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Tumor antigen presentation: changing the rules

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Abstract Cell-based tumor vaccines have been developed on the basis of the hypothesis that tumor cells can be genetically modified to present antigen to T lymphocytes directly. Contrary to expectations, cross-priming is the predominant pathway for activation of tumor-specific CD8+ T cells, while direct presentation of antigen dominates activation of tumor-specific CD4+ T cells. These results pose interesting paradoxes for the generation of immune responses, and have definite implications for the development of anti-cancer vaccines.

Key words Tumor antigen presentation · Immunotherapy · MHC class II · Cross-priming · Cell-based cancer vaccines

Introduction

Many recently developed cancer immunotherapeutic strategies use tumor cells transfected/transduced with genes encoding molecules that enhance immune responses. These approaches are based on the hypothesis that genetically modified tumor cells will be effective antigen-presenting cells (APC) of tumor-associated antigens (TAA), and that immunization with the modified cells will stimulate a potent antitumor immune response in tumor-bearing individuals. Most approaches focus on activating tumor-specific CD8+ T cells. Some strategies use tumor cells transfected/transduced with cytokine genes to enhance CD8+ T cell development, while other strategies use tumor cells transfected with costimulatory molecules to deliver the antigen-specific signal and the second, costimulatory, signal concomitantly to CD8+ T cells (reviewed in [6]). These approaches have shown promising results in animal systems, and several are being tested in clinical trials (http://cancernet.nci.nih.gov).

Despite their capacity to enhance cytotoxic T cell (CTL) activity and prolong immune memory, activation of tumor-specific T helper (T_h) cells has been less extensively pursued. Immunotherapeutic strategies that have targeted tumor-specific T_h cell activation, however, have yielded significant antitumor activity [11, 17, 21, 26]. Several of these studies have used tumor cells transfected with syngeneic MHC class II genes as immunogens to protect naive mice [11, 17, 26] or to treat mice with primary tumors or metastatic disease [3, 32]. Immunotherapy with class-II-transfected tumor cells is based on the hypothesis that the tumor cells present endogenously synthesized tumor peptides in the context of MHC class II molecules and efficiently activate tumor-specific CD4+ T_h lymphocytes [25, 27]. Although MHC class II molecules usually present exogenously synthesized antigen, there is precedence for the presentation of endogenous antigen [36]. Furthermore, in vitro studies using class-II-transfected mouse tumor cells, demonstrate that, in the absence of the class-II-associated accessory molecules invariant chain and DM, MHC-class II-expressing tumor cells are efficient APC for endogenously synthesized molecules [1].

Activation of tumor-specific CD8+ T cells occurs mostly via cross-priming, while activation of tumor-specific CD4+ T cells is via direct and cross-priming

Since the genetic modifications were designed to enhance the antigen-presenting capacity of the tumor cells, it was anticipated that the modified tumor cells would directly present TAA to T lymphocytes (Fig. 1a). Direct antigen presentation by cytokine- and B7-modified tumor cells to CD8+ T cells was particularly expected, because endogenously synthesized antigens, such as TAA, normally intersect the MHC-class-I-processing pathway [39], and are pre-

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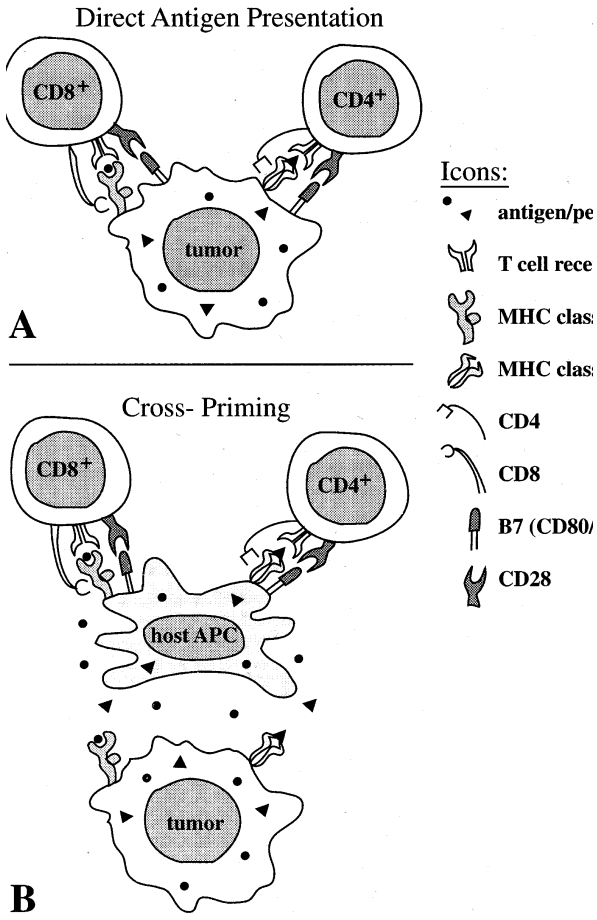

Fig. 1A Direct antigen presentation or direct priming occurs when the genetically modified tumor cell (in this case tumor cells transfected with syngeneic MHC class II genes) directly interacts with the responding T lymphocyte (in this case a CD4+ T helper cell) and initiates T cell activation. **B** Cross-priming or indirect antigen presentation occurs when the genetically modified tumor cell sheds or releases tumor antigens and the antigen is processed and presented by host-derived, professional antigen-presenting cells (*APC*). There is no direct interaction between the genetically modified tumor cells and the activated T lymphocytes

sented at the cell surface in the context of MHC class I molecules. Contrary to this expectation, however, host-derived cells, and not tumor cells, are the APC for CD8+ T cells when the immunizing tumors are cytokine-modified (cross-priming, Fig. 1b) [9, 15, 16, 31]. Antigen presentation to CD8+ T cells by B7.1-transfected tumor cells is less clear-cut. In one case, B7-transfected tumor cells directly presented tumor-encoded antigen to CD8+ T cells [9] while, in another study, cross-priming occurred [16]. Although cross-priming has been observed in other immunization systems [4, 5], direct presentation of complexes between MHC class I antigens and peptides is thought to be the predominant mechanism for CD8+ T cell activation. Cross-priming of tumor-specific CD8+ T cells following immunization with genetically modified tumor cells, therefore, is unexpected and unusual.

In contrast to activation of CD8+ T cells, immunization with MHC-class-II-transfected tumor cells activates tumor-specific CD4+ T cells by both direct and cross-priming pathways, although the direct pathway is used more frequently [2]. In one sense, this result is not surprising, since the class II transfectants were designed to present endogenously synthesized tumor antigen directly. However, since MHC class II molecules usually present exogenously synthesized antigens, it is an unusual antigen-presentation pathway for MHC class II molecules.

Are the pathways of antigen presentation to tumor-specific CD4+ and CD8+ T cells fundamentally different, or can the experimental results of these studies be reconciled?

There are several possible explanations for the apparent dichotomy between activation of CD4+ and CD8+ T cells by genetically modified tumor cells. A major deviation in the antigen-presentation studies is the time at which CD4+ and CD8+ T cell activity was measured and whether a primary or secondary response was evaluated. The studies assessing CD8+ T cell activation were conducted at least 2 weeks after immunization and, in some studies, T cells were boosted or irradiated tumor cells were used [9, 15, 16, 31]. In contrast, activation of CD4+ T cells was assessed within 1 week of immunization, live cells were the immunogen, and no boosts were performed [2].

Direct antigen presentation by tumor cells requires intact tumor cells, while cross-priming should be favored if soluble tumor antigen or tumor debris is available to host APC. Since live tumor cells are likely to remain intact longer in vivo than irradiated tumor, immunization with irradiated cells may favor cross-priming. In addition, with increasing time in vivo, some tumor cells may be destroyed, releasing tumor antigens. Assaying 1 week after immunization, therefore, may reveal cross-priming due to the availability of soluble tumor antigen, while assaying earlier than 1 week after immunization may reveal direct presentation because viable tumor cells with intact peptide/MHC complexes are present. As the antitumor response matures, activated effector T cells will be generated that destroy intact tumor cells, releasing soluble tumor antigen. Therefore, at the earliest stages of the immune response, direct antigen presentation may predominate and, as the response matures, cross-priming may increase and dominate as soluble tumor antigen becomes available to host APC (Fig. 2). Since the CD8+ T cell activation studies were performed at late assay times (more than 2 weeks after immunization), while the CD4+ T cell activation studies were performed earlier (1 week after immunization), the differences in priming may reflect differences in maturity of the immune response, rather than fundamental differences in pathways of CD4+ and CD8+ T cell activation.

The differences between activation of CD4+ and CD8+ T cells may also be due to variations in the immunizing tumor cells. Most of the studies showing cross-priming were performed with tumor cells genetically modified to secrete cytokines [9, 15, 31] while those studies showing direct antigen presentation used tumor cells genetically modified

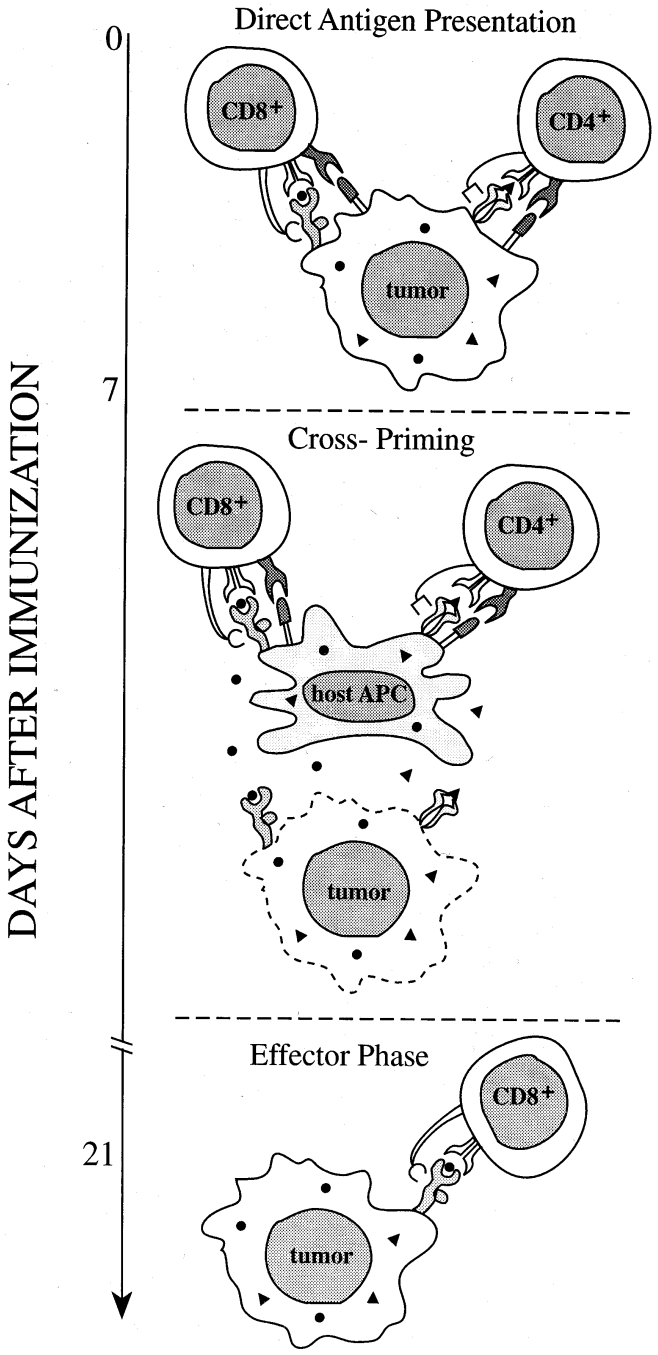

Fig. 2 Schematic drawing depicts the "maturing" of the antitumor immune response following immunization with MHC-class-II-transfected tumor cells. During the early stages of the response (up to 1 week after immunization) the class-II-transfected tumor cell is the predominant APC to CD4+ T cells. Later in the response (more than 1–2 weeks after immunization), soluble tumor antigens become available as a result of non-specific tumor cell lysis (e.g. via natural killer cell, macrophage, or inflammatory responses), and host-derived APC begin to present tumor antigens. As the immune response matures, host-derived APC become the predominant APC, and cross-priming is the principal pathway for T cell activation

to express integral plasma membrane proteins [2, 9]. For cytokine-modified tumors, cross-priming to CD8+ T cells may be favored, since cytokines are diffusible molecules and can act at a distance from their site of production. In addition, expression of cytokine receptors is not limited to particular cell types, so diffusible cytokines may affect a variety of target cell types. In contrast, integral membrane proteins may be more restricted in their action because they require cell-cell contact, and therefore, favor direct antigen presentation. As such, only cells in close proximity to the modified tumor cells would potentially be affected. Also, the corresponding receptors for the transfected integral membrane proteins (i.e. TcR and CD28) are limited to T cells, and direct interaction of the transgene products with their receptors is the only known process by which the transgenes can mediate their effect. Thus, the variation between CD4+ and CD8+ T cell activation may be the result of the specific tumor cell modifications rather than differences in the pathways of T cell activation.

Where does CD4+ T cell activation occur following immunization with MHC-class-II-transfected tumor cells?

During a conventional immune response, CD4+ T cells are activated within a lymph node. Soluble or particulate antigen is either trapped by APC in lymph nodes, or it is taken up in the periphery by APC and transported to a lymph node. In either case, the antigen is processed by the professional APC, and presented in the context of the APC MHC class II molecules [20]. Since lymph nodes contain the highest density of lymphocytes, this trafficking pattern favors exposure of antigen to the maximal number of CD4+ T cells, and subsequent activation of those CD4+ T cells with the appropriate receptors.

It is unclear if the same presentation pathway occurs when tumor cells are the APC for tumor antigens. Since the antigenic tumor peptide is bound to tumor-cell-encoded MHC class II molecules, it would be necessary for the intact tumor cell to migrate to the lymph node to be exposed to the maximal number of CD4+ T cells. Tumor cells traffic via the lymphatic system when they metastasize, so they home to draining lymph nodes [37]. The alternative situation, of CD4+ T lymphocytes trafficking to the tumor site seems unlikely, since it is difficult to envision a sufficient number of CD4+ T cells with the appropriate antigen receptors homing to the tumor site. CD4+ T cell activation, therefore, most likely occurs within the nearest draining lymph node.

As the antitumor immune response matures and effector T cells (presumably CD8+ T cells) are produced, tumor cells will be lysed, and soluble tumor antigen will be available to professional APC (Fig. 2). Further CD4+ T cell activation would then proceed as in conventional immune responses via presentation by professional APC in a lymph node. The therapeutic benefit of MHC-class-II-transfected tumor cells, therefore, may be their ability to initiate CD4+ T cell priming quickly and effectively, which, in turn, may activate direct antigen presentation to CD8+ T cells or cross-priming by professional host-derived APC.

If tumor antigens are presented both directly and indirectly by genetically modified tumor cells, what are the implications for the development of tumor vaccines?

The preceding studies raise several key points for the development of cancer vaccines targeted to the activation of tumor-specific T cells.

Optimal antitumor activity will be achieved if tumor-specific CD4+ and CD8+ T cells are activated

Although most immunotherapeutic strategies have focussed on activating tumor-specific CD8+ T cells, optimal antitumor activity is achieved if both CD4+ and CD8+ tumor-specific T cells are induced [3, 18, 19]. Even in situations where cross-priming of CD8+ CTL is sufficient for enhancing antitumor immunity (e.g. by immunization with tumor cells expressing interleukin-3 or granulocyte/macrophage-colony-stimulating factor), the immunity is dependent on CD4+ T cells [13, 30]. Likewise, if tumor immunity is to play a role in limiting the recurrence of primary tumor or the future onset of metastatic disease, then CD4+ and CD8+ immunological memory should be optimized. Future vaccine development strategies, therefore, should address activation of CD4+ and CD8+ tumor-specific effector and memory T cells.

Immunization with genetically modified autologous or syngeneic tumor cells may not be necessary for optimal CD8+ T cell activation

Genetically modified tumor cells are currently being tested in numerous clinical trials as cell-based vaccines for the activation of tumor-specific CD8+ T cells (http:\\cancer-net.nci.nih.gov). However, if cross-priming is the principal pathway for activation of tumor-specific CD8+ T cells, then the production of such modified tumor cells may not be necessary for optimal CD8+ T cell activation. In contrast, vaccine development should focus on optimizing class-I-restricted tumor peptide presentation by professional, host-derived APC. It is clear from transfection studies that cross-priming is facilitated by immunization with cytokine-transfected tumor cells [15, 31], and this approach should, therefore, be pursued. Immunization protocols for tumor vaccines using the following agents are also being tested in both animal systems and clinical trials: class-I-restricted, defined tumor antigen epitopes [23, 33]; peptide-pulsed professional APC, such as dendritic cells [10, 24, 27, 29, 40]; professional APC transduced with bulk tumor cell RNA or RNA encoding specific class-I-restricted tumor antigens [7]; heat-shock proteins [35]; and phagocytic cells fed with tumor-antigen-coated latex beads [14, 38]. These approaches are aimed at targeting defined or undefined tumor epitopes to the MHC class I molecules of professional APC, either via intersection with the intracellular class I processing pathway, or by peptide exchange with existing class I molecules. Regardless of the precise targeting method, if cross-priming is the most efficient pathway for activation of tumor-specific CD8+ T cells, then vaccine development should focus on maximizing class I epitope presentation by professional, host-derived APC.

Immunization with cell-based vaccines consisting of genetically modified autologous or syngeneic tumor cells may provide optimal CD4+ T cell activation

In contrast to CD8+ T cells, genetically modified tumor cells are efficient APC for activation of CD4+ T_h lymphocytes. Additional engineering of tumor cells so that they are more efficient presenters of endogenous tumor antigens may further improve their ability to activate tumor-specific T_h lymphocytes quickly and efficiently. CD8+ T cell activation may also be enhanced, because such vaccines should favor co-localization of tumor-specific CD4+ and CD8+ T cells, allowing for efficient delivery of T_h cytokines to CD8+ T lymphocytes. Further engineering could include expression of MHC class II and B7 molecules, plus additional accessory molecules that facilitate antigen presentation to CD4+ T cells, such as CD40 [8, 22] and/or 4-1BBL [12, 28], or molecules that quantitatively enhance T cell activation, such as superantigens [34].

These cell-based immunotherapeutic reagents have advantages and disadvantages. The major disadvantage is that autologous (or perhaps allogeneic) tumor is required, and customization for individual patients will be necessary. The major advantage is that characterization of specific tumor antigens is not required, so that such cells could be effective immunotherapeutics for a wide variety of malignancies. Genetically modified tumor cells, therefore, may be effective immunotherapeutic agents for maximizing CD4+ T_h cell generation, and they should be considered as potential cell-based vaccines.

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