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Molecular Mechanisms Used by Tumors to Escape Immune Recognition: Immunogenetherapy and the Cell Biology of Major Histocompatibility Complex Class I

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Summary

In this article, we explore the hypothesis that tumor cells can escape recognition by $CD8^+$ T cells via deficiencies in antigen processing and presentation. Aspects of the molecular and cellular biology of major histocompatibility complex class I are reviewed. Evidence for histology-specific molecular mechanisms in the antigen-processing and -presentation deficiencies observed in some human and murine tumors is presented. Mechanisms identified include down-regulation of antigen processing, loss of functional β_2-microglobulin, and deletion of specific α-chain alleles. Finally, we discuss studies using an antigen-presentation-deficient mouse tumor as a model for the immunogenetherapy of an antigen-presentation deficiency.

Keywords

Major histocompatibility complex class I; Tumor; Antigen processing; Antigen presentation; $CD8^+$ T cells; Vaccinia virus; recombinant

Tumor immunologists once vigorously debated the existence of tumor-associated antigens (TAAs). This debate has generally been resolved, since numerous researchers have shown, in a variety of systems, that tumors can be recognized and, in some cases, destroyed by elements of the immune system (1–4).

The reasons why tumor cells may express TAAs are beginning to be understood. For example, TAAs may be the result of the processes of carcinogenesis, which are generally thought to stem from damage to a large number of genes, some of which have a role in the molecular mechanisms regulating cell growth and division (5). This damage results in the uncontrolled cellular proliferation that defines the transformed cell. Thus, possible origins of TAAs include self-proteins, such as fetal antigens (6), oncogene products (including fusion proteins), mutated tumor-suppressor gene products, other mutated cellular proteins, or such foreign proteins as viral gene products (including but not limited to products of oncogenic viruses). Nonmutated cellular proteins may also be antigenic if they are expressed aberrantly (e.g., in an inappropriate

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subcellular compartment) or in supranormal quantities (7). Finally, totally normal proteins could be recognized by autoreactive T cells (8).

Given the numerous steps of cellular transformation and the sometimes bizarre genotypes observed in cancer cells, it could be argued that tumor cells are likely to contain many new antigens potentially recognizable by the immune system. Nevertheless, every death resulting from progressive cancer can be thought of as resulting from a tumor that has not been eradicated by the immune system. Why is this the case?

ESCAPE OF TUMOR CELLS FROM IMMUNE RECOGNITION

Escape from immune recognition by tumor cells could be the result of many mechanisms. Considered here are those possible mechanisms involving the target cell (i.e., tumor cell) and the antigens associated with it. Clearly, there could be loss of antigenic epitopes on tumor cells as a result of lack of expression of the entire protein in question. Alternatively, the epitope recognized could mutate to form a structure not recognized because of a "hole" in the repertoire of the immunologically relevant molecules. In some cases, this lack of recognition could be the result of self mimicry, or tolerance. In other cases, lack of recognition of an epitope could be the result of biophysical constraints to recognition. These mechanisms could be operative in the escape of tumors from elements of both the humoral and the cellular immune systems.

Because T cells recognize antigen only after it has been processed and presented by molecules of the major histocompatibility complex (MHC), there is another, entirely different set of occurrences that could play a part in the escape of tumor cells from recognition by T cells (9). An understanding of the molecular structures implicated in recognition of antigens by T cells (reviewed in ref. 10) led to the hypothesis that some tumor cells escape recognition by T cells not because of antigenic loss or mutation but because of a failure to process and present TAAs, Thus, a tumor might evade recognition by T cells by failing to present tumor antigens that were not absent but instead hidden intracellularly and not presented on the cell surface. The second part of this hypothesis is that if the deficiencies in antigen processing and presentation could be corrected, then tumor cells could be immunologically recognized by T cells.

Many investigators have shown that the recognition and destruction of tumor cells by T lymphocytes is due, in part, to the activity of $CD8^+$ T cells (T_{CD8^+}). T_{CD8^+} that specifically lyse human and murine tumor cells can be generated in vitro and can eliminate established tumor in vivo (1–4). Anti-tumor T_{CD8^+} have also been shown to specifically release cytokines when exposed to the appropriate tumor target (11). We chose to study T_{CD8^+} recognition for these reasons.

THE CELL BIOLOGY OF ANTIGEN PROCESSING AND PRESENTATION

The specificity of T-cell recognition is largely due to the T cell receptor (TCR). The molecular structures of TCR and immunoglobulin (Ig) molecules are very similar and are likely to have evolved from the same primordial molecule (12). However, these two classes of molecules recognize fundamentally different molecular targets. Ig molecules recognize antigen in native or denatured conformations. T cells generally can recognize only processed antigens that are presented by self–MHC molecules (Table 1). MHC molecules presenting peptide antigens designated immunogenic trigger a T-cell response that can consist of proliferation, up-regulation of surface molecules, activation of lytic processes, and/or secretion of cytokines.

T_{CD8^+} recognize MHC class I molecules bearing peptides of eight to 10 residues (13) derived from proteins located in the cytosol (14). Association of these peptides with class I α chains and β_2-microglobulin (β_2m) is thought to occur in the endoplasmic reticulum (ER) or in an

intermediate secretory compartment. Generation of peptide fragments eight to 10 residues in length is likely to require the unfolding of proteins containing antigenic epitopes, but this requirement for unfolding is entirely speculative. Even more speculative is the possible role of members of the heat-shock family of proteins in the unfolding process.

There is clear evidence that antigenic fragments presented by MHC class I molecules are generated by proteolysis, but very little is known about their molecular structures. Ubiquitin-targeted proteolysis may play a part. There is circumstantial evidence that a large nuclear and cytosolic proteolytic structure (termed the proteasome), physically associated with two MHC gene products called LMP-7 and LMP-2 (formerly known as RING 10 and RING 12, respectively), is involved in the production of antigenic peptides or their delivery to class I molecules. Two recent reports, however, indicate that the LMP-2 and LMP-7 gene products are not necessary for the presentation of some antigens (15,16). The function of these gene products in antigen processing, therefore, remains to be established.

MHC class I molecules clearly have some role in the generation of properly sized antigens. Falk and colleagues have shown that the peptide fragments recovered from an antigen-presenting cell are dependent on the class I molecules expressed by that cell (17). There are two competing explanations for this phenomenon. The first is that cytoplasmic proteins are broken down to generate extremely short-lived intermediates, and class I molecules “capture” the right fragments, protecting them from further degradation. Alternatively, cytoplasmic proteins could be broken down into longer peptide fragments that are held by class I molecules while cellular proteases trim them to the appropriate lengths. This could be called the “cookie cutter” hypothesis. Elements of both of these hypotheses may play a role in fitting the peptide pool to the available class I.

How are antigens transported into the ER? A great deal of evidence suggests that peptide antigen associates with class I and β_2m in the ER or in some post-ER compartment (10). Two gene products that are clearly involved, designated TAP-1 and TAP-2 (for transporter associated with antigen processing), are encoded in the MHC. Based on sequence homology, these genes are members of the ABC transporter family. TAP-1 (previously known as RING 4, Y3, and PSF-1) and TAP-2 (previously known as RING 11, Y1, and PSF-2) are clearly needed for cells to efficiently process antigen (reviewed in ref. 18). Based on size and predicted structure, it seems likely that these proteins directly transport peptide from the cytosol, but this theory is unproved.

One member of the heat-shock protein family of proteins (HSPs), called gp96, has been proposed by Srivastava (unpublished observations) to be capable of binding peptides that could subsequently be presented by class I molecules (19). These HSPs may help stabilize unfolded, class I heavy-chain molecules and then transfer antigenic peptides to these molecules, leading to the formation of the stable trimolecular complex of class I α-chain, β_2m, and peptide antigen. The genes coding for class I α-chains, the putative peptide transporters, and the proteasome component molecules appear to be very closely associated with the MHC region on chromosome 17 in the mouse, or chromosome 6 in the human (for map, see ref. 20). Each of these genes, along with β_2m and gp96, is inducible with interferon-γ (IFN-γ).

ANTIGEN-PROCESSING AND -PRESENTATION DEFICIENCIES IN HUMAN CANCERS

Several molecular mechanisms by which tumor cells fail to process and present endogenous antigens to T_{CD8^+} could be hypothesized (Table 2). Using isolates of tumor cells from patients at the National Institutes of Health to establish cell lines for in vitro studies and fresh tumor sections for immunohistochemical studies and in vivo correlates, we have found that at least

three mechanisms are operative. The first mechanism, identified in five melanoma cell lines (N. P. Restifo, unpublished observations), involves the loss of functional β_2m expression, similar to that seen by D'Urso et al. (21). The antigen-presentation deficit documented in these cell line studies is entirely correctable by gene insertion of β_2m. A second mechanism, also observed in melanoma, is the loss of expression of particular MHC class I alleles (F. Marincola, unpublished observations), and it, too, is correctable by insertion of the missing α-chain genes. The third type of defect, observed originally in small-cell lung cancer histology, stems from the down-regulation of certain molecules that putatively process antigens, including TAP and MHC-encoded proteasome components (22). This mechanism is correctable by treatment with exogenous IFN-γ or by insertion of the IFN-γ gene. It is this final mechanism that will be discussed in detail below.

In our attempts to examine the capacity of human tumor cell lines to process cytosolic antigens for T_{CD8^+} recognition, we were faced with the problem of the massive polymorphism of the MHC. Since class I molecules are among the most polymorphic genes in the human genome, we were able to consistently use one class I molecule, to present one antigenic epitope for recognition by one population of effector cells, to study the antigen-processing system. Instead, early studies focused on the cumbersome task of tailoring reagents for particular MHC alleles. We thus devised a method for screening a large number of tumor-cell isolates that was independent of both the human leukocyte antigen type of the tumor and the presence or absence of specific cellular proteins. This method exploits the capacity of recombinant vaccinia virus (rVV) to infect a wide variety of human tumor cells. Using an rVV encoding the mouse H-2 K^d class I molecule (K^d-VV), we could test human tumor cell lines for presentation of viral antigens to mouse K^d-restricted, VV-specific T_{CD8^+} populations and thus study the antigen-processing capabilities of human tumor cells per se.

One of the earliest observations made had nothing to do with the processing of intracellular antigens. Instead, five melanoma cell lines (of ~60) were found deficient in the expression of functional β_2m (N. Restifo, unpublished observations). As mentioned above, supplying an intact β_2m gene using an rVV completely restored the antigen-presentation function of these melanomas.

In another set of experiments (22), 26 tumor cell lines were infected with K^d-VV and tested for lysis by VV-specific T_{CD8^+}. In each experiment, the T2 cell line was included as a negative control. T2 cells lack a 1-megabase region of the MHC that contains the portion coding for the TAP genes and proteasome component molecules and are known to be deficient in their capacity to process viral antigens for T_{CD8^+} recognition (23,24). These experiments showed that human tumor cell lines vary widely in their capacities to process and present viral antigens to T_{CD8^+}. While some cell lines with a variety of tumor histologies were lysed at high levels by VV-specific cytotoxic T lymphocytes (CTLs), many were lysed at low levels. Notably, all six of the small-cell lung carcinomas (SCLCs) studied were consistently recognized at levels similar to or even lower than T2 cells. Figure 1 shows an example of the kinds of functional data obtained from these experiments.

The failure of VV-specific T_{CD8^+} to lyse various tumor cells could not be attributed to low levels of expression of VV gene products. SCLCs generally expressed high levels of viral antigens relative to the other tumor cell lines studied. The failure of T_{CD8^+} to lyse VV-infected SCLCs appeared to be due to a genuine defect in antigen presentation and not to other factors.

Pulse-chase methodology was used to study the biosynthesis and intracellular transport of class I molecules (22). These studies confirmed that class I molecules remained completely sensitive to digestion with endo H throughout an 80-min chase period. Thus, class I molecules were not transported through the Golgi complex to the cell surface. IFN-γ treatment of SCLC cells

greatly altered the intracellular trafficking of class I molecules, inducing the transport of a substantial portion of K^d molecules from the early portion of the secretory pathway through the Golgi complex. This was shown to be the case, since approximately half of the class I molecules immunoprecipitated became resistant to endo H digestion within 80 min of their synthesis. The acceleration of the intracellular transport of class I molecules was associated with higher steady-state class I expression on the cell surface, similar to that seen in the murine tumor system described below (25).

Retention of class I molecules in an endo H-sensitive form has been observed in cells that fail to express TAP or MHC–proteasome subunit gene products (26). When the RNA from the SCLC lines was probed for the expression of message from the TAP and MHC–proteasome subunit molecules, no mRNA could be detected. Low or absent steady-state levels of mRNA indicated either that transcription was down-regulated or that there was a shortening of the half-life of these messages. Our inability to detect these relevant mRNAs by Northern blot analysis did not indicate a deletion of the genes in these cells, since treatment of cells with IFN-γ for 48 h induced the expression of large quantities of mRNA encoding the four genes. Perhaps most importantly, IFN-γ greatly enhanced the antigen-presenting capacity of the SCLCs, but not T2 cells, in functional assays of antigen presentation (22). IFN-γ had no discernible effect on the presentation of peptide that was exogenously provided, despite the fact that it was clearly capable of enhancing presentation of VV antigens to VV-specific T_{CD8^+}.

Our studies with SCLC cell lines did not address the question of whether poor antigen processing was associated with transformation or outgrowth of the tumor or whether this poor processing was representative of the natural regulation of class I expression in the tissue of origin. It is, however, unlikely that the poor antigen-processing capacities exhibited by SCLC cells is an artifact resulting from cell culture, since immunohistochemical studies show that SCLCs express very low or undetectable levels of class I in vivo (27). Thus, poor expression of class I molecules on the cell surface could be associated with a profound incapacity to process endogenously synthesized proteins.

DEVELOPMENT OF A MOUSE MODEL

The great enhancement by antigen processing of IFN-γ treatment of SCLCs suggested that recognition of tumor cells by anti-tumor T_{CD8^+} could be enhanced by the specific up-regulation of the antigen-processing system in tumor cells. Antigen processing and presentation by tumor cells was conceivably a limiting step in the activation of antitumor T_{CD8^+}, especially in tumors expressing low levels of class I. In order to test a therapeutic intervention in animals, we developed a mouse model.

Of two large panels of methylcholanthrene-(MCA) induced sarcomas generated in our laboratory, seven have been extensively characterized (28,29) (summarized in Table 3). An eighth tumor, MC 38, a murine colon adenocarcinoma, has also been characterized. Of these tumors, MC 38, MCA 105, 203, 205, and 207 were described as immunogenic since after simple immunization of tumor in *C. parvum*, they protected against subsequent challenge with the same tumor. MCA 102 had an intermediate phenotype; it induced a CTL response but did not protect against subsequent challenge. MCA 106 was found to express low levels of class I but, nevertheless, was found to be immunogenic. The tumors studied differed widely in their expression of class I molecules on the cell surface (29). At opposite ends of the distribution were MC 38 and MCA 101 or MCA 106, which had a 100-fold difference in steady-state levels of cell-surface class I. Similar results were obtained using either fresh tumor explants or cells that had been maintained in tissue culture. MHC class II molecules were not detected on the

surfaces of any of these tumors by FACS analysis. Thus, MCA 101 expressed very low levels of class I and neither induced CTLs nor protected against tumor challenge.

In studies using model viral systems, in which tumor cell lines were infected with influenza A/PR/8/34 (PR8) virus, MCA 101 was found to be nearly incapable of presenting endogenous antigens to antiviral CTLs, despite the fact that MCA 101 was extremely well infected. The antigen-processing capability of MCA 101 was found to be as poor as RMA-S, which was included as a negative control. Several of the other tumors tested also presented endogenously generated viral antigens poorly. Since $CD8^+$ CTLs have a proven role in the immune response against MCA-induced tumors, it was not surprising that many of the tumors tested had a lowered capacity to present endogenously generated viral antigens. In fact, one would expect that tumor cells with antigen-processing or -presentation defects would enjoy a selective advantage. It should be noted, however, that decreased class I expression can make tumor lines more susceptible to lysis by natural killer (NK) cells in some cases (30). Thus, some of the mechanisms used by tumors to escape recognition by CTLs may increase their susceptibility to other cells in the immune system.

To examine the capability of MCA 101 to present individual influenza virus proteins to CTLs, we infected cells with VV recombinants expressing individual influenza A virus proteins that are known to be recognized by $H\text{-}2^b$ restricted CTLs (31). This test showed that while nucleoprotein (VV-NP), nonstructural 1, hemagglutinin, and acidic polymerase were presented by MCA 207 (an immunogenic tumor), none of the proteins were presented by MCA 101 over control levels obtained with a VV recombinant expressing the nucleocapsid protein of vesicular stomatitis virus. These findings indicated that MCA 101 was incapable of presenting a wide variety of proteins to CTLs via the endogenous route.

Our findings show that the nonimmunogenic murine sarcoma MCA 101 presented endogenously, generated influenza virus antigens poorly to CTLs. It should be noted, however, that it remained unclear from these studies whether a complete lack of presentation occurred or whether the number of MHC-peptide epitopes was insufficient to be detected by our polyclonal responder populations. The latter explanation seems more likely. It is important to note that as with the SCLC cell lines, the presentation of endogenous antigens by MCA 101 was made possible by up-regulating class I expression with IFN-γ.

AN IMMUNOGENETHERAPY DESIGNED TO CORRECT DEFICIENT ANTIGEN PROCESSING

The nonimmunogenic murine MCA-induced sarcoma MCA 101 had a very poor capacity to present endogenously generated viral antigens. It grew rapidly and lethally in nonimmunosuppressed mice. Furthermore, of seven MCA-induced tumors generated and characterized extensively in our laboratory, only MCA 101 could neither act as an immunogen in vivo nor generate CTLs in vitro (Table 3).

Our goal in the next series of experiments (25) was to genetically modify MCA 101 to convert it into a good presenter of endogenous antigens. We thought that this modification could be accomplished by transducing the cells with the cDNA for murine IFN-γ. Thus, our aim with the gene-insertion studies was not to use IFN-γ for its direct effects on cells of immune lineage, but instead for its effects on the antigen-presentation capabilities of the tumor cells before and after gene modification. We then hoped to correlate the in vitro antigen-processing capabilities with in vivo behavior of these cells as immunogens.

Using this system, it was thus possible to answer the question of whether MCA 101 was nonimmunogenic because it lacked TAAs or because of its failure to present some postulated,

but as yet unidentified, TAA. Since the amelioration of the capacity of MCA 101 to present endogenous antigens after treatment in vitro with exogenous mIFN-γ was transient and correlated with the duration of class I expression, we hypothesized that retroviral transduction of MCA 101 with the cDNA for mIFN-γ would have the effect of prolonging the processing and presentation of endogenous antigen by virtue of the stable insertion of the gene.

IFN-γ-transduced MCA 101 expressed much higher levels of surface class I molecules, as measured by FACS analysis, then the neomycin resistance gene (Neo^R) bulk-transduced cells and wild-type MCA 101. This pattern of very low or unmeasurable IFN-γ production with greatly increased class I production was similar to that seen by Gansbacher and colleagues (32). Increased class I expression was seen in both fresh and cultured tumor lines.

In an effort to test the effect of IFN-γ gene transduction on the capability of MCA 101 to present endogenous antigens, we infected wild-type MCA 101, IFN-γ gene-modified MCA 101, and MCA 101 tumor gene modified with the neomycin resistance gene alone with VV genetically engineered to express influenza A genes (25). Effector cells were splenocytes from B6 mice stimulated in vivo with VV-NP and in vitro with the NP peptide. Wild-type MCA 101 was not killed when it was sham-infected, when it was infected with the control VV containing the neuraminidase gene, or when it was infected with the VV engineered to express the influenza A nucleoprotein gene (VV-NP). IFN-γ gene-modified tumor cells were also not killed when sham-infected or when infected with a control virus, but they were specifically killed when they were infected with the VV-NP virus. Like wild-type MCA 101, MCA 101 modified with the neomycin resistance gene alone did not present viral antigens, indicating that the improvement in the capability of IFN-γ gene-modified MCA 101 to present antigen was not simply due to the effects of retroviral transduction. It thus appeared that by inserting the mIFN-γ gene into a bulk population of wild-type MCA 101, we could convert it from a poor presenter of antigen to a cell line capable of presenting antigen to a similar extent as our most immunogenic tumors (25). This finding led us to hypothesize that MCA 101 might be capable of presenting its own tumor antigens in vivo.

EFFECTS OF IFN-γ GENE TRANSDUCTION ON THE IMMUNOGENICITY OF MCA 101

Generation of successful cultures of tumor-infiltrating lymphocytes (TILs) from wild-type MCA 101 had not been achieved in our laboratory. In order to obtain pure cultures of uniformly high class I–expressing transductants, the bulk-transfected MCA 101 (101.NAT) was cloned. We found that the insertion of the cDNA for IFN-γ caused increased expression of steady-state surface class I, as measured by FACS analysis, on some but not all of the tumor clones tested. Despite their variable levels of class I expression, none of the clones secreted >5 U of mIFN-γ/10^6 cells/ml/24 h by ELISA. When clones were implanted subcutaneously into syngeneic B6 mice, no significant differences were found between the growth rates of high class I–expressing and low class I–expressing tumors in vivo.

Antitumor T cells could be grown only from high class I–expressing transductants (25). This was done by harvesting subcutaneous tumors after 10–20 days, then immunobeading single-cell suspensions of tumor digests using immunomagnetic beads coated with Thy 1.2^+ antibodies. Like other murine TILs generated in our laboratory, the TIL cultures used in vivo were found to be exclusively $CD8^+$ by FACS. These antitumor CTLs were then expanded in vitro in 20 U/ml of rhIL-2 and tested in vivo and in vitro.

In an effort to test whether the TILs generated from high class I–expressing mIFN-γ-transduced clones could be active in an adoptive immunotherapy model against established wild-type tumor in vivo, we used the 3-day lung metastases model. In these experiments, mice were

injected intravenously with fresh wild-type MCA 101 or IFN-γ gene-modified MCA 101 tumor cells. On day 3, mice were treated with saline alone, interleukin-2 (IL-2) (10,000 U) in saline twice daily for 5 days, or the same dosage of IL-2 plus varying dosages of TILs. Mice were killed on day 14, when their lungs were harvested and counted in a blinded fashion for the number of pulmonary tumor nodules. Most significantly, TILs generated from the mIFN-γ gene-modified MCA 101 tumor clones expressing high levels of class I were effective against pulmonary metastases from the wild-type MCA 101 tumor.

TILs generated from IFN-γ gene-modified tumor were tested for release of cytokines after coincubation with appropriate tumor cells. TILs derived from high class I–expressing, IFN-γ gene-modified tumor clones were tested by exposure to a variety of stimuli. Maximal release was gauged when TILs were stimulated with an anti-CD3 antibody, 2C11. When TILs were incubated alone, without stimulation, background levels of tumor necrosis factor-α (TNF-α) and IFN-γ were relatively low for both cytokines. Fresh tumor preparations alone were found to secrete little, if any, IFN-γ. Production of TNF was variable by fresh tumor preparations but was uniformly <20 U/10^6 cells/ml/24 h. Values obtained from tumor alone were subtracted from values obtained from tumor plus TILs. The most important finding was that wild-type MCA 101 stimulated release of IFN-γ and TNF-α almost as well as IFN-γ gene-modified MCA 101. Thus, TILs generated from mIFN-γ, gene-modified tumor clones could be triggered to secrete, in a relatively tumor-specific fashion, IFN-γ, and TNF-α not only against high class I–expressing tumor clones but also against the low class I–expressing wild-type tumor.

The MCA 101 tumor was not consistently lysed by therapeutic TILs. However, it did stimulate the release of cytokines with relative specificity. One possible explanation of this effect is that the measurement of cytokine release is more sensitive than assays of cytotoxicity. This finding agreed with previous data, which showed that $CD8^+$ TILs that were therapeutic in vivo were not in every case cytotoxic (11).

Increased antigen density on mIFN-γ gene-modified MCA may explain why a therapeutically useful $CD8^+$ T-cell response can be obtained from high class I–expressing clones and not from low class I–expressing mIFN-γ-transduced clones, control Neo^R transduced clones, or wild-type tumor. There is evidence in tumor immunology studies that an immunologically strong afferent tumor stimulus can elicit a response against an immunologically much weaker efferent stimulus (33).

While IFN-γ-transduced tumor lines and clones secreted little, if any, IFN-γ into culture supernatant, the effect of the cytokine could act primarily via intracellular IFN-γ receptors. We have inferred that the observed effects of IFN-γtransduction are due to the enhancement of the transduction of certain genes involved in the processing and presentation of endogenous antigen (25). However, IFN-γ has many effects and may be enhancing the outgrowth of antitumor T cells through an entirely different mechanism. For example, IFN-γ has been shown to be a potent up-regulator of certain accessory molecules, ICAM-1 among them, and while it was not specifically addressed in the studies presented here, the effect of IFN-γ on these molecules may play an important role in the phenomena described here. Another possibility is that IFN-γ acts primarily through up-regulation of class II expression on macrophages and dendritic cells, thereby enhancing antigen presentation to T-helper (Th) cells. IFN-γ may alter the responsiveness of other immune cells. For example, the observed effects of tumor transduction with the gene for IFN-γ may be due partly or entirely to the enhancement of Th1 responses with a concomitant diminution of Th2 responses. This conversion has been shown to occur under the influence of IFN-γ in other systems (34). The relative contributions of these mechanisms are currently being addressed in our laboratory.

CURRENT WORK AND FUTURE DIRECTIONS

Genetic or pharmaceutical therapies directed at the enhancement of antigen processing may exploit the beneficial effects of IFN-γ. However, IFN-γ has been shown in some cases to be antiproliferative to T cells and NK cells, and other ways of enhancing antigen processing and presentation might prove to be more therapeutically useful. It seems likely that the genes involved in antigen processing and presentation share common regulatory elements. When these regulatory elements are elucidated, new therapies could be developed for specific up-regulation of antigen processing in cancer and infectious disease. Conversely, down-regulation of these gene products may prove to be useful in tissue transplantation or in autoimmune disease.

Other investigators have recently shown the antimetastatic effects of vaccination of tumor-bearing mice by immunization with IFN-γ gene-modified tumor cells (35). Furthermore, the use of tumor cells gene modified with the cDNA for IFN-γ has been extended, and partially confirmed, in our own laboratory by Shiloni and colleagues (unpublished observations). In these studies, one high and one low class I–expressing clone of the nonimmunogenic MCA-102 fibrosarcoma, designated 4JK and 24JK respectively were retrovirally transduced with the cDNA encoding for murine IFN-γ. Retro viral transduction of tumor cells with the cDNA encoding for IFN-γ resulted in a substantial up-regulation of class I surface expression in the 24JK clone but little change in the 4JK clone. In an attempt to generate antitumor lymphocytes, these gene-modified cells were inoculated into mouse footpads, and draining lymph nodes (DLNs) were removed, dispersed, and cultured in vitro for 10 days with irradiated tumor cells and IL-2. DLNs from mice bearing either unmodified tumor or tumor transduced with cDNA encoding for Neo^R or IFN-γ were used to treat recipients harboring 3-day pulmonary metastases induced by the parental unmodified tumor. Treatment with DLN cells obtained after the injection of 24JK tumor cells modified with the gene for IFN-γ significantly reduced the number of pulmonary metastases in four separate experiments, compared with groups treated by DLN cells generated from inoculation of either the unmodified, parental 24JK clone or the same clone transduced with the Neo^R gene only. In contrast, DLN cells induced by either IFN-γ-transduced 4JK (high MHC class I expressing) tumor or an unmodified 4JK tumor (moderate MHC class I expressing) had significant but equal therapeutic efficacy. Although the in vitro growth rate of tumor cell lines was unaffected by the insertion of the mouse IFN-γ cDNA, their in vivo (s.c.) growth rates were significantly slower than those of the nontransduced tumors. Thus, after retroviral transduction of the murine IFN-γ cDNA into a nonimmunogenic tumor with a very low level of surface expression of MHC class I, modified tumor cells could elicit therapeutic T cells from DLNs capable of successfully treating established pulmonary metastases upon adoptive transfer. This strategy confirms previous observations on the potential therapeutic effects of gene modification of tumor cells with IFN-γ. Significantly, these studies further define the circumstances in which IFN-γ immunogenetherapy might be useful.

In conclusion, new immunogenetherapeutic strategies may be based on a more detailed understanding of the molecular mechanisms used by tumors to escape immune recognition. A more complete knowledge of the cell biology of MHC class I in health and disease can lead to new strategies aimed at enhancing processing and presentation of tumor antigens. These approaches may lead to the generation of immune responses against tumor histologies not previously thought to be susceptible to T-cell based immunotherapies of cancer.

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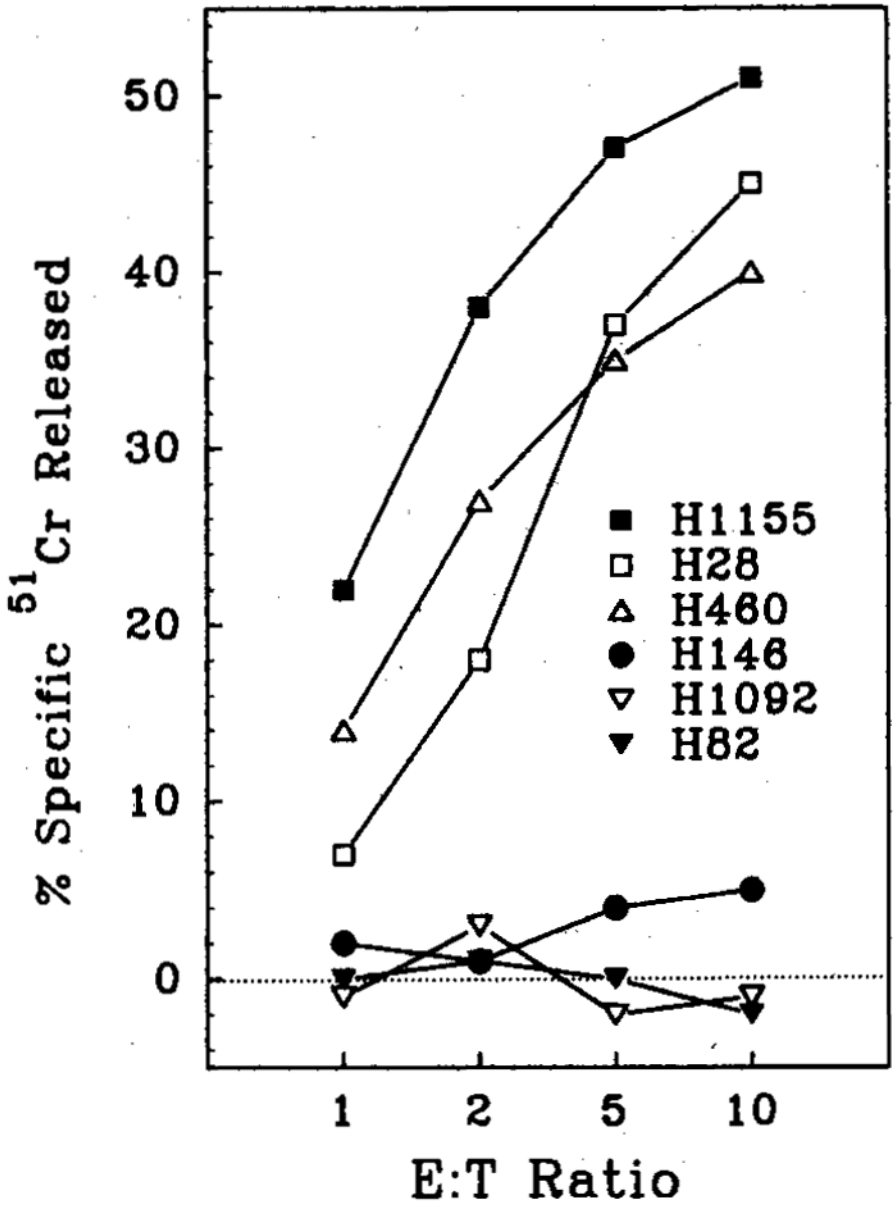

FIG. 1.

Screening of human tumor lines for antigen-processing defects. Cell lines studied were cultured human tumor cells tested for their capacity to present vaccina virus (VV) antigens to K^d-restricted, VV-specific T_{CD8}^+. The tumor cell lines were provided by J. Minna (University of Texas, Southwestern). H1155, H28, and H460 are all non-small-cell lung cancers. H146, H1092, and H82 are all small-cell lung cancers. In the representative experiment shown, all tumor cell lines processed and presented endogenous antigens poorly.

TABLE 1

Toward a molecular understanding of immune recognition of antigen

	Humoral	Cellular
Recognizing cell	B lymphocyte	T lymphocyte
Recognizing molecule	Immunoglobulin	T-cell receptor
"Self" molecules required?	No	Yes
Phase of antigen	Fluid or solid	Solid
State of antigen	Native or denatured	"Processed"

TABLE 2

Mechanisms of escape of human tumor cells from recognition by T_{CD8^+} relating to antigen processing and presentation

Gene lost, mutated, or Underexpressed	Result	Cell-surface class I (MAb W6/32)
TAP 1, 2 (LMPs, proteases?)	Poor processing of peptide antigens	Decreased
β_2-Microglobulin	α-Chain instability	Absent
α-Chain alleles	No presentation	Absent-normal

MAb, monoclonal antibody; LMP, low-molecular-weight proteins.

TABLE 3

Summary in vitro and in vivo characteristics of MCA-induced sarcomas

Tumor	Anti-tumor CTLs in vitro	Immunogen in vivo	Relative MHC class I
MC38	Yes	Yes	423 ± 12
MCA 207	Yes	Yes	249 ± 4
MCA 205	Yes	Yes	110 ± 3
MCA 203	Yes	Yes	64 ± 2
MCA 105	Yes	Yes	32 ± 9
MCA 102	Yes	No	28 ± 3
MCA 101	No	No	7 ± 1
MCA 106	Yes	Yes	4 ± 1