Immune Escape Associated with Functional Defects in Antigen-Processing Machinery in Head and Neck Cancer

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Abstract

Recent revival of interest in the role of immune surveillance in the pathogenesis and control of malignant diseases has focused attention on escape mechanisms used by tumor cells to evade immune recognition. Defects in the host’s tumor antigen – specific immune responses and abnormalities in tumor cell expression of HLA class I molecules and tumor antigen are known to contribute to tumor progression. However, the mechanism(s) responsible for the lack of tumor cell recognition by functional HLA class I antigen – restricted, tumor antigen – specific CTLs despite expression of the restricting HLA class I allele and targeted tumor antigen by tumor cells remain(s) unexplained. In squamous cell carcinomas of the head and neck (SCCHN), this type of tumor escape is a rule rather than the exception. Here, we discuss evidence pointing to functional defects in the antigen-processing machinery as one mechanism underlying resistance of SCCHN cells to recognition and lysis by HLA class I antigen – restricted, tumor antigen – specific CTL. In addition, based on the restoration by IFN-γ of SCCHN cell sensitivity to recognition by these CTL, we suggest strategies that may improve the clinical course of the disease by enhancing susceptibility of malignant cells to immune recognition.

The limited efficacy of conventional therapies, including surgery, radiotherapy, and chemotherapy, in most malignant diseases has stimulated interest in developing alternative therapeutic strategies. T cell–based immunotherapy for cancer has attracted much attention during the last 10 years (1, 2), largely because of its success in the control of tumor growth in animal model systems (3). The identification of human tumor antigens suitable for immunotherapy and immunomonitoring (4) and the optimistic expectations of basic tumor immunologists and clinical oncolgists eager to solve a major problem in current clinical medicine have driven the rapid translation of experimental findings to the clinic. Thus, a rather large number of patients with various types of malignant disease have been enrolled in clinical trials of T cell–based immunotherapy at many clinical centers. Surprisingly, the clinical responses have been disappointing (5), and in general, it has not been possible to correlate clinical responses with tumor antigen – specific CTL immune responses. This discrepancy has prompted investigations into mechanisms underlying the failure of tumor antigen–specific CTL to control tumor growth in cancer patients, especially those treated with immunotherapies.

T-cell dysfunction and abnormalities in molecules crucial for tumor cell recognition, such as HLA antigens and tumor antigen, have been identified as potential mechanisms responsible for disease progression or recurrence in spite of the well-documented presence of HLA class I antigen – restricted, tumor antigen – specific CTL in patients with cancer (6–8). However, to the best of our knowledge, no mechanism has been described to account for the lack of tumor cell recognition by HLA class I antigen – restricted, tumor antigen – specific CTL despite the expression of the targeted tumor antigen and of HLA class I molecules by tumor cells. Such an escape mechanism seems to be used by squamous cell carcinomas of the head and neck (SCCHN) cells and could account for disease progression in the presence of circulating tumor antigen – specific CTL in this malignancy (9). Therefore, SCCHN cells represent a useful in vitro model to identify the molecular mechanism(s) underlying the lack of correlation between immune and clinical responses in malignant diseases without detectable defects in HLA class I molecule and/or tumor antigen expression or effector cell functions.

In this article, following a short review of the antigen-processing machinery (APM), we will (a) describe APM defects as a mechanism underlying in vitro resistance of SCCHN cells to recognition by HLA class I antigen–restricted, tumor antigen–specific CTL; (b) review evidence supporting the clinical relevance of these in vitro findings; and (c) discuss strategies to overcome resistance of SCCHN cells to CTL recognition.

APM

Mostly, although not exclusively, endogenously derived antigens are processed via the HLA class I pathway in antigen-
presentation of tumor cells, including tumor cells (8). As shown in Fig. 1, proteins are marked for ubiquitination in the cytosol and subsequently degraded by the proteasome. This complex structure is composed of constitutive β subunits of the proteolytic core, δ, MB-1 (β5), and ζ. Their replacement with the β-type subunits LMP2, LMP7, and LMP10, which are induced by IFN-γ, leads to the assembly of the immunoproteasome. The latter generates a distinct set of antigenic peptides with increased affinity to MHC class I antigens. Peptides processed by the proteasome are transported to the endoplasmic reticulum by the transporter associated with antigen processing (TAP), which is composed of the two noncovalently associated subunits TAP1 and TAP2. In the endoplasmic reticulum, HLA class I heavy chain associates with β2m, which after being properly folded with the assistance of the chaperones BiP, calnexin, calreticulin and ERp57, β2m-HLA class I heavy chain complexes are brought into association with TAP and tapasin, which brings the dimeric complex into association with TAP and ensures peptide loading onto class I heavy chain-β2m complexes. Trimeric HLA class I-β2m-peptide complexes are transported to the cell surface.

The in vitro model we have used to investigate mechanisms of tumor cell resistance to HLA class I antigen–restricted, tumor antigen–specific CTL is shown in Fig. 2. It consists of (a) an HLA-A2+ SCCHN cell line PCI-13 established from a tumor biopsy (12) and expressing HLA-A*0201 molecules and the tumor antigen, MAGE-3 (The PCI-13 cell line is responsive to exogenous IFN-γ, which up-regulates TAP1, TAP2, and tapasin expression in the tumor cells.); (b) HLA-A2 antigen–restricted, MAGE-3271-279 peptide–specific CTL lines generated from peripheral blood mononuclear cells of normal donors or SCCHN patients by ex vivo stimulation with MAGE3271-279-pulsed autologous dendritic cells; and (c) enzyme-linked immunospot assays for IFN-γ secretion by the CTL challenged with HLA-A2+ MAGE-3+ tumor targets. As shown in Fig. 2, the PCI-13 SCCHN cell line is not recognized (i.e., does not induce IFN-γ secretion in CTLs in the enzyme-linked immunospot assay) by semiallogeneic HLA-A2+ MAGE-3271-279 peptide–specific CTL, although PCI-13 cells express the restricting HLA-A2 antigen and the target tumor antigen as detected by flow cytometry and by quantitative reverse transcription-PCR, respectively. Another HLA-A2+, MAGE-3+ SCCHN cell line (PCI-30) was not lysed in 4-hour 51Cr-release assays (Fig. 3G). However, the susceptibility of these SCCHN targets to CTL-mediated recognition was restored either by pulsing them with the cognate tumor antigen–derived peptide MAGE-3271-279 or by incubation with IFN-γ (100 units/mL for 72 hours at 37°C). The recognition was inhibited by HLA-A2- and HLA-A-B,-C
antigen–specific monoclonal antibodies (mAb) but was not affected by HLA-DR antigen–specific mAb. These results show that HLA-A2 molecules are not only expressed on PCI-13 cells but are also able to present the exogenous cognate peptide to CTL. Furthermore, these findings imply that CTL recognition of PCI-13 targets following incubation with IFN-γ is not the result of HLA-A2 antigen up-regulation but is likely to reflect other mechanisms, such as increased expression of APM component expression. This conclusion is corroborated by the results derived from the analysis of the mechanisms underlying the lack of recognition of the SCCHN PCI-30 cell line by HLA-A2 antigen–restricted, HER2369-377 peptide–specific CTL. Therefore, the lack of recognition of SCCHN cells by CTL in the absence of exogenously added tumor antigen peptide or IFN-γ probably reflects defect(s) in the generation, transport, and/or loading of the endogenous peptide on HLA-A2 molecules, most likely because of APM component down-regulation and/or dysfunction. This mechanism that has been occasionally described in small cell lung carcinoma, renal cell carcinoma, melanoma (13–15) is supported by the following lines of evidence. The lack of PCI-13 cell recognition by CTL is associated with a low level of TAP1, TAP2, and tapasin expression, as shown by the results of flow cytometry analysis of cells intracellularly stained with APM component–specific mAb. Furthermore, up-regulation of APM components LMP2, TAP1, TAP2, and tapasin in four HLA-A2+ SCCHN cell lines incubated with IFN-γ (Fig. 3) was associated with restoration of their recognition by HLA-A2 antigen–restricted, tumor antigen–specific CTL. Lastly and more conclusively, transfection of PCI-13 cells with wild-type TAP1 cDNA or with TAP1 and TAP2 cDNA restored their recognition by HLA class I antigen–restricted, tumor antigen–specific CTL (16).

These conclusions based on the in vivo analysis of interactions between a SCCHN target and tumor antigen–specific CTL have two important implications. First, they provide a mechanism for the unexpected clinical finding that the disease progresses despite the presence of functional tumor antigen–specific CTL and the lack of detectable defects in HLA class I molecule and/or in targeted tumor antigen expression in malignant lesions (15). Second, they emphasize the limitations of the analysis of HLA class I antigen expression on tumor cells for evaluating their susceptibility/resistance to recognition by HLA class I antigen–restricted, tumor antigen–specific CTL. Monitoring of the level of the trimolecular class I–β2m-peptide complexes on tumor cells is likely to be more informative to establish tumor cell sensitivity/resistance to immune intervention, because HLA class I molecule expression may not correlate

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**Fig. 2.** HLA class I molecule and APM component up-regulation in SCCHN PCI-13 cells and restoration of their tumor antigen–specific CTL recognition following incubation with IFN-γ or pulsing with exogenous MAGE-3271-279 peptide. A, HLA-A2 molecule up-regulation on SCCHN PCI-13 cells following incubation with IFN-γ but not following pulsing with tumor antigen peptide. Untreated PCI-13 cells (solid line) and PCI-13 cells incubated with IFN-γ (100 units/mL for 72 hours at 37°C; finely dotted line) and PCI-13 cells pulsed with the exogenous tumor antigen–derived peptide MAGE3271-279 (10 μg/mL for 1 hour at room temperature) were stained with HLA-A2 antigen–specific FITC-conjugated mAb (solid black line). B, HLA-A2 antigen up-regulation but not following pulsing with exogenous tumor antigen peptide. Untreated PCI-13 cells and T2 cells pulsed without IFN-γ but with the unrelated Flu58-66 peptide were used as a control. C, Monitoring of the level of the trimolecular class I–β2m-peptide complexes on tumor cells is likely to be more informative to establish tumor cell sensitivity/resistance to immune intervention, because HLA class I molecule expression may not correlate
with that of the trimolecular complex recognized by CTL. This possibility emphasizes the need for probes to measure HLA class I antigen–tumor antigen–peptide complexes on tumor cells.

**Clinical Significance of Abnormalities in HLA Class I Antigen or APM Component Expression in SCCHN Lesions**

The question remains whether the described in vitro findings have an in vivo counterpart and are of clinical significance. Information about APM component expression in SCCHN lesions and in other types of tumors is scanty. Only a limited number of malignant lesions have been investigated by immunohistochemistry, and the analysis has been restricted to a few APM components in the majority of the studies (17, 18). The paucity of information reflects, at least in part, the limited availability of mAb suitable for immunohistochemical detection of APM components in tissues and, especially, in formalin-fixed, paraffin-embedded tissue sections. Only recently, a panel of APM component-specific mAb, which meet these requirements has become available (19, 20). These reagents have facilitated the analysis of APM component expression in tumor biopsies, and the studies done thus far have shown down-regulation of several APM components in malignant lesions (17, 18, 21–23). A representative example of the staining of SCCHN lesions with the LMP-2-specific mAb SY-1 is shown in Fig. 4. The frequency of APM component down-regulation varies in SCCHN lesions, ranging from 18% to >80% for LMP2, TAP1, TAP2, and tapasin (21–23). Furthermore, an association has been found between APM component down-regulation in SCCHN lesions and clinical course of the disease (21) as well as patient survival, as illustrated in Fig. 5 (22, 23). This association is likely to reflect the negative effect of APM dysfunction on the recognition of tumor cells by HLA class I antigen–restricted, tumor antigen–specific CTL because of defects in the generation and/or expression of the trimolecular class I–IgG–peptide complexes. This hypothesis is supported by the association found in maxillary sinus carcinoma between APM component down-regulation and reduced patient survival as well as reduced CD8+ T-cell infiltration into malignant lesions (21). Furthermore, our

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3 R.L. Ferris, unpublished data.
4 R.L. Ferris, unpublished results.
T-cell infiltration into the tumor and poor prognosis (18), significantly (studies in progress indicate that LMP2 down-regulation is with reduced patients’ survival. Adjusted P values are shown for the log-rank test used to compare the groups. Reproduced with permission from Meissner et al. (22).)

Fig. 5. Association of APM component down-regulation in primary SCCHN lesions with reduced patients’ survival. A, LMP2 (1), LMP7 (2), TAP1 (3), and TAP2 (4) down-regulation was correlated with reduced patients’ survival. Adjusted P values are shown for the log-rank test used to compare the groups. Reproduced with permission from Ogino et al. (23).

studies in progress indicate that LMP2 down-regulation is significantly (P < 0.005) correlated with the extent of CD8+ T-cell infiltration into the tumor and poor prognosis (18). Taken together, these findings argue in favor of the possibility that APM component defects responsible for in vitro resistance of SCCHN cells to CTL recognition also represent the mechanism underlying the association between APM component expression in SCCHN lesions and clinical course of the disease. Thus, APM defects in tumor cells may be emerging as a prognostic biomarker of disease.

Strategies for Counteracting the Lack of SCCHN Cell Recognition by CTL

T-cell recognition of tumor cells plays a major role in the control of tumor growth by the host’s immune system and in the success of T cell–based immunotherapy. Therefore, the development of strategies to counteract the escape of tumor cells from T-cell recognition is a priority. The in vitro data we have described indicate that the lack of SCCHN cell recognition by HLA class I antigen–restricted, tumor antigen–specific CTL reflects defects in the presentation of the targeted tumor antigen–derived peptides because of APM dysfunction. The latter most likely results from abnormalities in peptide transport and/or loading on β2m-associated HLA class I heavy chains, because transfection of SCCHN cells with wild-type TAP1 cDNA is effective in restoring their T-cell recognition (16).

As observed in almost every other type of malignant cell described in the literature (13, 14), the defects in APM components identified in SCCHN cells are functional and not structural, because TAP function can be restored by cytokines, especially IFN-γ (17, 18). These findings suggest the potential use of intralosomal IFN-γ administration as a strategy to up-regulate APM component expression in SCCHN cells in vivo and to restore their recognition by HLA class I antigen–restricted, tumor antigen–specific CTL, provided that the tumor-associated microenvironment does not counteract the effect of IFN-γ. The concentration of IFN-γ required to up-regulate APM component expression in situ is not known at present, although 100 units/mL of IFN-γ seem to be sufficient to correct APM dysfunction in SCCHN cells in vitro. Because previous clinical trials have not reported significant side effects associated with the systemic administration of IFN-γ (24, 25), we would predict no or few side effects associated with the intralosomal administration of the dose of IFN-γ, which is expected to be required to up-regulate APM components in SCCHN cells. Moreover, the validity of this therapeutic approach is supported by in vivo studies in experimental tumor models (26) and by the up-regulation of APM components we have observed in human SCCHN lesions transplanted in nonobese diabetic/severe combined immunodeficient mice following intralosomal IFN-γ administration.5

Besides the modulation of APM components, IFN-γ is expected to have proapoptotic effects on tumor cells and to bias immunologic responses toward cytolytic and T helper type 1 (Th1) immunity, which seems to be more effective at inducing inflammatory and antitumor effects. Direct proapoptotic effects of IFN-γ on tumor cells have been long recognized (27). IFN-γ mediates tumor cell death by the induction of a number of apoptosis-related genes, and it also sensitizes cancer cells to signals delivered by the tumor necrosis factor family members (27, 28). More recent evidence indicates that caspase-8 is cleaved in IFN-γ–treated cancer cells, and that together with cispalatin, IFN-γ exerts more powerful antiproliferative effects (29). Previous demonstrations that human IFN-γ can directly inhibit the growth of human tumor cells in vitro and in human cancer xenografts in nude mice have led to its clinical application in patients with malignancy (30). A number of clinical studies have shown that IFN-γ has activity against advanced cancer, especially in patients with ovarian carcinoma or melanoma (24, 25, 30–32). When used in combination with chemotherapy, IFN-γ administration was associated with a significant increase in progression-free survival and only mild toxicity (30). When systemic IFN-γ (100 μg/m2 s.c. once weekly for up to 6 months) was used for adjuvant therapy of metastatic melanoma, it was found to be well tolerated, induced up-regulation of HLA class I and II molecules on the tumor in up to 43% of the 19 patients, and resulted in three complete clinical responses (31). In head and neck cancer, IFN-γ has shown some early clinical promise (25) but has not been evaluated more extensively in clinical trials.

5 A.B. DeLeo and R.L. Ferris, unpublished data.
Clinical grade human recombinant IFN-γ is now commercially available (Actimmune, Intermune, Brisbane, CA) and is Food and Drug Administration approved to treat patients with the immunodeficiency syndrome, chronic granulomatous disease. Therefore, IFN-γ is available for human clinical trials in cancer patients with Food and Drug Administration approval. Our data indicate that the induction of SCCHN cells recognition by CTL occurs after pretreatment with as little as 100 IU IFN-γ for 72 hours, a dose easily achievable clinically. In an orthotopic murine tumor model established in nude mice, using s.c. implanted PCI-13 cells, four intralymphal injections of 50,000 IU per injection of IFN-γ led to detectable up-regulation of TAP1 and TAP2 in tumor cells. The doses of recombinant IFN-γ used in our in vitro and in vivo experiments to up-regulate APM component expression are well within the pharmacologic range of the doses used previously (1,000,000 IU) for s.c. therapy of patients with melanoma (31). Indeed, this study used a 100-fold higher dose that we believe is necessary for delivery to the tumor site. Thus, intralymphal injections of IFN-γ seem to be a feasible approach for achieving sufficient local levels of the cytokine to restore APM component expression in vivo and to reproduce the biological effects we have observed in our experiments.

We would predict that the combination of intralymphal IFN-γ administration with T cell–based immunotherapy should improve its efficacy by counteracting an escape mechanism used by tumor cells to avoid T-cell recognition. Our predictions are supported by reports of tumor regression in mice following the systemic administration of TAP (33–35). All the available preclinical and clinical information justify proof-of-principle clinical studies to prove that intralymphal IFN-γ administration may have a beneficial effect on the clinical course of SCCHN through multiple mechanisms.

References

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