

Reduction in susceptibility to natural killer cell-mediated lysis of human FO-1 melanoma cells after induction of HLA class I antigen expression by transfection with B2m gene.

M Maio, ... , R A Zeff, S Ferrone

J Clin Invest. 1991;**88**(1):282-289. <https://doi.org/10.1172/JCI115289>.

Research Article

Induction of HLA class I antigens on cultured melanoma cells FO-1 after transfection with a human or a mouse B2m gene was associated with a statistically significant reduction in their susceptibility to natural killer (NK) cell-mediated lysis. These results indicate that the structural differences between human and mouse beta 2-mu do not abolish the ability of the HLA class I molecular complex to modulate NK cell-mediated lysis of melanoma cells FO-1. The role of HLA class I antigens in the phenomenon is corroborated by the ability of anti-HLA class I MAb to enhance, although to a different extent, the susceptibility of transfected FO-1 cells to NK cell-mediated lysis. Gamma interferon (IFN-gamma) and tumor necrosis factor-alpha (TNF-alpha) significantly reduced the susceptibility to NK cell-mediated lysis of transfected FO-1 cells. Surprisingly, TNF-alpha reduced the extent of lysis more than IFN-gamma, although the latter cytokine enhanced HLA class I antigen expression more than the former one. This finding, in conjunction with a reduction in the susceptibility to NK cell-mediated lysis of untransfected FO-1 cells incubated with IFN-gamma or TNF-alpha, suggests that the two cytokines reduce NK cell-mediated lysis of transfected cells by modulating not only the expression of HLA class I antigens, but also that of other structures. Induction of HLA class I antigens and their modulation with IFN-gamma did not affect the [...]

Find the latest version:

<https://jci.me/115289/pdf>



Reduction in Susceptibility to Natural Killer Cell-mediated Lysis of Human FO-1 Melanoma Cells after Induction of HLA Class I Antigen Expression by Transfection with B₂m Gene

Michele Maio,* Maresa Altomonte,* Revati Tatake,[§] Richard A. Zeff,[§] and Soldano Ferrone*

*Division of Experimental Oncology 2 and Immunology Section, Centro di Riferimento Oncologico, Aviano, Italy 33081; [‡]Department of Microbiology and Immunology, New York Medical College, Valhalla, New York 10595; and [§]Department of Pathology, University of Connecticut, Farmington, Connecticut 06032

Abstract

Induction of HLA class I antigens on cultured melanoma cells FO-1 after transfection with a human or a mouse B₂m gene was associated with a statistically significant reduction in their susceptibility to natural killer (NK) cell-mediated lysis. These results indicate that the structural differences between human and mouse $\beta_2\text{-}\mu$ do not abolish the ability of the HLA class I molecular complex to modulate NK cell-mediated lysis of melanoma cells FO-1. The role of HLA class I antigens in the phenomenon is corroborated by the ability of anti-HLA class I MAb to enhance, although to a different extent, the susceptibility of transfected FO-1 cells to NK cell-mediated lysis. Gamma interferon (IFN- γ) and tumor necrosis factor- α (TNF- α) significantly reduced the susceptibility to NK cell-mediated lysis of transfected FO-1 cells. Surprisingly, TNF- α reduced the extent of lysis more than IFN- γ , although the latter cytokine enhanced HLA class I antigen expression more than the former one. This finding, in conjunction with a reduction in the susceptibility to NK cell-mediated lysis of untransfected FO-1 cells incubated with IFN- γ or TNF- α , suggests that the two cytokines reduce NK cell-mediated lysis of transfected cells by modulating not only the expression of HLA class I antigens, but also that of other structures. Induction of HLA class I antigens and their modulation with IFN- γ did not affect the susceptibility to lymphokine-activated killer (LAK) cell-mediated lysis of transfected FO-1 cells. Characterization of the molecular mechanism(s) underlying abnormalities in HLA class I antigen expression by melanoma cells and of the role of these molecules in the interactions of melanoma cells with various types of effector cells may suggest novel immunotherapeutic approaches to melanoma. (*J. Clin. Invest.* 1991. 88:282-289.)
Key words: IFN γ • monoclonal antibody • LAK cells • TNF- α

Introduction

Malignant transformation of melanocytes may be associated with changes in HLA class I antigen expression (for review, see reference 1). These changes may have clinical relevance, be-

Address reprint requests to Soldano Ferrone, M.D., Department of Microbiology and Immunology, Basic Sciences Building, Valhalla, NY 10595.

Received for publication 23 October 1990 and in revised form 14 January 1991.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/91/107/0282/08 \$2.00

Volume 88, July 1991, 282-289

cause a statistically significant, although not absolute, association has been found between levels of HLA class I antigen expression in melanoma lesions and clinical course of the disease (2). The mechanism underlying this phenomenon is not known but is of great interest, because its characterization may contribute to our understanding of the role of HLA class I antigens in the biology of melanoma cells and in their interactions with host's immune system. This information may eventually suggest novel therapeutic approaches to melanoma.

Besides their role as restricting elements in the lysis of target cells by cytotoxic T cells (3), HLA class I antigens have been shown to regulate susceptibility to natural killer (NK)¹ cell-mediated lysis of tumor cells in some, although not all, the systems investigated (for review, see reference 4). Scanty and conflicting information is available about the role of HLA class I antigens in NK cell-mediated lysis of melanoma cells. To the best of our knowledge, only Lobo and Spencer (5) and Versteeg et al. (6) have investigated this phenomenon. Lobo and Spencer (5) have reported that cultured melanoma cells SK-MEL-1 are particularly resistant to NK cell-mediated lysis; at variance with results obtained with other cell lines, anti-HLA class I MAb did not increase the extent of NK cell-mediated lysis. Versteeg et al. (6) have shown that a spontaneous HLA class I negative variant isolated from the melanoma cell line 518A after long time culturing and the melanoma cells IGR30 with a reduced HLA class I antigen expression after transfection with the *c-myc* oncogene display an increased susceptibility to NK cell-mediated lysis. Although suggestive, these results do not prove a cause-effect relationship between reduction in HLA class I antigen expression and increased susceptibility to NK cell-mediated lysis of melanoma cells IGR30 and 518A, because prolonged in vitro culturing or transfection with *c-myc* oncogene may modify the expression of unrelated antigenic structures which modulate susceptibility to NK cell-mediated lysis. Furthermore, the results obtained with most of the tumor systems investigated might not be valid for melanoma cells, because the latter express molecules, such as HLA class II antigens and intercellular adhesion molecule-1 (ICAM-1) (7, 8), which may play a role in the interaction of NK cells with target cells (5; Maio, M., and S. Ferrone, unpublished results). Therefore, to eliminate the potential interference of unrelated structures in the analysis of the role of HLA class I

1. Abbreviations used in this paper: ADCC, antibody dependent cell mediated cytotoxicity; $\beta_2\text{-}\mu$, β_2 -microglobulin; ICAM-1, intercellular adhesion molecule-1; IFN- γ , gamma interferon; IIF, indirect immunofluorescence; LAK, lymphokine activated killer; NK, natural killer; PBMC, peripheral blood mononuclear cells; TNF- α , tumor necrosis factor- α .

antigens in NK cell-mediated lysis of melanoma cells, in the present study, we have characterized the effect of the induction of HLA class I antigens on the susceptibility to NK cell-mediated lysis of melanoma cells FO-1 which do not express HLA class I antigens because of a structural defect in B_2m gene (9, 10). Furthermore, because NK cell-mediated lysis of human tumor cells cannot be modulated by H-2 class I heavy chains associated with human β_2 -microglobulin ($\beta_2\text{-}\mu$) (11), we have compared the susceptibility to NK cell-mediated lysis of FO-1 cells transfected with a human and a mouse B_2m gene to determine the effect of differences in $\beta_2\text{-}\mu$ structure (12–14) on the lytic phenomenon.

Methods

Cells. The melanoma cell line Colo 38, the melanoma cell line FO-1 (9), and the cell line FO-1C transfected with a mouse B_2m gene (10) were grown in RPMI 1640 medium (Flow Laboratories Inc., McLean, VA) supplemented with 10% heat-inactivated FCS (Flow Laboratories Inc.) and 2 mM L-glutamine.

Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood of healthy volunteers by Ficoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) density gradient centrifugation (400 *g* for 30 min). After three washings with HBSS (Flow Laboratories Inc.), they were used either as a source of NK cells or for lymphokine activated killer (LAK) cell generation. The latter were generated by culturing PBMC in the continuous presence of interleukin-2 (IL-2) (1,000 U/ml) for at least 10 d; half of the medium was changed every 48 h with an equal volume of fresh medium containing IL-2. At the end of the culture more than 95% of cells were stained by anti-CD3 MAb OKT3 in indirect immunofluorescence (IIF). The functional activity of LAK cell preparations was monitored by testing their ability to lyse the NK cell-resistant cultured melanoma cells Colo 38.

Human B_2m gene. Human $\beta_2\text{-}\mu$ clone p β_2m13 in the vector pEMBL9 (14) was purified on a CsCl gradient cushion and linearized with the restriction enzyme Sal I before transfection.

MAB and conventional antisera. The MAb W6/32, TP25.99.1, and CR11-115 to distinct monomorphic determinants of HLA class I antigens, the MAb Q6/64 and H2-89-1 to determinants restricted to the gene products of the HLA-B locus, the anti- $\beta_2\text{-}\mu$ MAb NAMB-1, the anti-HLA-DR, DQ, DP MAb Q5/13 and the anti-ICAM-1 MAb CL203.4 were developed and characterized as described elsewhere (15–20). Anti-CD3 MAb OKT3 was purchased from Ortho Diagnostic Systems (Milan, Italy). MAB were purified from ascitic fluid by sequential precipitation with caprylic acid and with ammonium sulfate (21). F(ab')₂ fragments were prepared by incubating purified immunoglobulins (5 mg/ml) in 100 mM sodium citrate (pH 3.5) with 25 mg of pepsin (Sigma Chemical Co., St. Louis, MO) for 18 h at 37°C. Digestion was stopped by adding 1/10 volume of 3 M Tris buffer (pH 8.8). Samples were then extensively dialyzed against PBS, pH 7.2. The purity of MAB and F(ab')₂ fragment preparations was monitored by SDS-PAGE (22) under reducing and nonreducing conditions. The activity of purified MAB and F(ab')₂ fragment preparations was monitored by IIF staining of target cells.

The mixture of mouse IgG, MslgG, used to monitor nonspecific staining of melanoma cells in IIF was purchased from Coulter Immunology, Hialeah, FL. Rabbit anti-mouse IgG (H + L) antibodies and fluorescein isothiocyanate conjugated (FITC) F(ab')₂ fragments of goat anti-mouse IgG (H + L) antibodies were purchased from Jackson Immunoresearch Laboratories, Inc. (West Grove, PA).

Cytokines. Human recombinant gamma interferon (IFN- γ) and IL-2 were obtained from Hoffman-LaRoche Inc., Nutley, NJ; human recombinant TNF- α was obtained from Cetus Corp., Emeryville, CA.

Transfection of FO-1 melanoma cells with human B_2m gene. FO-1 melanoma cells were transfected by electroporation as described elsewhere (10). Briefly, cells (5×10^7), linearized human B_2m DNA (100

μ g) and Eco RI digested pSV2 neo-DNA (5 μ g) were mixed in cold sterile PBS (1 ml). Electroporation was performed in a Bethesda Research Laboratories (Gaithersburg, MD) Cell-Porator using an electric pulse of 1 kV at 330 μ F capacitance. Cells were then resuspended in DMEM supplemented with 10% FCS at 1×10^6 /ml, seeded in eight 100-mm tissue culture dishes and incubated at 37°C in a 5% CO₂ humidified atmosphere. Nonadherent cells were removed after overnight incubation. Cells were fed with DMEM supplemented with 10% FCS and with G418 sulfate (2 mg/ml) (Gibco Laboratories, Grand Island, NY). Cells from each plate were grown to confluence. To identify cells transfected with human B_2m gene which express HLA class I antigens, cells were sequentially incubated with the anti-human $\beta_2\text{-}\mu$ MAB NAMB-1 and with FITC conjugated antimouse Ig xenoantibodies and analyzed with a cytofluorograph. FO-1H cells, which were stained by MAB NAMB-1, were sterile sorted, subcloned, and expanded. The expression of HLA class I antigens was corroborated by staining with MAB TP25.99.1 in IIF.

Serological assays. IIF was performed by incubating cells (5×10^5) with an excess of MAB at 4°C for 30 min. After three washings with PBS supplemented with 0.5% BSA and 0.01% (wt/vol) NaN₃ (PBS-BSA-AZ) cells were incubated for an additional 30 min at 4°C with FITC-F(ab')₂ fragments of goat anti-mouse IgG (H + L) antibodies. After three washings with PBS-BSA-AZ, cells were resuspended in PBS-BSA-AZ and analyzed for surface fluorescence utilizing a FAC-Star flow cytometer (Becton Dickinson Co., Mountain View, CA) equipped with a 9153B computer (Hewlett-Packard Co., Palo Alto, CA). Fluorescence was collected by using a four-decade logarithmic amplifier. Mean fluorescence intensity was converted from a logarithmic to a linear scale using a calibration factor. Viable cells (1×10^4 , volume gated) were collected in a list mode fashion for data analysis. The latter was performed with a Consort C30 software (Becton-Dickinson Co.).

Radiolabeling of cells, indirect immunoprecipitation, and SDS-PAGE. These procedures were performed as described elsewhere (10). Briefly, cells (2×10^7) were labeled with ¹²⁵I (Amersham International, Amersham, UK) utilizing the lactoperoxidase method. They were then incubated for 30 min at 4°C in lysis buffer containing 1% NP40, 10 mM Tris-HCl (pH 8.2), 0.5 M NaCl, 1 mM EDTA, 1 mg/ml BSA, and 1 mM PMSF. The cell extract was incubated for 12 h with MAB bound to protein A-Sepharose (Pharmacia Fine Chemicals) precoated with rabbit anti-mouse IgG (H + L) antibodies. One-dimensional SDS-PAGE was performed on 10% polyacrylamide slab gels under reducing conditions using the buffer system described by Laemmli (22). Gels were processed for autoradiography using a Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY).

Cytotoxicity assays. All experiments were performed in triplicates in round bottom microtiter plates (Costar, Cambridge, MA). Target cells (2×10^6) were resuspended in 1 ml of RPMI 1640 medium supplemented with 20% FCS and incubated with 200 μ Ci of Na₂⁵¹CrO₄ (Amersham International) for 1 h at 37°C in a 5% CO₂ humidified atmosphere with occasional gentle shaking. After four washings with HBSS, target cells (1×10^4) were incubated for 4 h at 37°C in a final volume of 200 μ l of RPMI 1640 medium containing 10% FCS with different numbers of effector cells to obtain effector/target cell ratios of 100, 50, 25, 12.5:1 and of 20, 10, 5, 2.5:1 for NK and LAK cell-mediated lysis, respectively. Then plates were centrifuged and supernatant (100 μ l) was harvested from each well and counted in a gamma counter. Percent of cytotoxicity was calculated as (experimental ⁵¹Cr release – spontaneous ⁵¹Cr release / maximum ⁵¹Cr release – spontaneous ⁵¹Cr release) \times 100. Spontaneous release represents the radioactivity released by 1×10^4 ⁵¹Cr labeled target cells during a 4-h incubation; maximum release is the radioactivity released by 1×10^4 target cells lysed with NP40. Only experiments with a spontaneous release lower than 15% were used for the statistical analysis.

Statistical analysis. Data were analyzed with the Student's paired *t* test utilizing the Statworks statistical package from Cricket Software, Inc. (Philadelphia, PA). Differences with *P* values of at least 0.05 were considered statistically significant.

Results

Expression of HLA class I antigens on FO-1 melanoma cells transfected with a mouse or a human B₂m gene. The untransfected melanoma cells FO-1, the melanoma cells FO-1C transfected with a mouse B₂m gene and the melanoma cells FO-1H transfected with a human B₂m gene were sequentially incubated with the anti-HLA class I MAb TP25.99.1 and with FITC-anti-mouse IgG (H + L) xenoantibodies and analyzed with a cytofluorograph. No specific staining of FO-1 cells was detected, because the mean fluorescence intensity value of 42 was similar to that obtained with FO-1 cells incubated with the negative control MsIgG. FO-1C and FO-1H melanoma cells were both stained by anti-HLA class I MAb TP25.99.1, with mean fluorescence intensity values of 95 and 156, respectively (Fig. 1 A). The mean fluorescence intensity values of FO-1C and FO-1H cells stained with the negative control MsIgG were similar to that obtained with FO-1 melanoma cells. The three melanoma cell lines were brightly stained by anti-HLA class II MAb Q5/13 and by anti-ICAM-1 MAb CL203.4 with mean fluorescence intensity values for the three cell lines of ~140 and 145, respectively (data not shown).

The expression of HLA class I antigens on FO-1C and FO-1H cells was corroborated by the results of SDS-PAGE analysis of antigens immunoprecipitated by the anti-HLA class I MAb TP25.99.1 from ¹²⁵I-labeled FO-1C and FO-1H cells. Two components with the characteristic electrophoretic mobility of the two subunits of HLA class I antigens were found in the immunoprecipitates; the intensity of the components in the immunoprecipitate from FO-1C cells was lower than that of those in the immunoprecipitate from FO-1H cells. Representative results are shown in Fig. 2 which also presents for comparison purposes the SDS-PAGE analysis of antigens immunoprecipitated by anti-ICAM-1 MAb CL203.4 from ¹²⁵I-labeled FO-1,

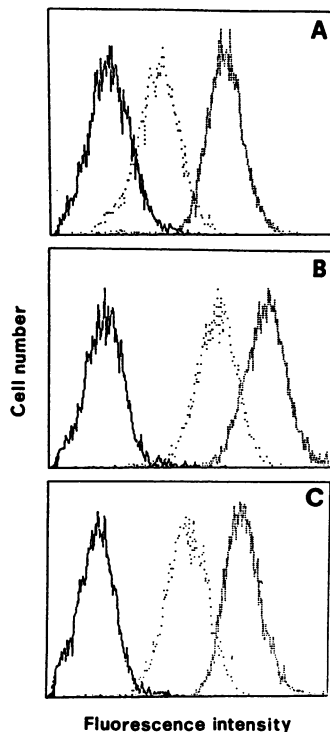


Figure 1. Expression and modulation by cytokines of HLA class I antigens on FO-1, FO-1C, and FO-1H melanoma cells. FO-1 (—), FO-1C (· · ·), and FO-1H (· · · · ·) cells were incubated for 42 h at 37°C with IFN- γ (1,000 U/ml) (B) or with TNF- α (1,000 U/ml) (C). Cells incubated under the same experimental conditions, but not exposed to cytokines were used as controls (A). At the end of the incubation, cells were washed and sequentially incubated with anti-HLA class I MAb TP25.99.1 and with FITC conjugated F(ab')₂ fragments of goat anti-mouse IgG xenoantibodies. Then cells (1×10^4 volume gated) were analyzed by flow cytometry.

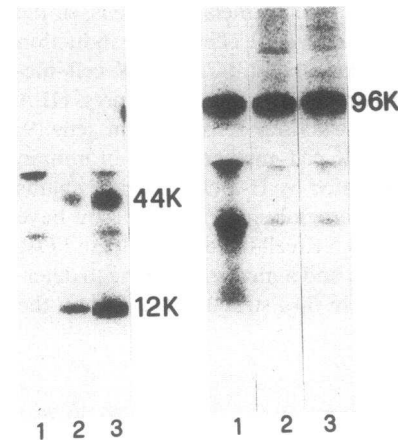


Figure 2. SDS-PAGE analysis of antigens immunoprecipitated by anti-HLA class I MAb TP25.99.1 from FO-1, FO-1C, and FO-1H melanoma cells. FO-1 (lane 1), FO-1C (lane 2), and FO-1H (lane 3) cells were labeled with ¹²⁵I and solubilized with NP40. After indirect immunoprecipitation with MAb TP25.99.1, antigens were eluted from the immunoadsorbent and analyzed by SDS-PAGE in a 10% slab gel in the presence of 2% β -mercaptoethanol (left). Gels were then processed for autoradiography. Antigens immunoprecipitated by the anti-ICAM-1 MAb CL203.4 from the three melanoma cell lines were used as controls (right).

FO-1C, and FO-1H cells. The intensity of ICAM-1 was similar in the immunoprecipitates from the three melanoma cell lines.

Modulation by IFN- γ and TNF- α of HLA class I antigen expression by melanoma cells FO-1, FO-1C, and FO-1H. Incubation for 42 h with IFN- γ (1,000 U/ml) (Fig. 1 B) and TNF- α (1,000 U/ml) (Fig. 1 C) strongly enhanced the reactivity of FO-1C and FO-1H melanoma cells with anti-HLA class I MAb TP25.99.1 in IIF. The coefficients of variance (t) obtained with three experiments were 70.2 ($P = 0.0002$) and 20.3 ($P = 0.002$) for FO-1C cells treated with IFN- γ and TNF- α , respectively, and 62.3 ($P = 0.0002$) and 48.6 ($P = 0.0004$) for FO-1H cells treated with IFN- γ and TNF- α , respectively. The increase in HLA class I antigen expression on FO-1C and FO-1H cells induced by IFN- γ was more marked than that induced by TNF- α . The coefficients of variance (t) obtained with three experiments were 11.2 ($P = 0.007$) and 20.4 ($P = 0.002$) for FO-1C and FO-1H cells, respectively. The level of HLA class I antigens on FO-1C cells treated with cytokines was significantly lower than that on FO-1H cells treated with cytokines. The coefficients of variance (t) obtained with three experiments were 87.1 ($P = 0.0001$) and 14.3 ($P = 0.005$) for IFN- γ and TNF- α treated cells, respectively. Neither IFN- γ nor TNF- α induced HLA class I antigen expression on FO-1 cells. The coefficients of variance (t) obtained with three experiments were 3.1 ($P = 0.09$) and 2.5 ($P = 0.12$) for FO-1 cells treated with IFN- γ and TNF- α , respectively. A 40-channel increase in the mean fluorescence intensity values was observed when the three melanoma cell lines were stained with the anti-HLA class II MAb Q5/13, after incubation with IFN- γ (data not shown). On the other hand, TNF- α did not upregulate the expression of HLA class II antigens. Incubation with IFN- γ and TNF- α induced a 40-channel increase in the mean fluorescence intensity values on FO-1, FO-1C, and FO-1H cells stained with the anti-ICAM-1 MAb CL203.4 (data not shown).

Susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to NK cell-mediated lysis. FO-1 cells were susceptible to NK cell-mediated lysis. A sixfold variation was observed in the extent of lysis obtained with PBMC isolated from 17 donors, because the ⁵¹Cr release ranged between a minimum of 11% and a maximum of 69% at the effector/target cell ratio of 100:1.

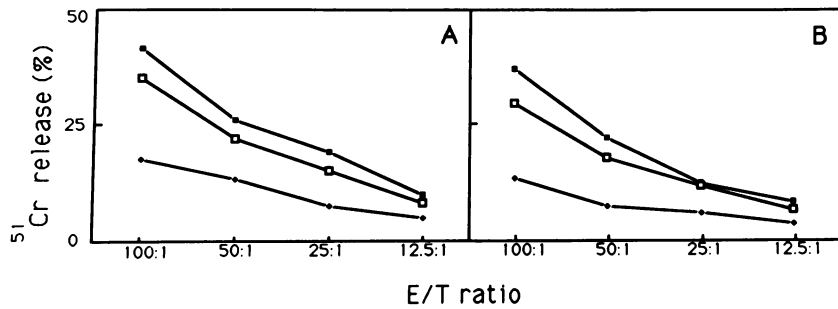


Figure 3. Susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to NK cell-mediated lysis. FO-1 (■), FO-1C (□), and FO-1H (◆) cells were labeled with ⁵¹Cr and mixed with PBMC from donor MM (left) and from donor SF (right). At the end of a 4 h incubation at 37°C supernatant was harvested and released radioactivity was measured in a γ -counter. Data are expressed as percentage of specific ⁵¹Cr release.

The mean \pm SD was 37.8 \pm 13.7. Induction of HLA class I antigen expression on FO-1 cells by transfection with a mouse or a human B₂m gene significantly reduced their susceptibility to NK cell-mediated lysis. The mean \pm SD of ⁵¹Cr release from FO-1C and FO-1H cells tested with PBMC from 17 donors at the effector/target cell ratio of 100:1 were 30 \pm 15 with ranges of 4 and 53 and 20 \pm 11 with ranges of 4 and 51, respectively. Both values are significantly lower than those obtained with FO-1 cells with *P* values of 0.00001 and 0.00000004, respectively. In addition, the ⁵¹Cr release from FO-1C cells was significantly higher (*P* = 0.0006) than that from FO-1H cells.

FO-1, FO-1C, and FO-1H cells were also compared in their susceptibility to lysis by PBMC from six of the 17 donors utilizing various effector/target cell ratios, to determine the effect of this variable on the role of HLA class I antigens in NK cell-mediated lysis of FO-1 cells. Results obtained with PBMC from two donors are shown in Fig. 3. As shown in Table I, the level of NK cell-mediated lysis of FO-1 cells was significantly higher than that of FO-1H cells at all the effector/target cell ratios tested and significantly higher than that of FO-1C cells only at the effector/target cell ratios of 100:1 and 50:1. Furthermore, the extent of NK cell-mediated lysis of FO-1H cells was significantly lower than that of FO-1C cells at all the effector/target cell ratios tested, but that of 12.5:1.

Table I. Effect of HLA Class I Antigen Induction on the Susceptibility of Cultured Human Melanoma Cells FO-1 to NK Cell-Mediated Lysis

Cells	Effector/target cell ratio			
	100:1	50:1	25:1	12.5:1
FO-1	39 \pm 7*	25 \pm 9	15 \pm 7	10 \pm 5
FO-1C	31 \pm 3	20 \pm 9	13 \pm 7	7 \pm 5
<i>P</i> <	0.005 [‡]	0.006	NS [§]	NS
FO-1H	21 \pm 7	14 \pm 7	9 \pm 5	6 \pm 3
<i>P</i> <	0.001	0.001	0.004	0.03
FO-1C	31 \pm 8	20 \pm 9	13 \pm 7	7 \pm 5
FO-1H	21 \pm 7	14 \pm 7	9 \pm 5	6 \pm 3
<i>P</i> <	0.007	0.009	0.02	NS

FO-1, FO-1C, and FO-1H cells were labeled with ⁵¹Cr and incubated at 37°C for 4 h with PBMC. The supernatant was then harvested and released radioactivity was measured in a γ -counter. Data are expressed as percentage of specific ⁵¹Cr release. * Mean \pm SD of percentage of specific ⁵¹Cr release obtained in at least six experiments. [‡] Data were analyzed utilizing a paired *t* test. [§] *P* > 0.05.

Effect of anti-HLA class I MAb on NK cell-mediated lysis of FO-1, FO-1C, and FO-1H melanoma cells. To corroborate the role of HLA class I antigens in the reduction of NK cell-mediated lysis of FO-1C and FO-1H cells and to investigate the role of distinct determinants of HLA class I antigens in this phenomenon, the effect of MAb to five distinct determinants of HLA class I antigens on the NK cell-mediated lysis of FO-1C and FO-1H cells was analyzed. Incubation for 30 min of melanoma cells with MAb TP25.99.1 and W6/32 to monomorphic determinants of HLA-A, B, and C antigens and with anti-HLA-B antigen MAb H2-89-1 and Q6/64 markedly enhanced the NK cell-mediated lysis of FO-1C and FO-1H cells. On the other hand, the MAb CR11-115 to a monomorphic determinant of HLA-A, B, and C antigens had no detectable effect on the extent of lysis. Furthermore, the five anti-HLA class I MAb did not affect the extent of NK cell-mediated lysis of FO-1 cells. Results of a representative experiment are shown in Table II. Each MAb has been tested at least twice with reproducible results. Furthermore, similar results were obtained

Table II. Differential Effect of MAb to Distinct Determinants of HLA Class I Antigens on the Susceptibility of FO-1C and FO-1H Melanoma Cells to NK Cell-Mediated Lysis

MAb	FO-1		FO-1C		FO-1H	
	⁵¹ Cr release	%	⁵¹ Cr release	%	⁵¹ Cr release	%
None	36.5	—	24.0	—	15.8	—
H2-89-1 (IgG)	40.8*	11 [‡]	38.7	61	38.6	144
F(ab) ₂	37.1	2	36.3	51	39.4	149
Q6/64 (IgG)	39.5	8	33.2	38	31.7	100
CR11-115 (IgG)	38.5	5	26.1	8	16.1	2
TP25.99.1 (IgG)	38.5	5	33.8	40	25.1	63
F(ab) ₂	39.0	7	31.6	32	23.8	51
W6/32 (IgG)	34.4	—	27.6	15	26.7	68

FO-1, FO-1C, FO-1H cells were incubated for 30 min at room temperature with MAb H2-89-1 and Q6/64 to determinants restricted to HLA-B antigens and with MAb CR11-115, TP25.99.1, and W6/32 to monomorphic determinants of HLA class I antigens (final concentration, 20 μ g/ml). Cells incubated under the same experimental conditions with mouse immunoglobulins were used as controls. At the end of the incubation, cells were washed, labeled with ⁵¹Cr, and incubated at 37°C for 4 h with PBMC from donor MM at the effector/target cell ratio of 100:1. The supernatant was then harvested and released radioactivity was measured in a γ -counter. Data are expressed as percentage of specific ⁵¹Cr release. * Percentage of specific ⁵¹Cr release. [‡] Percentage increase of specific ⁵¹Cr release as compared with ⁵¹Cr release in the presence of mouse immunoglobulins.

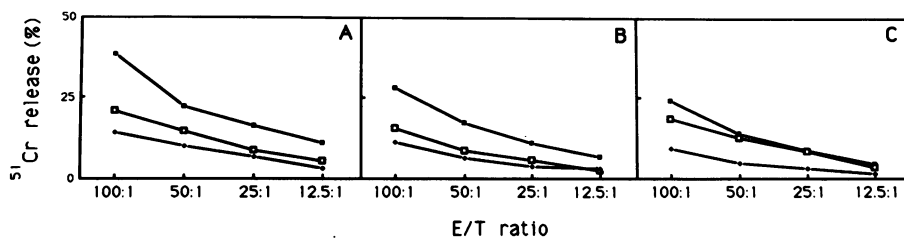


Figure 4. Effect of IFN- γ and TNF- α on the susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to NK cell-mediated lysis. FO-1 (\blacksquare), FO-1C (\square), and FO-1H (\blacklozenge) cells were incubated for 42 h at 37°C with IFN- γ (1,000 U/ml) (B) or with TNF- α (1,000 U/ml) (C). Cells incubated under the same experimental conditions, but not exposed to cytokines were used as controls (A). At the end of the incubation, cells were

washed, labeled with ^{51}Cr , and incubated at 37°C for 4 h with PBMC from donor MA. The supernatant was then harvested and released radioactivity was measured in a γ -counter. Data are expressed as percentage of specific ^{51}Cr release.

when MAb were added to the mixture of melanoma cells and PBMC. The anti-HLA class I MAb did not enhance NK cell-mediated lysis of FO-1C and FO-1H cells by mediating an antibody-dependent cell mediated cytotoxicity (ADCC) phenomenon, because their F(ab')₂ fragments were as effective as the whole IgG (Table II). No marked difference was found between FO-1C and FO-1H cells incubated with anti-HLA class I MAb in terms of extent of NK cell-mediated lysis. On the other hand, the percentage of increase of NK cell-mediated lysis of FO-1H cells induced by MAb H2-89-1, Q6/64, TP25.99.1, and W6/32 was greater than that of FO-1C cells. The anti-HLA class I MAb differed in their ability to enhance the NK cell-mediated lysis of FO-1C and FO-1H cells. The effect of the anti-HLA class I MAb tested did not correlate with the level of expression of the corresponding determinants: the MAb H2-89-1 which recognizes a determinant restricted to HLA-B antigens displayed a higher enhancing effect than MAb TP25.99.1 and W6/32 which recognize determinants expressed on the gene products of HLA-A, B, and C loci (Table II).

Effect of IFN- γ and TNF- α treatment on the susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to NK cell-mediated lysis. To determine whether cytokines modulate NK cell-mediated lysis of melanoma cells and whether the effect requires HLA class I antigen expression by target cells, FO-1, FO-1C, and FO-1H cells were first incubated with IFN- γ (1,000 U/ml) or TNF- α (1,000 U/ml) for 42 h and then tested for susceptibility to NK cell-mediated lysis. Results of an experiment with PBMC from one donor are shown in Fig. 4. A summary of the results obtained with PBMC from six donors is presented in Fig. 5. IFN- γ -treated FO-1C and FO-1H cells displayed a significantly lower susceptibility to NK cell-mediated lysis than their untreated counterparts, with *P* values of 0.01 and 0.04, respectively. IFN- γ -treated FO-1 cells were also less susceptible to NK cell-mediated lysis than their untreated counterparts; the difference, however, did not reach the level of statistical significance. Furthermore, a comparison of the susceptibility to NK cell-mediated lysis of IFN- γ -treated untransfected and B₂m-transfected FO-1 cells showed that at the effector/target cell ratio of 50:1 the extent of lysis of FO-1H cells was significantly lower than that of untransfected FO-1 cells (*P* = 0.001), but not significantly different from that of FO-1C cells. The latter displayed a significantly (*P* = 0.01) lower susceptibility to NK cell-mediated lysis than untransfected FO-1 cells. At the effector/target cell ratio of 25:1, only the difference between the NK cell-mediated lysis of FO-1H and FO-1 cells remained statistically significant with a *P* value of 0.01.

Incubation with TNF- α (1,000 U/ml) for 42 h induced changes in the susceptibility to NK cell-mediated lysis of FO-1C, FO-1H, and untransfected FO-1 cells similar to those in-

duced by IFN- γ . The reduction in the susceptibility to NK cell-mediated lysis of FO-1 and FO-1H cells induced by TNF- α was more marked than that induced by IFN- γ . However, the difference between TNF- α -treated FO-1 and FO-1H cells and their IFN- γ -treated counterparts did not reach the level of statistical significance.

Lack of effect of HLA class I antigen induction on the susceptibility of FO-1 melanoma cells to LAK cell-mediated lysis. FO-1 cells were highly susceptible to lysis by LAK cells. Induction of HLA class I antigen expression after transfection of FO-1 cells with a human or a mouse B₂m gene reduced their susceptibility to LAK cell mediated lysis. Results of an experiment with LAK cells from one donor are shown in Fig. 6 A. The difference, however, in the extent of lysis of FO-1, FO-1C, and FO-1H cells by LAK cells generated from five donors did not reach the level of statistical significance. Furthermore, incubation of FO-1, FO-1C, and FO-1H cells with IFN- γ (1,000 U/ml) for 42 h had only a slight effect on the susceptibility of the three cell lines to LAK cell-mediated lysis. Results of an experiment with LAK cells from one donor are shown in Fig. 6 B.

Discussion

Transfection with a human or a mouse B₂m gene induced HLA class I antigen expression on cultured human melanoma cells FO-1 which lack these antigens (9) because of a B₂m gene de-

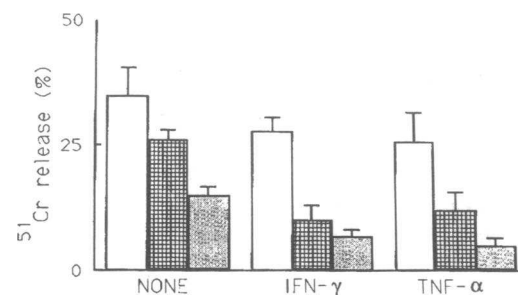


Figure 5. Effect of IFN- γ and TNF- α on the susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to NK cell-mediated lysis. FO-1 (\square), FO-1C (\blacksquare), and FO-1H (\blacksquare) cells were incubated for 42 h at 37°C with IFN- γ (1,000 U/ml) or with TNF- α (1,000 U/ml). Cells incubated under the same experimental conditions, but not exposed to cytokines were used as controls. At the end of the incubation, cells were washed, labeled with ^{51}Cr , and incubated at 37°C for 4 h with PBMC from six donors of the effector/target cell ratio of 50:1. The supernatant was then harvested and released radioactivity was measured in a γ -counter. Data are expressed as the mean \pm SD of the percentage of specific ^{51}Cr release obtained in six experiments.

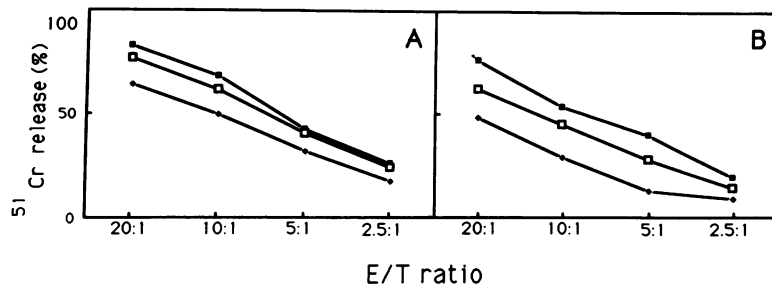


Figure 6. Effect of IFN- γ on the susceptibility of FO-1, FO-1C, and FO-1H melanoma cells to LAK cell-mediated lysis. FO-1 (\blacksquare), FO-1C (\square), and FO-1H (\blacklozenge) cells were incubated for 42 h at 37°C with IFN- γ (1,000 U/ml) (B). Cells incubated under the same experimental conditions, but not exposed to IFN- γ were used as controls (A). At the end of the incubation, cells were washed, labeled with ^{51}Cr , and incubated at 37°C for 4 h with LAK cells from donor CY. Then the supernatant was harvested and released radioactivity was measured in a γ -counter. Data are expressed as percentage of specific ^{51}Cr release.

fect (10). The level of HLA class I antigens on FO-1 cells transfected with a mouse B_2m gene is lower than that on cells transfected with a human B_2m gene. Although the number of copies of human and mouse B_2m gene transfected into FO-1 cells is not known, the difference in HLA class I antigen expression is not likely to result from a limiting amount of $\beta_2\text{-}\mu$ mRNA, because the intensity of the component hybridizing with $\beta_2\text{-}\mu$ probe in Northern blot experiments is similar in cells transfected with the human and mouse B_2m gene (Tatake, R., S. Ferrone, and R. A. Zeff, unpublished results). On the other hand, the different HLA class I antigen expression may reflect the higher efficiency in the association of HLA class I heavy chains with human than with mouse $\beta_2\text{-}\mu$ (23, 24). Assembly of the two subunits and transport and insertion into the cell membrane of the HLA class I molecular complex are enhanced by IFN- γ and to a lower extent by TNF- α . A similar observation has been made in two mouse tumor cell lines (25).

The susceptibility of FO-1 cells to NK cell-mediated lysis was significantly reduced after induction of HLA class I antigen expression by transfection with a human or a mouse B_2m gene. The significant reduction in the susceptibility to NK cell-mediated lysis of FO-1 cells transfected with a mouse B_2m gene is noteworthy, because it shows that the structural differences between human and mouse $\beta_2\text{-}\mu$ (12–14) do not abolish the ability of the HLA class I molecular complex to reduce the susceptibility to NK cell-mediated lysis of FO-1 melanoma cells. On the other hand, replacement of HLA class I heavy chain with the mouse counterpart is associated with the loss of the ability to modulate NK cell-mediated lysis of a cultured lymphoid cell line (11). These results altogether strongly suggest that the heavy chain of the HLA class I molecular complex plays the major role in the modulation of NK cell-mediated lysis of tumor cells.

The increase induced by anti-HLA class I MAb in NK cell-mediated lysis of FO-1C and FO-1H cells corroborates the role of HLA class I antigens in this phenomenon. Furthermore, the lack of effect of anti-HLA class I MAb on NK cell-mediated lysis of untransfected FO-1 cells indicates that they modulate the extent of lysis by interacting with the target cells and not with the effector cells. The latter conclusion is in agreement with data by Sturmhofel and Hammerling (26). An anti-H-2 class I MAb did not affect the NK cell-mediated lysis of a variant of mouse thymoma EL4 cells which does not express H-2 class I antigens, but enhanced that of the cells which had acquired H-2 class I antigens after transfection with a mouse B_2m gene.

The differential susceptibility to NK cell-mediated lysis of FO-1C and FO-1H cells suggests a relationship between extent of NK cell-mediated lysis of tumor cells and their level of HLA class I antigen expression. This possibility is supported by the

inverse relationship between the susceptibility to NK cell-mediated lysis and the level of expression of major histocompatibility complex (MHC) class I antigens by various human and mouse tumor cell lines (11, 26–31). The latter include clones of cell lines with spontaneous different expression of MHC class I antigens, clones which acquire different levels of MHC class I antigens after correction of the structural abnormality of the defective gene and cells with differential loss of MHC class I antigens after mutagenesis and selection with antibodies and complement. However, in our system, we cannot exclude the role of antigenic differences of the HLA class I molecular complex expressed by FO-1C and FO-1H cells, because association with xenogeneic $\beta_2\text{-}\mu$ may change the antigenic profile of HLA class I heavy chain (32) and FO-1C cells differ from FO-1H cells in the reactivity pattern with a panel of anti-HLA class I MAb (Tatake, R., S. Ferrone, and R. A. Zeff, unpublished results). Nevertheless, the role of quantitative differences in the level of HLA class I antigen expression by FO-1C and FO-1H cells is suggested by the additional reduction in their susceptibility to NK cell-mediated lysis associated with the enhancement of HLA class I antigen expression by IFN- γ and TNF- α . The effect was more marked on FO-1C cells than on FO-1H cells, because the difference in the susceptibility to NK cell-mediated lysis between these two types of targets was lost after incubation with the cytokines. The latter results support Storkus et al.'s (33) suggestion that the extent of NK cell-mediated lysis is correlated to HLA class I antigen expression only within certain ranges of expression. No significant change is detected in the extent of NK cell-mediated lysis, when HLA class I antigen expression is below a threshold level or above a plateau level.

The increase in NK cell-mediated lysis of melanoma cells induced by anti-HLA class I MAb parallels similar results obtained with human T cell lymphomas, breast carcinoma cells (34) and lung small cell carcinomas (5) and with mouse thymoma cells (26). On the other hand, these results are at variance with the lack of effect of anti-MHC class I antibodies on the NK cell-mediated lysis of human (5) and mouse (35) melanoma cells and of human lymphoblastoid cells (27). Furthermore, our results differ from those in the literature in two aspects; in Lobo and Spencer's (5) experiments, only target cells which do not express HLA class II antigens were susceptible to increase in NK cell-mediated lysis by anti-HLA class I antibodies, whereas in our studies, the phenomenon was observed with a melanoma cell line which expresses HLA class II antigens. Whether this discrepancy reflects technical reasons or the differential expression by the target cells used of adhesion molecules like ICAM-1, which play a role in the interaction of target cells with effector cells (Maio, M., and S. Ferrone, unpublished results), remains to be determined. Furthermore, in

Jabrane-Ferrat et al.'s experiments (34), the whole IgG of the anti-HLA class I MAb tested enhanced the extent of NK cell-mediated lysis, whereas the F(ab')₂ fragments did not, therefore suggesting that the increase mediated by anti-HLA class I MAb may reflect an ADCC phenomenon. On the other hand, in our experiments, the whole IgG of anti-HLA class I MAb were as effective as F(ab')₂ fragments in enhancing NK cell-mediated lysis of melanoma cells FO-1C and FO-1H. Whether the conflicting results reflect differences in the characteristics of the MAb tested and/or in the antigenic profile of the cells used as targets in the two studies remains to be determined. In agreement with the results obtained by Lobo and Spencer (5) who utilized cultured lymphoid cells as targets, we have found a differential effect of MAb to distinct monomorphic determinants of HLA class I antigens on NK cell-mediated lysis of melanoma cells. This finding does not appear to correlate with the affinity constants of the MAb tested and/or with the level of expression of the corresponding determinants on target cells. On the other hand, the mapping to the $\alpha 1/2$ domains of HLA class I heavy chains of the epitope(s) required for protection against NK cell-mediated lysis (33) suggests that the differential effect of the anti-HLA class I MAb tested may reflect differences in the distribution of the corresponding determinants on the three domains of HLA class I heavy chains. In this regard, testing with mouse cells transfected with H2/HLA class I hybrid genes has shown that the determinant recognized by MAb H2-89-1 with the strongest modulatory effect is expressed on the $\alpha 2$ domain, whereas that recognized by MAb CR11-115 with no detectable effect is expressed on the $\alpha 2$ and $\alpha 3$ domain (Takiguchi, M., and S. Ferrone, unpublished results).

The results with IFN- γ or TNF- α -treated melanoma cells deserve some comments, because they may suggest some explanations for the conflicting information about the effect of cytokines on NK cell-mediated lysis of tumor cells. Although not statistically significant, the reduction in the susceptibility to NK cell-mediated lysis of FO-1 cells after incubation with IFN- γ or TNF- α suggests that the two cytokines modulate the expression not only of HLA class I antigens but also of other structures which are involved in the phenomenon. The role of the latter structures which will be referred to as non-HLA-NK cytotoxicity related structures is also suggested by the more marked effect of TNF- α than of IFN- γ on NK cell-mediated lysis of melanoma cells FO-1H, although the latter cytokine enhances HLA class I antigen expression more than the former one. In addition, these results suggest that the non-HLA-NK cytotoxicity related structures are more susceptible to modulation by TNF- α than by IFN- γ . A precedent for the latter possibility is represented by the higher susceptibility of ICAM-1 to modulation by TNF- α than by IFN- γ (36). The differential expression of the non-HLA-NK cytotoxicity related structures and of HLA class I antigens may account for the conflicting data about the effect of IFN- γ on NK cell-mediated lysis of various types of human tumor cells (37-44). Furthermore, the modulation of the non-HLA-NK cytotoxicity-related structures may account for the reduction in the susceptibility to NK cell-mediated lysis of tumor cells after incubation with IFN- γ , but not after transfection with HLA class I genes, although the two approaches induce similar levels of HLA class I antigens (43, 45). The reduction in the susceptibility to NK cell-mediated lysis of FO-1 cells after incubation with IFN- γ is similar to the slight decrease in NK cell-mediated lysis of a cultured B lymphoblastoid cell line after incubation with IFN- γ , although the

low HLA class I antigen expression did not change (37). These results are at variance with the lack of effect of IFN- γ on the NK cell-mediated lysis of variants of the mouse cell lines EL4 (26) and YAC (46), both of which do not express H-2 class I antigens. If not caused by technical factors, the different results may reflect differential expression by various tumor cell lines of non-MHC-NK cytotoxicity-related structures. Furthermore, the lack of statistical significance in the difference of NK cell-mediated lysis between control and IFN- γ treated FO-1 cells suggests that the modulation by IFN- γ of the non-HLA-NK cytotoxicity-related structures is not sufficient to change the susceptibility of target cells to NK cell-mediated lysis.

The lack of effect of HLA class I antigen expression and modulation by IFN- γ on the susceptibility of melanoma cells FO-1 to LAK cell-mediated lysis parallels similar findings with a cultured B lymphoid cell line which acquires HLA class I antigens after transfection with HLA class I genes (11). These findings are in conflict with results obtained with other cell lines. Quillet et al. (41) have described a reduction in susceptibility to LAK cell-mediated lysis of cultured B lymphoid cells Daudi which have acquired HLA class I antigens after transfection with a human B_{2m} gene. Furthermore, Ohlen et al. (30) have reported an inverse relationship between the level of HLA class I antigen expression by HLA loss variants of a cultured B lymphoid cell line and their susceptibility to LAK cell-mediated lysis. Lastly, Jabrane-Ferrat et al. (34) utilizing human breast carcinoma cells and Tsai et al. (47) utilizing mouse lymphoma and melanoma cells have shown that enhancement of MHC class I antigen expression on target cells incubated with IFN- γ reduces their susceptibility to LAK cell-mediated lysis.

The results we have shown indicate a differential role of HLA class I antigens in the lysis of melanoma cells FO-1 by various types of effector cells. This information in conjunction with the characterization of the molecular basis of abnormalities in HLA class I antigen expression by melanoma cells may suggest novel immunotherapeutic approaches to melanoma.

Acknowledgements

The authors thank Dr. H. L. Ploegh, Department of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, for providing a genomic human $\beta_2\text{-}\mu$ clone and acknowledge the excellent secretarial assistance of Mrs. Edwina L. Jones, Mrs. Harriett V. Harrison, Mrs. Donna D. James, and Miss Gail D. Price.

This work was supported by the Associazione Italiana per la Ricerca sul Cancro, by the Public Health Service grant CA37959 and CA39559 awarded by the National Cancer Institute, Department of Health and Human Services, by grants IM-500 and IM-554 awarded by the American Cancer Society, and by grant CT-219-8809 awarded by the American Heart Association. M. Maio was supported by a fellowship from an Italia-United States exchange program awarded by the Consiglio Nazionale delle Ricerche and National Institutes of Health. R. A. Zeff is an Established Investigator of the American Heart Association.

References

1. Ruiter, D. J., V. Mattijssen, E.-B. Broecker, and S. Ferrone. 1991. MHC antigens in human melanomas. *Semin. Cancer Biol.* In press.
2. van Duinen, S. G., D. J. Ruiter, E. B. Broecker, E. A. van der Velde, C. Sorg, K. Welvaart, and S. Ferrone. 1988. Level of HLA antigens in locoregional metastases and clinical course of the disease in patients with melanoma. *Cancer Res.* 48:1019-1025.

3. McMichael, A. J. 1980. HLA restriction of human cytotoxic T cells. *Springer Semin. Immunopathol.* 3:3-22.
4. Ljunggren, H.-G., and K. Karre. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today.* 11:237-244.
5. Lobo, P. I., and C. E. Spencer. 1989. Use of anti-HLA antibodies to mask major histocompatibility complex gene products on tumor cells can enhance susceptibility of these cells to lysis by natural killer cells. *J. Clin. Invest.* 83:278-287.
6. Versteeg, R., L. T. C. Peltenburg, A. C. Plomp, and P. I. Schrier. 1989. High expression of the *c-myc* oncogene renders melanoma cells prone to lysis by natural killer cells. *J. Immunol.* 143:4331-4337.
7. Wilson, B. S., F. Indiveri, M. A. Pellegrino, and S. Ferrone. 1979. DR (Ia-like) antigens on human melanoma cells. Serological detection and immunohistochemical characterization. *J. Exp. Med.* 149:658-668.
8. Maio, M., B. Gulwani, J. A. Langer, R. S. Kerbel, G. J. Duigou, P. B. Fisher, and S. Ferrone. 1989. Modulation by interferons of HLA antigen, high-molecular-weight melanoma-associated antigen, and intercellular adhesion molecule 1 expression by cultured melanoma cells with different metastatic potential. *Cancer Res.* 49:2980-2987.
9. Guarini, L., M. Temponi, G. M. Edwalds, J. R. Vita, P. B. Fisher, and S. Ferrone. 1989. In vitro differentiation and antigenic changes in human melanoma cell lines. *Cancer Immunol. Immunother.* 30:262-276.
10. D'Urso, C. M., Z. Wang, Y. Cao, R. Tatake, R. A. Zeff, and S. Ferrone. 1991. Lack of HLA Class I antigen expression by cultured melanoma cells FO-1 due to a defect in B_2m gene expression. *J. Clin. Invest.* 87:284-292.
11. Storkus, W. J., J. Alexander, J. A. Payne, J. R. Dawson, and P. Cresswell. 1989. Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes. *Proc. Natl. Acad. Sci. USA.* 86:2361-2364.
12. Suggs, S. V., R. B. Wallace, T. Hirose, E. H. Kawashima, and K. Itakura. 1981. Use of synthetic oligonucleotides as hybridization probes: isolation of cloned cDNA sequences for human β_2 -microglobulin. *Proc. Natl. Acad. Sci. USA.* 78:6613-6617.
13. Parnes, J. R., and J. G. Seidman. 1982. Structure of wild-type and mutant mouse β_2 -microglobulin genes. *Cell.* 29:661-669.
14. Gussow, D., R. Rein, I. Ginjaar, F. Hochstenbach, G. Seemann, A. Kottman, and H. L. Ploegh. 1987. The human β_2 -microglobulin gene. Primary structure and definition of the transcriptional unit. *J. Immunol.* 139:3132-3138.
15. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens: new tools for genetic analysis. *Cell.* 14:9-20.
16. Turco, M. C., M. De Felice, L. Corbo, G. Morrone, R. Mertelmann, S. Ferrone, and S. Venuta. 1985. Regulatory role of monomorphic determinant of HLA class I antigens in T cell proliferation. *J. Immunol.* 135:2268-2273.
17. Quaranta, V., M. A. Pellegrino, and S. Ferrone. 1981. The monoclonal xenobody Q6/64 recognizes a determinant expressed by certain gene products of the A and B loci of the HLA region. *Immunogenetics.* 14:403-413.
18. Pellegrino, M. A., A.-K. Ng, C. Russo, and S. Ferrone. 1982. Heterogeneous distribution of the determinants defined by monoclonal antibodies on HLA-A and B antigens bearing molecules. *Transplantation.* 34:18-24.
19. Quaranta, V., L. E. Walker, M. A. Pellegrino, and S. Ferrone. 1980. Purification of immunologically functional subsets of human Ia-like antigens on a monoclonal antibody (Q5/13) immunoadsorbent. *J. Immunol.* 125:1421-1425.
20. Matsui, M., M. Temponi, and S. Ferrone. 1987. Characterization of a monoclonal antibody-defined human melanoma-associated antigen susceptible to induction by immune interferon. *J. Immunol.* 139:2088-2094.
21. Temponi, M., T. Kageshita, F. Perosa, R. Ono, H. Okada, and S. Ferrone. 1989. Purification of murine IgG monoclonal antibodies by precipitation with caprylic acid: comparison with other methods of purification. *Hybridoma.* 8:85-95.
22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680-685.
23. Rein, R. S., G. H. A. Seemann, J. J. Neeffes, F. M. H. Hochstenbach, N. J. Stam, and H. L. Ploegh. 1987. Association with β_2 -microglobulin controls the expression of transfected human Class I genes. *J. Immunol.* 138:1178-1183.
24. Perarnau, B. M., A. C. Gillet, R. Hakem, M. Barad, and F. A. Lemonnier. 1988. Human β_2 -microglobulin specifically enhances cell-surface expression of HLA class I molecules in transfected murine cells. *J. Immunol.* 141:1383-1389.
25. Klar, D., and G. J. Hammerling. 1989. Induction of assembly of MHC class I heavy chains with β_2 -microglobulin by interferon- γ . *EMBO (Eur. Mol. Biol. Organ.) J.* 8:475-481.
26. Sturmhofel, K., and G. J. Hammerling. 1990. Reconstitution of H-2 class I expression by gene transfection decreases susceptibility to natural killer cells of an EL4 class I loss variant. *Eur. J. Immunol.* 20:171-177.
27. Storkus, W. J., D. N. Howell, R. D. Salter, J. R. Dawson, and P. Cresswell. 1987. NK susceptibility varies inversely with target cell class expression. *J. Immunol.* 138:1657-1659.
28. Algarra, I., C. Ohlen, M. Perez, H.-G. Ljunggren, G. Klein, F. Garrido, and K. Karre. 1989. NK sensitivity and lung clearance of MHC-class-I-deficient cells within a heterogeneous fibrosarcoma. *Int. J. Cancer.* 44:675-680.
29. Carlow, D. A., U. Payne, N. Hozumi, J. C. Roder, and A. A. Czitzrom. 1990. Class I (H-2K^b) gene transfection reduces susceptibility of YAC-1 lymphoma targets to natural killer cells. *Eur. J. Immunol.* 20:841-846.
30. Ohlen, C., M.-T. Bejarano, A. Gronberg, S. Torsteinsdottir, L. Franksen, H.-G. Ljunggren, E. Klein, G. Klein, and K. Karre. 1989. Studies of sublines selected for loss of HLA expression from an EBV-transformed lymphoblastoid cell line. Changes in sensitivity to cytotoxic T cells activated by allostimulation and natural killer cells activated by IFN or IL-2. *J. Immunol.* 142:3336-3341.
31. Shimizu, Y., and R. DeMars. 1989. Demonstration by class I gene transfer that reduced susceptibility of human cells to natural killer cell-mediated lysis is inversely correlated with HLA class I antigen expression. *Eur. J. Immunol.* 19:447-451.
32. Ferrier, P., C. Layet, D. H. Caillol, B. R. Jordan, and F. A. Lemonnier. 1985. The association between murine β_2 -microglobulin and HLA Class I heavy chains results in serologically detectable conformational changes of both chains. *J. Immunol.* 135:1281-1287.
33. Storkus, W. J., J. Alexander, J. A. Payne, P. Cresswell, and J. R. Dawson. 1989. The $\alpha 1/\alpha 2$ domains of class I HLA molecules confer resistance to natural killing. *J. Immunol.* 143:3853-3857.
34. Jabrane-Ferrat, N., F. Calvo, A. Faille, J. F. Lagabrielle, N. Boisson, A. Quillet, and D. Fradelizi. 1990. Recombinant gamma interferon provokes resistance of human breast cancer cells to spontaneous and IL-2 activated non-MHC restricted cytotoxicity. *Br. J. Cancer.* 61:558-562.
35. Gorelik, E., Y. Gunji, and R. B. Herberman. 1988. H-2 antigen expression and sensitivity of BL6 melanoma cells to natural killer cell cytotoxicity. *J. Immunol.* 140:2096-2102.
36. Temponi, M., G. Romano, C. M. D'Urso, Z. Wang, U. Kekish, and S. Ferrone. 1988. Profile of intercellular adhesion molecule-1 (ICAM-1) synthesized by human melanoma cell lines. *Semin. Oncol.* 15:595-607.
37. Harel-Bellan, A., A. Quillet, C. Marchiol, R. DeMars, T. Tursz, and D. Fradelizi. 1986. Natural killer susceptibility of human cells may be regulated by genes in the HLA region on chromosome 6. *Proc. Natl. Acad. Sci. USA.* 83:5688-5692.
38. Gronberg, A., R. Kiessling, and W. Fiers. 1985. Interferon- γ is a strong modulator of NK susceptibility and expression of β_2 -microglobulin but not of transferrin receptors of K562 cells. *Cell. Immunol.* 95:195-202.
39. Gronberg, A., M. T. Ferm, J. Ng, C. W. Reynolds, and J. R. Ortaldo. 1988. IFN- γ treatment of K562 cells inhibits natural killer cell triggering and decreases the susceptibility to lysis by cytoplasmic granules from large granular lymphocytes. *J. Immunol.* 140:4397-4402.
40. Main, E. K., D. S. Monos, and L. A. Lampson. 1988. IFN-treated neuroblastoma cell lines remain resistant to T cell-mediated allo-killing, and susceptible to non-MHC-restricted cytotoxicity. *J. Immunol.* 141:2943-2950.
41. Quillet, A., F. Presse, C. Marchiol-Fournigault, A. Harel-Bellan, M. Benbunan, H. Ploegh, and D. Fradelizi. 1988. Increased resistance to non-MHC-restricted cytotoxicity related to HLA A, B expression. Direct demonstration using β_2 -microglobulin-transfected Daudi cells. *J. Immunol.* 141:17-20.
42. Leiden, J. M., B. A. Karpinski, L. Gottschalk, and J. Kornbluth. 1989. Susceptibility to natural killer cell-mediated cytotoxicity is independent of the level of target cell class I HLA expression. *J. Immunol.* 142:2140-2147.
43. Stam, N. J., W. M. Kast, A. C. Voordouw, L. B. Pastoors, F. A. van der Hoeven, C. J. M. Melief, and H. L. Ploegh. 1989. Lack of correlation between levels of MHC class I antigen and susceptibility to lysis of small cellular lung carcinoma (SCLC) by natural killer cells. *J. Immunol.* 142:4113-4117.
44. Pena, J., C. Alonso, R. Solana, R. Serrano, J. Carracedo, and R. Ramirez. 1990. Natural killer susceptibility is independent of HLA class I antigen expression on cell lines obtained from human solid tumors. *Eur. J. Immunol.* 20:2445-2449.
45. Maziarz, R. T., S. J. Mentzer, S. J. Burakoff, and D. V. Faller. 1990. Distinct effect of interferon- γ and MHC Class I surface antigen levels on resistance of the K562 tumor cell line to natural killer-mediated lysis. *Cell. Immunol.* 130:329-338.
46. Ljunggren, H.-G., K. Sturmhofel, E. Wolpert, G. J. Hammerling, and K. Karre. 1990. Transfection of β_2 -microglobulin restores IFN-mediated protection from natural killer cell lysis in YAC-1 lymphoma variants. *J. Immunol.* 145:380-386.
47. Tsai, L., C. Ohlen, H.-G. Ljunggren, K. Karre, M. Hansson, and R. Kiessling. 1989. Effect of IFN- γ treatment and in vivo passage of murine tumor cell lines on their sensitivity to lymphokine-activated killer (LAK) cell lysis in vitro; association with H-2 expression on the target cells. *Int. J. Cancer.* 44:669-674.