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HLA expression in cancer: implications for T cell-based immunotherapy

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Abstract HLA class I expression is altered in a significant fraction of the tumor types reviewed here, reflecting either immune pressure or, simply, the accumulation of pathological changes and alterations. However, in all tumor types analyzed, a majority of the tumors express HLA class I, with a general tendency for the more severe alterations to be found in later-stage and less differentiated tumors. These results are encouraging for the development of specific immunotherapies, especially considering that (1) the relatively low sensitivity of immunohistochemical techniques might underestimate HLA expression in tumors, (2) class I expression can be induced in tumor cells as a result of local inflammation and lymphokine release, and (3) class I-negative cells would be predicted to be sensitive to lysis by natural killer cells.

Keywords HLA · Class I · Tumor · Epitope · Immunotherapy

Disease progression in cancer and infectious disease

A dynamic interaction exists between host and disease, both in the cancer and infectious-disease settings. In the latter, pathogens evolve during disease. For example, the viral sequences that predominate early in HIV infection differ from those associated with AIDS and the terminal disease stages. Pathogen forms that are effective in establishing infection are believed to differ from those most effective in terms of replication.

The pathological process by which an individual succumbs to a neoplastic disease is a complex phenomenon. During the course of disease, many changes occur within the cancer cells. The tumor accumulates alterations which in part related to a seemingly random, vicious circle of dysfunctional regulation of growth and differentiation, but are also related, to some degree, to maximizing its growth potential, and to escaping drug treatment and/or the body’s immunosurveillance.

An in-depth discussion and review of these topics is beyond the scope of the present article. Here, we emphasize that neoplastic disease is a complex and dynamic process, which results in the accumulation of several different biochemical alterations of cancer cells as a function of disease progression. It also results in significant levels of intra- and intercancer heterogeneity, particularly in the late metastatic stage. This situation ultimately translates into a significant heterogeneity of approaches and efficacies of drugs targeting neoplastic diseases.

All too familiar examples of cellular alterations affecting treatment outcomes include the outgrowth of radiation- or chemotherapy-resistant tumors during the course of therapy. Significant heterogeneity of responses also appears to be associated with other newer approaches to cancer therapy, including anti-angiogenesis drugs, passive antibody immunotherapy, and active T cell-based immunotherapy. These examples are analogous to the unfortunate emergence of drug-resistant viral strains as a result of aggressive chemotherapy of chronic hepatitis B virus and HIV infection, and the current resurgence of drug-resistant tuberculosis and malaria.

The interplay between disease and the immune system

One of the main factors contributing to the dynamic interplay between the host and disease is the immune
response mounted against the pathogen. In infectious diseases, the relationship between immune responses and the appearance of escape mutants has been well characterized in a variety of disease settings, including HIV, SIV, hepatitis C virus, leishmaniasis, and malaria. Immune responses can, in certain conditions, control these infections. Several different animal model systems and prospective studies of natural infection in humans suggest that immune responses against the pathogen of interest can lead to prevention and/or therapeutic elimination of disease. A common motif from this work is the requirement for a multispecific T-cell response, and the lower effectiveness of narrowly focused responses. These observations have spurred significant enthusiasm, and guide the development of various specific immunotherapies and vaccines.

In the cancer setting, there are several indications that immune responses can impact neoplastic growth. First, the studies in many different animal models have demonstrated that antitumor T cells, restricted usually by MHC class I, can prevent or treat tumors. Second, encouraging results have been obtained from immunotherapy trials. Third, observations made in the course of natural disease have correlated the type and composition of T-cell infiltrate within tumors with positive clinical outcomes (Coulie et al. 1999). The presence of nonspecific cytotoxic lymphocytes (CTLs) was also correlated with control of tumor growth, until antigen loss emerged (Marchand et al. 1999; Riker et al. 1999). Similarly, loss of β2-microglobulin was detected in 5/13 lines established from melanoma patients after receiving immunotherapy at the National Cancer Institutes (Restifo et al. 1996). Finally, HLA class I is frequently altered in various tumor types, leading to the speculation that this phenomenon might reflect immune pressure exerted on the tumor by means of class I-restricted CTLs. Several studies have proposed that the extent and degree of alteration in HLA class I expression not only reflect past immune pressures, but might also have prognostic value (van Duinen et al. 1988), even though this issue is still controversial. Taken together, these observations provide a rationale for immunotherapy of cancer, and at the same time suggest that effective strategies need to account for the complex series of pathological changes associated with neoplastic disease. In the following sections we will focus on one such change, namely the decrease in HLA expression in tumors, especially as it relates to antigen-specific T-cell immunotherapy.

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**Assaying HLA expression in tumor cells and lines**

Various approaches have been utilized over the years to assay for expression of HLA class I on tumor cells. By far the most widely utilized method is immunohistochemical staining of formalin-fixed or frozen tumor sections. The lack of standardized protocols that can be applied consistently by different investigators has rendered it difficult to compare results obtained in different studies.

Analysis using fluorescence-activated cell sorting (FACS) appears to be the most accurate and reliable method. It is routinely performed utilizing well-standardized protocols, and allows one to simultaneously quantitate expression on normal versus tumor cells, separated by the use of staining for specific tumor markers. Analysis using FACS is of higher sensitivity than immunohistochemistry, and several recent publications have emphasized that staining of formalin sections can lead to serious underestimation of class I expression, both in terms of levels of expression and numbers of cells positive for expression. Recent studies indicate that some tumors, which would have been scored as class I negative by immunohistochemistry, do express low levels of class I molecules when analyzed by FACS (Diederiksen et al. 1998; Koopman et al. 2000; Tait 2000).

In terms of reagents, anti-class I, pan-reactive antibodies, such as W6/32, have been widely utilized. This approach although generally useful cannot detect allele-specific losses or decreases. To overcome this limitation, allele-specific antibodies are also routinely utilized. The use of allele-specific reagents is limited by the availability of well-characterized specific antibodies, and by their consistent use by different laboratories. To overcome these limitations, a section of the 13th International HLA Workshop, chaired by Dr. S. Ferrone, will specifically address the issues of quality control of protocols and reagents utilized for tumor cell staining.

Another hurdle to standardization and interpretation of immunohistochemistry assay are the classification and definition of what represents a significant loss/decrease in expression. A large fraction of cells within a given tumor can exhibit total loss, while the remaining cell population might exhibit normal expression. In most cases, this might be classified as an instance of total loss, while in functional terms, the tumor would still be subject, in large part, to direct attack from the immune response. Furthermore, in the case of heterogeneous expression, both normally expressing and defective cells are predicted to be susceptible to immune destruction based on “bystander effects” (see below). Related to these issues, a study from Esteban and co-workers (1996) investigated a total of 60 tumors of laryngeal origin.

Alternative assays are also available but are utilized less frequently. These involve assaying for the presence of specific mRNAs as well as gel electrophoresis of specific HLA products. Nucleic acid-based assays have the potential for higher sensitivity. Klein and co-workers (1996), for example, noted that HLA class I expression is not lost from colorectal cancers, despite their scoring for negative expression at the level of detection by antibody staining.