Characterization of Cloned Class I MHC-restricted, CD8⁺ Anti-Meth A Cytotoxic T-Lymphocytes: Recognition of an Epitope Derived from the Meth A gp110 Tumor Rejection Antigen

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Pittsburgh Cancer Institute, Division of Basic Research and Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213 [M. A. F., M. A. F., Meth A gpl10 Tumor Rejection Antigen Characterization of Cloned Class I MHC-restricted, CD8⁺ Anti-Meth A Cytotoxic

ABSTRACT

Meth A gp110 has been tentatively identified as a tumor rejection antigen. Following isolation of a class I major histocompatibility complex (MHC)-restricted, CD8⁺ anti-Meth A cytotoxic T-lymphocyte (CTL), we sought to determine whether the determinant recognized by this CTL was: (a) functional in tumor rejection of Meth A sarcoma; and (b) derived from Meth A gp110.

Initially, we isolated an anti-Meth A CTL-resistant variant of Meth A sarcoma, Meth A4R, by immunoselection. The results of the subsequent analysis of Meth A4R cells showed the CTL-defined determinant as having a functional role in transplantation rejection of Meth A sarcoma.

Walker et al. (Proc. Natl. Acad. Sci. USA, 89: 7915–7918, 1993) showed that the cationic lipid, N-[1-(2,3-dioleoyloxy)propyll]N,N,N-trimethylammonium-methyl sulfate, mediated delivery of a recombinant glycoprotein into the cytosol of target cells, making it available for processing and presentation by class I MHC molecules. As a result, the cells were sensitized for cytolyis by a class I MHC-restricted CD8⁺ CTL, which recognized an epitope expressed by the glycoprotein. In a similar manner, we treated the SV40-transformed HAI cell line, SVBalb, which is relatively insensitive to cytolyis by the anti-Meth A CTL, with Meth A gp110 and N-[1-(2,3-dioleoyloxy)propyll]N,N,N-trimethylammonium sulfate. The sensitivities of the treated cells and control cell lines to the anti-Meth A CTL were then examined. The results of these experiments permit us to conclude that the determinant recognized by the anti-Meth A CTL line is derived from Meth A gp110.

INTRODUCTION

The immunogenicity of chemically induced sarcomas in tumor rejection assays has been attributed to their expression of antigenic determinants, commonly referred to as tumor-specific transplantation antigen or TRA² (1–5). A major goal of tumor immunology is the identification of these antigens in the hope that this knowledge might facilitate the identification of human tumor antigens and provide a more rational approach to tumor vaccines and immunotherapy (6).

We recently reported an analysis of the tumor rejection-inducing activity which can be isolated from the Con A Sepharose-binding protein fraction of the Meth A sarcoma (7). The results indicated that this activity was associated with a M₁ 110,000 glycoprotein, gp110. The lack of cross-reactivity for gp110 of serological reagents raised firmly implied that gp110 was antigenically distinct from these other proteins. Together with the demonstration of the restricted immunogenicity of Meth A gp110 in tumor rejection assays, these results strongly suggested that gp110 might be the putative tumor-specific transplantation antigen of chemically induced sarcomas.

Class I MHC-restricted CD8⁺ CTL are usually considered the critical effector cells involved in antitumor responses (12–16). It is well established that these cells recognize antigenic peptides bound to class I MHC molecules on the cell surface of target cells (17–21). In general, these peptides are derived from proteins synthesized by the target cell, rather than exogenously synthesized proteins. Under these constraints, determining whether a protein contains an epitope recognized by a class I MHC-restricted CTL requires the availability of the cDNA encoding the protein and/or peptides synthesized on the basis of protein sequences. These probes permit the analysis of CTL reactivity against transfected or peptide-pulsed target cells, which facilitates the identification of CTL-defined epitopes, as has recently been demonstrated in the identification of CTL-recognized p53 peptides (22, 23).

The role of CD8⁺ CTL in tumor rejection of Meth A sarcoma, unlike in other tumor model systems (24), has not been studied by analyzing the effect of in vivo immunodepletion of CD8⁺ cells on the ability of Meth A-immunized mice to reject a Meth A challenge. Instead, immunodepletion of Lyt-2⁺ cells from sensitized T-cells obtained from Meth A-bearing or -immune mice was shown to abrogate the ability of sensitized T-cells to cause tumor regression when adoptively transferred to irradiated BALB/c mice bearing 3-day-old, locally growing Meth A sarcoma (25). This result strongly implicates CTL in tumor rejection of Meth A sarcoma. Following the isolation of a cloned H-2Kd-restricted anti-Meth A CTL, CTLMA-9C, we sought to: (a) define the relevancy of the determinant recognized by this CTL in tumor rejection of Meth A sarcoma; and (b) determine whether this determinant was expressed on Meth A gp110. The demonstration that the CTL-defined determinant had a functionally significant role in tumor rejection of Meth A sarcoma and was derived from gp110 would provide additional support for its designation as a TRA.

The role of CTLMA-9C-defined determinant in tumor rejection of Meth A sarcoma was indirectly studied with an analysis of a CTL-resistant variant of Meth A, Meth A4R, which was isolated by immunoselection. The results of in vivo and in vitro analyses of Meth A4R are supportive of the CTL-recognized epitope playing a significant role in defining the immunogenicity of Meth A sarcoma.

It has recently been demonstrated that a transfection reagent, the cationic lipid DOTAP, when complexed with a glycoprotein-mediated delivery of protein to the cell cytosol, allowing processing and presentation of derived peptides. Cells treated with recombinant herpes simplex virus glycoprotein B residues 23–718 complexed with DOTAP were sensitized to cytolyis by class I MHC-restricted anticylglycoprotein B CTL (26). This process was abrogated by treatment of the cells in the presence of BFA, a fungal antibiotic which blocks the transport of class I MHC molecules from the ER (27).

In our evaluation of whether the anti-Meth A CTL recognized a determinant expressed on Meth A gp110, an SV40-transformed BALB/c cell line, SVBalb, was used as the target cell. This cell line is relatively insensitive to cytolyis by the anti-Meth A CTL. Follow-
ing treatment with Meth A gp110 and DOTAP, these cells were evaluated for their sensitivity to the antitumor CTL. The results strongly suggest that Meth A gp110 expresses the anti-Meth A CTL-defined epitope.

MATERIALS AND METHODS

Animals. The BALB/c and CB6F1 (BALB/c × C57BL/6)F1 mice, used in this study were obtained from The Jackson Laboratory (Bar Harbor, ME) and the Small Animal Facility, National Cancer Institute (Frederick, MD). All animals were maintained in a specific pathogen-free facility.

Tumors. The following chemically induced BALB/c sarcomas were used in these experiments: C1300, CMS1, CMS4, CMS5, Meth A, CMS4, and MSMS were negative in the non-good laboratory practices mouse antibody production (16 viruses) test (Microbiological Associates, Inc., Rockville, MD). Additional tumors used in this study included the C57BL/6 melanoma, JB/RH (29), and the DBA/2 mastocytoma, P815 (30).

Antibodies. Culture supernatants of hybridomas producing anti-H-2Kd (HB159, IgG2a), anti-H-2Dd (HB102, IgG2a; HB76; IgM), and anti-H-2Ld (HB27; IgG2a) mAbs were used in this study. FITC-anti CD3 mAb (HB159, IgG2a; B-392, IgM), anti-H-2Dd (HB102, IgG2a; HB76; IgM), and anti-H-2Ld (HB27; IgG2a) mAbs were obtained from Boehringer Mannheim (Indianapolis, IN), and PE-anti-CD3 (CD4) and FITC-anti-Lyt-2 (CD8) were obtained from Pharmingen (San Diego, CA). An extensive panel of anti-mouse TCR mAbs were obtained from the Experimental Immunology Branch, National Cancer Institute.

Purification of gp110. HPEC-purified Meth A gp110 was obtained from Mono Q fast performance liquid chromatography fractions of Meth A cytosol (7) using a Model 230A HPEC (Applied Biosystems, Foster City, CA). The samples (∼400 µg) to be chromatographed were concentrated in the presence of 0.1% SDS, heated 2 min in the presence of 2-mercaptoethanol, and applied to a HPEC gel (5% acrylamide, 3.5 mm x 5 cm). The samples were run at 1 mA for 4 h and 5 min, and 100-µl fractions were collected. Aliquots of the fractions were analyzed by SDS-PAGE, and the gp110-containing fractions were pooled and concentrated by membrane ultraltration. The protein concentrations of gp110 preparations were estimated based on a comparison of the relative intensity of silver staining of the protein bands in an SDS-PAGE gel of the preparation versus a known amount of gp110, whose concentration was determined by amino acid analysis.

MLTC. Mice were immunized against Meth A sarcoma by three weekly i.p. injections of 5 × 10⁶ irradiated Meth A sarcoma, followed by repeated challenges s.c. with 2 × 10⁶ nonirradiated Meth A sarcoma (31). nylon-wool nonadherent PEC (12), obtained from Meth A-immune CB6F1 mice, were cultured in 24-well plates at a density of 8 × 10⁶ cells/well/0.2 ml RPMI 1640 supplemented with 5 units/ml recombinant interleukin 2 (Cetus), 0.1% sodium pyruvate, 3 mm l-glutamine, 5 × 10⁻⁵ M 2-mercaptoethanol, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% fetal bovine serum (complete medium) were combined in the presence of 4 × 10⁶ irradiated (10,000 rad) tumor cells/well as stimulator (7).

Isolation of the anti-Meth A CTL Cell Line, CTLMA-9C. The antitumor effector cells generated in the MLTC were passaged several times in 96-well plates at a cell density of 1.6 × 10⁶ effector cells/well/0.2 ml complete medium in the presence of irradiated Con A-stimulated splenocytes (1 × 10⁶ cells/well) and tumor cells (4 × 10⁶ cells/well) (31). Once an anti-Meth A CTL cell line was established, the cells were maintained for several passages in 24-well plates under growth conditions described above. The effector cells were cloned by limiting dilution (10 cells/well to 0.5 cell/well) in wells of 96-well plates on a feeder layer consisting of irradiated Con A-stimulated splenocytes and tumor cells in the presence of 5 units/ml recombinant human interleukin 2, as described above. Every 3 days, 100 µl of conditioned medium were removed from each well, and 100 µl of fresh complete medium were added. The cells growing in the wells which had been seeded at the lowest cell densities were harvested, grown in 24-well plates, tested for reactivity, and cloned as described above. In general, the cloned CTL cells could be cultured in the presence of irradiated tumor cells and splenocytes for extended periods of time (up to 4 months) without significant loss of reactivity and/or specificity (31).

Cell-mediated Cytotoxicity Assay. Cytotoxicity was measured in a 4-h ⁵¹Cr release assay using 96-well, round-bottomed plates (7). Target cells were labeled with 100 µCi of Na₂⁵¹CrO₄ per 2 × 10⁵ cells for 1 h at 37°C; then the cells were washed and resuspended at 10⁵ cells/ml in complete media. Target cells were then seeded at 5 × 10⁵ cells/well in 50 µl of medium. Suspensions of effector cells were added to triplicate wells to give various effector:target ratios in a final volume of 200 µl. Plates were centrifuged and incubated at 37°C for 4 or 18 h. Plates were then centrifuged and 100 µl of supernatant removed from each well and counted in a gamma counter. In the microcytotoxicity assay, 500 target cells/well were plated, and the supernatants removed from the wells were counted by liquid scintillation. The spontaneous release as well as maximum amount of cpm was determined, and the percentage of specific cytotoxicity was calculated by the standard procedure.

Immunoselection of CTL-resistant Meth A Variant, Meth A4R. Based on the procedure described by Topalian et al. (32), a CTLMA-9C-resistant variant of Meth A sarcoma was isolated following four successive rounds of immunoselection, starting at an effector:target ratio of 1:8 and progressing to 64:1. The CTL-resistant cells were cloned by limiting dilution (∼0.5 cell/well) in 96-well plates, and several clones were expanded and analyzed for their resistance to cytolyis by CTLMA-9C cells. One clone, designated Meth A4R, was selected for further analysis.

Tumor Rejection Assays. Groups of three BALB/c female mice each received two weekly injections i.p. of 5 × 10⁴ irradiated, in vitro-grown Meth A or Meth A4R cells. Seven days after the last immunization, mice were challenged bilaterally with in vivo-grown Meth A sarcoma injected s.c. (7, 33). Tumor growth was determined 21 days after tumor challenge. Statistical analyses were based on Student’s t test.

Sensitization of SVBalb with Meth A gp110. SVBalb cells were treated with gp110 complexed with the cationic lipid, DOTAP (Boehringer Mannheim) using a modification of the procedure described by Walker et al. (26). Briefly, SVBalb cells (H-2Kd), kindly provided by Dr. Christopher Walker (Chiron Corp., Emeryville, CA), were adapted to growth on reduced serum media (1 part conditioned medium and 3 parts serum-free hybridoma medium (GIBCO), and then plated at a density of 3 or 4 × 10⁶ cells/well/1 ml serum-free hybridoma medium in 24-well plates. The plates were incubated overnight at 37°C, the medium was removed, and 300 µl serum-free hybridoma medium supplemented with DOTAP (1.6 µl) alone or complexed with gp110 or OVA (100 ng/ml) was added to duplicate or triplicate wells. The treated cells were cultured overnight at 37°C, harvested using EDTA, and radiolabeled for use as target cells. In general, due to the low numbers of target cells recovered, the sensitivity of these cells to cytotoxicity was determined in a 4-h microcytotoxicity assay as described above.

RESULTS

Characterization of the Cloned anti-Meth A CTL Cell Line, CTLMA-9C. The anti-Meth A effector cells established from PEC obtained from Meth A-immune CB6F1 mice following a 5-day MLTC showed relatively restricted cytolytic reactivity against the Meth A sarcoma and were H-2Kd restricted (data not shown). Anti-Meth A CTL were cloned by limiting dilution from these anti-Meth A effector cells. Several cloned anti-Meth A CTL were isolated and evaluated for their reactivity against Meth A sarcoma and the antigenically unrelated CMS4 sarcoma (29, 34), and one clone, designated CTLMA-9C, was selected for further characterization. The phenotype of the CTLMA-9C cell line was determined to be V₆.₈₃ CD₃⁺ CD₄⁺, CD₈⁻, and NK-1.1⁻; and its cytolytic reactivity against Meth A target cells was inhibited by anti-CD8 and anti-H-2Kd mAb but not by anti-CD4 or anti-H-2Dd mAb (Fig. 1A).

The reactivity of the CTLMA-9C cells against a panel of ten chemically induced sarcomas of BALB/c origin in the standard 4-h cell-mediated cytotoxicity assay was shown to be uniquely restricted to Meth A. The results from an evaluation of CTLMA-9C reactivity against Meth A and nine other chemically induced BALB/c sarcomas are shown in Fig. 1, B and C. In addition, no significant reactivity was detected against mitogen-stimulated BALB/c splenocytes, the SV40-transformed BALB/c cell line, SVBalb (26), C57BL/6 melanoma JB/RH, or the lymphokine-activated NK cell-sensitive P815 tumor cell line (34) (data not shown).
Characterization of Meth A4R. Resistance of cells to cytolysis by a class I MHC-restricted CTL might be the result of one or more of several distinct mechanisms. These include alterations in expression of antigen and/or class I MHC molecules, loss of ability to process and present antigen, or general resistance to cell-mediated cytolysis (24, 32). To rule out the possibility that the resistance of Meth A4R to cytolysis by CTLMA-9C cells was due to alteration in expression of class I MHC molecules, in particular H-2Kd (the restriction element of CTLMA-9C cells), the variant sarcoma cells were analyzed for expression of class I MHC molecules by flow cytometry. As indicated in Table 1, the levels of expression of H-2Kd and H-2Dd by Meth A4R were comparable to those expressed by the parental Meth A sarcoma. The level of expression of H-2Ld by the variant, however, was higher than that of the parental cell line. This was a consistent observation; the variant was found to express 1.3 to 2.1 times more H-2Ld than that of the parental cell line. The basis for this is presently unknown. It might, however, be a consequence of variation in the levels of H-2Ld expressed by Meth A sarcoma cells from which the variant was selected and cloned. We also determined that cytolysis of Meth A4R cells by CTLMA-9C-mediated lectin-dependent cytolysis, as well as by cytotoxic granules isolated from these CTL, was comparable to that of parental Meth A sarcoma (data not shown). These results imply that the resistance of Meth A4R cells probably involves a decrease in tumor antigen expression, rather than a low level of H-2Kd expression or a general resistance to cell-mediated cytolysis.

This conclusion was supported by: (a) the inability of Meth A4R cells, unlike the parental cells, to significantly protect mice from a subsequent challenge of the parental Meth A sarcoma (Table 2); and (b) inability of Meth A4R to restimulate in vitro anti-Meth A effector cells from PEC obtained from Meth A-immune mice (Table 3). These results strongly suggest that the CTLMA-9C-defined epitope plays a role in defining the immunogenicity of Meth A sarcoma.

Sensitization of SVBalb Cells with Meth A gp110 and DOTAP. In order to test the possibility that the determinant recognized by the anti-Meth A CTL derives from Meth A gp110, SVBalb cells were treated in serum-free medium with various concentrations of HPEC-purified Meth A gp110 (12 ng/ml to 200 ng/ml) complexed with DOTAP (26). Representative results of a HPEC preparation of gp110

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**Table 1** Flow cytometry analysis of expression of class I MHC antigens on Meth A and Meth A4R sarcomas

<table>
<thead>
<tr>
<th></th>
<th>Mean fluorescence channel number</th>
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<tbody>
<tr>
<td></td>
<td>Kd</td>
</tr>
<tr>
<td>Meth A</td>
<td>111.34</td>
</tr>
<tr>
<td>Meth A4R</td>
<td>110.05</td>
</tr>
</tbody>
</table>

**Table 2** Inability of Meth A4R to cross-immunize against Meth A in in vivo tumor rejection assaya

<table>
<thead>
<tr>
<th>Immunizing tumor</th>
<th>Experiment 1b</th>
<th>Experiment 2c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meth A</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Meth A4R</td>
<td>2/6</td>
<td>3/6</td>
</tr>
<tr>
<td>None</td>
<td>6/6</td>
<td>2/4</td>
</tr>
</tbody>
</table>

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a In each experiment, three groups of three BALB/cAnNIH female mice each were immunized three times at 7-day intervals with 5 × 10⁷ irradiated tumor cells.

b In experiment 1, mice were challenged bilaterally s.c. with 5 × 10⁶ Meth A sarcoma.

c In experiment 2, mice were initially challenged bilaterally s.c. with 1 × 10⁶ Meth A sarcoma; however, no progressive growth occurred. Two months later, these mice were rechallenged with 5 × 10⁶ Meth A sarcoma, and the resulting tumor growth of the rechallenged mice was recorded. Tumor incidence in control mice for the second tumor challenge of 5 × 10⁶ Meth A sarcoma was 8 of 8.

d Tumor incidence was determined 21 days after tumor challenge.
Table 3 Comparison of effects of Meth A and Meth A4R on restimulation of anti-Meth A effector cells obtained from Meth A-immune mice

<table>
<thead>
<tr>
<th>Mice*</th>
<th>Meth A</th>
<th>Meth A4R</th>
<th>100:1</th>
<th>50:1</th>
<th>25:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>+</td>
<td>−</td>
<td>10.4 ± 1.0</td>
<td>5.8 ± 0.6</td>
<td>5.8 ± 3.4</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>10.9 ± 2.4</td>
<td>6.2 ± 1.4</td>
<td>6.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Meth A immune</td>
<td>+</td>
<td>−</td>
<td>26.2 ± 0.5</td>
<td>14.7 ± 1.6</td>
<td>15.1 ± 2.5</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>12.2 ± 1.4</td>
<td>7.1 ± 0.9</td>
<td>7.4 ± 4.5</td>
<td></td>
</tr>
</tbody>
</table>

*Mice were immunized twice with 5 × 10^6 irradiated Meth A sarcoma i.p. on a 7-day interval. Seven days following the last immunization, the mice were challenged s.c. with 1 × 10^5 Meth A sarcoma.

**PEC were obtained from surviving mice and appropriate control mice and restimulated in vitro in the presence of Meth A or Meth A4R for 5 days. Cells were harvested, and their reactivity against Meth A target cells was determined in the standard cell-mediated cytotoxicity assay at the designated effector:target ratios. Values presented are percentage cytotoxicity (mean ± SE).

The transfection of target cells with a glycoprotein complexed to the cation lipid, DOTAP, as described by Walker et al. (26) represents a novel method for evaluating the potential antigenicity of a protein with respect to recognition by class I MHC-restricted CTL. It is particularly suitable for those proteins for which neither a cloned cDNA nor extensive protein sequence data are available. Following the tentative identification of Meth A gp110 as a Meth A TRA and the isolation of a cloned H-2K^d-restricted, anti-Meth A specific CTL, “protein transfection” seemed ideal for evaluating the potential antigenicity of Meth A gp110. The results of the analysis of SVBalb cells, which had been treated with Meth A gp110 and DOTAP, indicate that the treatment sensitized the cells to cytolysis by the anti-Meth A CTL cell line, CTLMA-9C. Increasing levels of cytolysis were evident for cells treated with DOTAP and concentrations of Meth A gp110 ranging from 12 to 100 ng/ml. At antigen concentrations of 200 ng/ml or more, however, lower levels of lysis were observed. This situation is similar to that reported by Walker et al. (26), who noted that...
treatment of cells with DOTAP and high concentrations of glycoprotein B antigen resulted in less sensitization, and hypothesized that large amounts of protein might interfere with delivery and/or processing of the antigen.

Two lines of evidence indicate that sensitization of SVBalb cells to cytolysis by anti-Meth A CTL was due to presentation in association with H-2K<sup>d</sup> molecules of a gp110-derived peptide. It was demonstrated that: (a) cytolysis of sensitized cells was blocked by antibodies against H-2K<sup>d</sup> molecules but not by antibodies against H-2D<sup>d</sup> molecules; and (b) the sensitization of the SVBalb cells was abrogated by treatment of the cells in the presence of BFA, an antibiotic which blocks exit of class I MHC molecules from the endoplasmic reticulum (26, 27).

Since the presence of antigenic peptides bound to a “carrier” protein has been proposed to account for the observed immunogenicities of several previously isolated, heat shock protein-related Meth A TRA (35-38), the Meth A gp110 used in our experiments was isolated by HPEC under reducing conditions. Sequence analyses of the NH<sub>2</sub>-terminal and internal peptides of independently isolated preparations of HPEC-purified Meth A gp110 failed to detect sequence heterogeneities. It is highly unlikely, therefore, that these preparations of gp110 contained unrelated proteins or peptides.

Although the results of the study by North and Bursuker (25) did involve CD8<sup>+</sup> T-cells in tumor rejection of Meth A sarcoma, Ward <em>et al.</em> (24) have pointed out that there is no evidence that these cells are commonly required for rejection of chemically induced sarcomas. While the restricted specificities of the immune responses against chemically induced sarcomas reflect the specificity of reactivity normally attributed to T-cell-mediated responses, other immune cells, such as natural killer cells and macrophages, might also play critical roles in effective tumor eradication. As an approach to defining the role of the CTLMA-9C-recognized determinant in tumor rejection of Meth A sarcoma, we immunoselected for a CTL-resistant variant of Meth A. This variant, designated Meth A4R, was isolated following four rounds of immunoselection. Its resistance to cytolysis by CTLMA-9C did not appear to be due to an alteration in expression of class I MHC molecules or general resistance to cell-mediated cytolysis.

The subsequent indications that Meth A4R is less immunogenic and less antigenic than the parental tumor suggest that its resistance is due to a decrease in the level of its expression of the CTL-defined tumor

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3 Loftus, K. Sakaguchi, A. B. De Leo, and E. Appella, unpublished results.
peptide and that this determinant plays a functionally significant role in tumor rejection of Meth A sarcoma.

The basis for the apparent decrease or loss of tumor antigen expression by Meth A4R is presently unclear, since Meth A4R cells express gp110 at a level comparable to that of the parental tumor. The pattern of migration of Meth A4R gp110 upon 7.5% SDS-PAGE, however, is distinct from that of Meth A gp110. While Meth A gp110 electrophoreses as a discrete doublet with the lower band being the predominating species (7), Meth A4R gp110 migrates as a "doublet" with a discrete, predominant lower band but a diffuse upper "band" (data not shown). This observation suggests that posttranslational modification of gp110 might account for the apparent loss or decrease in tumor antigen expression by Meth A4R cells.

Since the cloned anti-Meth A CTL cell line used in this study was isolated from mice immunized with Meth A sarcoma, not a purified antigen preparation, its specificity was not influenced by either immunization of mice with a particular antigen or restimulation of lymphocytes from Meth A-immune mice with that antigen. Consequently, the apparent recognition by CTLMA-9C of an epitope expressed by Meth A gp110 is an independently derived characteristic of this CTL.

The focus of our future research will be to demonstrate the antigenic polymorphisms of gp110 isolated from antigenically distinct sarcomas using the protein transfection technique as well as by molecular cloning of gp110 cDNA. In an effort to extend our present results, we have recently isolated a cloned H-2Kb-restricted, anti-CMS4-specific CTL cell line. We intend to determine by protein transfection experiments whether this CTL cell line recognizes an epitope expressed on CMS4 gp110. In the future, these antitumor CTL cell lines will be valuable probes for analyzing target cells transfected with CDNAs encoding gp110 isolated from these antigenically unrelated sarcomas, thereby confirming the expression by these molecules of tumor-specific CTL-defined tumor peptides and facilitating their identification.

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REFERENCES


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