31 Tumor Immune Escape Mechanisms

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Summary

The immunosurveillance theory postulates that the immune system is able to identify transformed cells and eliminate them. The theory predicts that the incidence of cancer would increase, or the latency period of cancer would decrease, in the absence of a functional immune system. However, the fact that the incidence of only some cancers increases in immunosuppressed patients shows that not all cancers abide by this theory. Most cancers escape immunosurveillance because they are fundamentally "self," and autoreactive immune cells are usually deleted or anergized so that they do not attack self. The tumors that do face immune pressure are virus-associated cancers and cancers expressing immunogenic tumor antigens. These tumors have, however, evolved mechanisms to escape immune eradication. An effective way of escaping immune eradication is to prevent detection. The expression of tumor-associated antigens enhances the immunogenicity of a tumor, and if it is able to reduce the presentation of such markers, then the tumor remains relatively invisible to the immune system and escapes detection. If the tumor does not manage to escape detection, then it can evolve to prevent the activation of the immune response. The immunosuppressive effects of cancer cells are mediated by the secretion of soluble factors, by the expression of inhibitory molecules,

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and by turning the cellular infiltrates into tolerizing cells that can in turn suppress other potentially tumor-specific immune cells. Some tumor cells have evolved to become resistant to the death effector mechanisms of the immune system. Finally, some tumors have evolved to turn the immune system against itself by causing the death of the immune cells through an activation-induced cell death mechanism that normally functions to limit the immune response under physiological conditions. These immune escape mechanisms in combination make the tumor a formidable foe for the immune system. Therefore, a well thought out immunotherapy strategy would keep in mind the escape mechanisms the tumor could adopt under immune pressure to direct the most propitious strike.

Key Words: Immunosurveillance; immunogenicity; escape mechanisms; tumor antigens; immunodetection; tumor microenvironment.

1. INTRODUCTION: IMMUNOSURVEILLANCE OF CANCER

The immunosurveillance hypothesis states that a physiologic function of the immune cells is to recognize and destroy transformed cells. The concept of immunosurveillance was first introduced in 1909, when Paul Ehrlich proposed that immunity against cancer was mediated by "cellular forces" that kept tumors in check *(1)*. The theory was later appended by Thomas Lewis and Sir MacFarlane Burnet, who proposed that immunological recognition of transformed cells was a form of homeostatic surveillance that could allow the body to guard against malignancies *(2,3)*. Implicitly, the hypothesis predicts that the incidence of cancer would increase or tumor latency periods would be reduced in the absence of immunosurveillance. Epstein-Barr virus (EBV)-related neoplasms are examples of cancers usually controlled by immunosurveillance that increase in incidence in immunosuppressed individuals. EBV is a lymphotropic herpes virus that affects the majority of individuals *(4)*, and causes little significant disease in a healthy immunocompetent person. It establishes itself within the nucleus of B-lymphocytes expressing the CD21 molecule during the initial infection, and remains in the body in a state of latency for that individual's lifetime. This latent state is associated with the production of viral proteins like Epstein-Barr nuclear antigen and latent membrane proteins that protect the B-cell from apoptosis and allow intermittent low-grade viral replication *(5)*. The viral replication is usually held in check by cytotoxic T-cells (CTLs) driven EBV-specific immunosurveillance in healthy individuals. However, in the immunosuppressed transplant recipient, the impaired EBV-specific CTL response leads to an increase in viral replication and ultimately, to B-cell transformation. EBV-induced posttransplant lymphoproliferative disorder (PTLD) is the most common neoplasm found in pediatric renal transplant recipients *(6)*. The incidence of PTLD is four times higher amongst pediatric than adult transplant recipients *(7)*, possibly because of the fact that a larger proportion of the children are EBV naive pretransplant and therefore have little immunity towards the virus. The significant increase in virus-associated cancers in immunocompromised patients, and the finding that preemptive antiviral therapy in hematopoietic stem cell transplantation prevented EBV-associated PTLD *(8)* supports that immunosurveillance reduces the incidence of virus-induced tumors in an immunocompetent host.

Additional evidence supporting the role of immunosurveillance comes from studies on the incidence of neoplasms amongst transplant patients under long-term immunosuppression and immunocompromised human immunodeficiency virus (HIV)-infected patients. Skin cancer was noted to be increased in patients receiving long-term immuno-

suppression because of a solid-organ transplant *(9)*; also, the risk of malignancy in renal transplant patients receiving long-term immunosuppression was considerably higher *(10)*. HIV-associated immunosuppression has been linked to a greater increase in cases of Kaposi sarcoma, non-Hodgkin's lymphoma, and invasive cervical cancer *(11,12)*. Most of the malignancies observed in the immunosuppressed and immunocompromised patients were noted to be associated with viral-infections, such as B-cell lymphomas (EBV), Kaposi sarcoma (human herpes simplex virus 8) and cervical cancers (human papilloma virus). This makes the virus-associated cancers excellent targets for immunotherapy.

1.1. Most Cancers Slip Through the Immunosurveillance Net

It is important to note that the cancers that immunosuppressed patients are at an increased risk of developing are not the same as those that are most commonly found in the general populace. This implies that most cancers are not covered under the immunosurveillance theory, and many cancers develop simply because the immune system does not recognize them as foreign in the first place. Cancer cells are basically "altered self-cells" and may not be very immunologically different from normal cells. In fact, most cancer cells escape immunosurveillance, because they simply do not satisfy the primary condition of the immunosurveillance theory, which requires the distinction of transformed cells from normal cells. Central and peripheral tolerance mechanisms such as the clonal deletion by ubiquitous self-antigens and clonal inactivation by tissue-specific antigens presented in the absence of costimulatory signals ensure that the immune system does not attack self. Apart from the virus-associated cancers, most cancers are not immunogenic, because the antigens that they express are self-antigens against which the immune system has been tolerized. However, this does not mean that immunotherapy cannot work on these cancers; it just has to be achieved by breaking immunological tolerance to self-antigens and at the cost of autoimmunity. Therefore, such immunotherapy strategies can only be done in tissues that can be spared because all cells of these tissues, including the nontransformed cells will be susceptible to immune destruction.

1.2. Immunotherapy—the Need to Pick the Right Target

The success and specificity of immunotherapy strategies is absolutely contingent on the choice of the target antigen. Focusing the immune response on antigens truly unique to the tumor increases the specificity of the response and reduces the chances of developing autoimmunity. In contrast, directing the immune attack at tumor-associated antigens detected in both tumor and normal cells could lead to the immune destruction of the self tissues. Naturally, there exists the probability of tumor escape by various means of downregulating the expression of the target antigen because of the immune pressure exerted on the tumor. Therefore, a greater number of available antigenic targets would allow immunotherapy strategies to cast a wider net to counteract tumor escape mechanisms. The academy of cancer immunology has a website that contains links to several databases set up with the purpose of characterizing tumor antigens that elicit immune responses in humans (http://www.cancerimmunity.org/statics/databases.htm). The characterization and identification of novel tumor antigens is also fundamental to the design of improved therapeutic or prophylactic cancer vaccination schemes. Although most interest has been focused on identifying antigens that could be good targets for $CD8^+$ CTLs that kill transformed cells expressing antigenic peptides in the context of major histocompatibility complex (MHC) class I molecules, currently however, efforts have

been turned to identify antigens recognized by $CD4^+$ T-helper (Th) cells that enhance and amplify the immune response through costimulation and the local production of cytokines. A consensus exists that a combined vaccine based on $CD8^+$ and $CD4^+$ T-cell epitopes would improve the efficacy of therapeutic cancer vaccines substantially.

The cells within any particular tumor may contain their own individual mutations; therefore, the tumor is rather heterogeneous in its susceptibility to any sort of therapy. This accounts for the escape variants that evolve after chemotherapy or immunotherapy. To conduct immunotherapy, in addition to choosing an antigenic target that provides specificity, the knowledge of how cancers react in response to immune pressure would help to make the proposed treatment plan more encompassing so that it does not fail because of tumor escape variants.

2. TUMOR ANTIGENS

2.1. Tumor Antigens: How to Identify the Enemy

Tumor antigens are processed and presented to the adaptive immune system as short peptide fragments known as epitopes on major MHC class I and MHC class II molecules. The MHC class I molecules are expressed by nearly all nucleated cells of the body, and normally present peptides that are generated endogenously in the cells. It is imperative that the cancer cell presents some form of immunogenic antigen in order for the $CD8^+$ CTLs to recognize the tumor cell and destroy it. The $CD8^+$ T-cells are the key effectors of antitumor immunity mediated by the adaptive immune system, and they recognize antigenic epitopes presented in the context of MHC class I molecules. $CD4^+$ Th cells also play an important role in antitumor immunity *(13)*, as they enhance and amplify the immune response through costimulation and the local production of cytokines. Th cells recognize antigens presented in the context of MHC class II molecules whose expression is limited to professional antigen-presenting cells (APCs) such as dendritic cells (DCs). The presentation of antigenic epitopes derived from the tumor cells allows the immune system to distinguish between normal and transformed cells and direct the immune attack based on these antigens. Tumor antigens can be classified into five major groups based on their expression patterns: mutational antigens, shared tumor-specific antigens, differentiation antigens, overexpressed antigens, and viral antigens *(14)*.

2.1.1. Mutational Antigens

Mutational antigens are derived from ubiquitous proteins that are mutated in tumor cells. Point mutations, chromosomal translocations, deletions, or gene insertions can lead to the generation of unique tumor antigens distinct for each tumor. The mutational antigens are highly tumor-specific, and some may also be involved in the transformation process. Chronic myelogenous leukemia is characterized by the presence of Bcr-Abl, a fusion product resulting from the translocation of the of cellular Abelson tyrosine kinase from chromosome 9 to a 5.8-kb breakpoint cluster region on chromosome 22 *(15)*. The detection of Bcr-Abl junctional epitopes that bind to both MHC class I human leukocyte antigen (HLA)-A2 *(16)* and MHC class II DR4 *(17,18)* demonstrate that mutational antigens can potentially induce potent immune responses and may be involved in the natural antitumor response in patients. On the other side of the high specificity of mutational antigens is that their potential value as generic cancer vaccines in immunotherapy is limited, as such mutations may not be shared by many patients.

2.1.2. Shared Tumor-Specific Antigens

Shared tumor-specific antigens are antigens whose expression is usually silenced in normal tissues but are activated in tumors of various histological types. Expression of these antigens on normal tissues has only been detected on placental trophoblasts and testicular germ cells that do not express MHC class I molecules. Hence, these antigens are usually not presented to the immune system and can be considered tumor-specific and are also known as cancer–testis antigens. The prototype shared tumor-specific antigens are the melanoma antigen genes, which are normally expressed in testis and placenta and overexpressed in melanoma, bladder cancer, breast cancer, lung cancer, and prostate cancer *(19)*.

2.1.3. Tissue-Specific Differentiation Antigens

Differentiation antigens lack the specificity of tumor-specific shared antigens, as they are differentiation markers that are expressed not just by the malignant cells, but also by the normal cells of the same origin as the cancer cells. Tyrosinase, for example is expressed by both normal melanocytes and most melanoma cells. Targeting such antigens would also result in the autoimmune destruction of the normal tissue as has been demonstrated by the vitiligo *(20)* induced after vaccination against tyrosinase in melanoma patients. Immunotherapy strategies based on such antigens should be reserved to tissues that are not vital for survival, as exemplified by the targeting of the prostate-specific antigen that could lead to the destruction of the prostate tissues in prostate cancer.

2.1.4. Overexpressed Antigens

T-cell activation is dependent on a minimum number of T-cell receptor/peptide/MHC contacts *(21)*; therefore, the overexpression of many proteins in cancer cells could lead to the generation of an immune response to these self-proteins. The high levels of mutant or wild-type p53 expressed in many cancers make it a potential immunotherapy target, and it has been used against colorectal cancer without inducing autoimmunity *(22)*. However, because these overexpressed proteins are expressed by many normal tissues, it is difficult to assess the safety threshold for each antigen that does not result in widespread autoimmunity.

2.1.5. Viral Antigens

Viral antigens are foreign and are only found on infected cells, thereby making them ideal targets because of their high specificity. Although viruses have evolved their own set of immune evasion strategies, immunotherapy of virus-associated cancers can be directed against viral-antigens vital for viral replication or growth. The human papilloma virus (HPV) E6 and E7 proteins interfere with normal cell-cycle regulation *(23,24)* and are required for the viral life cycle *(25,26)*. Diverse immunotherapy strategies directed against HPV E7 and HPV E6 *(27)* have led to promising results *(28)*.

2.2. Tumor-Associated Antigens Can Induce Tolerance

Qualitative and quantitative changes have been observed in the glycolipids and glycoproteins on the cell surface of tumor cells *(29,30)*. The cell surface location of these antigens make them good candidates for therapeutic and diagnostic purposes, because they are accessible to both the cellular and humoral components of the immune system. The mucins are the most extensively studied group of glycoproteins. Mucins are large

glycoproteins with high-carbohydrate content expressed by a variety of normal and malignant epithelial cells. The mucins CA-125 and CA-19-9 have been detected in ovarian carcinomas *(31,32)*, whereas mucin (Muc)-1 has been found in breast carcinomas *(33)*. Under physiological conditions, Muc-1 is expressed on the apical surface of breast ductal epithelium and is inaccessible to the immune system. In ductal carcinomas of the breast however, Muc-1 looses its apical polarization and displays new carbohydrate and peptide epitopes, thereby becoming an accessible target for the immune cells. Muc-1 can be easily detected by monoclonal antibodies, and also contains T-cell epitopes and has been used as a target for tumor vaccination schemes *(34)*. However, it has been shown recently that tumor-derived Muc-1 mucins were responsible for the impaired maturation and function of monocyte-derived DCs. Tumor derived-Muc-1 changed the cytokine repertoire of the DCs and resulted in their development into interleukin (IL)-10^{high} IL-12^{low} regulatory APCs *(35)* as a novel mechanism of tumor immune evasion.

3. IMMUNODETECTION

3.1. Stealth and Camouflage—Escaping Immunodetection

Two arms of the immune system work complementarily in immunodetection. The adaptive immune system detects the presence of a transformed cell by scanning for altered self-cells. The innate immune system detects the presence of a transformed cell by looking out for missing self. Therefore, in order to escape successfully both arms of the immune system, cancer cells have evolved a joint strategy of both stealth and camouflage. They have to hide the tumor antigens they express and disguise themselves as something that the body will not reject. The CTLs of the adaptive immune system recognize antigens bound on MHC class I molecules expressed by nearly all nucleated cells of the body. If the MHC class I molecule on the tumor cells presents a viral or aberrant peptide, then the antigen-specific CTLs eliminate the tumor cell. The fetus is an allograft that survives within the maternal host despite its low expression of allogenic MHC molecules that would usually result in immune destruction by the natural killer (NK) cells of the innate immune system. The same immune evasion strategies utilized by the fetus "camouflage" the cancer cells and enable them to escape the NK cells. Together, this stealth and camouflage strategy described in the following two subheadings enables the cancer cells to evade detection.

3.1.1. Evading the CTLs

Tumor cells often have an altered expression pattern of class I molecules, as a consequence of profound defects in the antigen processing pathway. This promotes poor expression or loss of class I peptide presentation, which permits tumor cell escape from CTL killing. Different mechanisms that lead to loss of class I molecules have been described so far *(36)*. Production of immunosuppressive molecules that downregulate the expression of MHC class I on nucleated cells and defects in the antigen processing machinery have been clearly demonstrated by examining tissue samples from several cancers. Recently, by microdissection and reverse transcription-polymerase chain reaction, a problem in the presentation of class I peptide was detected in transformed colon cells *(37,38)*. Profound defects in the processing and presentation of peptides were found to be caused by an accumulation of the HLA class I heavy chain in the cytoplasm of neoplastic cells, biallelic inactivation of the β-2 microglobulin, downregulation of the low-molecular-weight protein (LMP)7, and deregulation of the transporter associated with antigen

processing (TAP) 2. All these defects allow the colon carcinoma cells to become "invisible" to the adaptive immune system. In addition, histological samples showed down-regulation of the proteosome multicatalytic complex subunits LMP-2 and LMP-7 in prostate and renal carcinoma, small cell lung carcinoma, and non-small cell lung cancer *(39–42)*. All these examples indicate that class I down-regulation is an important mechanism of tumor escape.

3.1.2. Tricking the NK Cells

Despite the reduction in MHC class I expression, tumors are still susceptible to attack from immune cells. Tumor cells that lack MHC class I expression are an attractive target for the NK cells of the innate immune system. NK cells bind to the polymorphic determinants of the MHC class I molecules through killer-cell inhibitory receptors (KIRs) *(43)*. The interaction between KIRs and MHC class I molecules is inhibitory in nature, and on ligation leads to inhibition of NK-cell cytotoxicity, maintaining tolerance towards self-tissue. The downregulation of MHC class I molecules on the cell surface of tumor cells will therefore normally lead to the NK-mediated killing of the tumor cells. Another way the NK cells keep track of MHC class I expression is through the heterodimer CD94-NKG2A, which recognizes nonclassical MHC class I molecules such as HLA-E *(44)*. HLA-E presents the signal peptides from the classical MHC class I molecules (HLA-A, -B, and -C), and downregulation of any haplotype molecule in particular would normally result in a reduction of cell surface HLA-E and an increase of the susceptibility of tumor cells to NK-mediated killing.

In order to escape NK-mediated killing, cancer cells have evolved to establish tolerance using similar mechanisms as those found in fetal–maternal interactions. HLA-G is a nonclassical MHC class I molecule expressed in the placenta and helps to maintain tolerance to the fetus. It is expressed by many cancers like melanoma, renal carcinoma, lung carcinoma, glioblastoma, and ovarian cancer. It is upregulated through the local expression of environmental factors such as cytokines, stress factors, and chemotherapeutic agents *(45,46)*. HLA-G exerts its immunoinhibitory effects through at least three KIRs expressed by nearly all cells of the immune system *(47,48)*, and therefore has powerful immunosuppressive effects *(49,50)*. In renal carcinoma, HLA-G expression on tumor cells blocks the cytolytic activity of lymphocyte activated killer cells and CTLs, promoting the evasion of the immune response *(51)*. Soluble HLA-G has also been detected in the plasma of patients suffering from malignant melanoma, glioma, breast, and ovarian cancer *(52)* and can result in local or systemic immunosuppressive effects. However, the signal peptide for HLA-G also serves as a peptide ligand for HLA-E. The interaction between the CD94-NKG2 and HLA-E presenting a nonamer from the the HLA-G signal peptide can lead to inhibition or activation of NK-cytotoxicity, depending on the inhibitory or activating nature of the CD94–NKG2 heterodimer *(53–55)*.

Stress and cellular transformation causes some malignant cells to express MHC class I chain-related (MIC) molecules and UL16-binding protein 1 that are ligands for the NK-activating NKG2D receptor *(56)*. The triggering of NK-activating receptors can result in NK-mediated cytotoxicity of cell types that still express significant level of MHC class I molecules in vitro. NKG2D is also expressed by CTLs and results in their activation when triggered. To avoid being killed by NK cells, tumor cells can produce soluble MICs (sMICs) that block the activating NKG2D receptor. sMICs bind to NKG2D, inducing its endocytosis and degradation, resulting in a reduced expression of NKG2D on tumor-infiltrating and peripheral blood T-cells in cancer patients *(57)*. In colorectal patients,

NKG2D downregulation by sMICs resulted in the decreased expression of another NK-activating receptor, the natural cytotoxicity receptor, and the CXCR1 and CCR7 chemokine receptors. This resulted in homing defects and inactivation of the NKG2D NK population *(58)*.

4. IMMUNOMODULATORY MECHANISMS

4.1. Immunological Regulatory Processes Exploited by the Tumor Cells

Cancer cells are basically self-cells that are no longer regulated by normal cellular processes and proliferate without control. These aberrant cells are predisposed to accumulating genetic errors that place them in a better position to adapt to changes in their environment. Like organisms predicted by Darwin's Theory of Natural Selection to adapt to the environment or suffer extinction, immune pressure selects for tumor variants that are resistant to immune eradication. Apart from the immune evasion strategies listed, modulation of the immune response to incapacitate the antitumor response is a powerful evolutionary adaptation of the cancer cells. Most of the immunomodulatory mechanisms found in tumors are based on normal homeostatic control processes of the immune response set in place to prevent unbridled proliferation of the immune cells, or to maintain tolerance towards self-tissues.

4.1.1. Disrupting Cell–Cell Interaction

To establish a strong and productive interaction, immune cells are required to reinforce their cellular communication through the induction of adhesion molecules on their surface. The intercellular adhesion molecule (ICAM)-1 is crucial for the formation of the immunological synapse. ICAM-1 participates in the cell–cell interaction between the NK cell and the malignant cell. Transformed cells have been shown to disrupt this cellular interaction by producing the matrix metalloproteinase 9, which results in ICAM-1 shedding and resistance to NK cell killing *(59)*.

4.1.2. Requirement for a Second Signal—a Chance to Turn Off the Immune Response

Recognition is only the first step in triggering an immune response. The productive interaction leading to activation requires a second costimulatory signal. The costimulatory signal is provided by the ligation of B7.1 (CD80) or B7.2 (CD86) molecules on the surface of APCs to CD28 on the T-cells or NK cells *(60)*. Although CD28 plays a vital role in the induction of T-cell activation, other members of the CD28 family such as CTL-associated antigen (CTLA)-4, programmed death (PD)-1, and inducible costimulator (ICOS) have opposite functions. Engagement of B7 family members with CTLA-4, PD-1, and ICOS leads to inhibition instead of activation of T-cells *(60)*. Accumulating data suggest that CTLA-4 functions predominantly to regulate activation of naive T-cells in lymphoid organs; ICOS and PD-1 regulate activation and effector phases within and outside lymphoid organs *(61)*.

PD-1 is a negative regulatory receptor expressed by activated T-cells, B-cells, and macrophages, which binds to B7-H1 or B7-DC *(62,63)* expressed on activated DCs, B-cells and monocytes *(64,65)*. B7.H1 plays an important role in the regulation of the humoral and cellular immune responses, promoting the apoptosis of activated B-cells and T-cells that express the ligand PD-1. B7-H1 has been detected in human lung carcinomas, ovary carcinomas, colon carcinomas, and melanomas *(66)*. The expression of B7-H1 on transfected P815 tumor cells increased the apoptosis of tumor-reactive T-cells and facili-

tated the growth of highly immunogenic B7.1$^+$ tumors in vivo, demonstrating its role in tumor-mediated immunosuppression *(66)*.

B7-H4 is a recently discovered B7 family member that causes detrimental effects on T-cell immunity: inhibiting T-cell proliferation, cytokine production, and cell cycle progression. The expression of the putative ligand of B7-H4 is inducible on T-cells, but has yet to be identified. B7-H4 is not expressed in normal tissues, but is constitutively expressed in 85 and 31%, respectively, of ovarian cancer and lung cancer tissues *(67)*. B7-H4 may have an important role in the immune evasion of these tumors.

4.1.3. CD40—Providing a "Helping" Hand

Most solid tumors are able to escape immunosurveillance, simply because naive T-cells normally circulate between the blood and the secondary lymphoid organs and do not encounter the tumor cells. Tumor-specific protective CTLs can therefore only be induced if sufficient tumor cells reach the secondary lymphatic organs. Therefore, professional APCs that can prime naive T-cells within the lymphoid organs are indispensable in the activation of natural antitumor response. Immature DCs can pick up antigens derived from apoptotic cells, virus infected cells or neoplastically transformed cells and present them on MHC class I molecules in a process known as crosspresentation. Crosspresentation can either activate or suppress the immune response and has been termed "crosspriming" or "crosstolerance," respectively. Although crosspriming has been demonstrated to be inefficient and insufficient in inducing protective CTLs *(68)* on its own, the ability of DCs to present antigens to CD4$^+$ Th cells through MHC class II molecules remains very important, because the presence of Th cells during the priming phase of CTLs contribute significantly to antitumor immunity. Maturation of DCs is mostly dependent on exposure signals resulting from inflammation such as exposure to necrotic cells *(69)* or Toll-like receptor signaling *(70,71)*. CD4$^+$ T-cells and DCs can provide reciprocal "help" to each other. Immature DCs can present antigens on MHC class II molecules to the CD4$^+$ T-cells that express CD40 ligand (CD40L). The CD40L–CD40 interaction enables the maturation of DCs *(72)*. Mature DCs express high levels of costimulatory molecules that provide the costimulation needed for the naive T-cells to proliferate and differentiate. Like the CTLs, the Th cells also require costimulation in order to be fully activated. Absence of the second costimulatory signal can lead to a state of anergy or tolerance in the CTL and the Th cells *(73)*. CD40 ligation of DCs has the capacity to induce high levels of the cytokine IL-12, which polarizes CD4$^+$ T-cells toward a Th1 type, enhances proliferation of CD8$^+$ T-cells, and activates NK cells *(74,75)*.

CD40 is also expressed by B-cells and rescues low-affinity antigen-binding and autoreactive B-cells in germinal centers from Fas–Fas ligand (L)-mediated apoptosis *(76)*. The apoptotic signal is dependent on the activation of the death-inducing signaling complex (DISC) that can be inhibited by the Fas-associating protein with death domain-like interleukin 1 converting enzyme inhibitory protein (FLIP). CD40 signaling leads to the stabilization of FLIP and to the rescue of Fas-mediated apoptosis *(77)*. CD40 has been detected on a variety of human cancer cells, from various origins such as bladder, ovarian, colorectal, liver, lung, pancreas, prostate, cervical, and breast *(78–80)*. It has been shown that CD40 activation on bladder and human gastric carcinoma cells inhibits apoptosis mediated through Fas using a similar mechanism to the one in the B-cell apoptosis rescue *(81,82)*.

In addition, CD40 activation is able to induce an increase in the motility of gastric carcinoma cells *(83)*, and its expression has been detected in the tumor vasculature of

renal and breast carcinoma as well as in Kaposi's sarcoma *(84)*, suggesting a potential role of CD40 in the angiogenesis and metastasis of cancer. Elevated plasma levels of soluble CD40L also correlated with metastatic spread in human lung cancer *(83,85)*. The high serum levels of soluble CD40L have proangiogenic effects *(86)*, as it can induce the increased transcription of vascular endothelial growth factor (VEGF) by endothelial cells expressing CD40 *(87)*.

5. TUMOR MICROENVIRONMENT

5.1. The Effects of the Tumor Microenvironment on the Antitumor Response and Tumor Growth

The pleiotropic effects of cytokines can function to support or suppress the immune system. Tumor cells have evolved to produce cytokines that suppress the immune response and to profit from the proangiogenic effects of some cytokines. Cytokines present in the milieu when naive $CD4^+$ T-cells are activated can skew the balance of development into Th1 or Th2. Th1 and Th2 cytokines have reciprocal inhibition on the development of the type of Th response. IL-12 and interferon (IFN)-γ lead to the development of Th1 cells that augment cell-mediated immune responses crucial for antitumor immunity. IL-4 induces the development of Th2 cells *(88,89)*, which promote humoral responses and inhibit the formation of a Th1 response.

The local production of type 1 cytokines like IFNγ, IL-2, and tumor necrosis factor (TNF)-α favor cell-mediated immunity and is important in the control of tumor growth. Tumor cells have been shown to produce *(90)* or to induce the production of type 2 cytokines through tumor-infiltrating lymphocytes. It has also been suggested that the hypoxic conditions found around tumors may bias the immune response towards a type 2 response *(91)*. Type 2 cytokines downregulate the expression of type 1 cytokines, inactivating the cell-mediated antitumor response. Analysis of the cytokine microenvironment from the fresh pleural effusions and tissue samples from several cancers has revealed the predominant expression of type 2 cytokines like IL-4 or immunosuppressive cytokines such as transforming growth factor (TGF)-β and IL-10 *(92,93)*. TGF-β and IL-10 suppress the type 1 and proinflammatory responses of the immune system *(94,95)*.

5.1.1. Interleukin 10

IL-10 has potent immunosuppressive effects on APCs and effector T-cells. IL-10 reduces the expression of type 1 cytokines, inhibits antigen-specific T-cell proliferation *(96)*, and inhibits the production of proinflammatory cytokines by macrophages *(97)* and APCs *(98)*. DCs matured in vitro in the presence of IL-10 are impaired in their ability to produce type 1 cytokines, leading to the development of Th2 cells in vivo *(99)*, resulting in the development of a humoral response instead of a cellular response that is more beneficial for antitumor immunity. IL-10 has also been shown to turn DCs into tolerogenic DCs. Pretreatment of DCs with IL-10 induces an antigen-specific anergy in CTLs (100). In tumors, local production of IL-10 has also been associated with an increase in the expression of HLA-G, resulting in the induction of tolerance towards the tumor in addition to general immunosuppression *(101,102)*. The exclusion of APCs from the tumor mass has also been attributed to the local production of IL-10 *(103,104)*.

5.1.2. Transforming Growth Factor-β

TGF-β is commonly overexpressed in many cancers and has many immunosuppressive effects, including the inhibition of T-cell proliferation and their development into

CTLs and Th cells *(105)*. TGF-β-overexpressing tumors are particularly aggressive, and have been correlated with a more malignant phenotype. Apart from its role in tumor-mediated immunosuppression, TGF-β also regulates cellular proliferation, differentiation, extracellular matrix production, cell motility, and apoptosis *(106,107)*. Tumor cells have exploited the pleiotropic effects of TGF-β to its full advantage. *Ras* is a commonly activated oncogene and the cooperation of TGF-β receptor and the *Ras* oncogene signaling pathway has been implicated in the oncogenic and metastatic process in a mammary epithelial carcinogenesis model *(108–111)*. TGF-β has also been detected in epithelial compartment and in tumor stroma *(112,113)*, where it may have an important role in controlling stromal formation within a developing tumor by increasing the synthesis of matrix proteins such as collagen, fibronectin, laminin, and tenascin *(114)*. TGF-β is also able to induce integrins production important to mediate adhesion and cell migration through the extracellular matrix and induce angiogenesis by inducing PA-1, which inhibits the conversion of plasminogen into angiogenesis inhibitor; angiostatin *(115)*, thereby contributing to the metastatic ability of tumor cells.

TGF-β also mediates cell cycle arrest and theoretically, should also inhibit tumor growth. Binding of TGF-β to the ternary TGF-β receptor complex activates a cascade of signal transduction pathways regulated by mothers against DPP homolog (SMAD)2, SMAD3, SMAD4, and mitogen-activated protein kinase *(116,117)* that negatively regulate the transcriptional levels of c-Myc and inhibit retinoblastoma protein phosphorylation *(108–120)*, resulting in cell cycle arrest. Tumor escape from TGF-β-mediated cell cycle arrest is accounted for by point mutations, homozygous deletions, gene rearrangements, and aberrant transcripts in the RI and RII *(121–123)* of the TGF-β receptor complex. Deletions and mutations of components of the TGF-β receptor signaling pathway like SMAD3 and SMAD4 *(124)* have also been detected. It is yet unknown what the molecular mechanisms are that allow the tumor cells to become insensitive to TGF-β cell cycle arrest effects while remaining sensitive to its induction of migration/invasion.

5.1.3. Effects of IFN-γ on Antitumor Immunity

IFN-γ is important for the generation of an effective Th1 response as well as for NK cell-mediated antitumor immunity *(125)*. IFN-γ is a key mediator of antitumor immunity, as it is able to induce the upregulation of many genes containing the IFN response sequence element. In addition, it has been shown to be essential to tumor rejection mediated by both $CD4^+$ T-cells and $CD8^+$ T-cells through induction of angiostasis *(126,127)*. IFN-γ exposure can sensitize breast cancer cells to apoptosis by upregulation of caspase 8 *(128)*. Expression of the antigen presentation machinery is also regulated by IFN-γ. IFN-γ upregulates the transcription of transporter associated with antigen processing and the proteasome subunits low-molecular-weight protein 2 *(129)*. Tumors may become unresponsive to the effects of IFN-γ through defective IFN-γ signaling, allowing them to gain resistance to IFN-γ-mediated apoptosis *(130)* and maintain low MHC class I expression levels *(131)*. In hepatocellular carcinoma, there is a correlation between the degree of metastasis and the poor expression of IFN-γ receptor on tumor cells. In metastatic cases, the decreased expression of IFN-γ receptor on tumor cells causes a considerable reduction of MHC class I molecules and Fas on these cells, impairing IFN-γ control of tumor growth *(132)*.

IFN-γ secretion can also lead to the suppression of the immune response indirectly through the upregulation of IFN-γ-inducible genes. Indoleamine 2,3-dioxygenase (IDO) is an IFN-γ-inducible enzyme *(133)* that catabolizes tryptophan and causes proliferation

arrest of T-lymphocytes because of tryptophan degradation *(134,135)*. IDO-expressing cells create a tryptophan-depleted microenvironment around themselves, as tryptophan crosses the plasma membrane readily through specific transporters to be degraded in the cytosol. Its expression by the placenta is important in the prevention of allorejection of the fetus by maternal T-cells *(136)*. IDO is expressed by DCs following ligation of B7.1/B7.2 *(137)*, and may be a mechanism by which DCs regulate T-cell responses *(138)*. Tumor cells can express IDO, and tumor cell lines transfected with IDO in vitro suppress T-cell proliferation *(139)*, and it has been proposed that tumor cells may be able to recruit APCs and induce tolerogenic IDO-expressing APCs *(140)*. These APCs would then be able to home to draining lymph nodes and tolerize naive T-cells to tumor-derived antigens. The discovery of accumulation of IDO-positively staining cells in immunohistochemistry studies of lymph nodes from melanoma patients supports this hypothesis *(141)*. IFN-γ production is often taken as a favorable indicator in the antitumor response. In a setting where the tumor cells have evolved to become less sensitive to IFN-γ-induced apoptosis, the IFN-γ could simply have a negative effect by inducing IDO production and tolerizing the immune system to the tumor.

Another example of the difficulty in accessing the outcome of IFN-γ production on antitumor immunity is illustrated by the interaction between IFN-γ-inducible chemokines and inducible nitric oxide synthase (iNOS). IFN-inducible CXC chemokines are powerful inhibitors of angiogenesis *(142)*. Intratumoral production of IFN-inducible chemokines like CXCL9 and CXL10 is associated with reduced angiogenesis and increased recruitment of $CD8^+$ T-cells in renal carcinoma *(143)*. IFN-γ also causes the upregulation of iNOS that leads to the production of nitric oxide. Nitric oxide is able to upregulate the production of angiogenic molecules like IL-8 and VEGF, and downregulate the expression of antiangiogenic chemokines like CXCL9 and CXL10 *(144)*. In hepatocellular carcinoma, iNOS expression was associated with increased microvascular density, resistance to apoptosis mediated by Bcl-2 synthesis, and cell proliferation of malignant cells *(145)*. This illustrates the complexities in trying to predict the outcome of IFN-γ-inducible products on angiogenesis and immune modulation in the tumor microenvironment.

5.1.4. Constitutive Signal Transducer and Activator of Transcription 3 Signaling

Many of the cytokine-activated signaling pathways converge on the signal transducer and activator of transcription (STAT)3 signaling molecule. STAT3 is involved in the regulation of cell differentiation, survival, cytokine, and chemokine production, and is required for DC maturation and activation *(146,147)*. The constitutive activation of STAT3 has been reported in many cancers *(148–150)*. STAT3 signaling in tumor cells has been shown to lead to the tumor immune evasion by inhibiting the activation of proinflammatory cytokines and chemokines, leading to a reduction in the number of inflammatory infiltrates like macrophages and neutrophils in the tumors *(151)*. STAT3 signaling also drives the secretion of factors that lead to the inhibition of DC maturation, thereby preventing the induction of an antitumor T-cell response *(151)*. Constitutive STAT3 activity also confers apoptosis resistance to the tumor cells *(152)* and upregulates VEGF expression to stimulate tumor angiogenesis *(153)*. Constitutive STAT3 signaling in tumors results in tumor immune evasion from both the innate and adaptive immune system, protects tumors from apoptosis, supports tumor growth through activation of angiogenesis, and is a clear example of how the tumors can utilize the pleiotropic functions of cytokines by the simple dysregulation of a key signaling molecule involved in cytokine signaling.

5.2. Cellular Infiltrates in the Tumor: Allies or Enemies?

The quantitative and qualitative analysis of the cellular infiltrates in the tumor microenvironment can lead to a greater understanding of the outcome of the immune modulatory effects of the tumor. A reduction in the number of tumor-infiltrating DCs in advanced malignancies can lead to impaired priming and generation of tumor-specific T-cells in the local environment and can be considered as mechanism of immune evasion *(154)*. High numbers of tumor-infiltrating cells may not necessarily be a favorable indicator of an effective antitumor response. Some of the tumor-infiltrating cells can be tolerogenic cells that can actively downregulate the cellular immune response through the production of immunosuppressive molecules. Among these cells, the regulatory T-cells and NK T-cells are considered key players in the negative regulation of tumor immunity through their production of type 2 and immunosuppressive cytokines like IL-4, IL-10, IL-13, and TGF-β *(155)*. Tumor-infiltrating macrophages in the Lewis lung carcinoma model produce considerable quantities of IDO *(156)* that suppresses the local T-cell response through antigen-specific anergy.

6. ACQUIRING RESISTANCE TO DEATH EFFECTOR MECHANISMS

The immune eradication of tumor cells is mediated by apoptosis that can be induced by the release of cytotoxic granules or death receptors. Tumors have evolved ways to become resistant to the death effector mechanisms, thereby becoming truly impervious to immune attack. The perforin/granzyme and Fas/FasL pathways are the two main effector mechanisms by which CTLs and NK cells mediate antitumor immunity *(157–158)*. The downstream effects of both pathways are similar, as they both lead to activation of the caspase cascade and mitochondrial-dependent cell death. The caspases and cytochrome *c* released from the mitochondria further synergize by enhancing each others' activation.

6.1. The Perforin/Granzyme B Pathway

In the granule-mediated pathway, CTLs and NK cells package specialized cytotoxic granules containing pore-forming perforins and granzymes. Perforins polymerize in response to calcium, and are inserted into the target cell membrane to create a channel that results in cellular necrosis through disruption of osmotic stability *(159)*. In addition to the cytolytic effect of the perforins, the granzymes in the granules can also induce cellular apoptosis through the activation of caspases. Human CTLs contain five different granzymes that have different substrate specificities and modes of action to induce cell death *(160)*. Granzyme A-induced apoptosis results from single-strand DNA breaks, independent of caspase activation *(161)*. Granzyme B is able to activate both caspase-dependent and caspase-independent pathways of cell death in the target cell *(162)*. Caspase 3 and caspase 8 are direct substrates of granzyme B, and activation of the caspase cascade leads to apoptosis and activation of caspase-activated deoxyribonuclease (CAD) leading to DNA fragmentation *(163–165)*. CAD is normally found in the cytpoplasm in an inactive form bound to its inhibitor ICAD. Caspase 8 cleaves ICAD to release CAD, leading to DNA fragmentation. Granzyme B can also activate the proapoptotic Bcl-2-family member, Bcl-2-interacting domain (Bid) *(166,167)*, through cleavage. Activation of Bid leads to the oligomerization and insertion of proapoptotic Bcl-2-associated X protein (Bax) and Bcl-2 antagonist killer 1 into the pore and outer mitochondrial membrane *(168,169)*. This eventually results in the release of cytochrome *c*, mitochondrial

collapse *(170)*, and subsequent release of mitochondrial-derived activator of caspase that bind to the inhibitors of apoptosis and releases the suppression on caspases for their full activation *(171,172)*. The release of cytochrome *c* can result in the formation of an apoptosome that includes apoptotic protease-activating factor 1 and procaspase 9 *(173)*. The apoptosome is able to activate caspase 9 *(174)* in the presence of andenosine triphosphate and activate more caspase 3, augmenting caspase-mediated apoptosis. Overexpression of a serine protease inhibitor, PI-9/SPI-6, was found in a variety of human and murine tumors. PI-9/SPI-6 inactivates granzyme B and protects cells against CTL-mediated perforin killing *(175)*.

6.2. Fas-Mediated Apoptosis

The interaction between the death receptor, Fas and its ligand, FasL, leads to the trimerization of Fas to bring together death domains (DDs) in the cytoplasmic portion of the molecules. The DDs then recruit adaptor proteins that form a DISC capable of activating initiator caspases like caspase 8 and caspase 10. Caspase 8 activation leads to activation of CAD and activation of the mitochondrial-induced death through Bid cleavage. Mutations in the *fas* gene, leading to a reduction in Fas expression, have been reported in many cancers *(176–178)* as a mechanism of gaining resistance to Fas-mediated apoptosis. Fas can also be secreted by tumor cells to bind to the FasL on tumor-specific CTLs to protect tumor cells from apoptosis *(179)*.

Decoy receptors containing functional extracellular ligand-binding domains but lacking intracellular DD have been found that regulate sensitivity to death-receptor-mediated apoptosis. DcR3 is a soluble decoy receptor secreted by tumor cells *(180,181)* and overexpressed in malignant glioma, pancreatic adenocarcinoma, colon, prostate, lung, and gastrointestinal tumors *(182–186)*. DcR3 binds to FasL and allows tumor cells to gain resistance to Fas/FasL-mediated apoptosis. DcR3 also suppresses the activation and differentiation of DCs *(187)* and macrophages *(188)* and downregulates T-cell proliferation. The FasL signaling pathway also serves as a local chemoattractant, and the production of DcR3 results in defective homing by reducing the recruitment of microglial macrophages, neutrophils, $CD4^+$, and $CD8^+$ T-cells *(189,190)* as a means of immune evasion. DcR3 has proangiogenic effects and is able to promote endothelial cell proliferation, migration, and the expression of matrix metalloproteinases *(191)*. Altogether, the immunosuppressive, antiapoptotic, and angiogenic activities of DcR3 can make it an important player in not just immune evasion but also in tumor growth.

Caspase 8 is the key initiator cell death protease in the death receptors pathway. Its activation is dependent on its recruitment to DISC following death receptor engagement. c-FLIP can bind DISC and prevent the activation of caspase 8 *(192)*. c-FLIP is expressed by many cancer cells and represents yet another way by which cancer cells gain resistance to death-receptor-mediated apoptosis *(193)*.

6.3. Production of Antiapoptotic Molecules

Tumor cells can also gain resistance to apoptosis through the production of antiapoptotic molecules. Members of the Bcl family have either proapoptotic functions or antiapoptotic functions and control the mitochrondrial-component of apoptosis. Bcl-2 and Bcl-X_L are commonly overexpressed in cancers and protect cells against apoptosis by preventing cytochrome *c* release *(194)*. Survivin is involved in the downregulation of apoptosis in malignant cells. In a prostate cancer cell line PC3, the increased production

of survivin protects cells against apoptosis mediated by TNF-α by preventing the activation of caspase 9 *(195)*. Survivin was also found to cause the upregulation of FasL in colon cancer cells *(196)*.

7. COUNTERATTACK BY THE TUMOR CELLS

Activation-induced cell death is a homeostatic mechanism that controls the magnitude of the immune response that has been exploited by tumor cells in their counterattack against the immune system. Contraction of the immune response after activation is coordinated Fas-FasL interactions that result in the death of activated cells. FasL expression on tumor cells has been documented in several cancers: hilar cholangiocarcinoma *(197)*, intrahepatic cholangiocarcinoma *(198)*, renal cell carcinoma *(199)*, cervical adenocarcinoma *(200)*, and melanoma *(201)*. The expression of FasL on malignant cells can lead to the *in situ* elimination of tumor-specific T-cells that express Fas on their cell surface *(202)*. TNF-related apoptosis-inducing ligand is another member of the TNF super-family that mediates cell death. TNF-related apoptosis-inducing ligand has been detected in metastatic gastric carcinoma cells from malignant ascites *(203)*, resulting in the death of tumor-infiltrating lymphocytes that bear the counter-receptors DR4 and DR5.

Soluble FasL can be released by tumor cells systemically, inducing the death of circulating lymphocytes in the periphery. Astrocytomas are known to produce high levels of soluble FasL *(204)*, which can be cytotoxic to Fas-expressing T-cells. This particular phenomenon has also been detected in colon cancer cells that shed their membrane associated FasL into the environment *(205)*. Tumors can also combine death-resistance mechanisms with counterattack on the immune system. Renal carcinomas were reported to decrease the expression of membrane-bound Fas, and secrete soluble FasL *(206)*.

8. ANGIOGENIC PROCESSES THAT FACILITATE TUMOR IMMUNE EVASION

Angiogenesis is a vital process in tumor survival. However, some of the angiogenic factors can indirectly facilitate tumor immune evasion because of their immunosuppressive effects. VEGF is a key mediator in both vasculogenesis and angiogenesis *(207)*. VEGF expression is associated with poor prognosis and increased metastatic spreading in ovarian cancer *(208)*. In addition, VEGF also inhibits T-cell development and contributes to tumor-mediated immune suppression *(209)*. Cyclooxygenase (COX)-2 is overexpressed in many cancers *(210,211)*, and is implicated in the angiogenic process *(212)*. COX-2 contributes to the production of prostaglandins by catalyzing the oxygenation of arachidonic acid to the common precursor of all prostanoids. The various prostaglandins are synthesized by distinct synthases in different tissues. The local production of prostaglandin (PG)E2 leads to immunosuppression in the tumor microenvironment through inhibition of T-cell and B-cell proliferation and diminished cytotoxicity of NK cells *(213,214)*. PGE2 is a powerful inhibitor of TNF-α and type 1 cytokine production and causes the downregulation of the cellular antitumor immune response. Another prostaglandin that can negatively affect antitumor immunity is PGD2. PGD2 is the ligand for the PGD2 receptor expressed on effector memory Th2 cells. An increased COX-2 activity and subsequent PGD2 production could promote the trafficking and activation of Th2 cells into tumor, suppressing the production of type 1 cytokines as a form of tumor immune evasion.

9. CONCLUSION

The myriad ways by which cancer cells escape immune eradication could be an indication of the immune pressure it faces. Cancer cells are usually successful in escaping immunodetection, because many of them are not particularly immunogenic. Cancer immunotherapy is therefore most successful in situations where the immune system is able to distinguish the transformed cells from surrounding normal cells with which it shares antigens against which the immune system is tolerant. Tumor antigens therefore serve as the first signals to alert the immune system. Vaccination schemes in cancer immunotherapy are distinctly different from classical vaccination that is prophylactic. Cancer cells may have already modulated the immune response and therefore nullified the potential therapeutic effects of a vaccine. The accumulation of data on immunogenic tumor-derived antigens will increase the arsenal of targets against which efforts can be directed. It is imperative for researchers and physicians venturing into cancer immunotherapy to pick their targets carefully, because no immunization scheme can be successful against an enemy that the immune system cannot "see." The inability of most naive T-cells to encounter tumor cells early enough in the blood and secondary lymphoid organs contributes to the lack of immunosurveillance for most types of cancers. Vaccination allows for the activation of tumor-specific T-cells and lowers their threshold of activation, allowing the activated CTLs to eradicate the tumor cells despite their low MHC class I expression. This argues for cancer immunotherapy, even for cancers that are not covered by the immunosurveillance theory, so long as they express antigens that can be targeted with minimal consequence of autoimmunity. The tumor environment shaped by angiogenic processes, chemokines, cytokines, and cellular infiltrates plays a huge determinant in the eradication of the tumor. The presence of T-lymphocytes specific for tumor antigens may not be a good enough indicator for the success of a potential vaccine. Although every tumor is different in itself, understanding the evasion strategies based on tumor type may enable us to support vaccination strategies with other immune modulators in order to conduct successful immunotherapies. The immune evasion strategies that tumors are able to adopt and their immunomodulatory effects as a direct consequence of immune pressure or as an indirect effect of angiogenesis (Fig. 1), pose as hurdles to existing natural antitumor activity and therapeutic vaccination schemes. An effective cancer vaccine needs to create optimal activation conditions, such as adequate costimulation and a cytokine environment conducive for the Th1 response at the priming phase to prevent antigen-specific anergy or Th2-suppression of the Th1 response. Existing tolerance will have to be broken toward antigens that the immune system is already tolerant. Activation of the immune system is the result of the integration of activating and inhibitory signals. Tolerance can be broken by providing "help" in the form of cytokines and costimulation and by inhibiting tolerogenic stimuli such as immunosuppressive cytokines and inhibitory costimulation. A recent paper outlines strategies to potentiate cancer vaccines by inhibiting the immunosuppressive factors *(215)*. Autoimmune diseases are the proof that low levels of autoreactive cells do exist and can turn into potent antigen-specific killers. With the appropriate adjuvants and vaccination strategies, these cells can be unleashed against the cancer cells to eradicate these altered "self" cells. There is great promise for cancer immunotherapy, but there is a need to pick the right targets and strengthen the immune attack in order to break down the tolerogenic obstacles put up by the tumor cells.

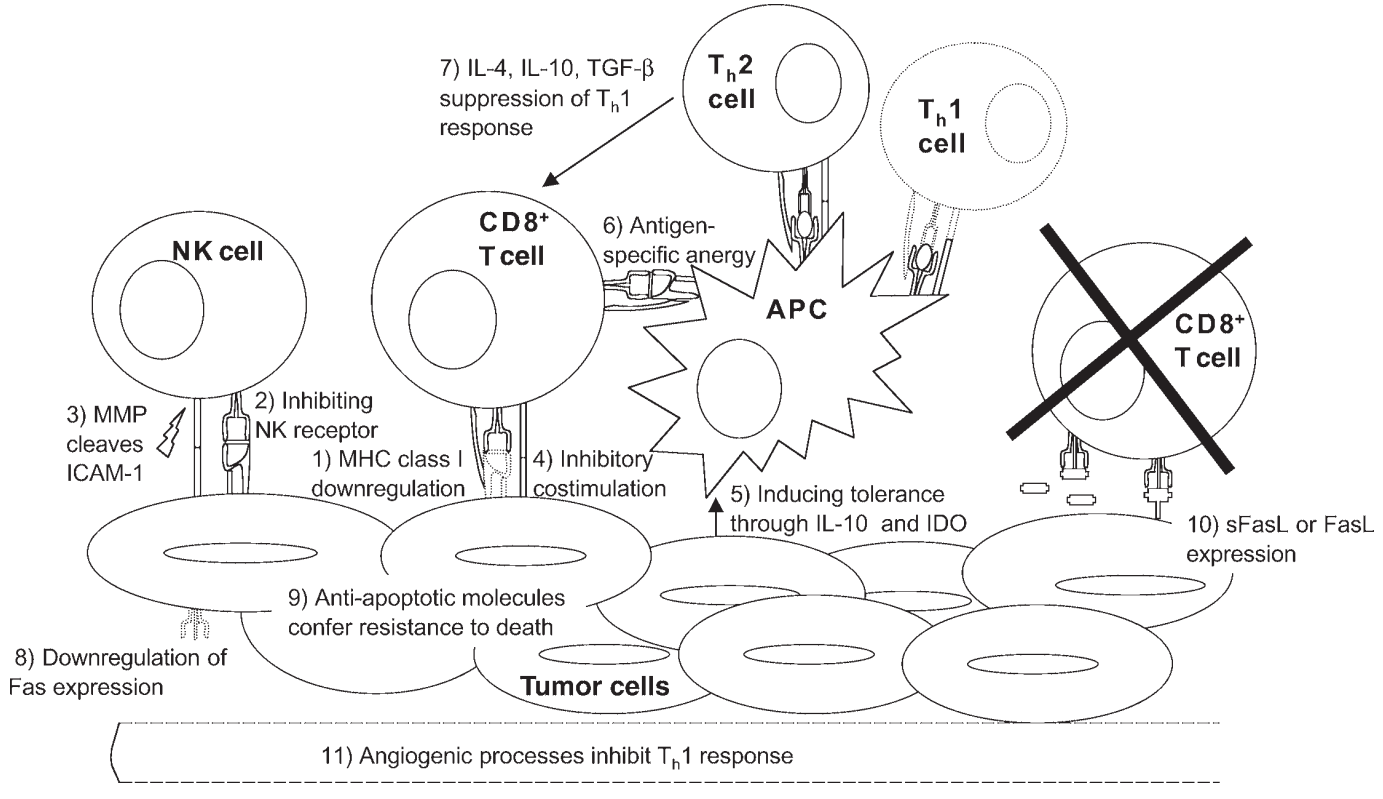

Fig. 1. Tumor immune invasion strategies. Tumor cells can evade immunodetection by (1) downregulation of major histocompatibility complex (MHC) class I molecules and by (2) inhibiting the natural killer (NK) cell receptor. They can inhibit NK cell-mediated killing by (3) disrupting cell–cell interactions. (4) The expression of inhibitory members of the CD28 costimulatory family leads to inhibition of the priming or effector phases of T-cell response. (5) Interleukin (IL)-10 and indoleamine 2,3-dioxygenase (IDO) secreted by tumor cells or by tumor-infiltrating cells can lead to the generation of tolerogenic antigen-presenting cells (APCs) that can (6) induce antigen-specific anergy of T-cells when they home to the lymph nodes, or (7) lead to the development of a type 2 response that suppresses the T-helper (Th) cells response. Tumors can (8) downregulate the expression of Fas and (9) produce antiapoptotic molecules to escape from death effector mechanisms. (10) Soluble (s)Fas and cell surface Fas ligand(L) expression by the tumor cells result in apoptosis of Fas-expressing T-cells. (11) Angiogenesis induced by the tumor cells indirectly results in inhibition of the Th1 response.

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