# Identification of Human Cancers Deficient in Antigen Processing

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## Summary

Intracellular antigens must be processed before presentation to $CD8^+$ T cells by major histocompatibility complex (MHC) class I molecules. Using a recombinant vaccinia virus (Vac) to transiently express the $K^d$ molecule, we studied the antigen processing efficiency of 26 different human tumor lines. Three cell lines, all human small cell lung carcinoma, consistently failed to process endogenously synthesized proteins for presentation to $K^d$-restricted, Vac-specific T cells. Pulse-chase experiments showed that MHC class I molecules were not transported by these cell lines from the endoplasmic reticulum to the cell surface. This finding suggested that peptides were not available for binding to nascent MHC molecules in the endoplasmic reticulum. Northern blot analysis of these cells revealed low to nondetectable levels of mRNAs for MHC-encoded proteasome components *LMP-7* and *LMP-2*, as well as the putative peptide transporters TAP-1 and TAP-2. Treatment of cells with interferon $\gamma$ enhanced expression of these mRNAs and reversed the observed functional and biochemical deficits. Our findings suggest that downregulation of antigen processing may be one of the strategies used by tumors to escape immune surveillance. Potential therapeutic applications of these findings include enhancing antigen processing at the level of the transcription of MHC-encoded proteasome and transporter genes.



It has long been known that elements of the cellular immune system are capable of specifically recognizing and destroying tumor cells (1, 2). In part, this reflects the activity of $CD8^+$ T cells ($T_{CD8+}$) (3–7), which recognize class I molecules of the MHC-bearing peptides of 8–10 residues derived from proteins located in the cytosol (8–11). There are now numerous examples of both mouse and human $T_{CD8+}$ that specifically recognize tumor cells and have therapeutic activity after adoptive transfer, in some cases inducing a complete remission (12–16).

Despite the potential for T cells to eradicate neoplasms, it is obvious from the progressive growth of most cancers that many tumors escape recognition by $T_{CD8+}$. The reasons for this are only partly understood. There is evidence that some tumor cells express low levels of class I molecules in vivo and in vitro (17). Poor class I expression by tumor cells in mice and humans have generally been attributed to low levels of class I $\alpha$ chain gene transcription (18, 19). Attempts have been made to enhance $\alpha$ chain transcription by transfection or transduction of class I $\alpha$ chains into tumors (20, 21), or by use of DNA-hypomethylating or -alkylating agents (22). These studies, directed at increasing the immunogenicity of the tumors involved, have met with mixed success. Studies by Weis and Seidman (23) reported that after the transfection of tumor cells with MHC class I genes there was no increase in cell surface expression of class I molecules despite a 20–60-fold increase in mRNA for the inserted class I genes. Based on recent discoveries, it seems possible that this was due to limiting amounts of antigenic peptides, which are required for proper assembly of class I $\alpha$ chains with $\beta_2$-microglobulin ($\beta_2$-m)[1] in the endoplasmic reticulum, before transport of the complex to the plasma membrane.

Antigenic peptides are believed to be generated from a cytosolic pool of proteins. Association of these peptides with class I $\alpha$ chains and $\beta_2$-m is thought to occur in an early secretory compartment (24–26). There is circumstantial evidence that two MHC gene products, called *LMP-7* and *LMP-2* (formerly known as RING 10 and RING 12, respectively; see reference 27), physically associated with a large nuclear and cytosolic proteolytic structure (termed the proteasome), somehow alter the function or location of the proteasome to favor either the production of antigenic peptides or their

[1] *Abbreviations used in this paper:* $\beta_2$-m, $\beta_2$-microglobulin; CM, culture media; HA, hemagglutinin; NP, nucleoprotein; SCLC, small cell lung carcinomas.

delivery to class I molecules (28–30). Two other gene products, now designated TAP-1 and TAP-2 (for transporter associated with antigen processing), are encoded in the region of the MHC, and are members of the ABC transporter family (31–36). 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. Whether these proteins directly transport peptide from the cytosol, or act in another manner, remains to be established.

To examine the capacity of human tumor cell lines to process cytosolic antigens for $T_{CD8+}$ recognition, we devised a method for screening a large number of tumor cell isolates that is independent of both the HLA type of the tumor and the presence, or absence, of specific cellular proteins. This method exploits the capacity of vaccinia virus (Vac) to infect a wide variety of human tumor cells (37). Using a recombinant Vac encoding the mouse H-2 $K^d$ class I molecule ($K^d$-Vac), we could test human tumor cell lines for presentation of viral antigens to mouse $K^d$-restricted, Vac-specific $T_{CD8+}$ populations and thus study antigen processing capabilities of human tumor cells per se.

## Materials and Methods

*Tumor Cells.* All cell lines used in these studies were generated by A. F. Gazdar, J. D. Minna, and their coworkers (University of Texas Southwestern, Dallas, TX), with the exception of SW480, LS174T, HT-29, WiDr, MDA-231, MCF7, BT-20, Hs578T, SK-BR-3, and MDA-468, which were obtained from American Type Culture Collection (Rockville, MD), and CY 6T, which was generated in our laboratory.

*Viruses.* The production of a Vac recombinant containing the $K^d$ gene ($K^d$-Vac) has been described (38). Note that this recombinant differs from a previously published Vac-$K^d$ (39) at position 114. The gene we used represents the corrected version of the $K^d$ gene in plasmid pH33 in which the wild-type GLN at position 114 is replaced by a HIS. This residue is located in the floor of the $K^d$ Ag binding site, and greatly influences the ability of $K^d$ to present viral antigens. The p*KCK*$^d$*wt* construct was provided by Jaulin Kourilsky (Institut Pasteur, Paris, France). The A/Puerto Rico/8/34 (PR8) influenza virus infectious stock was generated using the allantoic cavity of embryonated hen eggs and virus concentration using chicken red cell agglutination. The Vac recombinants containing the PR8 nucleoprotein (NP) and hemagglutinin (HA) genes have been described (40).

*Effector Cells.* Polyclonal $T_{CD8+}$ populations were generated from 6–8-wk-old female BALB/c mice by intravenous injection of $5 \times 10^6$ PFU of Vac or Vac-NP virus. After at least 2 wk, spleens were removed, dispersed to single cell suspensions with a homogenizer (Dounce), and stimulated in vitro with either Vac- or PR8-infected BALB/c splenocytes at a ratio of 2:1. Cells were then cultured in culture media (CM) consisting of Iscove's modified medium with 7.5% FCS (Biofluids, Rockville, MD) to generate $T_{CD8+}$. LAK cells were prepared as previously described (41).

*Cytotoxicity Assays.* Target cells were infected with 10 PFU/cell of vaccinia virus expressing the $K^d$ class I molecule for 60–90 min, incubated in CM at 37°C for 3 h, then labeled with $Na^{51}CrO_4$ for 1 h. Target cells were then mixed with $T_{CD8+}$. After 4 h of incubation the amount of released $^{51}Cr$ was determined by $\gamma$ counting. Percent specific lysis was calculated as follows: 100× [(experimental cpm − spontaneous cpm)/(maximal cpm − spontaneous cpm)].

*FACS® Analysis and Antibodies.* Cytofluorographic analysis was done using a FACScan 440® (Becton Dickinson & Co., Mountain View, CA). Cultured tumor cell lines were harvested with 0.02% EDTA, washed, then stained for 30 min with culture supernatant containing mAb supernate from H28-E23 (anti-HA antigen). Where designated, cells were stained with the mAb TW2.3, which recognizes an intracellular vaccinia-specific antigen (J. Cox et al., manuscript in preparation). To enable antibody access to the antigen, cells were fixed with paraformaldehyde and made permeable by including 0.1% saponin (wt/vol) during all manipulations. In all cases, cells with the appropriate isotype-matched control antibody were used. mAb binding to cells was followed by binding with goat anti-mouse FITC-conjugated antibody (Boehringer Mannheim Biochemicals, Indianapolis, IN).

*Metabolic Radiolabeling Experiments.* Pulse-chase experiments were performed as previously described (42). Briefly, $6 \times 10^6$ cultured tumor cells were incubated in methionine-free DMEM for 30 min at 37°C, then incubated with 100 $\mu$Ci [$^{35}$S]Met (Amersham Corp., Arlington Heights, IL) for 10 min. Ice-cold PBS was then immediately added to a portion of the cells. These cells represent the 0-min time point. Warmed medium containing 2 mg/ml of unlabeled Met was added to the remaining cells, which were then incubated at 37°C for the amount of time specified. Detergent extracts from radiolabeled cells were then normalized to contain equal amounts of acid-precipitable counts, and incubated with protein A–Sepharose previously loaded with the mAb specified. One half of each sample was digested overnight with 5 mU Endo H (Boehringer Mannheim Biochemicals). The other half of the sample was mock digested. Samples were then analyzed by SDS-PAGE using a 12% polyacrylamide gel and the buffer system of Laemmli. Gels were fixed, incubated with Amplify (Amersham Corp.), dried, and exposed to preflashed Kodak XAR-5 x-ray film (Kodak, Rochester, NY) for autoradiography.

*Northern Blot Analyses.* To generate specific probes for transporter and proteasome genes, total RNA was isolated by the guanidine isothiocyanate–cesium chloride centrifugation method. Specific probes were generated from RNA isolated from an EBV-transformed B cell line, or from the 501 melanoma cell line (both cell lines were established in our laboratory). First-strand cDNA was synthesized from 10 mg total RNA with an oligo(dT) primer. 30–35 cycles of PCR amplification was performed using the conditions: 94°C for 30 s, 60°C for 30 s, 72°C for 1 min followed by an extension cycle of 10 min at 72°C. The specific primers used had sequences as follows: GACAAGAGCCACAGGTATTTGG and TGATGAGAAGCACTGAGCGG for TAP-1 (formerly RING 4, Y3, or PSF-1), TACCTGCTCATAAGGAGGGTGC and ATTGGGATATGCAAAGGAGACG for TAP-2 (formerly RING 11, Y1, or PSF-2), TCGCCTTCAAGTTCCAGCATGG and CCAACCATCTTCCTTCATGTGG for *LMP-7* (formerly RING 10), and TTGTGATGGGTTCTGATTCCCG and CAGAGCAATAGCGTCTGTGG for *LMP-2* (formerly RING 12). PCR products were subjected to electrophoresis in 1.5% agarose gels, the correct sizes of bands were isolated, purified by glass powder methods (Geneclean; BIO 101, La Jolla, CA), and used as probes for Northern hybridizations. PCR product identity was confirmed by cutting with restriction enzymes and comparing predicted fragment sizes with those found in our preparations. The $\beta$-actin cDNA probe was purchased from Clonetech (Palo Alto, CA). The probes were labeled by the random priming method using random hexamers. For Northern blot, 10 $\mu$g of total RNA was subjected to electrophoresis in a 1% agarose formaldehyde gel and transferred to a nylon membrane (Zeta-Probe; Bio-Rad Laboratories, Richmond, CA). Hybridization was done in a 40% formamide hybridization solution (Northern hybridization buffer; 5 Prime → 3 Prime, Inc., West Chester, PA) at 42°C

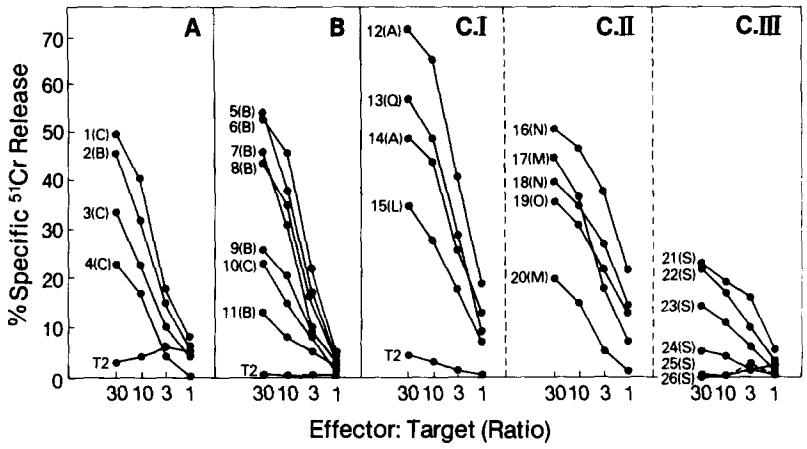


**Figure 1.** Screening of human tumor lines for antigen processing defects. Cell lines studied were cultured human tumor cells tested for their ability to present Vac antigens to $K^d$-restricted, Vac-specific $T_{CD8+}$. Numbers correspond to the following tumor lines and histologies: *C*, colon; *B*, breast; *A*, adenocarcinoma of the lung; *Q*, squamous cell carcinoma of the lung; *L*, large cell carcinoma of the lung; *N*, neuroendocrine tumor of the lung; *M*, mesothelioma; *O*, carcinoid of the lung; and *S*, SCLC of the lung. Tumor designations are as follows: *1*, SW480; *2*, LS174T; *3*, HT-29; *4*, WiDr, T2 (see reference 9); *5*, MDA-231; *6*, MCF7; *7*, BT-20; *8*, Hs578T; *9*, SK-BR-3; *10*, CY 6T; *11*, MDA-468; *12*, H23; *13*, H157; *14*, H358; *15*, H1334; *16*, H1155; *17*, H28; *18*, H460; *19*, H720; *20*, Hmeso1; *21*, H187; *22*, H510A; *23*, N417; *24*, H146; *25*, H1092; *26*, H82.

overnight. Membranes were then washed three to four times in 2× SSC at 60°C for 30 min, and autoradiography was then performed.

*Peptide Pulsing Experiments.* The peptide used (NP 147–155 from influenza A/Puerto Rico/8/34) was synthesized on a peptide synthesizer (Milligen/Biosearch, Burlington, MA) and HPLC purified with confirmation of sequence by fast atom bombardment (M-Scan Inc., West Chester, PA). $3 \times 10^6$ tumor cells in a volume of 1 ml of CM were pulsed with 1 $\mu$M of peptide during $^{51}Cr$ labeling for 90 min at 37°C. Cells were then washed three times and used in a $^{51}Cr$ release assay as described above.

## Results

*Screening the Antigen Processing Capabilities of Human Tumor Lines.* Fig. 1 depicts a composite of three experiments (*A*, *B*, and *C*) in which 26 different tumor cell lines were infected for 4 h with $K^d$-Vac, and tested for lysis by Vac-specific $T_{CD8+}$. In each experiment, the T2 cell line was included as a negative control. T2 cells lack a one-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 ability to process viral antigens for $T_{CD8+}$ recognition (43, 44). These experiments revealed that tumor cell lines vary widely in their abilities to process and present viral antigens to $T_{CD8+}$. While some cell lines from a variety of tumor histologies were lysed at high levels by Vac-specific CTLs, a number of the lines were lysed at low levels. These included tumor cell lines derived from several histologies, including colon (WiDr and CY6T), breast (MDA 468), lung mesothelioma (Hmeso1), and most consistently, lung cancers of the small cell histology. Notably, three of these small cell lung carcinomas (SCLC), H82, H146, and H1092, were consistently (six/six experiments) recognized at levels similar to, or even lower than, T2 cells (Fig. 1 *C.III*).

The failure of Vac-specific $T_{CD8+}$ to lyse the various tumor cells cannot be attributed to low levels of expression of Vac gene products. Cytofluorography after indirect immunofluorescence staining was used to assess whether tumor lines were infected with Vac and productive of viral protein. As $K^d$ on the cell surface was potentially dependent on the function of antigen processing machinery, the surface expression of a marker gene, the PR8 HA glycoprotein (Vac-HA), was studied. The HA molecule was transported by the infected cell to the cell surface where its expression was assessed using an HA-specific mAb. Representative results are depicted in Fig. 2. Tumor lines poorly recognized by $T_{CD8+}$ expressed similar or more viral antigens than those well recognized by $T_{CD8+}$, and no obvious correlation between viral gene expression and degree of $T_{CD8+}$ recognition was observed. Indeed, by this analysis, SCLC generally expressed high levels of viral antigens relative to the other tumor cell lines studied. Evidence that SCLC were well infected by Vac was corroborated by a second approach (not shown). In several $^{51}Cr$ release assays, a portion of target cells were assayed for binding

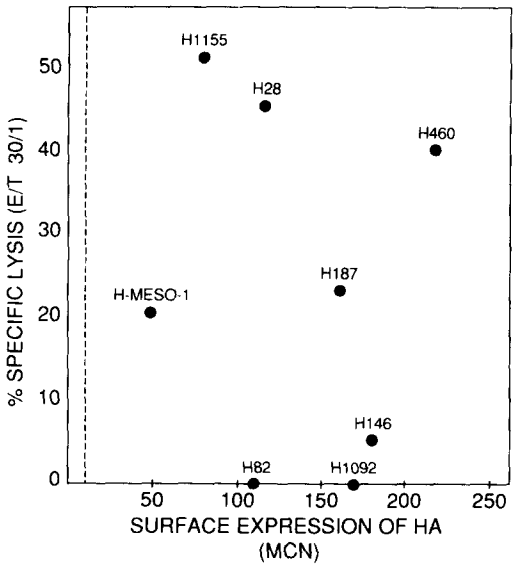


**Figure 2.** Representative experiment showing that expression of viral antigen does not correlate with lysability of lung cancer lines. In this experiment, cells were infected with 10 PFU/cell of the Vac-HA virus or with Vac-$K^d$ virus. Vac-HA-infected cells were stained for surface expression of the HA molecule and the mean channel numbers (MCN) by FACS® analysis are plotted on the *x*-axis. Isotype-matched control Ab staining was always below a MCN of 10 (*dotted line*). Plotted on the *y*-axis is the percent specific $^{51}Cr$ released (E/T 30:1) by cells infected by Vac-$K^d$. Note that the SCLC cell lines H82, H146, and H1092 were productive of the viral gene product HA, indicating that these cells are infectable by Vac, but that they were not lysed by anti-Vac $T_{CD8+}$ cells.

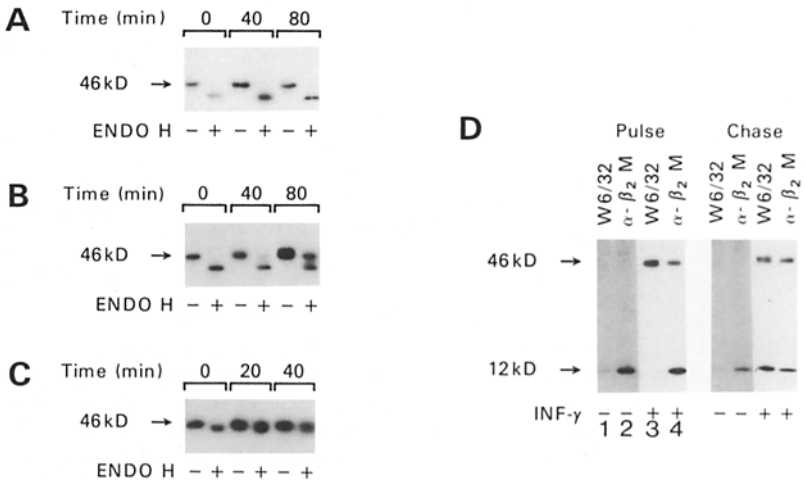


**Figure 3.** Assembly and transport of MHC class I molecules in the H82 SCLC cell line. (*A*) Experiment to study synthesis and transport of $K^d$ molecules. Detergent extracts from [$^{35}$S]Met-labeled, $K^d$-Vac-infected cells were immunoprecipitated with the HB-159 $K^d$-specific mAb, and left untreated, or digested with Endo H as indicated before analysis by SDS-PAGE. Cells were chased for 0, 40, or 80 min after pulse labeling to monitor class I exocytosis. (*B*) Experiment to examine synthesis and transport of $K^d$ molecules after SCLC line was pretreated with IFN-γ. Experiment was performed as in *A* but cells were pretreated with 1,000 U/ml of rhIFN-γ for 48 h before assay. (*C*) Experiment to study the behavior of native class I molecules. Experiment was as in *B*, except cells were uninfected and were immunoprecipitated with W6/32. (*D*) Experiment to test assembly of native class I molecules, with and without pretreatment with IFN-γ. Uninfected cells radiolabeled as in *A* and chased for 0 (*pulse*) or 120 min (*chase*) were immunoprecipitated with the W6/32 mAb, specific for α chains complexed with $\beta_2$-m or the $\beta_2$-m-specific mAb L368 (ATCC designation HB 149). Where designated, cells were pretreated with 1,000 U/ml of rhIFN-γ for 48 h before assay.

of mAb TW 2.3, which recognizes an intracellular vaccinia virus protein (Yewdell, J. W., unpublished observation). All SCLC lines were found to be well infected with Vac relative to controls. Additionally, all of the SCLC lines were efficiently lysed relative to other cell lines by LAK cells, as determined by $^{51}$Cr release assay (not shown). This demonstrates that the failure of virus-specific $T_{CD8+}$ to lyse SCLC is not due to a general resistance of the cells to lysis by immune cells, or their failure to release $^{51}$Cr after a lethal hit has been received. Thus, the failure of $T_{CD8+}$ to lyse Vac-infected SCLC appeared to be due to a genuine defect in antigen presentation, and not other factors.

*Assembly and Transport of Class I Molecules in SCLC Cell Lines.* To further characterize the antigen presentation deficit in SCLC, pulse-chase methodology was utilized to study the biosynthesis and intracellular transport of class I molecules. Class I molecules were immunoprecipitated from detergent extracts from cells pulse radiolabeled with [$^{35}$S]Met for 10 min, and chased for up to 80 min. In Fig. 3 *A*, detergent extracts from $K^d$-Vac-infected cells were immunoprecipitated with the anti-$K^d$-specific mAb SF1-1.1.1 (ATCC designation HB 159), which is directed against the α chain of the $K^d$ molecule. One half of each immunoprecipitate was then digested with endo-β-*N*-acetylglucoseaminidase H (Endo H), which cleaves N-linked oligosaccharides in the simple, high-mannose forms that exist before the modifications associated with transport of class I α chains through the later portions of the Golgi complex. Samples were then analyzed by SDS-PAGE for the characteristic increase in α chain mobility observed after removal of N-linked oligosaccharides. As seen in Fig. 3 *A*, $K^d$ molecules remained sensitive to digestion with Endo H throughout the 80-min chase period.

*Northern Blot Analysis of TAP and LMP.* 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 (45–47). It was therefore of interest to measure the expression of these genes in SCLC. Lacking suitable antibody reagents to directly measure the expression of these gene products, we performed Northern blots prepared from total mRNA that were then probed for the expression of the genes indicated. As seen in Fig. 4, mRNA for each of the four genes was detected in a cell line with normal antigen presentation capacity, but not in T2 cells, whose deletion encompasses all four genes. mRNA was also not detected in each of the SCLC cell lines deficient in antigen presentation. Low or absent steady-state levels of mRNA indicates either that transcription is downregulated or that there was a shortening of the half-life of these messages. Lack of detection of message by Northern blot analysis, however,

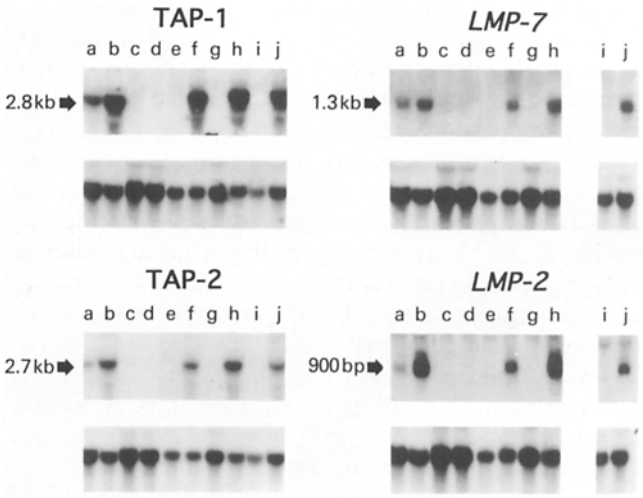


**Figure 4.** Northern blot analyses of TAP and LMP genes. Lanes correspond to various tumor cells as follows: (*a*) H1155 (positive control), (*b*) H1155 treated with 1,000 U IFN-γ for 48 h, (*c*) T2 (negative control), (*d*) T2 with IFN-γ, (*e*) H82, (*f*) H82 with IFN-γ, (*g*) H146, (*h*) H146 with IFN-γ, (*i*) H1092, and (*j*) H1092 with IFN-γ. The same blots stripped and reprobed with β-actin are shown below. Note that mRNA levels for TAP and proteasome components were not detected in SCLC lines unless these lines were pretreated with IFN-γ.

clearly does 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. Note also that IFN-γ increased mRNA levels in control cells (H1155 is shown), but not in T2 cells. These findings are consistent with prior reports that expression of these genes, like class I α chain genes, are IFN-γ inducible (34, 48).

*Effect of IFN-γ on Class I Assembly and Transport.* In addition to enhancing mRNA levels of *LMP* and TAP genes, IFN-γ treatment of SCLC cells greatly altered the intracellular trafficking of class I molecules. As seen in Fig. 3 *B*, IFN-γ induced the transport of a substantial portion of $K^d$ molecules from the early portion of the secretory pathway through the Golgi complex, since approximately half of the class I molecules immunoprecipitated became resistant to Endo H digestion within 80 min of their synthesis (Fig. 3, *C* and *D*). In the absence of IFN-γ, SCLC cells do not synthesize class I molecules reactive with the W6/32 mAb, which recognizes only α chains associated with $\beta_2$-m (Fig. 3 *D*). Consistent with this finding, a mAb specific for $\beta_2$-m precipitates only $\beta_2$-m without any complexed α chains. Thus, these cells either fail to assemble class I α chains, fail to produce α chains (18), or both. After treatment of cells with IFN-γ, a large amount of assembled molecules reactive with W6/32 or anti-$\beta_2$-m mAbs are detected (Fig. 3 *D*), and these molecules rapidly ($t_{1/2}$ < 20 min) acquire Endo H resistance (Fig. 3 *C*). The near absence of radiolabeled $\beta_2$-m at time 0 in class I α chain immunoprecipitated by W6/32 (Fig. 3 *D*, lane *3*) was consistently observed. This may be due to the binding of nascent, radiolabeled α chains to a relatively large pool of unlabeled $\beta_2$-m already present in the endoplasmic reticulum. Thus, the vast majority of the W6/32-reactive class I appears to be bound to nonradiolabeled $\beta_2$-m at time 0, although the pool of radiolabeled $\beta_2$-m is ultimately bound by W6/32-reactive α chains after 120 min (Fig. 3, *D chase*). The radiolabeled $\beta_2$-m in Fig. 3, lane *4*, coprecipitates radiolabled class I α chain, which is apparently not recognized by the W6/32 mAb, and thus may not yet be fully folded at time 0.

*IFN-γ Enhances Presentation of Endogenously Produced Antigen.* We next tested the effect of IFN-γ on the capacity of SCLC to present viral antigens to $K^d$-restricted, Vac-specific $T_{CD8+}$. As seen in Fig. 5, IFN-γ greatly enhanced the antigen-presenting capacity of the SCLC, but not T2, cells. In this experiment, the $K^d$ restriction of $T_{CD8+}$ recognition was demonstrated by the failure of $T_{CD8+}$ to lyse IFN-γ-treated cells infected with a Vac recombinant that does not express $K^d$. Thus, IFN-γ did not act by making the cells more susceptible to lysis by NK cells that might be present in the secondary splenic effector populations.

In addition to the interaction between TCR and MHC class I–peptide complexes, interactions between other molecules on T cell and target cell surfaces can contribute to delivery of the lytic signal (49). While such interactions might be expected to be minimized in the interaction of mouse $T_{CD8+}$ with human target cells, it remained possible that the effect of IFN-γ on SCLC antigen presentation reflected the enhanced expression of accessory adhesion molecules. We therefore examined the effect of IFN-γ on the presentation of an exogenously added synthetic peptide to $K^d$-restricted $T_{CD8+}$ specific for the peptide. As seen in a representative experiment using H82 cells, IFN-γ had no discernible effect on peptide presentation (Fig. 6, *left*), despite the fact that it was clearly able to enhance presentation of Vac antigens to Vac-specific $T_{CD8+}$ (Fig. 6, *right*).

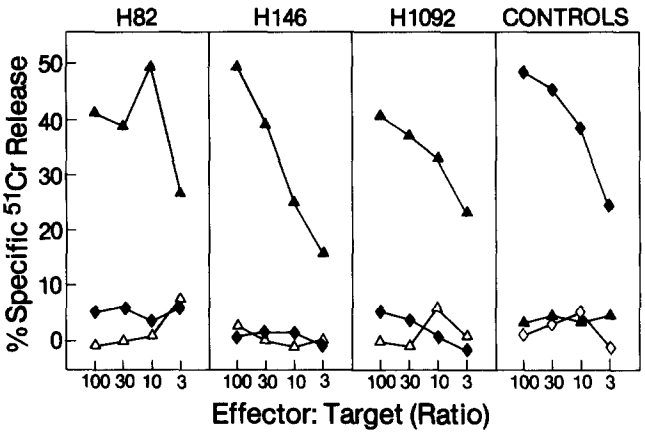


**Figure 5.** IFN-γ enables SCLC cells to present endogenous antigen. Vac-$K^d$-infected SCLC lines H82, H146, and H1092 (◆) were not lysed by $K^d$-restricted, anti-Vac $T_{CD8+}$, although a control cell line was lysed (represented by H1155 in this experiment [*controls*]). Treatment with 1,000 U IFN-γ for 48 h before infection enabled SCLC cells (▲), but not T2 cells (*control*) to present viral antigens. Cell lines pretreated with IFN-γ but infected with a control Vac not expressing $K^d$ (△) were not killed, controlling for nonspecific killing induced by IFN-γ pretreatment alone. (*Control*) H1155 without IFN-γ pretreatment after infection with wild-type Vac is shown (◇).

While the ability of non-IFN-γ-pretreated, Vac-$K^d$-infected cells to present exogenously provided peptide seems paradoxical since $K^d$ is inefficiently transported to the cell surface under these circumstances, a similar phenomenon is observed in other antigen processing–deficient cells. This observation has been attributed to surface expression of "empty"

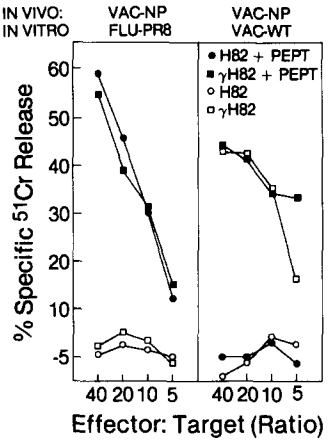


**Figure 6.** IFN-γ pretreatment does not enhance presentation of exogenously provided peptide. In this experiment, H82 cells (± exogenously pulsed peptide) were tested for lysis by $T_{CD8+}$ specific for influenza virus NP peptide (*left*, effector cells generated by in vivo priming with Vac-NP and in vitro secondary stimulation with FLU-PR8) or Vac antigens (*right*, effector cells generated by in vivo priming with Vac-NP and in vitro secondary stimulation with Vac-WT). (+ *pept*) Cells were pulsed with a synthetic peptide corresponding to NP residues 147–155 that represent the bona fide determinant recognized by $K^d$-restricted, NP-specific $T_{CD8+}$. (γ) Cells were pretreated with IFN-γ for 48 h before the assay. Similar results were obtained in a repeat assay. The same experiment was performed twice in experiments that included other SCLC cell lines (H146 and H1092) with similar results.

class I molecules (i.e., devoid of natural peptides) (50), the idea being that the transport of such molecules, while too low to detect by FACS® staining or immunoprecipitation, is sufficient to present the 200 or so peptides required for a $T_{CD8+}$ to lyse a target cell (51).

## Discussion

Class I molecules are poorly expressed on many different types of human tumor cells in vivo (17). Our findings indicate that this poor expression can be accompanied by a markedly decreased capacity to present endogenously synthesized proteins, and for SCLC at least, greatly diminished expression of four MHC genes that encode proteins thought to be necessary for efficient antigen processing. Although it is generally considered that most cell types are capable of constitutively processing and presenting antigens to $T_{CD8+}$, this has been directly established only with cells of immune lineage. The present studies do not address the question of whether poor antigen processing is associated with transformation or outgrowth of the tumor, or whether this poor processing is representative of the natural regulation of class I expression in the tissue of origin. This question may be particularly difficult to answer with SCLC since the identity of the precise cell of origin is not known. Whatever the case may be, the fact remains that SCLC cell lines processed antigen poorly in the studies reported here. The therapeutic implications of these findings are that poor class I expression and poor antigen processing capability must be meliorated if SCLC and other possible tumor histologies sharing these characteristics are to be made susceptible to $T_{CD8+}$-based immunotherapy. It is unlikely that the poor antigen processing capacity exhibited by SCLC cells is an artifact resulting from cell culture, since immunohistochemical studies show that SCLC expresses very low or undetectable levels of class I in vivo (52).

The great enhancement of antigen processing upon IFN-$\gamma$ treatment of SCLC suggests that $T_{CD8+}$ recognition of tumor cells could be enhanced by the specific upregulation of the antigen processing machinery in tumor cells. Such $T_{CD8+}$ might recognize tumor-specific antigens, or perhaps tissue-specific antigens in cases where tumors arise from tissues that are deficient in antigen processing. We recently described a mouse tumor (MCA 101) whose antigen processing capacity was similarly enhanced by IFN-$\gamma$ treatment (53). Expression of IFN-$\gamma$ in this tumor from a transfected gene resulted in the autocrine enhancement of antigen processing, and allowed the cells to induce a tumor-specific $T_{CD8+}$ response. Most importantly, $T_{CD8+}$ cells grown out of IFN-$\gamma$-transduced tumors were therapeutic against nonmodified tumor cells (54). Thus, it might be possible to obtain a beneficial $T_{CD8+}$ response by enhancing antigen processing machinery in a subset of tumor cells in vivo, or by injection of tumor cells that have been gene modified to enhance antigen processing in vitro. Such strategies might also be appropriate for infectious diseases in cases where the foreign organism infects cells with low antigen processing capability.

Genetic or pharmaceutical therapies directed at the enhancement of antigen processing may exploit the beneficial effects of IFN-$\gamma$. However, since IFN-$\gamma$ has been shown to be antiproliferative to T and NK cells, 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 upregulation of antigen processing in cancer and infectious disease. Conversely, downregulation of these gene products may prove useful in tissue transplantation or in autoimmune disease.

In addition to SCLC, we found in recent studies that some tumor cell lines of other histologies were unable to present viral antigens to $T_{CD8+}$. In a number of lines studied the defects have turned out to be nonfunctional $\beta_2$-m (Restifo, N. P., manuscript in preparation). The underlying deficits in other cell lines being studied remain to be established. The robust nature of T2 cells (and other antigen processing–deficient mutants) suggests that none of the antigen processing machinery is required for cell viability. In this event, it is to be expected that some tumor cells will possess mutations or deletions resulting in a functional deficiency in one or more of the dedicated components of antigen processing. Characterization of naturally occurring antigen processing mutants may help to identify novel gene products that function in the efficient processing of endogenously synthesized proteins.

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*Note added in proof:* Two recent reports indicate that the *LMP-2* and *LMP-7* gene products are not necessary for the presentation of some antigens (55, 56). The function of these gene products in antigen processing, therefore, remains to be established.

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