conclusion that tumor antigen cross-presentation is of little relevance for the induction of CD8 T cell responses against tumor antigens (6). Indeed, there are many examples in which tumors fail to cross-prime, as originally noted by Bevan in 1976 using p815 mastocytoma cells (7), and as shown by Ochsenbein et al. for a wider range of tumors (6). However, lack of cross-priming does not exclude cross-presentation, it may simply indicate an opportunity for therapeutic intervention (20).

**Implications for Therapy**

Cross-presentation does not necessarily lead to cross-priming, and even when cross-presented tumor antigens do prime antitumor immune responses, it seems that these responses are rarely adequate to stop tumor growth. Thus, the challenge for therapeutic intervention is to change the context in which tumor antigens are cross-presented into a more inflammatory one. This could be achieved by activating Toll-like receptors with, for example, CpG-containing oligonucleotides or poly I:C, by using cytokines such as IL-12 or IFN-α/β or by providing additional costimulatory signals such as agonistic anti-CD40 antibodies. All of these approaches have been tested with some success in animal models and, to some extent, in human clinical trials.

A different strategy is to use such immunotherapeutic strategies in combination with conventional therapeutic modalities, such as chemotherapy, radiotherapy, and surgery (4). This approach has two advantages. A first, practical, advantage is that it takes into account the clinical reality. Integration of immunotherapy into standard treatment regimens should facilitate its implementation. Second, conventional treatment could enhance the efficacy of subsequent immunotherapy. For instance, many chemotherapies kill cells by apoptosis and apoptotic cells are a good source of cross-presented antigen (3). Indeed, work from our laboratory revealed that the massive tumor cell apoptosis induced by chemotherapy leads to increased levels of cross-presentation (4, 22). This process could unmask additional tumor neo-antigens by increasing the amount of material available for cross-priming (Fig. 1). In these studies, subsequent immunotherapy, using an agonistic anti-CD40 antibody, cured 80% of tumor-bearing mice, when neither therapy was effective on its own (23). Although this combination of therapies was not effective for very large tumors, the same 80% rate of cure was achieved when partially resected large tumors were then treated with the chemotheraphy/immunotherapy combination (24). These experiments suggest that the increased levels of tumor neo-antigen cross-presentation after chemotherapy could be exploited by immunotherapy. Importantly, these results contest the notion that cross-presentation of antigen from apoptotic cells is a priori a tolerogenic event (25). We hypothesize that the massive apoptosis induced by chemotherapy could release proinflammatory mediators such as Hsps and IL-6, which promote cross-priming. Our current understanding of antigen cross-presentation predicts that any therapy that delivers higher levels of cross-presented tumor antigens to the draining lymph nodes could synergize with immunotherapy (Fig. 1). As a case in point, it has recently been shown that tumor destruction by radiofrequency ablation creates a source of antigen for the induction of antitumor immunity by immunotherapy (26).

In conclusion, the therapeutic exploitation of cross-presented tumor neo-antigens to boost antitumor immune responses shows that a tumor could be its own cellular vaccine. This idea represents a paradigm shift because the historical emphasis in tumor immunotherapy has been on the identification of and vaccination with defined tumor-associated antigens (27). We propose that in order to transform a tumor into its own vaccine, we need to crank the immunologic engine by using chemotherapy to increase the level at which tumor antigens are cross-presented (Fig. 1). The spark that fires the motor could be supplied by proinflammatory adjuvants that are directed at the APC (4).

**Acknowledgments**

Received 8/19/2005; revised 9/22/2005; accepted 11/18/2005.
References

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