PROTECTIVE MECHANISMS OF HEAD AND NECK SQUAMOUS CELL CARCINOMAS FROM IMMUNE ASSAULT

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Abstract: Head and neck squamous cell carcinoma (HNSCC) is an aggressive malignancy that is the sixth most common neoplasm in the world. Despite advances in treatments involving surgery, radiation, and chemotherapy, the 5-year survival has remained at less than 50% for the past 30 years, primarily because of local recurrences. Thus, the possibility of immunotherapeutic approaches for patients with HNSCC has gained interest. Unfortunately, patients with HNSCC have profound immune defects that are associated with increased recurrence. This review aims to provide an overview of both the defensive and immune subversive mechanisms by which patients with HNSCC can protect themselves from immune antitumor assault.

KEYWORDS: head and neck cancer; HNSCC; immune; immunosuppression; suppressor cells

PROTECTIVE MECHANISMS OF HNSCC FROM IMMUNE-MEDIATED DESTRUCTION

The approaches by which cancers evade immune destruction are at least as numerous as the number of host anticancer immune defense mechanisms. One way of considering these protective defenses of tumors is in clusters: (1) escape from immune recognition, (2) direct inhibition of immune defenses, and (3) indirect immune inhibition by induction of host immune suppressor mechanisms (Table 1). These approaches are not mutually exclusive and most likely occur concurrently to various degrees, although the relative contribution of one approach versus another in protecting head and neck squamous cell carcinoma (HNSCC) from immune-mediated destruction has not been systematically investigated.

ESCAPE FROM IMMUNE RECOGNITION

The expression of human leukocyte antigen (HLA) class I molecules on the cell surface is necessary for the presentation of peptide antigens to cytotoxic CD8+ T lymphocytes. One way that cancers, including HNSCC, shield themselves from immune surveillance is by the downregulation of HLA class I gene expression (Figure 1). Approximately 50% of HNSCC cases have been shown to have a loss of class I HLA molecules, and this has been correlated with regional lymph node metastasis. However, the demonstration in this and other studies that the downregulated HLA expression is not consistent among HNSCC specimens would suggest additional means by which...
HNSCCs evade immune surveillance. Reduced HLA class I expression has also been seen for other tumor types, such as pancreatic carcinoma, and was associated with a decline in tumor-infiltrating T cells. Interestingly, a study with gastric cancers showed selective downregulation of class I HLA antigens, with approximately half of these cancers being deficient in HLA-A and HLA-B while expressing nonclassical HLA molecules. This prompted the possibility that cancers might evade T cells that recognize tumor in the context of class I major histocompatibility complex (MHC) and also evade natural killer (NK) cells whose lytic activity is inhibited by self-HLA molecules.

Occupancy of the Fas receptor by its counterpart FasL leads to activation of the apoptotic pathway and, consequently, cell death. Fas–FasL interactions contribute to both the immune reactivity against malignant cells and tumor evasion of immune defenses. This dichotomy has recently been reviewed. Cytolytic T cells have the capacity to kill tumor cells through this pathway, although the reverse also occurs. However, when activated, T cells have increased expression of Fas, which increases their vulnerability to apoptotic death. Apoptotic death of the active T cells expressing Fas can then be triggered by HNSCC and other types of malignancies that express FasL. More recent studies have shown that plasma of patients with oral cancer contains FasL+ membranous vesicles having the capacity to induce T-cell death. This approach to immune evasion is not unique to HNSCC, because soluble FasL that is released by uveal melanoma cells through a metalloproteinase-dependent pathway has been demonstrated to provide a shield from T-cell killing. Of interest is that similar mechanisms contribute to the immune privilege of the fetus. Namely, fetal protection from immune recognition has been suggested to be mediated through trophoblast secretion of microvesicular FasL.

### IMMUNE DYSFUNCTION IN CANCER PATIENTS

Malignant cells, such as HNSCC, escape from immune-mediated destruction not only by evading immune recognition but also by directly inhibiting antitumor immune defenses. The impact of HNSCC on immune function is underscored by the reduced peripheral blood levels of CD3+, CD4+, and CD8+ T cells, with CD3+CD4+ cell levels being more prominently reduced in patients with active disease. Surprisingly, these depressed T-cell levels persist several years after curative surgery. This could be of clinical importance, because a reduced T-cell proliferative capacity to mitogenic stimulation has been associated with a poorer outcome for patients with HNSCC. Within HNSCC tissue, plasmacytoid dendritic cells have been shown to be defective in their capacity to produce interferon (IFN)-α, a cytokine that is important for antitumor reactivity. In addition, the maturation of dendritic cells in patients with HNSCC is impaired.

Attempts to overcome the immune dysfunction of patients with HNSCC have included combining in vivo immunization with autologous, irradiated HNSCC plus granulocyte-macrophage colony-stimulating factor (GM-CSF), followed by in vitro activation and expansion of lymph node cells draining into the vaccination site. Such treat-
ments of patients with recurrent and metastatic HNSCC disease have shown the capacity to stimulate in vitro antitumor immune reactivity and, in some instances, favorable clinical responses or stabilization of disease. Other approaches to stimulate immune reactivity to tumor have included vaccination with virus-modified HNSCC cells after surgical treatment of patients with HNSCC, which is consistent with the view that immunotherapy would be most effective when tumor burden is lessened.

**DIRECT INHIBITION OF IMMUNE DEFENSES**

Among the means by which HNSCC can directly inhibit antitumor immune reactivity is the production of soluble mediators (Figure 2). HNSCC and a murine HNSCC model produce soluble mediators, such as transforming growth factor (TGF)-β and interleukin (IL)-10, which can interfere in immune reactivity to cancer. In fact, blocking the inhibitory effects of TGF-β has been suggested as a means to enhance immune function of cancer patients. The impact of HNSCC-derived IL-10 on immune antitumor reactivity and tumor growth has not been examined. However, studies with human non-small cell lung cancer showed IL-10 inhibits generation of tumor-reactive T cells, and studies in colon carcinoma animal models showed tumor expression of IL-10 increases in vivo tumor growth. HNSCC also produce high levels of immune inhibitory prostaglandins, such as prostaglandin E2 (PGE$_2$). The effectiveness of reducing PGE$_2$ levels to lessen the immune inhibitory effects that are mediated by tumor-derived prostaglandins has long been suggested, although relatively few such studies have been conducted with patients with HNSCC. However, limited studies with patients with HNSCC have shown that treatment with cyclooxygenase-2 inhibitors to block production of PGE$_2$ restores immune functions and increases T-cell infiltration into the cancer mass. These studies also showed that reducing PGE$_2$ levels stimulates monocyte migration, which is necessary for their antitumor activity. These studies suggest prostaglandin production to be a mechanism that contributes to HNSCC-induced immune dysfunction. The role of tumor-derived PGE$_2$ on immune dysfunction is not unique to HNSCC. For example, breast cancer tissue has been shown to release more PGE$_2$ than adjacent normal tissue, and its production was associated with reductions in T-cell proliferation, expression of Th1 cytokines, dendritic cell expression of costimulatory molecules, and antigen uptake. PGE$_2$ produced by gliomas diminishes the capacity of dendritic cells to stimulate immune reactivity to tumor, because it reduces dendritic cell production of IL-12 and, instead, induces the production of IL-10. Dendritic cell function in patients with malignancies, including HNSCC, has also been shown to become defective as a result of the inhibition of their maturation by tumor-derived vascular endothelial growth factor (VEGF). There have been, however, some opposing studies that have shown no effect of HNSCC presence on dendritic cells.

**SUPPRESSOR CELL POPULATIONS**

In addition to evading immune responses and producing soluble mediators that directly inhibit antitumor immunity, an intriguing way in which tumors inhibit antitumor immune reactivity is by capitalizing on the immune inhibitory cell mechanisms of the host (Figure 3). In fact, some of
the ways in which tumors induce host cells to inhibit immune responses are through the same soluble mediators and those by which tumors directly cause immune inhibition. Although not all of the tumor-induced host immune suppressor mechanisms have been demonstrated and defined for head and neck cancers, it is reasonable to speculate that similar immune inhibitory cells are induced by HNSCC as by other solid malignancies.

Over time, studies have favored differing inhibitory cells as contributors to the immune inhibition of cancer patients. Two classes of host cells that have been recognized for several decades to inhibit antitumor immunity in tumor bearers include inhibitory macrophages and inhibitory T cells. However, views on how they induce immune inhibition and the prominence of various inhibitory subpopulations have varied.

The presence of macrophages within tumors was originally viewed with positive enthusiasm, because activation of macrophages induces their ability to kill tumor cells. However, these same activated macrophages and tumor-associated macrophages can also inhibit the reactivity of immune effector cells against tumors. Macrophages can produce many of the same immune inhibitory mediators as do tumors. This includes PGE$_2$ and IL-10. The importance of macrophage-derived prostaglandins in subverting immune responses has been shown by the effectiveness of cyclooxygenase inhibitors such as indomethacin in overcoming the immune inhibitory effects of suppressor macrophages, thus allowing T-cell reactivity. Less studied has been specifically how macrophages are induced by tumor to produce these inhibitory mediators. However, some studies have shown that production of TGF-β and PGE2 by human carcinoma cell lines and murine fibrosarcoma cell lines induces macrophages to become inhibitory toward T-cell activity through their production of nitric oxide and tumor necrosis factor (TGF)-α.

More recent studies have shown that macrophages from tumor-bearers can inhibit immune reactivity toward cancer by inducing apoptosis of T cells. Murine tumor-associated macrophages can induce T-cell apoptosis through their production of arginase and nitric oxide. Similarly, studies with patients with renal cell carcinoma showed increased arginase activity but showed that the arginase-producing immune inhibitory cells were neutrophils, rather than monocytic. Macrophages isolated from draining lymph nodes of patients with gastric cancer induced T-cell apoptosis through production of H$_2$O$_2$. Whether macrophages of patients with HNSCC suppress antitumor immune reactivity through these latter mechanisms is not clear and has not been adequately addressed.

Over time, enthusiasm for studying inhibitory macrophages has shifted to other cell types. Other cell types that have been known for a significant amount of time to have the potential to inhibit immune responses are T cells. Like macrophages, T-cell subpopulations can enhance or inhibit antitumor immune reactivity. Earlier studies suggested that CD8$^+$ T cells contribute to the immune inhibition of cancer patients. For a while, the term suppressor T cell was synonymous with inhibitory CD8$^+$ T cells. During the same time, it was recognized that CD4$^+$ T cells could also be inhibitory. These inhibitory cells were shown to be present in animal tumor models and in cancer patients and were sensitive to low-dose cyclophosphamide. The sensitivity of suppressor T cells to cyclophosphamide provides the rationale for including low-dose cyclophosphamide in some strategies for enhancing antitumor immune reactivity in patients, including those with HNSCC.

After an initial flurry of activity, interest in CD4$^+$ inhibitory T cells lost favor until their
return as T-regulatory (Treg) cells. The definition for Treg cells has undergone some refinement and now includes a combination of phenotypic and functional characteristics. Phenotypically, Treg cells express CD4 and CD25, although CD25 expression on CD4+ cells is not necessarily indicative of Treg cells, because it can also be indicative of activated CD4+ cells. However, Treg cells also express forkhead/winged helix transcription factor (FoxP3), which is a specific marker that is important for the function of Treg. Functionally, Treg cells inhibit T-cell activity through their production of soluble inhibitory mediators such as TGF-β and IL-10. CD4+CD25+ Treg not only inhibit T-cell reactivity but can expand the total level of inhibitory cells by converting CD4+CD25− cells into inhibitory CD4+CD25+ cells. The extent to which Treg cells contribute to the immune depression of patients with HNSCC has not been studied. However, increased levels of cells that have the phenotypic characteristics of Treg (CD4+CD25+FoxP3+) have been demonstrated in the peripheral blood of patients with HNSCC. Studies with patients with hepatocellular carcinoma showed increased levels of Treg within tumor tissue compared with normal tissue and increased levels of TGF-β expression in the peripheral blood of these patients compared with controls. These Treg cells were shown to inhibit T-cell proliferation and cytokine secretion. The potential importance of Treg cells in preventing antitumor immune reactivity was suggested in animal tumor models by the regression of melanoma patients, with the Th2 bias being incomplete and indicative of the presence, rather than the extent, of malignant disease.

Although tumors induce type 2 cytokine skewing, this is accentuated through several means. Soluble mediators in the peripheral blood of cancer patients can skew T-cell reactivity toward a Th2 profile. Over and above that, type 2 cytokines such as IL-4 or IL-10 are inhibitory to production of type 1 cytokines, such as IFN-γ. This, then, further diminished the generation of protective immune responses to cancer. These interrelationships between type 1 and 2 cytokines further complicate any attempts to facilitate a Th1 response so as to induce effective antitumor immune reactivity. Consequently, experimental therapeutic approaches are attempting to use select type 1 cytokines to redirect the overall immunologic cytokine profile toward a type 1 phenotype.
For example, IL-12 administration has been tested in patients with HNSCC. This treatment diminished Th2 cytokine levels and, instead, stimulated a Th1 phenotype. Although relatively few trials of this type have been conducted with patients with HNSCC, they show the feasibility of enhancing the immune defenses of patients with HNSCC.

Our studies with patients with HNSCC and with murine tumor models have shown the appearance of an additional immune inhibitory cell population in patients with HNSCC and in tumor-bearing mice. These immune inhibitory cells can be recognized by intense surface expression of CD34 and they mediate most of their suppressive activity through production of TGF-β. The CD34⁺ suppressor cells are progenitor cells having clonogenic capabilities in soft agar and whose mobilization results from tumor production of GM-CSF. Levels of the immune suppressive CD34⁺ progenitor cells are increased in the peripheral blood of patients with HNSCC and in the bone marrow, spleen, and blood of tumor-bearing mice. Of greater importance is that the immune inhibitory CD34⁺ cells are also present within the cancer mass of patients with HNSCC and tumor-bearing animals, and that they inhibit the activity of intra-tumoral T cells. Surprisingly, the immune inhibitory CD34⁺ cells persist in high numbers even after surgical excision of cancer. The increased presence of immune inhibitory CD34⁺ progenitor cells could impact not only antitumor immune reactivity of patients with HNSCC but also progression of HNSCC disease. This was suggested by the reduced levels of active intra-tumoral T cells and the reduced 2-year survival of patients with HNSCC whose primary tumors contained high levels of CD34⁺ cells.

Our studies showing an increase in the frequency of progenitor cells having immune suppressive activity are consistent with the blockage in maturation of dendritic cells and accumulation of immature myeloid cells that has been described by others for cancer patients, including those with HNSCC. Together, these studies show a defect in the differentiation and maturation of dendritic precursor cells that could otherwise be harnessed to stimulate reactivity against cancer. Consequently, approaches were examined through which to induce differentiation of the immune inhibitory CD34⁺ cells of patients with HNSCC into immune stimulatory dendritic cells. Studies had previously shown the capacity of vitamin D analogs to stimulate cellular differentiation. On the basis of this differentiation-inducing ability of vitamin D analogs, we tested whether vitamin D analogs could induce differentiation of tumor-mobilized immune suppressive CD34⁺ progenitor cells into antigen-presenting dendritic cells and whether this would enhance immune reactivity. Preclinical studies showed that treatment of tumor-bearing mice with vitamin D3 reduced the levels of immune inhibitory CD34⁺ progenitor cells and enhanced immune responses, including antitumor immunity. Coupling vitamin D3 differentiation-inducing treatment with adoptive immunotherapy enhanced the effectiveness of the adoptively transferred tumor-reactive T cells at limiting metastasis and postsurgical tumor recurrence. These animal studies were advanced to studies with peripheral blood cells of patients with HNSCC. In vitro studies showed that 1,25-dihydroxyvitamin D3 could enhance the differentiation of HNSCC-mobilized CD34⁺ progenitor cells into dendritic cells. Furthermore, these resultant dendritic cells were functional and able to present antigen to autologous T cells. Consequently, the effect of the vitamin D metabolite 25-hydroxyvitamin D3 was tested in patients with advanced HNSCC. This trial, which focused on the immunologic effects of treatments in the peripheral blood, showed a resultant decline in CD34⁺ cell levels, increased levels of cells expressing class II MHC, and increased functional capability of the peripheral blood T cells. Currently, studies are ongoing to assess how treatment with noncalcemic vitamin D analogs affect CD34⁺ cells within the tumor mass and the resulting effect on the intratumoral immune infiltrate.

THE INTERPLAY AMONG SUPPRESSIVE MECHANISMS AND CONCLUSIONS

The ways in which tumors induce host immune suppressor mechanisms and the types of suppressor mechanisms that are induced do not seem to be linked to any one cell type or malignancy, but considerable overlap exists. For example, tumor production of type 2 cytokines or other mediators that are inhibitory to antitumor immune reactivity can upregulate Th2 and downregulate Th1 cells. However, this can occur indirectly through host cell interactions. One example is the production of PGE₂ by glioma cells, which inhibits dendritic cell production of IL-12 and stimulates IL-10 production. This, in turn, stimulates the appearance of immune inhibitory Treg cells. Another example is also mediated through a den-
Immune Defenses of HNSCC


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