Introduction

Our immune system has evolved to identify and destroy microbial pathogens that may be detrimental to our health. Its activity is a balancing act of activation and inhibition with the ultimate goal to eliminate the intruder, while avoiding illicit self-destructive reactions. In addition to microbes, our immune system seems to play a role in the prevention and elimination of tumors (1). This process is today called cancer immunosurveillance, and it delineates the concept that immune system, similarly to protection against pathogens, should be able to recognize aberrant (dysplastic and neoplastic) cells and destroy them so that tumors will not form (1). Despite well-defined and immunogenic tumor antigens, and even in the presence of tumor-antigen–specific cytotoxic cells, the immune system does not seem to be very effective in eradicating malignant cells. Tumor cells are, albeit accumulated changes, not truly foreign but autologous cells that have become transformed in a subtle way, enabling them to evade host immune system in different ways.

The cancer immunoediting concept consists of three phases: designated elimination, equilibrium, and escape (Fig. 1). The “elimination” phase of cancer immunoediting corresponds to the same process, initially described as cancer immunosurveillance, whereby the immune system recognizes and destroys tumor cells that have developed as a result of failed nonimmunologic, intrinsic surveillance (e.g., DNA repair and control of apoptosis; refs. 1–3). The consequence of the elimination phase may be complete (all tumor cells are destroyed) or partial eradication of tumor cells (only a portion of tumor cells are destroyed). In the latter case, “equilibrium” may develop between the tumor and the immune system. During this period, developing tumor may remain dormant or continue to evolve and accumulate further changes (e.g., epigenetic modifications, DNA mutations, and related changes in gene expression). These changes can modulate the expression of tumor-specific and stress-induced antigens on tumor cells, rendering the sensitive/resistant to elimination by the host immune system. During this process, immune system exerts selective pressure by eliminating susceptible tumor cell clones whenever possible. This type of pressure once again may sufficiently control tumor progression, but if it fails, it contributes to the selection of tumor cell variants that are able to resist, suppress, or escape the antitumor response. During the “escape” phase, the immune system is no longer able to restrain tumor growth, which results in the progressively growing tumor. The cells with reduced immunogenicity are now more capable of surviving and growing in an immunocompetent host, which explains the apparent paradox of tumor formation in immunologically intact individuals.

Structural and functional alterations of the human leukocyte antigen (HLA) class I antigens represents a frequent event in cancer and serve to circumvent antigen-specific T-cell response (4). Cells lacking HLA class I molecules are susceptible to elimination by natural killer (NK) cells. Disengaged from the inhibitory effect of HLA molecules on their ligand [killer cell inhibitory receptors (KIR)], activated NK cells should be capable to eliminate HLA-negative tumor (1). Despite HLA class I loss, tumor cells continue to grow and are not being efficiently destroyed by NK cells. The reason for this may be more specific HLA loss that could provide escape from cellular immunity, while retaining at the same time the inhibitory effect on NK cells. Alternatively, tumor cells may express a HLA molecule that primarily delivers inhibitory signals. Based on its primarily immunoinhibitory properties, a nonclassic class Ib HLA molecule, HLA-G, became a suitable candidate for this role. Under physiologic conditions, expression of HLA-G is confined to immunoprivileged sites such as fetomaternal barrier. There, HLA-G was proposed to induce functional silencing of the immune response, allowing the tolerance of semi-allogeneic fetus during the pregnancy. In addition to its “nonclassic” attributes, such as limited polymorphism, unique processing of the primary transcript that creates 7 isoforms, its protein structure, and restricted cellular and tissue distribution, HLA-G affects profoundly almost every aspect of human innate and adaptive immunity (5, 6). In 1998, Paul et al. (7) provided the first evidence that functional HLA-G protein expression protects melanoma cell lines from NK-mediated cell lysis and may therefore play a role in tumor escape from the immune system of the host. After almost a decade of intensive research, HLA-G protein expression was assessed in 16 tumor types of either ectodermic, mesodermic, or endodermic origin (5, 6). HLA-G protein expression patterns observed in these studies seem to be very heterogeneous, reflecting not only different biology of individual tumors but also the differences in study populations (study design) and/or sensitivity of the methods used to detect HLA-G protein. HLA-G was preferentially detected in the tumor tissue and only rarely in the adjacent normal tissue, suggesting its specific association with tumor growth and progression.

Human Leukocyte Antigen–G and Cancer Immunoediting

Mirjana Urosevic and Reinhard Dummer

Department of Dermatology, University Hospital Zurich, Zurich, Switzerland

Abstract

Immunosurveillance is an extrinsic mechanism of cancer suppression that eliminates nascent tumors. However, the selection imposed by immunosurveillance can drive tumor evolution and the emergence of clinically apparent neoplasms. Mechanisms of immune escape acquired by less immunogenic variants during this process, termed immunoediting, may contribute significantly to malignant progression. In this review, we summarize the evidence that up-regulation of the nonclassic human leukocyte antigen (HLA) class I molecule HLA-G in tumor cells plays an important role in cancer and immune escape. [Cancer Res 2008;68(3):627–30]

HLA-G in Elimination Phase

The process of elimination, as already mentioned, is represented in the joined action of the effectors of innate (NK, NK T cells, γø

Requests for reprints: Mirjana Urosevic, Department of Dermatology, University Hospital Zurich, Gloriastrasse 31, 8091 Zurich, Switzerland. Phone: 41-44-255-11-11; Fax: 41-44-255-44-18; E-mail: mirjana.urosevic@usz.ch.
T cells, and dendritic cells) and adaptive (antigen-specific T and B cells) immunity to sense and destroy nascent-transformed cells. Given panoply of HLA-G to immune cell interactions, it is likely that expression of HLA-G on tumor cells could considerably weaken the immune response of the host in this phase.

Cell surface HLA class I expression on tumor cells is presumed to be intact or not drastically changed during the elimination process. Activity of tumor-infiltrating lymphocytes and NK cells in this phase is associated with local T_h1 cytokine profile and the production of IFNγ, which is one of the cytokines known to up-regulate HLA-G expression in tumor cells. Once HLA-G is up-regulated on such HLA class I–expressing cells, it becomes the major NK inhibitory ligand. To date, three HLA-G–recognizing KIRs have been identified: immunoglobulin-like transcript (ILT)-2, ILT-4, and KIR2DL4/p49. In addition to their expression on NK cells (KIR2DL4 and ILT-2), these receptors have also been detected on all T and B cells (ILT-2), monocytes/macrophages (ILT-2 and 4), and dendritic cells (ILT-2 and 4). Although ILT-2 and 4 have other HLA class I ligands, they seem to have the highest binding affinity for HLA-G. KIR2DL4/p49, on the other hand, is expressed by all NK cells and is thought to be a HLA-G–specific receptor. HLA-G expression by tumor cells seems to be a powerful way to switch off NK cells by bypassing activating signals delivered by, for example, MHC class I–related chain A coexpressed on the same cells (4). The actual coexpression of HLA-G on HLA class I–positive cells has already been shown in on several occasions in tumor tissue ex vivo (8), thus making this scenario a possible reality. Furthermore, via its ILT receptors on dendritic cells, HLA-G can affect their maturation, migration, and trafficking as well as down-regulate antigen presentation to naïve T cells (9). Apart from innate effectors, HLA-G is capable of suppressing proliferation of CD4+ T lymphocytes and of inhibiting allogeneic proliferative and antigen-specific HLA-restricted T-cell response. In this way, HLA-G expression would enable a certain proportion of tumor cells to pass unharmed through the cancer immunosurveillance.

**HLA-G in Equilibrium Phase**

As the adaptive immunity of a host continues to eliminate tumor cells, it simultaneously exerts immune selection pressure leading to the sculpting and production of less immunogenic tumor cell clones (e.g., selective or complete loss of class I HLA molecules and/or tumor antigens). Genetic instability due to defective intracellular control mechanisms as well as frequent epigenetic

**Figure 1.** HLA-G and cancer immunoediting. This figure depicts probable points of HLA-G activation and up-regulation during tumorigenesis and with the process of cancer immunoediting. Modulation of HLA-G expression during chemotherapy and immunotherapy is highlighted in italics.
changes contribute further to the development of a nonimmuno-
genetic phenotype. There are, however, very little experimental data to support this concept. Genome-wide hypomethylation is a frequent epigenetic event in cancer that leads to gene activation and is supposed to take place not only throughout tumorigenesis but also later on during the equilibrium phase (Fig. 1). In vitro studies imply that demethylation and histone acetylation may also be responsible for ectopic activation of HLA-G gene in cancer (10). On the other hand, HLA-G expression is associated with down-regulation of HLA class II molecules on several cell types, including dendritic cells, contributing additionally to already disturbed presentation of tumor antigens (9, 11).

HLA-G may also have an indirect effect by stabilizing cell surface expression of HLA-E, another nonclassic class I molecule (5, 6). In damaged cells, such as virally infected or tumor cells, down-regulation of classic class I molecules prevents stabilization of HLA-E on the cell surface. However, through the expression of HLA-G and its leader peptide, HLA-E can be stabilized at the surface and contribute additionally to the resistance of the tumor cells to NK cell lysis.

**HLA-G in Escape Phase**

Finally, as the immune system is no longer able to restrain tumor cell growth, which is leading to an increase in tumor size detectable by imaging techniques. The “escape” phase is the best characterized of the three in both mice and humans. There are numerous described mechanisms that enable tumor cells to efficiently evade antitumor immune response by, for example, modulating antigen expression, by producing immunosuppressive cytokines, or by inducing peripheral tolerance. Tumors seem to be able to generate appropriate microenvironment that allows them to skew the immune response such that tumor growth rather than elimination is favored.

Tumor cells that have lost molecules important for the immune recognition now tend to express only HLA-G on the cell surface. Numerous studies in cancers of different origin show preferential up-regulation of HLA-G in advanced disease, rather than in the initial tumor lesions, supporting its role the final phase of immunoediting. As described above, HLA-G is involved in alterations in antigen processing, and its expression also renders tumor cells resistant to different effector arms of the immune system. Moreover, soluble HLA-G also induces apoptosis of activated CD8+ T cells by activating Fas/Fas ligand pathway. Through a negative feedback mechanism, soluble form of HLA-G inhibits the proliferative response of allogeneic CD4+ T cells that secrete it. Increased serum levels of soluble HLA-G in patients with advanced cancer have been reported to correlate with tumor load (12). Whereas membrane-bound HLA-G acts locally at the site of its expression, secreted soluble HLA-G molecules could, apart from local effects, also have systemic inhibitory activity due to their distribution via circulation. Some HLA-G-positive tumor cells secrete exosomes (membrane vesicles) containing HLA-G molecules that are also likely to have a systemic effect.

Rapidly growing tumors often have disturbed balance between cell proliferation and oxygen supply that leads to a decrease in partial oxygen pressure, a physiologic stress condition termed hypoxia. Hypoxia results not only in cell death by necrosis, but it also profoundly affects overall gene expression and promotes angiogenesis, invasion, metastases, dedifferentiation, and enhanced glycolytic metabolism. Hypoxia also seems to induce HLA-G expression that is dependent on hypoxia-inducible factor 1 (Fig. 1; ref. 13). Similarly, chronic inflammation is supposed to promote tumor growth through constitutive activation of nuclear factor-κB (NF-κB) in transformed cells by favoring antipoptotic (survival) cell cycle progression and invasive phenotype (14). NF-κB activators also affect HLA-G by increasing its proteolytic shedding in tumor cells (Fig. 1; ref. 15). Soluble HLA-G molecules produced in this way may contribute to the systemic anergy through its specific interactions with immune cells described above. Indeed, HLA-G expression was observed more frequently in tumor lesions associated with inflammation (8). It is conceivable that tumor cells exposed to hypoxia undergo additional epigenetic changes, such as DNA demethylation and histone acetylation, which lead to opened chromatin and HLA-G transcriptional activation. Additional inflammatory stimuli (via NF-κB) could further enhance the levels of HLA-G transcripts/proteins in tumor cells, providing them protection from the host immune system (Fig. 1).

Tumors as well as tumor-infiltrating cells often produce interleukin (IL)-10 that exerts variety of biological effects (e.g., down-regulation of HLA class II and costimulatory molecules, functional impairment of antigen presenting cells, and diminishment of production of Th1 cytokines; ref. 16). IL-10 expression often localizes in the vicinity of HLA-G-expressing cells and seems to be one of the major factors responsible for HLA-G up-regulation in cancer (Fig. 1; ref. 8). Recognition of membrane-bound HLA-G results in the induction of Th2-type cytokines (such as IL-10), which could also contribute to the switching of the local cytokine profile toward immunosuppressive arm. Soluble HLA-G, on the other hand, increases IL-10 production irrespective of the presence of HLA-G-expressing cells. It is conceivable that metastasized tumors, through the release of soluble HLA-G into the bloodstream, may induce systemic anergy by inducing apoptosis of T cells or by inducing systemic production of IL-10. Another HLA-G-mediated mechanism cooperates to the generation of the immunosuppressive microenvironment around the tumor. Through inhibitory ILT-2 receptor on T cells, HLA-G impairs proximal T-cell receptor signaling by decreasing phosphorylation of CD3ζ and ZAP-70 and leading to the decreased IL-2 production (17). Decrease in Th1-type cytokines (e.g., IL-2 and IFN-γ) is reported to be correlated with increased levels of IL-10 and transforming growth factor-β in cancer. Quite an interesting issue is the induction of HLA-G expression (both membrane bound and soluble) by IFN-α, which is often used for immunotherapy of cancer (18). In case of failure to eradicate the tumor, immunotherapy using IFNs would help select less immunogenic tumor clones that are resistant to immune attack and would also result in HLA-G up-regulation, all of which might irredeemably enhance escape potential of the tumor cells (membrane-bound form) and contribute to systemic anergy to the tumor (soluble form; Fig. 1). Similarly, approaches using demethylating agents, such as 5-aza-2′-deoxycytidine, to up-regulate overall class I expression (and HLA-G) and to improve immunotherapy of cancer, as suggested by Fonsatti E et al. (19), should by meticulously scrutinized in the preclinical evaluation, especially taking into account the inhibitory effects mediated by HLA-G.

Not only tumor cells, but also tumor-infiltrating immune cells such as macrophages, lymphocytes, and dendritic cells were shown to express HLA-G (8). T cells expressing HLA-G seem to exhibit potent immunosuppressive properties (20). HLA-G presence of antigen-presenting cells, such as dendritic cells, induces suppressor regulatory T cells that also produce IL-10 (the same shown for soluble HLA-G; ref. 21). T cell can also acquire HLA-G from dendritic cells by trogocytosis (cell-to-cell transfer of membrane fragments) and change their phenotype from effector to suppressor (22).
Furthermore, signaling through ILT-4 promotes generation of tolerogenic immature dendritic cells that in turn inhibit maturation of T-cell activation (2, 9). By modulating cytokine microenvironment, HLA-G may not be only recruiting immune cells with HLA-G-specific ligand, but it may also induce expression of the respective HLA-G ligand (such as ILT-2) on already present infiltrating cells (8). KIR repertoire on NK and T cells can be modulated by cytokines (e.g., IL-10) and may further contribute to immune lethargy.

In conclusion, presented evidence supports involvement of HLA-G in every phase of cancer immunoediting, in (a) elimination, by disabling effectors of innate and adaptive immunity; in (b) equilibrium, by altering antigen presentation and by contributing to less immunogenic phenotype (plus processes described in the previous phase); and in (c) escape, by inducing immunosuppressive cytokines and peripheral tolerance to the tumor (plus processes described in the previous phases). The exact time point of HLA-G activation and up-regulation in tumor cells remains elusive—the hallmark of cancer is genetic instability, so the activation of HLA-G could take all the way through the process of cancer immunoediting. Given the notion that inflammation may promote tumor development, up-regulation of HLA-G during chronic inflammation would enable unnoticed growth of single-transformed cells beyond the immunosurveillance of the host. Future studies and continuous effort in this field will help us to better comprehend the versatility of HLA-G functions in cancer. It is with the elucidation and understating of the role of HLA-G in immunosculpting mechanisms and its importance in cancer immunotherapy that we will be able to develop the most optimal immunotherapeutic strategies that can lead to total tumor eradication.

Acknowledgments

Received 7/17/2007; revised 10/22/2007; accepted 10/29/2007.

References

Human Leukocyte Antigen–G and Cancer Immunoediting

Mirjana Urosevic and Reinhard Dummer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/3/627

Cited articles
This article cites 21 articles, 6 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/3/627.full#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/3/627.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/68/3/627.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.