# Histocompatibility Antigens on Murine Tumors

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*Summary.* Recent advances in tumor immunology suggest that the expression of the histocompatibility antigens, encoded by the major histocompatibility complex, is important in controlling the metastatic growth of certain murine tumors. The anomalous expression of histocompatibility antigens in many neoplasms appears to be associated with the ability of these cells to evade the immune system and progress to metastasis. This review examines some of the underlying molecular and immunobiological interactions that might determine the metastatic outcome of cellular transformation.

## The H-2 Antigens of the Major Histocompatibility Complex

The major histocompatibility complex (MHC) of the mouse consists of three classes of genes that encode cell surface and secreted products involved in immune regulation and function (*1*). Within the MHC, the class I genes represent a large multigene family of distinct, but related, sequences encoding several types of products. These products include the transplantation antigens, which were first identified on the basis of their role as the targets of graft rejection. The class II genes, encoding the immune response–associated or Ia antigens, are involved in the regulation of antibody responses to certain foreign antigens. The class III genes encode several components of the complement cascade.

There are approximately 32 class I genes in the mouse, which map either to the H-2 or Tla regions of the MHC (*1*). Each inbred mouse strain expresses a distinct set of MHC antigens (the haplotype). The products of the H-2 loci include the highly polymorphic histocompatability antigens H-2K, D, and L. On the other hand, the Qa and Tl genes (which map to the Tla region) are much less polymorphic among different strains of mice. Although the products of some of these genes appear to be expressed on certain lymphoid subsets and in the liver, their function has not been determined (*2, 3*).

The H-2 antigens are cell surface glycoproteins that function as targets directing the attack of cytolytic T lymphocytes (CTL's) against virally infected or neoplastic cells (Fig. 1). Although the precise molecular basis of T-cell recognition remains unknown, CTL function exhibits an absolute requirement for associated recognition between foreign antigens and self MHC components (*4–6*). As the products of specific class I genes are required for the restricted recognition of different viral antigens, the H-2 molecules are often referred to as restriction or control elements for CTL function. In addition, some CTL's can recognize non-self class I antigens as distinct targets and this phenomenon—known as allorecognition—is the cellular basis of the transplant rejection that occurs between histoincompatible strains of mice (*7*). The properties of CTL's contrast with those of natural killer (NK) cells. NK cells comprise a distinct subset of lymphoid cells able to kill certain tumors without preimmunization or MHC restriction. NK cells are believed to be important in the surveillance and elimination of cancer ( *4, 8*).

Many tumors appear to express cell surface molecules that can be recognized in association with self H-2 as foreign and become the object of an immune response (*4*). Thus, it is possible that the host immune response against a nascent neoplasm has a significant effect on tumor survival and metastasis. Although it is difficult to estimate to what extent such immune surveillance is responsible for the elimination of incipient tumors in healthy animals, recent evidence suggests that the level of the immune response against transplanted tumors is a major factor in determining tumor growth and metastatic behavior (*9–13*). Therefore, any process perturbing immune recognition, for example modulation of class I expression (*14*), might facilitate the escape of these target cells from immune destruction in the host (Fig. 2).

The class I alterations that have been observed on tumors can be grouped into the following three classes: (i) the loss or quantitative attenuation of the expression of specific class I antigens; (ii) the enhanced expression of H-2 or the activation of unexpressed class I loci mapping to the Qa/Tla region of the MHC; and (iii) the generation of novel class I antigens by mutation or recombination. While these phenomena are not observed in all tumor systems, tumor-associated alterations of MHC components may be one of several decisive factors determining the course of certain neoplastic diseases. The immunobiology associated with these phenomena has been recently reviewed (*4*). We will focus our discussion on the insights afforded by the recent application of molecular technology to the analysis of class I expression on certain metastatic tumors.

## Attenuated Expression of Class I Products on Metastatic Tumors

Tumors, especially those with virally or chemically induced etiologies, frequently exhibit an altered profile of class I products on their surface (*4, 15*). For example, the virally induced AKR T-cell leukemia line K36.16 expresses normal levels of H-2D antigen but negligible H-2K, as compared to normal lymphocytes (*14*). Unlike related AKR tumors that express H-2K and are rejected in immunocompetent, histocompatible mice, the K36.16 cell line is resistant to H-2K–restricted killing by T cells and readily progresses when transplanted into normal hosts (*14*). Presumably, the H-2K antigen, but not the H-2D antigen, is capable of acting as a restriction element for CTL recognition of the Gross/AKR murine leukemia viral antigen expressed on these cells, such that reduced H-2K expression allows the cells to escape immune destruction. To establish whether attenuation of the products of the H-2K locus was responsible for the altered growth behavior, Festenstein and co-

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workers (*9*) introduced the normal AKR H-2K gene into K36.16 cells. Clones of the transfected tumor cells expressing the transferred H-2K sequences at high levels were rejected when transplanted into normal, histocompatible mice. The same cells were able to progress to tumors when the hosts had been immunosuppressed by moderate doses of radiation. Thus, the attenuated H-2K expression on these cells correlates directly with their ability to avoid immune rejection and grow in immunocompetent hosts.

In the case of K36.16, H-2–restricted CTL killing may not be sufficient to account for the immunologic behavior and metastatic potential of the H-2K transfectants. For example, mice preimmunized with H-2K–positive K36.16 transfectants were later able to reject a second challenge with the original H-2K–negative K36.16 cells (*9*). Although H-2K expression appears to be required to initiate an adequate immune response for the rejection of the tumor, mice primed with tumor cells that express H-2K are able to reject a subsequent challenge by H-2K–negative cells. Thus, this response probably includes additional humoral as well as nonspecific components, such as NK cells, capable of eliminating the parental CTL-resistant tumor.

The relationship between class I expression and the metastatic behavior of tumors has also been examined by Katzav, Segal, Feldman, and their colleagues in studies of variants of the methylcholanthrene (MCA)-induced sarcoma T10 (*12*). Like many MCA- and virally-induced sarcomas, T10 expresses a tumor-associated antigen (TAA). The TAA's expressed on these tumors are antigenically diverse and have been characterized on the basis of their ability to immunize histocompatible mice against the tumor of origin (*16, 17*). These molecules appear to be unrelated to the class I antigens but can be recognized in the context of self-MHC as the target of a CTL response against the tumor (*18*).

T10 is derived from an F1 hybrid of two distinct inbred mouse strains and should express the allelic products of both parental H-2K and H-2D loci. The genotype of this tumor thus facilitated the analysis of the effects of the expression of the individual H-2 antigens on the metastatic progress of the tumor in hosts of the same F1 origin. Although a highly metastatic variant of this tumor, IE7, and a moderately metastatic variant, IC9, express H-2D–encoded class I products, neither type of cell expresses either H-2K allele (*19*). Hammerling and his colleagues (*20*) introduced each parental H-2K allele separately into IE7 and IC9 and found that expression of either of the parental H-2K antigens severely reduced the metastatic phenotype of the transplanted cells. The H-2K products appeared to function as classical CTL restriction elements in this system: CTL's generated against T10 cells that expressed one of the parental alleles could only recognize target cells expressing that same antigen. These studies suggest that a CTL response is responsible for the rejection of the H-2K–expressing T10 transfectants, at least in the context of the transplantation assay, and that H-2K loss allows the tumor to escape immune destruction. Such conclusions are only possible given the ability to specifically manipulate the profile of class I expression of a tumor by transfection with cloned class I genes (*21*).

The role of class I expression in the immunosurveillance of tumors has recently been highlighted by the analysis of several adenovirus-transformed cell lines having different metastatic potentials. Several strains of adenovirus (for example, Ad2 and Ad5) can transform rodent cells in culture, but these cells are unable to progress to tumors when transplanted into histocompatible hosts—presumably because of immune rejection. However, cells transformed with the highly oncogenic adenovirus strain Ad12 are able to grow and metastasize (*22*). One difference between Ad5- and Ad12-transformed rat cells that could explain the oncogenicity of Ad12 is the apparent absence of class I antigens on the Ad12 transformants (*23*). Low levels of class I transcripts were detected in these cells. These results have been confirmed with adenovirus-transformed mouse cells (*24*). H-2 expression was low or absent on the surface of the Ad12 transformants, as determined by means of monoclonal antibodies to H-2. However, residual levels of class I transcripts are present.

Although it is possible that such a reduction in class I expression could have a significant impact on the efficiency of CTL recognition, it is not clear that evasion of T-cell recognition is an adequate explanation for the oncogenic character of the Ad12 transformants. The oncogenic potential of hamster and rat cells transformed with the different adenovirus strains appears to correlate more meaningfully with their resistance to killing by macrophages and NK cells than with their resistance to CTL lysis (*25, 26*). In fact, the various sets of adenovirus transformants do not appear to differ significantly in their sensitivity to killing by either alloreactive CTL's or, more relevantly, CTL's generated against adenovirus-transformed tumors (*25, 27*). It is not clear, however, whether NK function alone is sufficient for the rejection of an incipient tumor, since nude mice (which are CTL deficient but which express high levels of NK activity) are highly susceptible to tumorigenesis by cells transformed with all strains of adenovirus (*28*).

Whatever the immune component(s) responsible for the rejection of adenovirus-induced tumors, recent transfection experiments suggest that the level of class I expression on these tumors determines, in part, whether they will be oncogenic (*29*). In these experiments, an H-2L gene from the BALB/c mouse strain was transfected into Ad12-transformed cells of the C57BL/6 strain. The transfectants were much less oncogenic when transplanted into BALB/c mice than was the parental Ad12 tumor. Thorough biochemical and immunological characterization of these transfectants is necessary, however, before it will be possible to conclude that these cells are rejected by the same immune components that are responsible for the rejection of the nononcogenic Ad2-transformed cells in histocompatible mice. It is possible that tumor rejection in this case might be the result of phenomena more directly comparable to graft rejection and unrelated to the differences between Ad2- and Ad12-transformed cells. The C57BL/6 cells from which the tumor was derived undoubtedly express several polymorphic surface proteins that may be sufficient to elicit graft rejection even between H-2–compatible mice.

## Molecular Mechanisms Involved in Attenuation of Class I Expression

Since alterations in class I expression appear to be involved in the escape from immune surveillance and the growth of some tumors, it is of interest to determine whether these tumor-associated alterations are the direct result of some mechanism related to cellular transformation. Given the variety of altered class I profiles that have been observed in tumors of diverse etiologies, a single unifying mechanism would be difficult to discern. Furthermore, only a fraction of all tumors exhibit any obvious changes in class I expression. Although it is likely that specific molecular mechanisms will be implicated in the class I alterations observed on certain classes of tumors—

particularly those of lymphoid origin or viral etiology—we believe that most class I alterations arise from random events and are immortalized and amplified in the course of neoplastic progression. Just as the genotype of neoplastic cells in culture is extremely unstable, tumor cells in vivo probably progress via the sequential activation, mutation, and inactivation of a variety of gene products (*30–32*). Therefore, any molecular event in a tumor variant that results in the loss of the class I restriction element required for CTL recognition of the tumor cell could provide a selective advantage, leading to clonal expansion of this variant and fixation in the terminal phenotype of the metastasis.

Obviously, a number of molecular mechanisms might be responsible for the attenuation of class I expression seen on tumors of certain etiologies. These could include deletion, mutation, or recombination of class I sequences; transcriptional inactivation; defects in posttranslational processing; or even loss of $\beta_2$-microglobulin, a small protein associated with class I products on the cell surface (*10, 33–36*).

Activation or loss of specific transcriptional regulators might also be relevant, although there is, as yet, no molecular description of the factors that might be involved. One clue to the identity of these factors might be derived from the studies of the adenovirus system. Ad12-transformed cells express low levels of class I transcripts. However, expression can be restored by treatment with γ-interferon, an intercellular messenger that acts as a potent activator of class I expression (*24, 37*). Furthermore, repression of class I transcription in Ad12-transformed cells does not affect the expression of transfected class I genes (*29*). Surprisingly, Ad12 infection, in contrast to cellular transformation, results in a transient 10- to 15-fold increase in the expression of class I messenger RNA (*38*).

Tanaka *et al.* have shown that changes in the levels of DNA methylation surrounding class I genes might also be relevant (*39*). The activation of H-2K expression that occurs in differentiating F9 embryonal carcinoma cells was associated with an increase in the methylation state of the H-2K gene, as detected by a specific 3′ H-2K probe. These findings contrast with most other systems in which gene activation is correlated with a decrease in the state of DNA methylation (*40*). However, as the authors note, the behavior of the H-2K gene appears unique even among the class I genes of the F9 cells, most of which appear to become relatively hypomethylated during differentiation. The developmental changes in the methylation patterns of

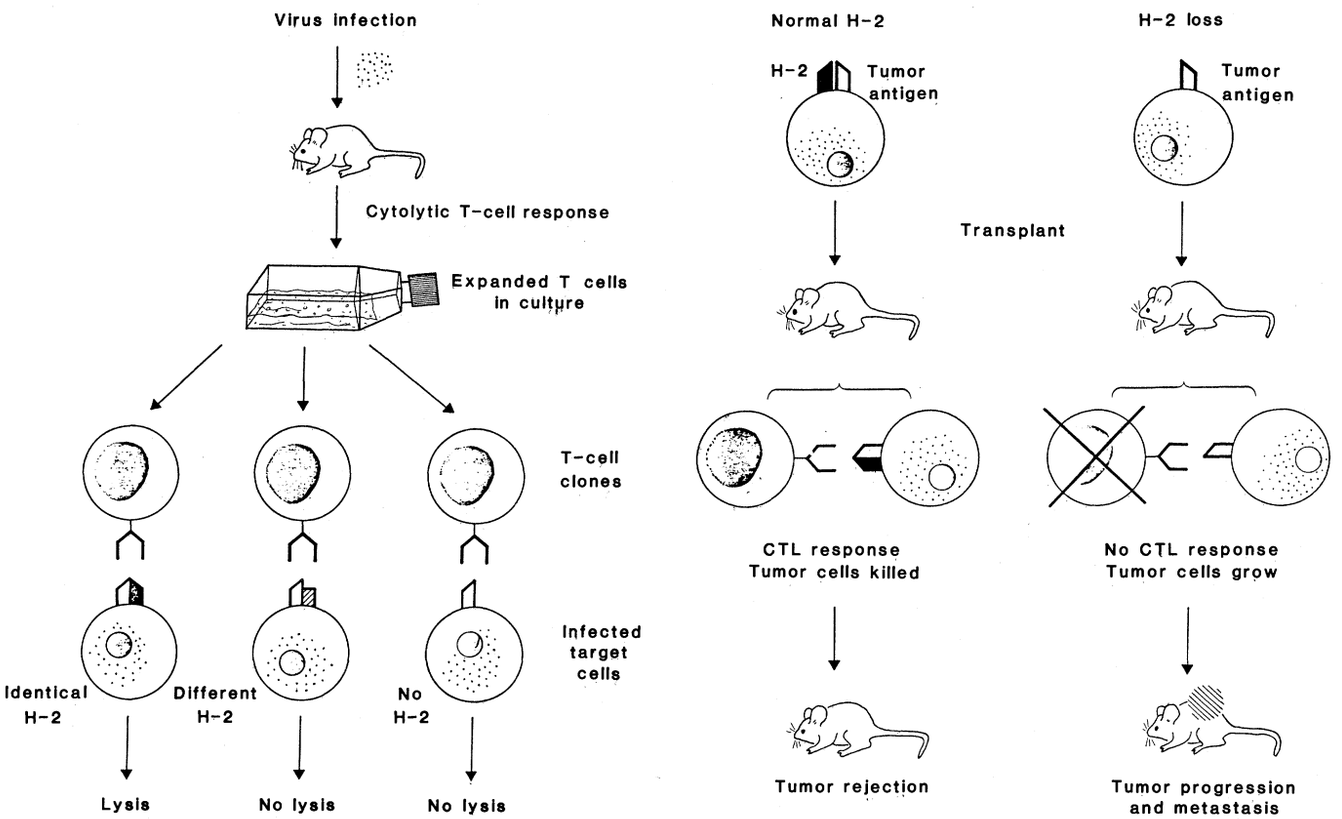


Fig. 1 (left). H-2–restricted T-cell recognition. Some of the major features characteristic of H-2–dependent CTL recognition of virally infected cells are shown. (Far left) CTL's generated during viral infection (shown expanded in culture) are able to lyse cells that express the viral antigen in association with the appropriate H-2 antigen. In the absence of H-2 (far right) or viral antigen, CTL recognition is blocked. This demonstrates the dual recognition of self plus nonself by cytolytic T cells. In the presence of an inappropriate H-2 molecule (for example, on target cells derived from a mouse strain different from that in which the CTL's are generated) the same cytolytic T cells are unable to recognize or lyse the target cell even in the presence of the viral antigen (center). Thus, the H-2 requirement is both absolute and specific. The T-cell receptor is represented as a single unit to depict the general features of H-2–restricted T-cell killing. Recent evidence suggests that the receptor may possess recognition sites for both self and foreign target antigens (*6*). Fig. 2 (right). Rejection of transplanted tumors based on class I–dependent T-cell responses. Transformed cells expressing a tumor-specific target antigen in association with the appropriate H-2 molecule on the cell surface can elicit a specific CTL-mediated immune response in transplanted recipients. In certain cases, the CTL's recognize the transplanted tumor cells as foreign, leading to the destruction of the transplanted tumor by the recipient mice (left). Such a CTL response, however, would not be generated when recipient mice are immunized with tumor cells that have lost the expression of the appropriate H-2 product (right). Although the tumor-specific target antigen is expressed, it would not be sufficient for CTL recognition. The transplanted tumor cells, left unchecked by the immune system of the recipient mouse, would be able to grow and progress to metastasis.

these genes are probably complex. Such changes in methylation patterns are generally believed to represent a response to changes in gene expression mediated by specific factors (for example, steroid hormone receptors) (*41, 42*). Since methylation patterns tend to be clonally maintained (*40, 43*) and DNA hypermethylation is capable of inactivating the expression of specific genes in other systems (*44*), it is possible that changes in the methylation pattern of specific class I genes might be responsible for the observed differences in class I expression on some tumors and their derivatives.

Methylation has been directly implicated in the regulation of class I expression in at least one tumor. Olsson and Forchhammer (*45*) have shown that both metastatic and nonmetastatic phenotypes could be imparted to subclones of the Lewis lung carcinoma by treatment with 5-azacytidine, a drug believed to specifically inhibit the enzymes responsible for the maintenance of DNA methylation patterns. The change in metastatic potential of the cells was associated with a change in expression of a particular class I molecule (*46*).

### Enhancement and Activation of Class I Sequences in Tumors

While various studies point to a clear relationship between loss of class I products and tumor growth and progression, in other tumor systems just the opposite relationship has been observed. The metastatic potential of certain variants of the H-2K–negative T10 sarcoma, for example, appears to be directly related to the expression of a particular H-2D gene product (*37*). Although transfectants expressing H-2K were nononcogenic (*20*), analysis of the H-2 expression of metastatic and nonmetastatic derivatives of the H-2K–negative parental tumor revealed that, in each case, metastatic potential in histocompatible hosts was correlated with the expression of the H-2D antigen from one of the parental strains (*47*). The expression of this antigen was enhanced several fold with respect to normal spleen cells (*47*). Expression of this antigen appeared to be causally related to the invasiveness of these cells since the metastatic potential could be reversed by selection of variants that had lost only one of the parental H-2D products (*47*). Moreover, H-2D–positive T10 subclones were more metastatic than variants that were completely H-2–negative (*47*). However, expression of the other H-2D parental allele tended to make the T10 variants less metastatic. Similar observations have been reported for other systems (*48*).

The expression of the H-2D antigen does not enhance the metastatic potential of all tumors. Generally, H-2D appears to be important in the resistance to the tumorigenic effects of murine retroviral infection. The H-2D molecule is the restriction element for CTL-mediated killing of BALB/c cells that have been transformed by certain retroviruses, including radiation leukemia virus and Friend murine leukemia virus (*4*). Therefore, it is surprising that H-2D can mediate such distinct immunological effects on derivatives of the T10 sarcoma. The H-2D expressed on T10 might function, not as a conventional CTL restriction element, but in yet another capacity. For example, the expression of H-2D, in association with tumor-associated target antigens, might exert a regulatory effect on the specific immune response against the tumor. This might occur through interactions with suppressive immune components such as suppressor T cells (*49*) or by induction of CTL's capable of recognizing T-cell receptor structures on CTL's specific for the tumor (*50*). Such specific immune suppression could explain the lack of immunogenicity associated with these subclones (*51*). Alternatively, the apparent H-2D enhancement might actually represent the activation of another class I molecule, antigenically related to but distinct from H-2D, since the recognition of Qa-Tla class I gene products by monoclonal antibodies specific for H-2 antigens has been reported (*52*). This is particularly important since class I activation has been observed on a large number of thymic leukemias (*2*), on rat cells transformed with polyoma virus (*53*), on some retrovirally transformed mouse cells (*54*), and on mouse fibroblasts transformed in vitro with simian virus 40 (*55*).

The de novo activation of a normally silent, presumably intact class I locus has been implicated in the enchanced invasiveness of several tumors, including the 5-azacytidine–induced metastatic variant of the Lewis lung carcinoma (*45, 56*). A monoclonal antibody generated against this variant may recognize a novel class I antigen, perhaps encoded within the Qa/Tla region of the MHC, which is specifically activated in this metastatic subclone (*45*). This product might exert an effect on the immune response to the tumor similar to that postulated for the H-2D antigen on T10. In addition, this category of altered H-2 expression may also affect the invasiveness of these cells through a nonimmunological mechanism. The H-2D products might be required for the functional assembly of membrane protein complexes essential for tumor localization and implantation (*57*).

### Molecular Mechanisms Responsible for Class I Activation

In each of the systems described above, the tumors exhibiting altered class I expression were derived from rare variants and selected by passage in vivo. To gain insight into the molecular mechanisms underlying enhanced or activated class I expression it might be necessary to study systems that operate independently of immune selection. BALB/c 3T3 cells, which normally express reduced H-2, have enhanced levels of class I transcripts after transformation in vitro with SV40 (*55*). Although Rigby and co-workers originally identified a complementary (cDNA) clone derived from SV40-transformed 3T3 cells as the product of an activated Tla gene (*55*), further analysis revealed that this clone was, in fact, derived from H-2D (*58*). However, SV40-transformed 3T3 cells do express de novo–activated class I transcripts in addition to enhanced H-2D. Robinson, Hunt, and Hood have isolated a cDNA clone from the metastatic SVT2 tumor and have shown by DNA sequence analysis that this cDNA is derived from the Tla class I gene, TL10 (*56*).

Analysis of SV40-transformed 3T3 cells reveals that a variety of class I genes, as identified by specific oligonucleotide probes, can be activated in these cells (59). However, no consistent pattern of activation has been reported. Expression of these antigens is dependent upon the function of SV40 large T antigen (60), a viral regulatory protein required for maintenance of cellular transformation. Many of the class I genes activated by SV40 appear to contain repetitive elements known as B2 repeats. Since transcription of these sequences is enhanced in SV40-transformed cells (*61*) it is possible that B2 repeats are involved in the regulation of class I transcription by SV40.

Although DNA sequence analysis of one of the Tla genes activated in SV40-transformed 3T3 cells indicates that the transcripts could encode functional molecules (*45*), it is necessary to demonstrate that the transcripts give rise to class I molecules before their function can be discerned. The generation of specific serological probes to peptides predicted from these gene sequences should aid in the identification and characteriza-

tion of such products and of their role in tumorigenesis. Since enhanced or activated class I expression in transformed cells might affect the immune response to such cells, it is also vital to identify the immunological factors underlying the phenomena. This includes not only biochemical characterization of the products of the activated class I genes but also the dissection of those immunological components (such as suppressor T cells) interacting with the tumor cells.

### Expression of Unique Class I Products on Tumors

There have been many reports in the literature of tumor-specific expression of histocompatibility antigens alien to the strain from which the neoplasm was derived (*4, 15*). Such reports generally stemmed from investigations of the anomalous growth of tumors in transplanted recipients, either in their rejection in syngeneic hosts or progression in histoincompatible strains of mice. At issue was whether expression of these novel antigens involved alteration of classical H-2 structures, perhaps through mutational or recombinational events, or the activation of normally silent and intact class I loci. Early attempts to identify these tumor-specific antigens were often complicated by a number of problems including the use of complex (and occasionally contaminated) antisera to characterize the unique antigens, the lack of biochemical characterization of the products involved, and the use of tumors of uncertain origin. For these reasons, many of the early reports describing the identification of novel class I antigens on tumors actually concerned the identification of antigens that were not class I, or that were the normal H-2 antigens of the strain from which the tumor was derived. Nonetheless, the concept of tumor-specific class I antigens remains intriguing, particularly in view of the possible functions of the MHC in tumorigenesis.

Structural alteration of a CTL restriction element might allow a tumor variant expressing only a fraction of its H-2 genes to evade the immune response against the parental tumor (*4*). Novel class I products might also act to suppress the immune response against the tumor, perhaps by mechanisms comparable to those discussed above for the effect of elevated levels of H-2D on the T10 sarcoma. Cell-surface expression of novel class I products might be a by-product of the expression of a secreted form of class I antigen that had been generated by alternative RNA splicing events, such as that reported in liver (*3, 62*). Secreted class I antigens have been proposed to function in the regulation of CTL activity (*63*). It could conceivably be advantageous in evolutionary terms for normal tissue to express novel class I antigens when transformed, given the efficiency with which CTL's identify and eliminate cells expressing foreign MHC.

Schreiber and his colleagues have extensively analyzed an ultraviolet (UV)-induced fibrosarcoma (designated 1591) to determine the basis of its extreme immunogenicity after transplantation into immunocompetent, histocompatible mice (*64*). Specific monoclonal antibodies generated against this C3H tumor identified some of the immunogenic molecules on 1591 as class I antigens (*65*). The novel antigens are similar to, but distinct from, the class I products normally expressed by C3H and other strains of mice on the basis of serological studies, tryptic peptide analysis, and two-dimensional gel analysis (*66*). Goodenow and co-workers have cloned the genes encoding these novel antigens and demonstrated that 1591 expresses, in addition to the normal complement of H-2K and H-2D, at least three novel class I antigens not normally expressed on C3H tissue (*67*), one of which appears to be derived from the H-2K gene (*68*). Analysis of mouse fibroblast lines transfected with the novel genes indicates that these unique class I antigens function as the targets of many of the CTL's generated against the tumor in normal mice (*69*). Furthermore, loss of the novel class I molecules from variants of 1591 correlates with their immunogenicity (*65*). Thus, these molecules appear to limit tumor progression in a transplant situation.

Southern blot and sequence analysis of the class I genes of 1591 and C3H tissue suggests that these novel antigens are generated by multiple recombination events among the endogenous class I genes of C3H (for example, H-2K) that might have occurred during the course of tumorigenesis (*67*). Recombination of class I sequences, as distinct from gene activation, might therefore be responsible for some of the tumor-specific transplantation antigens reported. Since recombination rather than simple mutation is believed to be the primary mechanism responsible for generating the enormous polymorphism seen among the H-2 antigens of different strains of mice (*1*), it is reasonable to implicate recombination in the generation of novel class I antigens. Events that could best be explained by recombination have also been observed by Martin *et al.* in their studies of the structural alterations of a variant class I antigen expressed on a C3Hf adenocarcinoma (*70*). C3Hf is a strain of mouse derived from C3H by mutational alteration of the H-2K gene. The alterations detected on the tumor antigen appear to represent a partial reversion of these original meiotic mutational events (*71*). Thus, somatic recombination of class I genes in tumors may be mechanistically comparable to events that occur normally in the germline (*72–74*).

Ordinarily, the expression of such strongly immunogenic molecules would be expected to cause rejection of a nascent tumor and prevent progression to metastasis. However, since up to 70 percent of all (UV-induced fibrosarcomas that progress in their original hosts are as immunogenic as 1591 as transplants, this is clearly not the case (*75*). The immunobiological basis for tumor susceptibility may involve both immunogenic and suppressive elements. This stems from the following observations: (i) UV-irradiated mice cannot reject UV-induced tumors, but continue to have the ability to limit the growth of other non–UV-induced tumors. (ii) Normal mice surgically linked to UV-irradiated mice lose the capacity to resist UV-induced tumors. This suggests that the UV treatment may elicit the production of suppressive immune components capable of specifically eliminating the T-cell response against UV-induced tumors. Moreover, this suppressor population is specific, distinguishing between transplants derived from UV-induced and, for example, MCA-induced tumors. In fact, these cells even appear to be able to distinguish between tumors induced by specific wavelengths of UV-irradiation (*75*). One possible explanation is that UV light induces a common, stably inherited antigenic change in all exposed cells that mediates suppression. Tumors arising from irradiated cells would then express this common antigen and be protected from immune rejection (*75*). Although it is unclear what relationship, if any, exists between the novel class I antigens of 1591 and UV-specific suppression, it is possible that these molecules were important to the progression of this tumor in the original irradiated host.

### Discussion

Neoplastic progression can be thought of as the evolution of the tumor through stages of sequential selection and adaptation, stages which can now be defined by means of molecular probes (*30, 31*).

Neoplastic development must be influenced by the selection of tumor variants best suited to growth, expansion, and metastasis. As a result, the interactions between the host and the tumor undoubtedly play a dynamic role in the pathology of the disease. Evidence for these interactions can be inferred from the phenotype of the tumor. Just as the elevated levels of plasminogen activator detected within metastatic clones of some tumors (*76*) might reflect the need for proteolytic activity at some time during the metastatic process, the altered class I expression frequently observed on metastatic tumors might imply that evasion of a CTL response is a common event during neoplastic progression.

Immune recognition could obviously have a dramatic effect on the evolution of many tumors through selection of tumor subsets capable of avoiding rejection. Careful analysis of the immunological behavior of the terminal metastatic cells should provide some insight into the nature of the selective pressures that had acted upon the parental tumor population. Unfortunately, most studies rely upon transplantation as the primary measure or indication of the immunogenicity and metastatic potential of a variant tumor, and the transplantation assay might not accurately reflect the unique immunological environment facing a variant cell in a nascent tumor. However, within the context of the transplantation assay, it is clear that modulation of class I expression can be considered a major factor in the ability of some tumors to evade immune destruction. It will be necessary to confirm that the phenomena defined by transplantation are relevant to the process of tumorigenesis.

The results discussed in this review have been derived from studies in rodent systems. It will be essential to determine whether any of the phenomena which we have described also apply to human tumors. However, the results obtained for the murine neoplasms are relatively unambiguous, since these studies take advantage of inbred mouse strains expressing well-characterized complements of MHC genes and products. In contrast, comparable human studies may be obscured by the complexities inherent in the human equivalent of the MHC, the HLA system. Furthermore, it may be impossible to make any meaningful judgments about the immunogenicity of human tumors without the aid of appropriate genetically characterized transplant hosts. The example of the highly immunogenic UV-induced murine tumors has shown that "if the only measure of antigenicity available were the immune response induced by the tumor in its primary host, we would conclude, incorrectly, that the tumors were not antigenic. This is, of course, precisely the situation with human cancers" (*75*). Despite the difficulties of studying human cancer, epidemiological evidence with respect to immunosuppressed patients indicates that the immune system plays an essential role, at least in the prevention of those human tumors with a viral etiology, such as Burkitt's lymphoma (*31*) and papilloma (*77*).

A number of issues remain unresolved concerning the extent to which alterations in class I antigen expression are responsible for the progression of neoplastic cells in the murine system. Foremost among these is a meaningful estimation of the generality of these phenomena in diverse tumor systems. Many metastatic tumors show no apparent alterations in the level or nature of the class I products expressed (*4*). However, without the appropriate antibody probes to rule out the possibility of expression of class I antigens with subtle structural alterations, anomalous expression may remain undetected. The discovery of serologically undefined class I products on some metastatic tumor variants emphasizes this possibility (*45*). However, class I anomalies might not be expected to be associated with tumors of all etiologies, since immune selection is probably only one of many factors involved in neoplastic development.

It is equally important to identify the molecular mechanisms responsible for the alterations in class I expression that occur during tumor progression. Since the events that modify class I expression in tumor variants probably occur only rarely in the tumor populations, their characterization may be extremely difficult.

The number of different relationships described between H-2 and the growth behavior of certain tumors points to the potential complexity of the interactions between a disseminating tumor and the immune system (Fig. 3). A tumor expressing a foreign antigen in association with an appropriate class I restriction element could be eliminated by the immune system of a healthy animal. Although any alteration in the class I expression of the tumor could profound-

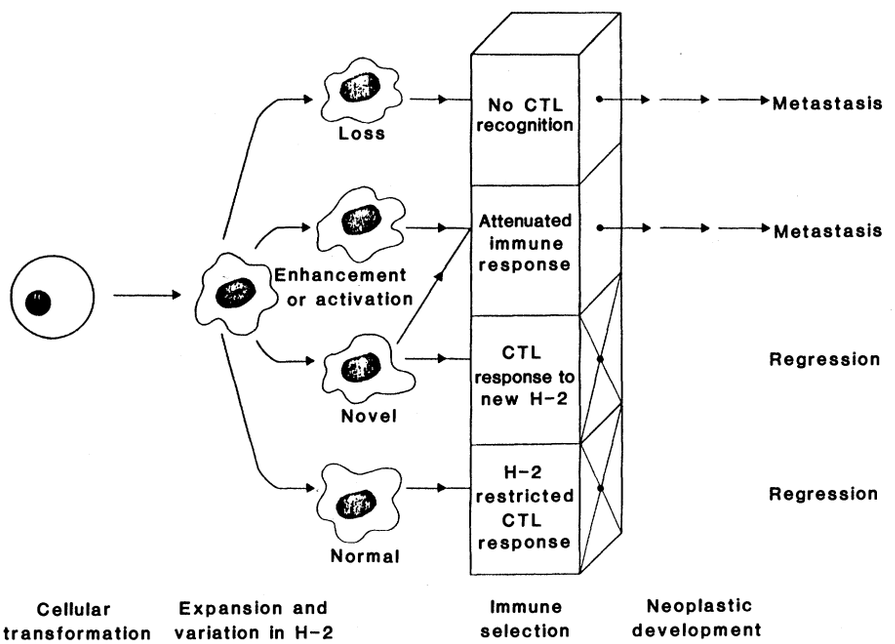


Fig. 3. Class I–mediated immune surveillance during tumorigenesis. The immunological fate of transformed cells displaying various H-2 expression profiles is depicted. Within the mouse, a cell may become transformed, allowing it to grow and expand beyond the limit permitted by the normal cellular regulatory processes. During expansion, the transformed cell can undergo changes in the expression of a number of genes, including those encoding class I antigens. The animal's immune system may or may not produce a CTL response to the altered class I phenotype of the tumor cell. Loss of expression of the appropriate H-2 antigen would allow the tumor cell to evade CTL recognition. Enhanced or activated class I expression may result in a reduced ability for the immune system to respond to the target antigens on the transformed cell. In both cases, such cells may undergo additional stages of selection, perhaps mediated by humoral or other cellular (for example, natural killer) responses, before reaching the final stage of metastasis. Novel class I antigens expressed on tumor cells may elicit a CTL response, in which case these transformed cells would be lysed and the tumor would regress. Similarly, a class I–restricted CTL response to a tumor cell expressing an unaltered H-2 profile would block neoplastic progression.

ly affect the efficacy with which the immune system recognizes a malignant cell, the oncogenic outcome of any specific alteration will depend upon the complex interactions of immune regulation. Additional studies may elucidate the multiplicity and generality of class I-dependent immunoselection during tumorigenesis. This will require the use of cloned genes, defined populations of lymphoid effector cells, and specific antibodies capable of identifying target molecules or distinguishing discrete differences in the expression of class I antigens. Future experiments, including tumor induction in transgenic mice that express class I mutations, are needed to describe some of the relevant molecular interactions between the T-cell network and the H-2 molecules and should facilitate the careful dissection of the molecular bases for MHC-dependent immune-responsiveness in tumorigenesis.

### References and Notes


1. L. Hood, M. Steinmetz, B. Malissen, *Annu. Rev. Immunol.* **1**, 529 (1983).
2. L. J. Old, *Cancer Res.* **41**, 361 (1981).
3. J.-L. Lalanne *et al.*, *Cell* **41**, 469 (1985)
4. P. C. Doherty, B. B. Knowles, P. J. Wettstein, *Adv. Cancer Res.* **42**, 1 (1984).
5. R. M. Zinkernagel and P. C. Doherty, *Adv. Immunol.* **27**, 51 (1979).
6. B. Pernus and R. Axel, *Cell* **41**, 13 (1985).
7. T. J. Braciale, M. E. Andrew, V. L. Braciale, *J. Exp. Med.* **153**, 1371 (1981).
8. N. Hanna and R. C. Burton, *J. Immunol.* **127**, 1754 (1981).
9. K. Hui, F. Grosveld, H. Festenstein, *Nature (London)* **311**, 750 (1984).
10. M. Imamura and W. J. Martin, *J. Immunol.* **129**, 877 (1982).
11. L. R. Gooding, *ibid.*, p. 1306.
12. P. De Baetselier, S. Katzav, E. Gorelik, M. Feldman, S. Segal, *Nature (London)* **288**, 179 (1980).
13. H. Festenstein and W. Schmidt, *Immunol. Rev.* **60**, 85 (1981).
14. W. Schmidt and H. Festenstein, *Immunogenetics* **16**, 257 (1982).
15. G. Parmiani *et al.*, *ibid.* **9**, 1 (1979).
16. G. Parmiani, *Transplant Proc.* XII, 50 (1980).
17. G. C. DuBois, E. Appella, L. W. Law, *Int. J. Cancer* **34**, 561 (1984).
18. L. Ährlund-Richter, E. Klein, F. Merino, *Immunogenetics* **15**, 53 (1982).
19. S. Katzav *et al.*, *Transplant. Proc.* XV, 162 (1983).
20. R. Wallich *et al.*, *Nature (London)* **315**, 301 (1985).
21. R. S. Goodenow, M. McMillan, A. Örn, M. Nicolson, N. Davidson, J. A. Frelinger, L. Hood, *Science* **215**, 677 (1982).
22. G. Huebner, in *Perspectives in Virology*, M. Pollard, Ed. (Academic Press, New York, 1967), vol. 5, p. 147.
23. P. I. Schrier, R. Bernards, R. T. M. J. Vaessen, A. Houweling, A. J. van der Erb, *Nature (London)* **305**, 771 (1983).
24. K. B. Eager *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5525 (1985).
25. K. Raška, Jr., and P. H. Gallimore, *Virology* **123**, 8 (1982).
26. A. M. Lewis, Jr., and J. L. Cook, *Science* **227**, 15 (1985).
27. G. H. Mellow *et al.*, *Virology* **134**, 460 (1984).
28. R. B. Herberman and H. T. Holden, *Adv. Cancer Res.* **27**, 305 (1978).
29. K. Tanaka, K. J. Isselbacher, G. Khoury, G. Jay, *Science* **228**, 26 (1985).
30. P. C. Nowell, *ibid.* **194**, 23 (1976).
31. G. Klein and E. Klein, *Nature (London)* **315**, 190 (1985).
32. G. Poste and I. J. Fidler, *ibid.* **283**, 139 (1980).
33. P. Gladstone, L. Fueresz, D. Pious, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1235 (1982).
34. B. Holtkamp, M. Cramer, K. Rajewsky, *EMBO J.* **2**, 1943 (1983).
35. H.-G. Burghert and S. Kvist, *Cell* **41**, 987 (1985).
36. F. Rosa *et al.*, *EMBO J.* **2**, 239 (1983).
37. D. Wallach, M. Fellows, M. Revel, *Nature (London)* **299**, 833 (1982).
38. A. Rosenthal *et al.*, *ibid.* **315**, 579 (1985).
39. K. Tanaka, E. Appela, G. Jay, *Cell* **35**, 457 (1983).
40. W. Doerfler, *Annu. Rev. Biochem.* **52**, 97 (1983).
41. J. B. E. Burch and H. Weintraub, *Cell* **33**, 65 (1983).
42. R. M. Grainger *et al.*, *Nature (London)* **306**, 88 (1983).
43. R. Stein, Y. Gruenbaum, Y. Pollack, A. Razin, H. Cedar, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 61 (1982).
44. M. Busslinger, J. Hurst, R. A. Flavell, *Cell* **34**, 197 (1983).
45. L. Olsson and J. Forchhammer, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3389 (1984).
46. L. Olsson, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 5414 (1985).
47. S. Katzav, P. De Baetselier, B. Tartakovsky, M. Feldman, S. Segal, *J. Natl. Cancer Inst.* **71**, 317 (1983); S. Katzav, S. Segal, M. Feldman, *Int. J. Cancer* **33**, 407 (1984).
48. L. Eisenbach, S. Segal, M. Feldman, *Int. J. Cancer* **32**, 113 (1983).
49. B. Payelle, G. Lespinats, S. Tlouzeau, *ibid.* **34**, 95 (1984).
50. P. M. Flood, M. L. Kripke, D. A. Rowley, H. Schreiber, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2209 (1980).
51. S. Katzav, S. Segal, M. Feldman, *J. Natl. Cancer Inst.* **75**, 307 (1985).
52. S. O. Sharrow, L. Flaherty, D. H. Sachs, *J. Exp. Med.* **159**, 21 (1984).
53. B. Majello, G. La Mantia, A. Simeone, E. Boncinelli, L. Lania, *Nature (London)* **314**, 457 (1985).
54. D. C. Flyer, S. J. Burakoff, D. V. Faller, *J. Immunol.* **135**, 2287 (1985).
55. P. M. Brickell, D. S. Latchman, D. Murphy, K. Willison, P. W. J. Rigby, *Nature (London)* **306**, 756 (1983).
56. R. Robinson, S. Hunt, L. Hood, in preparation.
57. M. Simonsen *et al.*, *Prog. Allergy* **36**, 151 (1985).
58. P. M. Brickell, D. S. Latchman, D. Murphy, K. Willison, P. W. J. Rigby, *Nature (London)* **316**, 162 (1985).
59. R. Robinson and S. Hunt, in preparation.
60. R. Robinson, personal communication.
61. K. Singh, M. Carey, S. Saragosti, M. Botchan, *Nature (London)* **314**, 553 (1984).
62. M. Kress, Y. Barra, J. G. Seidman, G. Khoury, G. Jay, *Science* **226**, 974 (1984).
63. M. Kress, D. Cosman, G. Khoury, G. Jay, *Cell* **34**, 189 (1983).
64. R. D. Wortzel, C. Philipps, H. Schreiber, *Nature (London)* **304**, 165 (1983).
65. C. Philipps *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5140 (1985).
66. M. McMillan, K. D. Lewis, D. M. Rovner, *ibid.*, p. 5485.
67. H. Stauss *et al.*, in preparation.
68. S. Watts, in preparation.
69. J. Forman, personal communication.
70. J. Martin *et al.*, *J. Immunogenetics* **5**, 255 (1978).
71. P. Callahan *et al.*, *ibid.* **130**, 471 (1983).
72. E. H. Weiss *et al.*, *Nature (London)* **301**, 671 (1983).
73. A. L. Mellor *et al.*, *ibid.* **306**, 792 (1983).
74. H. Sun, R. S. Goodenow, M. McMillan, L. Hood, *J. Exp. Med.*, in press.
75. M. Kripke, *Adv. Cancer Res.* **34**, 69 (1981).
76. L. Eisenbach, S. Segal, M. Feldman, *J. Natl. Cancer Inst.* **74**, 77 (1985).
77. H. Kirchner, *Immunol. Today* **5**, 272 (1984).


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