Chapter 6
Immunology of Head and Neck Cancer

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Abstract The immune system plays a key role in the progression of head and neck cancer. A greater understanding of the important contribution of the dysregulation and evasion of the immune system in the development and evolution of head and neck cancers should lead to improved therapies and outcomes for patients. Head and neck cancer evades the host immune system through manipulation of its own immunogenicity, production of immunosuppressive molecules, and promotion of immunomodulatory cell types. Also, the immune system can be exploited to promote metastasis, angiogenesis, and growth. In this chapter, we review basic immunology as it relates to head and neck cancer and discuss the theory of cancer immunosurveillance and immune escape. Current research on cytokines as biomarkers, cancer stem cell tumor antigens, and immunotherapeutic strategies are presented.

Keywords Immunology • Immunotherapy • Head and neck cancer • Biomarkers • Immune evasion • Immune surveillance • Monoclonal antibodies

Introduction

The immune system plays a key role in the progression of head and neck cancer. A greater understanding of the important contribution of the dysregulation and evasion of the immune system in the development and evolution of head and neck cancers should lead to improved therapies and outcomes for patients. In this chapter, we review basic immunology as it relates to head and neck cancer and discuss the theory of cancer immunosurveillance and immune escape.

There has been a recent renaissance in the idea that nascent cancer cells are destroyed by the immune system before tumor formation can occur (termed immune surveillance). Derangements in the immune system or alterations in the transformed cells may allow immune escape that allows the cancer to become manifest. Once tumor is established, there are a myriad ways in which it interacts with the immune system. Transcription factors such as NFκ(kappa)B (nuclear factor kappa-light-chain-enhancer of activated B cells) and STAT3 (signal transducers and activators of transcription), which are usually dysregulated in tumor-promoting inflammatory states in response to cytokine stimuli, are aberrantly activated in tumor cells and are intensively studied as possible targets for therapeutic intervention. Tumors themselves produce cytokines such as TGF-β(beta), IL-6, and IL-10, which suppress cell-mediated antitumor immunity. In response to inflammatory stimuli, head and neck cancer cells also can express receptors which are involved in lymphocyte and dendritic cell migration. Expression of these receptors by tumor cells, such as CCR7 and CXCR4, constitute immune exploitation of established signals intended for immune cells and have been associated with tumor invasion, metastasis, and cell survival, leading to treatment resistance. Another recently espoused theory is the idea that tumors are comprised of a heterogenous cell population in the tumor microenvironment that includes a special subpopulation of cancer stem cells (CSC) that are able to recreate the entire tumor phenotype and potentially evade immune recognition. These cells appear to be more resistant to conventional chemotherapy and radiation, and may not possess the same tumor antigen expression or T-cell recognition as non-CSC.

In head and neck cancer patients, there appear to be global alterations in the functional state of the immune system, as evidenced by changes in serum cytokines, chemokines and other immune-related biomarkers in cancer patients. There is considerable investigation focusing on the identification of serum biomarkers to monitor cancer progression, prognosis, treatment response, and relapse. Finally, we describe various immunotherapeutic strategies designed to utilize the immune system to stimulate elimination of cancer. These include cancer vaccines using tumor peptide antigens or viral, bacterial, and DNA-based vectors as well as tumor antigen-specific monoclonal antibodies (mAb). The recent clinical efficacy of these FDA-approved mAb, including cetuximab

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(anti-EGFR) and bevacizumab (anti-VEGF), has stimulated investigation into immunological mechanisms of action which may explain antitumor clinical activity.

Brief Overview of the Immune System

The immune system has traditionally been divided into two major arms: innate and adaptive immunity. This dichotomy is somewhat artificial since there is tremendous interaction between the two components. Innate immunity refers to the part of the immune system that provides antigen nonspecific, first-line protection. The effectors of innate immunity include NK cells and phagocytes such as neutrophils, macrophages, dendritic cells, and monocytes that ingest extracellular debris or pathogens. Innate immunity also utilizes pattern recognition systems that recognize molecules that are not normally present in the human body: double-stranded RNA, bacterial cell wall components, lipopolysaccharide, and microbial membranes. These pattern recognition systems can take the form of enzymes like lysozyme, antimicrobial peptides (defensins), soluble factors (complement, C-reactive protein, mannose-binding lectin), and cell surface receptors (Toll-like receptors, scavenger receptors). Innate immunity is static and nonspecific, and does not change in magnitude or efficacy after repeated exposure to antigenic challenges. However, innate immune signals effectively trigger the adaptive immune system. Dendritic cells (DC) and other antigen-presenting cells link the two systems. DC ingest and process tumor antigens, after effectors of innate immunity have destroyed the tumor cell. DC then present these antigens to cytolytic and helper T lymphocytes, causing clonal expansion of antigen-specific T cells. Activation of the adaptive immune system (T lymphocytes) provides immunologic memory responses against these antigens. Thus, key effectors in tumor immunology are NK cells, B cells, T cells, and DC.

B Lymphocytes

Early in the field of immunology, humoral immunity was believed to be the primary effector mechanism, in 1948 plasma cells were identified as the source of antibodies. Plasma cells are one of the two endpoints for B cells, the other being the memory B cell. B cells can be activated via T-cell-dependent or -independent antigens. Tumor antigens are T-cell dependent antigens which require binding of the antigen to the B cell receptor and a secondary activation signal via CD40 on an activated helper T cell. It is well established that B cells in cancer patients are capable of recognizing and producing antibodies to tumor antigens [1, 2]. In head and neck cancer, circulating serum antibodies have been found against p53 [3], MUC1 [4], p40 [5], p73 [6], and HPV E6 and E7 [7]. However, levels of circulating antibody have not been correlated with clinical outcome other than high postoperative levels of anti-p53 antibody which have been correlated with poor prognosis [8]. Interestingly, it has been noted that there is an increased frequency of IgE subtype immunoglobulins in head and neck cancer [2, 9]. The significance of this finding, if any, is unclear.

T Lymphocytes

T lymphocytes were defined in the early 1960s when mice were thymectomized in an attempt to prevent lymphoma. When the initial experiments in adult mice failed to have any effect, neonatally thymectomized mice were found to have profoundly decreased lymphocyte numbers and were unable to generate antibodies despite having plasma cells. Based on these data, Miller theorized that the thymus must be the source of a "helper" cell that is required to produce antibody [10–12]. In later experiments, depletion of CD8 abolished this destruction which identified CD8 T cells as a primary effector of specific tumor/allograft rejection.

T lymphocytes are defined by the presence of T-cell receptors (TCR) on their cell surface. TCR are part of the immunoglobulin superfamily and undergo germline DNA rearrangement to produce diversity much like immunoglobulin genes in B cells. TCR recognizes tumor antigens which are short peptide fragments bound to or "presented by" major histocompatibility complexes (MHC). There are two main classes of MHC: MHC I molecules found on the cell surface of all nucleated cells and MHC II is found only on professional antigen-presenting cells such as macrophages and dendritic cells. MHC class I and II binds with peptides, which are derived from tumor proteins and "processed" within the cell, and MHC then bind or present these tumor peptides on the cell surface for recognition by T cells. The TCR can only recognize peptide antigen when presented by a particular self-MHC molecule, a phenomenon known as MHC restriction, which led to the Nobel Prize in 1996 to Doherty and Zinkernagel. Therefore, CD8 T cells can recognize syngeneic (self) but not allogeneic (from someone else) tumor cells. MHC I binding tumor peptides are usually eight to ten amino acids in length, derived from endogenous proteins processed via the proteasome, and are presented to CD8 T cells. MHC II peptides are longer (11–16 amino acids), derived from exogenous proteins taken in by endocytosis, and are presented to CD4 T cells [13].

T lymphocytes are generally divided into $CD4^+$ or $CD8^+$ T cells. While it remains unclear how T cells are selected to become CD4 or CD8 cells, there are usually twice as many CD4 T cells as CD8 T cells released. Once antigen is encountered along with the appropriate costimulatory signals,

T cells become activated and differentiated. CD4 T help (T_H) cells usually differentiate into one of two major subclasses, T_H1 and T_H2, and this differentiation depends on the cytokine milieu in the environment at the time of activation. These two subsets of CD4 cells are differentiated by function and cytokine secretion profile. The T_H1 subset is responsible for most cell-mediated immune functions such as activation of CD8 T cells, inflammation, and delayed-type hypersensitivity as well as production of complement activating IgG antibodies. Macrophages or dendritic cells will produce IL-12 in response to intracellular pathogens. IL-12 along with IFN-γ(gamma) and IL-18 drive the T_H1 response. T_H1 cells secrete IL-2, IFN-γ, and TNF-α and are felt to be the most strongly antitumor subtype.

On the other hand, IL-4 drives a T_H2 response [14]. The T_H2 response drives B cells to produce IgM, IgE, and non-complement-activating IgG, as well as activating eosinophils, in response to parasitic invasion. T_H2 T cells are strongly implicated in allergy and are felt to be tumor permissive. T_H2 cells secrete GM-CSF, IL-3, IL-4, IL-5, IL-10, and IL-13. More recently, other subsets of CD4 T cells have been identified. T_H17 cells require TGF-β and IL-6 for differentiation and are defined by their production of IL-17. IL-17 is known to induce the production of several chemokines that attract proinflammatory cells and IL-17 expression is greatly increased in autoimmune diseases [15]. The final subset of CD4 T cells is the regulatory T cell (Treg) that was originally defined as a $CD4^+CD25^+Foxp3^+$ T cells. Tregs are thought to be a reciprocal subtype to T_H17 cells in that both are induced by TGF-β, but Tregs are immunosuppressive as opposed to T_H17 cells which are proinflammatory. Tregs have recently been strongly correlated with disease status in SCCHN patients [16, 17].

Natural Killer Cells

NK cells were discovered in 1975 when experiments studying tumor lysis by lymphocytes from immunized animals found lysis that was independent of previous immunization or activation [18]. This was thought to be an artifact until the NK cell was isolated and given the name "natural killer" cell for its ability to kill tumors without previous activation. NK cells kill much in the same way as cytotoxic T cells, through the interaction Fas ligand on their surface with Fas on target cells inducing apoptotic cell death. They also constitutively possess perforin and granzyme granules and degranulate causing cytolysis. Unlike T cells that are self MHC restricted and require self MHC for activation, NK cells are suppressed by the presence of self MHC via KIR receptors that inhibit NK killing when bound by self MHC [19]. These inhibitory signals can inhibit killing even when activating receptors on the NK cell are bound and therefore presentation of self MHC on the target's surface is protective. Activation receptors on the NK cell include NKD2D and FcγIII receptor. NKD2D binds ligands produced by cells stressed by DNA damage or infection. FcγIII receptor is a high affinity receptor for IgG which provides a mechanism by which NK cells can recognize targets bound by antibody. Activating Fcγ receptors mediate antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells, macrophages, monocytes, neutrophils, and eosinophils.

Dendritic Cells

Dendritic cells (DC) are antigen-presenting cells and as such are potent initiators of the immune response. DC efficiently take up antigen via several mechanisms including phagocytosis, macropinocytosis, and adsorptive endocytosis. After uptake, antigen is shunted into lysosomes and degraded for presentation on MHC II. DC also possess B7 molecules on their surface that provide a necessary secondary activation signal to T cells after engagement of the MHC–peptide complex with the TCR. Because DC are such potent activators of T cells and initiators of adaptive immunity, they have been intensely studied as a possible therapeutic for cancer immunotherapy.

Another important process mediated by DC is cross presentation of antigen derived from tumor cells or shed tumor products/vesicles. Exogenous antigen is processed via the exogenous pathway and presented to CD4 cells by DC via MHC II. However, DC are able to move exogenous antigen to the endogenous pathway and present these antigen to CD8 cells via MHC I. This surrogate presentation of exogenous antigen to the endogenous pathway is defined as cross presentation. Cross presentation serves a very important function because it allows DC to activate cytotoxic T cells against virally infected cells and tumor cells and have recently been harnessed in cancer vaccine trials.

Cancer Immunosurveillance and Immunoediting

The idea of immune control of malignant cells was first proposed by Paul Ehrlich in 1908, but it was not until the 1950s that greater understanding of the immune system gave rise to a formalized hypothesis. This "cancer immunosurveillance" hypothesis was introduced by Burnet and Thomas and stated that tumor cells must have recognizably different antigens than normal cells and therefore have the potential for immune clearance. Also at that time, the phenomenon of allograft rejection via cellular immunity was observed. Because grafting of allogeneic tissue is not a naturally occurring event, Thomas proposed that the actual primary function of cellular immunity was not to protect against allografts but rather to

protect against tumors. Conflicting experimental results led many to abandon the idea of cancer immunosurveillance for several decades, until several key discoveries have led to a revival of the hypothesis. First was the discovery of the NK cell in the late 1970s which seemed to provide innate immune protection from tumor [20]. The discovery of IFN-γ and its proapoptotic effect on tumor growth gave additional support to the potential for immune clearance of cancer cells [21]. Mice lacking IFN-γ receptors produced more tumors with decreased latency after methylcholanthrene challenge and addition of IFN-γ was protective against transplanted, spontaneous, and induced tumors in another experiment. Studies in mice lacking perforin, a key component of cytolytic granules in T cells and NK cells, recapitulated the results in IFN-γ receptor knockout mice with more frequent tumors and lower latency of formation [22]. Mice with genetically induced immunodeficiency were found to be more susceptible to both spontaneous and chemically induced tumors. In humans, epidemiologic data from AIDS patients demonstrate increased risk of lymphoma, Kaposi's sarcoma, and virally induced carcinomas of the genitourinary tract. There also appears to be a higher risk of HPV-associated HNC in HIV+ patients [23]. These data confirm the unchallenged idea that immune protection from viral infections reduces risks of cancer associated with viruses.

But what of tumors without viral etiology? Data gathered from transplant patients who are immunosuppressed to avoid organ rejection demonstrate increased risk of many tumors with no known viral etiology such as lung, head, and neck [24], pancreatic, endocrine, colon cancer, and melanoma [25]. The cancer immunosurveillance hypothesis has given rise to the theory of cancer immunoediting which is the idea that immune surveillance of cancers provides selective pressure on tumor cells and selects for cells that can evade the immune system. One study showed that many tumors grown in immunocompromised mice are rapidly cleared when injected into immunocompetent mice, whereas cancers from immunocompetent mice continue to grow when transplanted into immunocompetent mice, indicating a qualitative difference in the cancer cells that was dependent on the immune environment [26]. The theory contends that successful tumor formation can occur only after the cancer has discovered a means by which it can evade the immune system.

Immune Escape and Immunosuppression in Head and Neck Cancer

Cancer cells evade the immune system by two primary mechanisms: by reducing their innate immunogenicity or by suppressing the immune response (Fig. 6.1). Tumor cells can reduce T-cell-mediated recognition by altering HLA class I expression. It has been noted that some tumor cells have a complete loss of HLA expression due to defects in β_2-microglobulin expression or function. Alternatively, chromosomal defects in the HLA-encoding genes themselves can cause selective loss of HLA expression. This process has been noted in approximately 50%

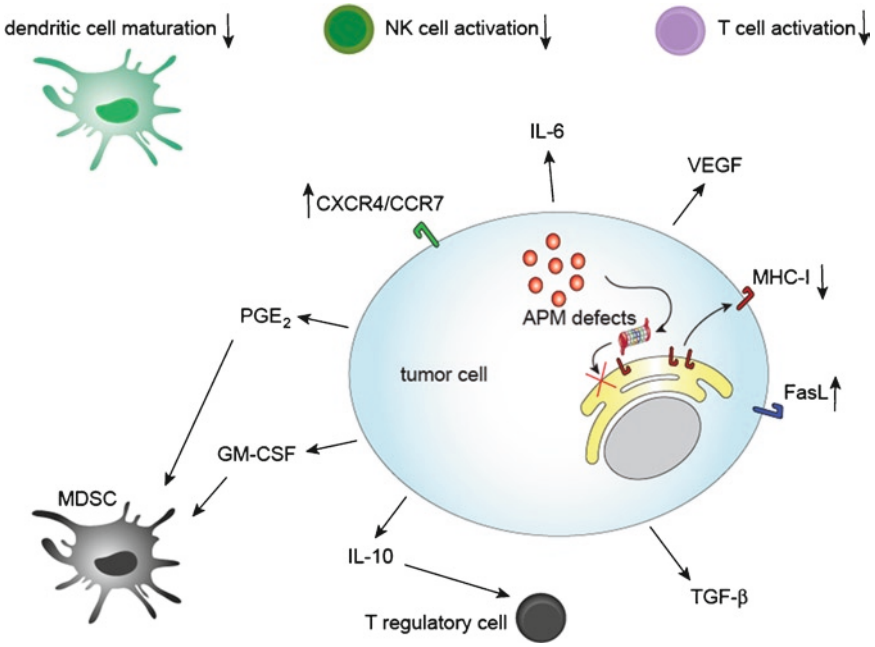

Fig. 6.1 Tumor cell immune evasion and exploitation. Tumor cells secrete several small molecules and cytokines that depress NK, DC, and T-cell function and induce immunosuppressive MDSC and regulatory T cells. MHC downregulation and defects in the antigen presentation machinery impairs T-cell recognition. Fas ligand is expressed which kills T cells. Chemokine receptors aid in metastasis of the cancer cell to lymph nodes

of head and neck squamous cell carcinomas [27] and was correlated with poor prognosis in esophageal squamous cell cancer [28] and laryngeal squamous cell cancer [29]. In other cancers, there is ample expression of HLA and tumor antigen but without recognition by T cells. Because HLA loss variants are killed by NK cells, one proposed explanation for the lack of NK cell killing is that cancer cells possess defects in their antigen presentation machinery (APM). This would reduce selectively tumor antigen-HLA peptide completely without reduction in overall surface HLA density.

Endogenous antigens are processed through the cytoplasmic immunoproteosome which consists of various subunits including low molecular weight proteasome (LMP) 2, LMP7, and LMP10. Antigenic peptides are transported to the endoplasmic reticulum by the transporter associated with antigen processing (TAP) where they are associated with HLA class I heavy chains by tapasin [30]. Thus, SCCHN cells that express HLA I and whole tumor antigen can evade T-cell recognition through decreased expression of LMP2, TAP1, TAP2, and tapasin. The observation that T-cell recognition could be reconstituted with either exogenous peptide or upregulation of APM expression [31] confirms the biological significance of this immune escape mechanism. In addition to decreased expression of HLA, SCCHN tumor cells express Fas ligand which can interact with Fas and transduce a powerful apoptosis signal to activated T cells allowing immune evasion [32] by eliminating tumor-infiltrating T lymphocytes.

As mentioned, decreased expression of HLA molecules is protective against T cells but increases NK cell-mediated cytolysis as the absence of HLA removes a key inhibitory signal for NK cells. Therefore, tumor cells must employ multiple mechanisms to suppress NK cell-mediated antitumor immunity. MICA, a ligand of NKG2D in NK and T cells, can be released in a soluble form to act as a competitive antagonist [33]. Cytokines and other molecules that suppress immune function such as IL-10, TGF-β, IL-6, PGE_2, VEGF, and GM-CSF are known to be produced by SCCHN cells. IL-10 reduces activation of cytotoxic T cells and has been correlated with advanced stage head and neck cancer [34]. TGF- β suppresses T cell and NK activation and is a key cytokine in the differentiation of regulator T cells [35]. TGF-β production is increased in preneoplastic oral cavity lesions and promotes angiogenesis and a protumorigenic microenvironment linking it to early tumor formation [36]. IL-6 signals via STAT3 to inhibit DC maturation, NK cell, T cell, neutrophil, and macrophage activation [37] and has been correlated with recurrence and survival in SCCHN [38]. Reduced DC numbers and function have been observed in this disease (Mueller-Burghaus paper). STAT3 is a transcription factor that is also involved several other immunosuppressive pathways such as IL-10 signaling [39], suppression of dendritic cells [40], downregulation of IL-12 [41], and generation of regulatory T cells [42]. PGE_2 is a prosurvival, proangiogenic molecule that is produced by many cancers including SCCHN [43, 44]. It is also a potent immunomodulator that decreases T-cell proliferation, inhibits Th1 T cells, decreases B-cell proliferation and inhibits maturation and antigen presentation of DC [45]. VEGF, which is primarily thought of as a promoter of angiogenesis, is overexpressed in 90% of SCCHN [46] and functions to increase the ratio of immature to mature DC in the tumor microenvironment which is thought to lead to T cell anergy [47]. GM-CSF when produced in large quantities by tumors recruit myeloid-derived suppressor cells (MDSC) [48, 49] which have been identified in SCCHN.

Myeloid-Derived Suppressor Cells

Myeloid-Derived Suppressor Cells (MDSC) are a diverse family of myeloid cells that are defined by $Gr1^+CD11b^+$ and in cancer patients they are usually also $CD33^+$ and $CD34^+$ [50]. They are increased in almost all cancer patients and, indeed, were first characterized in SCCHN [49] where their link to VEGF and GM-CSF was discovered. In addition to VEGF and GM-CSF, MDSC are induced by IL-6, IL-1β, PGE_2, and complement C5a. Initial studies in SCCHN found that MDSC inhibit IL-2 secretion by activated T cells which is a key step in T-cell proliferation and escalation of cell-mediated immunity. Also, they deplete the tumor microenvironment of arginine and cysteine which are essential for T-cell activation. MDSC produce nitric oxide and reactive oxygen species that catalyze the nitration of the TCR which inhibits TCR – MHC interactions and subsequent activation. Downregulation of the TCR zeta chain which also interferes with T-cell activation is mediated by MDSC along with downregulation of L-selectin which is important for migration of naïve T cells to lymph nodes. Data on the effect of MDSC on NK cells has been conflicting with reports of both enhancing as well as suppressive action on NK cells which may be a function of the heterogeneity of MDSC populations. MDSC also promote induction of Tregs via production of IL-10, TGF- β, and arginase [50]. Treatments such as antibody depletion, retinoic acid, gemcitabine, and STAT3 blockade that diminish MDSC restore immune surveillance, increase T-cell activation, and improve efficacy of immunotherapy. The basal levels of MSDC increase with age and may contribute to increased tumor frequency and growth rate increase with age [51].

T Regulatory Cells

Though it was long suspected that a subset of T cells were immunosuppressive, their characterization occurred relatively recently when it was found that this subpopulation

were $CD4^+$ cells that also expressed CD25 [52]. There are now four subtypes of regulatory T cells: naturally occurring thymus-derived $CD4^+CD25^{high}FoxP3^+$ Tregs, antigen-induced IL-10-dependent Tregs (Tr1), IL-4-dependent Tregs (Th3), and antigen-specific Tregs [16]. There is also a $CD8^+CD25^+$ variant which also appears to have immunosuppressive ability but their biological significance is unclear and they are thought to be overshadowed by the much more abundant $CD4^+$ Tregs [53]. Tregs cause anergy, apoptosis and cell cycle arrest of activated T cells via production of IL-10, TGF-β, and direct cell–cell contact [54]. They also inhibit the action of dendritic cells, NK cells, and B cells [55]. In SCCHN patients, Tregs are increased in frequency in peripheral blood and among T cells infiltrating the tumor and draining lymph nodes resulting in an immunosuppressed state [17, 56, 57]. Also, Treg numbers are inversely proportional to DC and $CD8^+$ T-cell numbers in SCCHN [58, 59]. Treg frequency as a prognostic indicator is unclear as one study linked increased Tregs with better locoregional control [60] while another study found increased Tregs associated with early recurrence [61]. Also interesting was the finding that Treg numbers were greater in SCCHN patients after treatment than before treatment indicating that oncologic treatment increases Treg numbers [17].

These data indicate that SCCHN induces an immunosuppressed state via multiple potent mechanisms which is a barrier to effective cancer immunotherapy. They secrete immunosuppressive cytokines and molecules. Cytokine levels are aberrant in SCCHN patients indicating deregulation or dysregulation of cytokine pathways [62]. There is increased frequency of immunosuppressive regulatory immune cells and there is a global dysfunction of almost every facet of the immune system in SCCHN patients.

Inflammation and Cancer

The strong link between inflammation and cancer is manifested by aberrant immune signals. The fact that some cancers arise at sites of chronic inflammation was first noted by Virchow over a century ago. Since then, chronic inflammatory states have been linked to a myriad of tumors: *Helicobacter pylori* infection and gastric cancer, inflammatory bowel disease and colon cancer, chronic irritation, and inflammation of the aerodigestive tract by tobacco and alcohol and SCCHN. Studies of the tumor microenvironment demonstrate infiltration of inflammatory mediators and a complex milieu of cytokines. Many of these cytokines have been previously discussed – TGF-β, IL-6, IL-10, GM-CSF – but also include cytokines such as IL-1β, IL-23, and TNF-α(alpha) as well as chemokines, which are "chemotactic cytokines" that direct immune cell migration.

Chemokines are a family of small heparin-binding cytokines that direct the movement and migration of leukocytes. There are four groups of chemokines based on the arrangement of cysteine residues near the N-terminus of the proteins: C, CC, CXC, and CX3C. The G-coupled transmembrane chemokine receptors are also divided into these four groups based on their cognate ligand [63]. SCCHN cells have aberrant expression of several chemokines. They overexpress CXCL1 which has been implicated in tumor angiogenesis, nodal metastasis, and leukocyte infiltration. CCL2 is also overexpressed in squamous cell cancer and is thought to have similar functions. CXCL5 is found in metastatic SCCHN and is involved in tumor migration and tumorigenesis. CXCL8, also found in metastatic SCCHN, promotes matrix metalloprotease secretion and subsequent extracellular matrix breakdown and tissue invasion.

Of the chemokine receptors, CXCR4 and CCR7 are of particular interest as these two receptors are overexpressed in malignant cells including SCCHN cells. Increased expression of CXCR4 and its ligand, CXCL12, in SCCHN cells is associated with nodal metastasis, tumor recurrence, and overall survival. Studies of CXCR4 activation have shown increased metastatic potential, induction of matrix metalloprotease and collagenase expression, decreased cell adhesion and increased cell mobility. CCR7 appears to have similar biological actions. High CCR7 expression is clinically associated with tumor stage, lymphatic invasion, nodal metastasis and poorer prognosis [64]. A study of chemokine receptor expression differences between primary and metastatic SCCHN cell lines found that only CCR7 was consistently upregulated in metastatic SCCHN [65]. CCR7 also provides tumor survival and invasion signals via the PI3 kinase signal transduction pathway [66]. These actions in tumor cells are similar to the action of CCR7 in dendritic and $CD8^+$ cells where they mediate chemotaxis to lymph nodes and antiapoptotic signals and may explain the predilection of SCCHN to metastasize to lymph nodes where there is a high concentration of chemokines. The production of chemokines and their receptors by SCCHN tumor cells represents exploitation of the immune system to promote tumor survival and metastasis.

A key regulator of the inflammatory response in cancer is the transcription factor NF-κB [67] which stimulates many cancer-promoting cytokines and chemokines in SCCHN [68]. NF-κB sits downstream of several soluble factors including TNF-α, IL-1, and reactive oxygen species that are produced by macrophages and granulocytes that infiltrate tumor. Of interest in relation to SCCHN, NF-κB activation can also be elicited by cigarette smoke condensate, betel nut extract, and EGFR signaling [69–71]. Activation of the NFκB pathway induces several tumor-promoting processes in SCCHN [72]. NF-κB is traditionally thought of as a stress response transcription factor because it controls expression of several prosurvival genes such as mdm2, TRAF1, TRAF2, IAP, and Bcl-XL. These act

as antiapoptotic signals for tumor cells and confer resistance to natural death pathways for aberrant cells. NF-κB also promotes tumor cell proliferation and expansion through regulation of a key cell cycle modulator, cyclin D1. Angiogenesis is promoted by NF-κB through VEGF production and several cytokines including TNF-α, IL-1, -6, and -8 are induced causing a positive feedback loop. Tissue invasion is promoted by the upregulation of heparinase, matrix metalloprotease, and urokinase. It has also been suggested that NF-κB mediates resistance to treatment with chemotherapy and radiation via regulation of GADD (growth arrest DNA damage) and glutathione-S-transferase [73]. The activation of NF-κB by inflammatory immune mediators demonstrates yet another subversion and exploitation of the immune system by cancer to promote key aspects of tumor formation and progression.

Cancer Stem Cells

Recently, there has been growing interest in the cancer stem cell hypothesis. Heterogeneity in tumor cells has long been accepted and this theory postulates the existence of a subpopulation of tumor cells that are pluripotent and are able to effectively recapitulate the entire heterogeneous tumor when transferred to another site. They are thought known to be more resistant than other tumor cells to chemotherapy as well as radiation [74]. Several defining markers of these stem cells have been proposed. The first marker proposed was CD44 [75], a cell surface glycoprotein which binds hyaluronate but may also inhibit the action of the p53 tumor suppressor in cancer cells [76]. However, CD44 expression is abundant in normal epithelia and its utility as a cancer stem cell marker is questionable [77]. Another proposed marker is aldehyde dehydrogenase 1 which is found in many embryonic stem cells and was identified as the responsible protein in conferring resistance to chemotherapeutic agents in stem cells [78]. Because these cancer stem cells are able to reconstitute the entire tumor, many believe that ultimately, it is treatment of this small population of resistant cells that determines the success or failure of oncologic therapy. If this is the case, it is important that these cells be addressed in any treatment regimen. Because aldehyde dehydrogenase 1 (ALDH1) is not highly expressed in normal tissues, its potential as a tumor antigen target has been recently explored [79].

Immune Mediators as Cancer Biomarkers

Because of the derangements in production of cytokines and other immunomodulatory molecules caused by cancer, there has been investigation into the possibility of using cytokine profiles as biomarkers. Biomarkers are of considerable interest because they could be useful in early detection of cancer, determination of prognosis, as a marker of treatment response and selection of optimal treatment regimen. Cytokines as biomarkers have been investigated in SCCHN in several studies. An older study found that serum TNF-α was 100-fold higher in cancer patients than in disease free controls [80]. A subsequent study linking serum TNF-α levels to cancer status was published but that paper found IL-6 to be a more sensitive marker than TNF-α [81]. Another cytokine commonly cited in papers as a possible biomarker for detection of tumor is IL-8 which is elevated in recurrent or metastatic cancer [82]. In a study of over 300 subjects encompassing those with active disease, no evidence of disease and healthy smokers 60 cytokines were measured and a panel of 25 including IL-8, IFN-α, IFN-γ, IL-1, and RANTES could correctly identify active disease with a sensitivity of 84.5% and a specificity of 92% [83]. This provided a proof-of-principle that the immune system may serve as a biosensor of malignancy and disease status. In another study, IL-6, IL-8, VEGF, and hepatocyte growth factor were elevated in cancer patients and decreases over treatment correlated with improved survival. Interestingly, elevated pretreatment VEGF was a good prognostic factor [84]. This is in contrast to a studies in non-small-cell lung cancer [85] and head and neck cancer (ASCO 2009 A6035) which demonstrated low pretreatment VEGF as a predictor of better treatment response and longer progression free survival. A large study of 444 patients found that high pretreatment IL-6 is an independent predictor of poor prognosis [38].

Head and Neck Cancer Immunotherapy

There are several strategies for delivering tumor vaccines with each having inherent advantages and disadvantages. All methods depend on delivering an antigen to the host in an effort to elicit an adaptive cellular immune response to the tumor antigen. Most methods require the use of a specific known tumor antigen but some can use entire tumor cells as part of the vaccine to activate the immune system against multiple unspecified and unknown tumor antigens.

DNA vaccines utilize delivery of naked DNA encoding a known tumor antigen to the patient. This DNA is taken up by cells and the antigen is expressed for subsequent processing and presentation by DC. DNA vaccines are safe, inexpensive, easy to deliver, and do not induce the formation of neutralizing antibodies allowing repeated administration. However, they have a low transfection efficiency and elicit a very weak immune response and therefore are often engineered to encode proteins that target DC or are given with adjuvant agents that increase DC activation. Currently in SCCHN,

DNA vaccines encoding a HPV-16 E6/E7 fusion protein is under development for HPV positive SCCHN [86] and another vaccine encoding Hsp65 has been tested in a phase I trial [87] and demonstrated clinical response in 4 out of 14 patients with recurrent unresectable SCCHN.

Bacterial/viral vaccines can deliver tumor antigen as well as functioning as an immune adjuvant because the immune system responses to a perceived infection. They are very immunogenic, relatively inexpensive, and easy to manufacture but have the downsides of potential toxicity, preexisting neutralizing antibodies, or the formation of antibodies against the bacterial or viral vector limiting repeat dosing or effectiveness. Also, these tend to elicit a stronger humoral rather than cellular immune response which is less desirable. Several such vaccine are currently under development: HPV-16 E7 Listeria vaccine [88], Vaccinia-based E6/E7 vaccine [89], and a Vaccinia-based E2 [90].

Peptide vaccines consist of synthesized peptides that have been designed to correspond to an epitope on a tumor antigen that binds well to the cleft of an HLA molecule. They are similar to DNA vaccines in that they are safe and inexpensive with low immunogenicity but have the added drawback of being restricted to the HLA subclass for which they were designed. The popular HLA subclass used in vaccine design is HLA-A2 as this is the most common subclass found in Caucasians. Clinical trials are underway with a MAGE-A3/HPV-16 peptide (NCT00257738) and a LMP-2 peptide for EBV-related nasopharyngeal carcinoma (NCT00078494).

To circumvent HLA restriction, whole proteins can be used as a vaccine. Whole proteins can be processed by the antigen-presenting cells and presented on self MHC to cause activation of T cells. However, the vast majority of identified tumor antigen proteins are self proteins and therefore the patient's immune system is tolerant to these proteins. Therefore, there is tremendous difficulty in producing an effective immune response with protein vaccines.

Tumor cell vaccines are similar to whole protein vaccines in that they are not HLA restricted and specific epitopes need not be known for their use. Often the tumor cells are given with adjuvant agents or modified by viral infection to improve their immunogenicity. A Newcastle disease virus infected tumor cell vaccine was found to induce a specific T-cell response and [91] that correlated with better clinical outcome. These vaccines tend to be labor intensive because tumor has to be isolated and processed before it can be used as a vaccine.

Dendritic cells are the most potent activators of antigen-specific T cells and consequently, DC vaccines are the most widely studied cancer vaccine strategy. This is an extremely labor-intensive method in which dendritic cells are isolated from each patient and they are loaded with tumor antigen ex vivo. This loading can be in the form of peptides, proteins, DNA transfection, tumor cell lysates, apoptotic tumors, necrotic tumors, or cell fusion. After DC are loaded with tumor antigen, they undergo maturation and activation with various cytokine cocktails to prime them for presenting the tumor antigen to T cells. These DC are then introduced to the patients usually into the tumor or into lymph nodes. Several DC-based vaccines are currently being developed for SCCHN: intratumoral injection of DC (NCT00492947), multivalent p53 DC vaccine [92], and lysyl oxidase like-4 transfected DC [93].

There are also efforts to reverse the immunosuppression associated with cancer. One method utilizes a cocktail of multiple cytokines delivered systemically to improve immune competence. Other strategies target specific inhibitory molecules. CTLA-4 is a receptor found on T cells which sends an inhibitory signal and leads to T-cell anergy. An anti-CTLA-4 antibody has been developed to block this inhibitory signal [94]. Another inhibitory cell surface protein on T cells is programmed death-1 [95] and antagonistic antibodies to this protein have demonstrated efficacy in phase II trials [96]. Anti-KIR antibodies remove the major inhibitory signal on NK cells. There are also monoclonal antibodies which act as agonists of various stimulatory receptors such as CD40, CD137, and glucocorticoid-induced tumor necrosis factor receptor [97–99] in various stages of development.

Monoclonal Antibody-Based Immunotherapy of SCCHN

Today the most widely used form of cancer immunotherapy is mAb therapy. Currently available mAbs that may have activity in head and neck cancer are listed in Table 6.1. The most extensively studied of these is cetuximab, a mouse–human chimeric IgG1 antiepidermal growth factor receptor (EGFR) mAb [100]. EGFR is an attractive target in SCCHN because it is overexpressed in 80–90% of SCCHN and leads to tumor cell proliferation, invasion, angiogenesis, tumor survival, and consequently, poor survival and prognosis [101]. The one mAb which does not target EGFR listed in Table 6.1 is bevacizumab which is a humanized IgG1 specific

Table 6.1 Currently available mAbs for investigation or clinical use in head and neck squamous cell carcinoma

Antibody	Subtype	Target
Cetuximab	Chimeric IgG1	Domain III of EGFR
Panitumumab	Human IgG2	Domain III of EGFR
Matuzumab	Humanized IgG1	Domain III of EGFR
Zalutumumab	Human IgG1	Domain III of EGFR
IMC-11F8	Human IgG1	Domain III of EGFR
Bevacizumab	Humanized IgG1	VEGF-A

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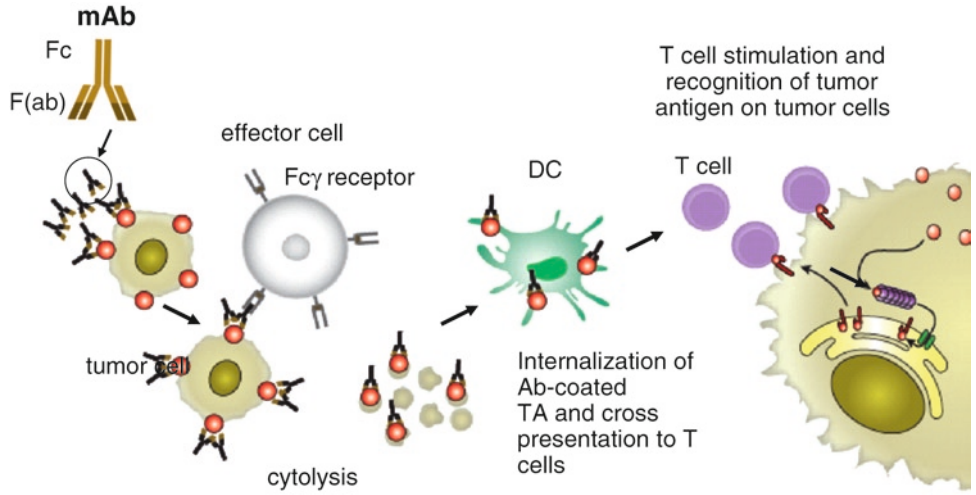

Fig. 6.2 Schematic representation of ADCC, the effector mAb has a constant fragment [Fc] that interacts with immune effector cells, and a variable fragment [F(ab)] that is antigen (EGFR) specific. During cross presentation, tumor antigens are degraded in the cytoplasm of dendritic cells (DC), and presented to T cells producing a cellular immune response. Reprinted from Lee, S., Lopez-Albaitero, A., and Ferris, R. L. (2009). Immunotherapy of head and neck cancer using tumor antigen-specific monoclonal antibodies. *Current Oncology Reports.* 11 (2), 156–162. With kind permission from Springer Science + Business Media

against VEGF-A. A phase II trial of a combination of bevacizumab and erlotinib in SCCHN demonstrated a response rate of 14.6% and an overall mean survival of 6.8 months [46] and several other phase II trials in combination with cetuximab and pemetrexed are pending. An Eastern Cooperative Oncology Group (ECOG) phase III trial studying bevacizumab in combination with chemotherapy is also currently underway.

It is becoming clear that anti-EGFR mAb mediate antigen-specific immune responses to targeted tumors (Fig. 6.2). There are two major mechanisms by which mAb can activate the immune system against a tumor target, direct killing via lytic immune cell (NK cell or monocytes) and complement fixation, or opsonization of tumor for phagocytosis and subsequent antigen processing. The latter would induce TA-specific cytotoxic T lymphocytes (CTL) to recognize and lyse tumor cells. One of the most direct methods by which antibodies can cause tumor lysis is via antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells and probably monocytes and neutrophils. Panitumumab and cetuximab both mediate ADCC [102] and the extent of ADCC is heavily influenced by genetic polymorphisms in FcγRIIIa, also known as CD16 [103]. Complement activation via the classical pathway is another major effector of humoral immunity and is activated by IgM, IgG1, IgG2, and IgG3. A combination of cetuximab and matuzumab can elicit complement-dependent cytotoxicity in vitro [104]. In addition to direct activation of NK cell lysis of tumor cells, TA-specific mAbs can elicit $CD8^+$ T-cell responses to tumor-derived antigens through interaction with FcγRs on antigen-presenting cells (APC). In human cells, there are three activating FcγRs, FcγRI, FcγRIIa, and FcγRIII and one inhibiting FcγR, FcγRIIB [105] with FcγRIIa being the dominant receptor on APC. This antigen-specific T-cell activation was noted in 78% of patients treated with trastuzumab for breast cancer and this activation seemed to correlate positively with clinical response [106]. Specific T-cell activation has recently been demonstrated in a model using glioma and cetuximab [107] and it is likely that similar T-cell activation also occurs in SCCHN patients treated with anti-EGFR mAbs (Lee, SC and Ferris, RL unpublished data).

The mechanism for TA-specific T-cell induction may actually be enhanced by ADCC and NK cell activation. In addition to their ability to mediate ADCC, activated NK cells, particularly $CD56^{bright}$ NK cells [108] have also been shown to secrete cytokines, such as IFN-γ, TNF-α, and chemokines, such as macrophage inflammatory protein-(MIP)-1α, MIP-1β, and RANTES, that inhibit tumor cell proliferation, enhance antigen presentation, and aid in the chemotaxis of T cells [103, 109]. Indeed, NK cells can interact with other innate immune cells that are present during the early phases of inflammatory responses [110]. This so-called NK cell–DC cross-talk follows the recruitment of both NK cells and DC to sites of inflammation [110, 111], resulting in potent activating bi-directional signaling. NK cells in the presence of cytokines released by DC become activated, regulating both the quality and the intensity of innate immune responses. Also, activated NK cells release cytokines that favor DC maturation and select the most suitable DC for subsequent migration to lymph nodes and efficient T-cell priming. In addition, IFN-γ secreting NK cells can be recruited directly to the lymph nodes to enhance T-cell

induction [112]. Elevated levels of the NK cell-derived chemokines IL-8, macrophage inflammatory protein-1, and RANTES have been detected within the sera of trastuzumab responding cancer patients [109]. These NK cell factors could induce the chemotaxis of naive and activated T cells, as indicated by the correlation of their presence with the infiltration of tumor tissue by $CD8^+$ CTL. These data suggest that NK cell cytokine and chemokine production may enhance DC cross presentation and T-cell induction, with the potential to spread it to other TA [113].

Conclusion

Cancer immunology is a rapidly evolving field and it is only recently that we have begun to understand the complex interaction between cancer and the host immune system. Tumor cells demonstrate several methods to exploit the immune system to help promote angiogenesis, derive prosurvival and proliferative signals, and induce metastasis and tumor progression. At the same time, cancers are able to cloak themselves from the immune system by self modification and by immunosuppression of the host. These insights and better understanding of the workings of the immune system have allowed the recent explosion of several promising immunotherapeutic agents that are currently in clinical use as well as under development.

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