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REVIEW

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How tumors escape immune destruction and what we can do about it

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Abstract There is strong circumstantial evidence that tumor progression in cancer patients is controlled by the immune system. As will be detailed below, this conclusion is based on observations that tumor progression is often associated with secretion of immune suppressive factors and/or downregulation of MHC class I antigen presentation functions. The inference is that tumors must have elaborated strategies to circumvent an apparently effective immune response. Importantly, a tumor-specific immune response cannot be detected in most individuals. While this failure is in part technical, it also suggests that the magnitude of the immune responses to which tumors have to respond is low. This raises the concern, which is the underlying theme of this commentary, that a more robust immune response elicited by deliberate vaccination will exacerbate the rate of immune escape and nullify the potential benefits of immune-based therapies.

Key words Cancer immunotherapy · Tumors · Immune escape

Tumor escape

I will start by making an assumption which at best is only partially correct: that the MHC class I-restricted $CD8^+$ cytotoxic T cell (CTL) effector arm of the adaptive immune response is best equipped to recognize the tumor as foreign and initiate the cascade of events resulting in tumor destruction. Therefore, discussion will be centered on vaccination strategies designed to enhance the CTL arm of the antitumor response and consequently on mechanisms of tumor escape from CTL.

It is convenient to classify various mechanisms of tumor escape from CTL into three categories: global, antigen-specific, and downregulation of the class I presentation pathway.

Global mechanisms

Secretion of immunosuppressive factors, or tumor growth in partially immunoprivileged sites, belong to this category. Secretion of immunosuppressive factors such as TGF-beta has been described in some instances, notably in gliomas [8]. Immunosuppressive factors could interfere with multiple steps and pathways in the generation of an effective immune response, including the activation or function of CTL and $CD4^+$ T helper cells. How effective are such mechanisms? It is conceivable that the in vivo selection process resulting in the secretion of a certain level of TGF-beta would lead to only a partial protection from immune recognition which is sufficient to provide a survival advantage in the face of a naturally occurring immune response. If so, effective vaccination that would generate an immune response considerably above what the tumors have encountered, could tilt the balance toward tumor destruction.

Antigen-specific mechanisms

Tumor antigen-loss variants

The emergence of tumor antigen-loss variants in the face of an effective immune response elicited by a vaccination protocol should be expected. Selection of viral variants resistant to CTL carrying mutations in the relevant T cell epitopes has been described [4]. Loss of the MART-1/Melan-A melanoma tumor-associated antigen has been seen in a patient with recurrent metastatic melanoma [17]. Furthermore, in a recent study of a melanoma patient vaccinated with a gp100-derived class I peptide, a meta-

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static lesion progressing after initial response lost gp100, but not MART-1, expression [15]. If this observation can be statistically substantiated, it will be the first evidence that a vaccination protocol can elicit a physiologically relevant antitumor immune response in cancer patients, but at the same time underscore the potential for selection of antigen-loss variants. This study notwithstanding, there is a paucity of documentation of CTL antigen-loss variants in cancer patients, likely reflecting the early stages of cancer vaccine development.

The solution to the emergence of antigen-loss variants is the use of polyvalent vaccines containing multiple tumor antigens. The problem at this moment is that tumor antigens have been identified in a few cancers, notably melanoma [23]. Moreover, it is far from clear to what extent the CTL response elicited by those antigens will contribute to the eradication of the antigen-bearing tumor [1]. The alternative approach is to vaccinate with unfractionated tumor-derived material as source of antigen, the assumption being that it will contain multiple tumor antigens, some of which in combination will elicit a sufficiently strong, and therapeutically beneficial, immune response [12, 26]. The concern with this approach is the increased likelihood of inducing unacceptable levels of autoimmune responses against "self" antigens.

The emergence of CTL antigen-loss variants is likely to be facilitated by the fact that tumor cells exhibit extensive heterogeneity [6, 7, 20]. It is prudent to assume that significant variations will exist between the antigenic profiles of metastatic lesions and the primary tumors or the tumor specimen used as source of antigens for vaccination. Effective vaccination, therefore, is predicated on the assumption that the metastatic lesions share at least some of the antigens used for vaccination. Clearly, the more antigens used in vaccine preparation the more likely that the metastatic lesion will not escape immune destruction. Until such time that multiple, shared, and effective antigens are discovered for a given cancer, the use of patient-specific tumor-derived antigenic mixtures would be the best approach to address this concern. (The reasonable yet unproven assumption is made here that effective tumor rejection antigens are mainly patient-specific.)

Tolerance

Under normal circumstances the immune system is not activated against self antigens, including antigens expressed in peripheral tissues with no thymic access. The functional tolerance exhibited against the latter class of antigens can be passive, where cognate T cells ignore the antigen, or active, where autoreactive T cells are either eliminated or anergized. Under certain conditions, the immune system can become also tolerant of foreign antigens. Whether tumor antigens are foreign or self, and what is anyway foreign and self as far as the immune system is concerned, are hotly debated and yet unresolved issues [18], and could be "dangerous" to consider here.

There is at present little evidence to suggest that tumor antigen-specific tolerance is a general and important phenomenon in human cancer, especially in the choice candidates for immunological intervention, i.e. clinically healthy patients with low tumor burden. The possibility that tumors could elicit a tumor antigen-specific state of tolerance has been shown in one study using a transgenic mouse model. In this study, B cell tumors expressing class II molecules and a model tumor antigen foreign to the host induced a rapid tolerance of cognate CD4 T cells carrying a transgenic TCR [27]. How generalizable this phenomenon is to human tumors, especially class II-negative tumors of non-B cell origin, is however unclear. In other transgenic models, expression of self antigens on tumor cells does not appear to induce unresponsiveness in the pool of antigen-specific autoreactive CTL [14, 25].

Downregulation of the class I presentation pathway

Mutations along the class I presentation pathway should be the simplest way for tumors to escape CTL elimination since it can be achieved by one or two mutational events (two mutations to inactivate both alleles or one mutation to create a dominant negative inhibitor). Since mutations along the class I presentation pathway will result in a significant reduction or total loss of MHC class I expression on the cell surface, the tumor cells may become more susceptible to elimination by NK cells. Thus, tumor cells may have to reach an uneasy compromise resulting in partial rather than complete inhibition of class I antigen processing, or undergo an additional selection to acquire NK resistance.

Complete loss of class I expression in murine tumors is not common. A typical example is the B16/F10.9 tumor line derived from the original B16 melanoma tumor by repeated selection for increased metastatic potential [21]. B16/F10.9 tumor cells express very few MHC class I molecules on the cell surface. Nevertheless, class I expression is not completely lost since the tumor cells can serve as targets for class I-restricted CTL. Importantly, by vaccination with tumor antigen-loaded dendritic cells, CTL responses and protective immunity can be induced in animals against the B16/F10.9 tumor cells [3, 19]. The B16/F10.9 example illustrates a very important and apparently general paradigm seen in murine studies that would hopefully apply to the cancer patient: the selection pressures prevailing in the tumor-bearing animals lead to reduction, but not complete elimination, of MHC class I expression to a level that is sufficient for the survival of the tumor as a metastatic lesion. Using an effective vaccination protocol, at least in animal models it is possible to generate an antitumor response that can overcome the tumor deficiency in antigen presentation and eliminate the tumor. The key here is of course the effectiveness of the vaccination protocol.

Downregulation of MHC class I expression is frequently seen in human tumors, and is particularly pro-

nounced in metastatic lesions [9, 10, 11, 24]. This is circumstantial but nevertheless compelling evidence of the role of CTL in controlling tumor progression in cancer patients. MHC class I expression has been mainly analyzed in surgically removed tumor specimens using immunohistochemical methods. Partial reduction or complete loss of MHC have been reported, encompassing all MHC molecules or limited to particular alleles. MHC loss can be seen in some but not all lesions of the same patient. Loss of MHC can be uniform where all cells within the lesion are affected, or heterogeneous where the extent of MHC reduction varies among the cells within the lesion. Downregulation of MHC class I expression has been attributed to mutations in β2-microglobulin (β2-m), transporter associated with antigen presentation (TAP) proteins, or the proteosomal LMP-2 and LMP-7 proteins [11, 24].

Owing to the limitations of immunohistochemical analysis, it is not clear if lack of staining reflects absolute or merely partial reduction of class I expression. This may be important because, as suggested from animal studies discussed above, if MHC class I expression is reduced but not completely eliminated, immunotherapy and CTL-mediated destruction of the low class I expressing tumor cells should still be feasible. In several instances, however, the absence of β2-m expression has been shown to result from complete loss of both β2-m alleles [2, 5, 22]. Such tumor cells should be completely resistant to CTL-mediated destruction, but sensitive to NK elimination.

Additional evidence implicating loss of MHC class I expression as a mechanism for tumor escape from CTL-mediated elimination comes from a longitudinal study of a melanoma patient. Tumor cells removed during initial surgery presented nine different antigens restricted to four separate HLA class I alleles to CTL clones established from the patient. The patient remained disease-free for 5 years after which a metastasis was detected. Notably, a cell line established from the metastatic lesion had lost all four alleles that had previously been shown to present melanoma antigens.

The frequent but variable and often partial loss of class I processing machinery could reflect several scenarios. First, the role of CTL in tumor elimination may be more limited than thought. Second, in the face of a low level of natural immunity, partial loss of class I processing may be sufficient for the survival of the progressing tumor as a metastatic lesion. Third, the partial loss of class I processing could reflect the role of NK cells in eliminating the class I-negative cells.

$CD4^+$ T-help

While this commentary has focused on tumor escape from CTL responses, the discussion will be inadequate without mentioning the role of $CD4^+$ T cells in this context. Induction of CTL responses in animals has been shown to require in some instances the concomitant induction of $CD4^+$ T cells, known as T-helper cells. T-help is generally required when the antigenic stimulus is weak, such as during the induction of antitumor CTL. On the other hand, maintenance of a CTL response (i.e. memory) always depends on T-help, including when the induction of CTL is T-help independent [28]. Furthermore, abortive induction of CTL responses in the absence of T-help can lead to antigen-specific unresponsiveness [13]. Since cancer is a chronic disease, maintenance of an antitumor immune (CTL) response is perhaps even more critical than the magnitude of the response generated upfront during vaccination. Hence, providing T-help during tumor vaccination may be more critical than many of us currently think. The question therefore arises as to whether progressing tumors have elaborated ways to downregulate CD4 T-help as a means to escape CTL-mediated destruction. While at present there is no direct evidence to support this hypothesis, the answer is likely to be in the affirmative.

Summary and conclusions

While the critical role of animal studies in focusing our attention on various mechanisms of tumor escape is often under-appreciated, the clinical impact of this phenomenon will be determined only in clinical studies. The extent of the tumor escape problem in cancer patients will be revealed not before potent vaccination strategies are in place. That tumors will respond to effective vaccination protocols by selection for immunological escape variants is certain. The remaining question is whether the rate of tumor escape will equal or exceed the beneficial impact of an otherwise effective vaccine. If the answer is 'yes', active immunotherapy of cancer is futile. If the answer is 'no' – and I truly (but also must) believe that the answer is 'no' – the question becomes how to prevent or reduce the rate of tumor escape in order to extend – potentially indefinitely – the duration of the therapeutic benefit of the vaccination protocol.

The two key parameters that will counter the negative impact of tumor escape are the effectiveness of the vaccination protocol and the use of multivalent vaccine preparations. An effective antitumor immune response will enhance tumor elimination and hence reduce the rate of selection for escape variants. Simultaneous vaccination against multiple tumor antigens will reduce the probability of selection for antigen-loss variants and will increase the likelihood that at least some of the tumor antigens used in the vaccine preparation will be represented in the disseminated metastatic lesions targeted for elimination.

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