Generation of Antigenic Variants from a Nonantigenic Murine Tumor Cell Line by Transfection with a Gene Encoding a Novel Tumor-specific Transplantation Antigen

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ABSTRACT

Tumors induced in mice by UV radiation often express highly immunogenic tumor-specific transplantation antigens (TSTA). The 216 gene, which encodes a TSTA of the C3H tumor UV-1591, has been cloned and characterized as a novel major histocompatibility complex Class I antigen. The purpose of this study was to determine whether the 216 gene-encoded TSTA can function as a tumor rejection antigen when expressed on unrelated, nonantigenic murine tumor cells or whether its function is restricted to UV-induced tumors. A cell line (10T-1) derived from a spontaneous transformant of C3H 10T/2 cells was cotransfected with DNA from p216 and pSV2-neo plasmids. About 2 wk after transfection, 216-positive colonies were isolated and tested for cell surface expression of the 216 gene product using a monoclonal antibody specific for 216 gene-encoded TSTA. Of 20 clones tested, four expressed high levels of 216 gene-encoded TSTA. These four clones were highly antigenic in that they were completely rejected in normal mice but grew progressively in nude mice. Furthermore, the 216-positive clones were immunologically cross-reactive with UV-1591, as determined by in vitro cytotoxic T-lymphocyte and in vivo immunization and challenge assays. Surprisingly, 216-positive 10T-1 transfecteds also cross-reacted with 10T-1 cells, both in vitro and in vivo. These results demonstrate that the product of a cloned TSTA gene from a UV-induced murine tumor is capable of functioning as a tumor rejection antigen when expressed on unrelated, nonantigenic tumor cells. In addition, these results indicate that this approach could be used to augment the immune response against poorly antigenic tumors.

INTRODUCTION

Most skin cancers induced in mice by UV radiation are highly antigenic in that they are immunologically rejected when transplanted into normal syngeneic recipients, but grow progressively in immunsuppressed mice (1–3). The molecular nature of TSTA expressed on UV-induced murine tumors is unknown at present with the exception of one tumor, UV-1591. Studies by Philippis et al. (4) have shown that UV-1591 tumor cells express novel MHC Class I antigens on their surface, as defined by their recognition by H-2-specific monoclonal antibodies, peptide mapping, cloning, and DNA sequencing. Moreover, the three novel Class I genes expressed on this tumor bear a striking homology to the H-2d and H-2" transplantation antigens.5,6 These novel Class I antigens are expressed on UV-1591 tumor cells in addition to the normal H-2K and H-2D antigens (4–6). The genes encoding these novel antigens have been cloned (6) and sequenced (7). Recently, Stauss et al. (8) showed that the product encoded by the gene designated 216 is in fact the product recognized as a tumor rejection antigen in the syngeneic rejection of 1591 tumor.

Variants of UV-1591 tumor that have lost expression of this novel TSTA (9) and the gene encoding it (8) have also been isolated. The loss of this gene product correlated with the ability of certain variants to grow in normal mice (4, 9), which indicated that it plays an essential role in limiting transplanted tumor growth consistent with their Class I nature and variation from normal C3H H-2 products. More important, transfection of this novel Class I gene into a UV-1591 progressor variant that had lost this antigen produced a reversion of the progressive growth behavior of the variant to the regressor phenotype, thus providing strong evidence that this novel Class I molecule functions as a tumor rejection antigen (8). However, it is not known whether this antigen can also function as a major tumor-rejection antigen when expressed on unrelated, nonantigenic tumors or whether its function is restricted to UV-1591, which expresses additional novel MHC Class I antigens. Therefore, we transfected this gene into an unrelated, nonantigenic murine tumor to demonstrate the alloantigenicity of these products.

MATERIALS AND METHODS

Animals. Six- to 8-wk-old specific-pathogen-free female C3H/HeN (mammary tumor virus negative) and athymic nude (nu/nu) mice were obtained from the Frederick Cancer Research Center Animal Production Area.

Tumor Cell Lines. UV-1591 is a fibrosarcoma induced in a mammary tumor virus-negative C3H/HeN mouse by chronic UV irradiation (2). The UV-1591 tumor is highly antigenic and regresses when transplanted into normal syngeneic mice. The 10T-1 cell line arose by spontaneous transformation of C3H mouse embryo fibroblast 10T1/2 cells.7 A highly tumorigenic clonal cell line of 10T-1 was established by serial transplantation in normal C3H mice. UV-2237 is a fibrosarcoma induced in a C3H/HeN mouse by chronic UV irradiation and is tumorigenic in normal C3H/HeN mice. MCA-113 is a fibrosarcoma induced in UV-irradiated C3H mice by s.c. injection of 3-methylcholanthrene on the unirradiated skin. The MCA-113 cell line is highly antigenic and is routinely rejected when transplanted into normal C3H mice. Tumor cells were grown in DMEM (Grand Island Biological Co., Grand Island, NY) containing 10% heat-inactivated FBS (HyClone Laboratories, Inc., Logan, UT), penicillin (100 units/ml), and streptomycin (100 μg/ml). All the cell lines were tested for and found to be free of Mycoplasma.

Plasmids. The complementary DNA clone (p216) encodes a novel tumor rejection antigen expressed on UV-1591 tumor cells (6). The pSV2-neo plasmid was obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, MD). Plasmid DNAs were isolated according to

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3 To whom requests for reprints should be addressed, at Department of Immunology, Box 178, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030.
4 The abbreviations used are: TSTA, tumor-specific transplantation antigen; MHC, major histocompatibility complex; Mab, monoclonal antibody; DNEME, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; RPMI, Roswell Park Memorial Institute; MLTC, mixed-lymphocyte tumor culture; GAM, goat anti-mouse; CTL, cytotoxic T-lymphocyte; FBS, fetal bovine serum.
5 D. R. Lee, R. J. Rubocki, W. Lie, and T. H. Hansen. The murine MHC complex (mammary tumor virus negative) and athymic nude (nu/nu) mice were obtained from the Frederick Cancer Research Center Animal Production Area.
6 Tumor Cell Lines. UV-1591 is a fibrosarcoma induced in a mammary tumor virus-negative C3H/HeN mouse by chronic UV irradiation (2).
7 The abbreviations used are: TSTA, tumor-specific transplantation antigen; MHC, major histocompatibility complex; Mab, monoclonal antibody; DNEME, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; RPMI, Roswell Park Memorial Institute; MLTC, mixed-lymphocyte tumor culture; GAM, goat anti-mouse; CTL, cytotoxic T-lymphocyte; FBS, fetal bovine serum.
8 R. S. Goodenow and R. Linsk, unpublished results.

7 H. N. Ananthaswamy, unpublished results.
the procedure described by Maniatis et al. (10). Superoiled plasmid DNA was purified on CsCl gradients.

DNA-mediated Gene Transfer. The DNA isolated from a plasmid containing the 216 gene (6) subcloned as a HindIII-Sal fragment in the vector was transfected into 10T-1 recipient cells along with DNA from the plasmid pSV2-neo (which confers resistance to the antibiotic G418), by the calcium phosphate precipitation technique of Graham and Van der Eb (11) and Southern and Berg (12). Twenty µg of p216 DNA plus 2 µg of pSV2-neo DNA were transfected onto four 60-mm plates containing 4 x 10^5 cells/plate. As controls, 10T-1 cells were transfected with pSV2-neo DNA alone to determine whether integration of foreign DNA had any effect on the antigenic properties of the recipient cells. About 24 h after transfection, cells from each dish were trypsinized and transferred to two 100-mm dishes. After another 16 to 20 h, G418 was added to the medium at a concentration of 400 µg/ml. The cells were fed with fresh medium containing G418 every 3 to 4 days. About 2 wk after transfection, individual G418-resistant colonies were isolated using sterile glass cloning cylinders and expanded in culture.

Indirect Immunofluorescence Assay. The individual transfected clones were analyzed for cell surface expression of 216 gene-encoded TSTA using monoclonal antibody Mab CP28 which specifically reacts with this gene product (6). The CP28-reactive antigen present on UV-1591 cells has been identified as the antigen that causes tumor rejection in normal syngeneic mice (8).

Single cell suspensions derived from different monolayer cultures by treatment with trypsin and EDTA were washed with PBS, and 1 x 10^6 cells were incubated with 100 µl of appropriately diluted CP28 on ice for 60 min. After 2 washes with PBS containing 5% FBS and 0.02% sodium azide, the cells were incubated on ice for 30 min with 50 µl of fluorescein isothiocyanate-conjugated GAM antibody (Cappel Laboratories, Cochrannie, PA). After additional washes, the cells were suspended in 1 ml of PBS containing 1% paraformaldehyde and then analyzed on a fluorescence-activated cell sorter (Ortho Cytofluorograph IIs; Ortho Diagnostics, Inc., Westwood, MA). Each cell line was stained with the second GAM antibody alone to determine the background fluorescence.

Rosetting Assay. Some of the clones that were positive for expression of 216 gene-encoded TSTA, as determined by indirect immunofluorescence assay, were subcloned by the rosetting technique described by Littman et al. (13), with slight modifications. Briefly, 5 x 10^4 cells were plated in DMEM in 150-mm dishes and grown until visible colonies were formed (about 1 wk). The medium was removed, and the cells were washed with PBS containing calcium and magnesium and incubated with anti-cell-surface-purified Mab CP28 (diluted 1:2000 in PBS containing 5% FBS) for 1 h at room temperature. The cells were washed 3 times with PBS and incubated with 7 ml of ovine erythrocytes conjugated with goat anti-mouse IgG (2% stock solution diluted 1:10 in PBS containing 5% FBS) (14). After incubating for 1 h at room temperature, free erythrocytes were gently aspirated and washed 3 times with PBS, and the rosette-positive red colonies were isolated using sterile glass cloning cylinders.

Antigenicity and Tumorigenicity Assays. To determine the antigenicity and tumorigenicity of 10T-1 transfecants expressing the 216 gene product, near-confluent cells were harvested by trypsinization and washed with serum-free RPMI 1640 medium. Viable cells (2 x 10^6) in 0.2 ml of serum-free RPMI were injected s.c. into groups of normal C3H mice, and 2 wk later the mice were exposed to 450 R of X-irradiation to prevent a primary response to tumor challenge while leaving the secondary response intact (16). As a control, groups of mice were given injections s.c. of 0.2 ml of RPMI medium alone. Forty-eight h later, 10 x 10^6 cells from G418-resistant clones of the antigenic transfecants, UV-1591 tumor, 10T-1 recipient cell line, or a syngeneic, non-cross-reactive tumor such as UV-2237 were injected s.c. on the contralateral side. In addition, groups of mice were also immunized with either UV-1591 or γ-irradiated (10,000 R) 10T-1 cells and challenged with various tumor cell lines. Tumor growth was monitored for 4 to 6 wk after injection.

RESULTS

Cells of the 10T-1 line were tested for tumorigenic potential and intrinsic antigenicity prior to their use as recipients in transfection experiments. Tumorigenicity of the 10T-1 cell line was assessed by injecting s.c. 1 x 10^6 or 2 x 10^6 cells into groups of 10 normal C3H mice. All mice developed tumors within 4 wk after injection (data not shown).

The intrinsic immunogenicity of 10T-1 cell line was determined by the procedure described by Prehn and Main (17). The results indicated that the 10T-1 tumor cell line was not detectably immunogenic, since extirpation of the primary tumor mass did not induce resistance against subsequent challenge with 10T-1 tumor.

In addition, the 10T-1 cell line did not react with the Mab CP28 specific for the 216 gene-encoded TSTA (Fig. 1) and thus is devoid of the epitope recognized by Mab CP28. However, the 10T-1 cell line expressed normal levels of H2-K^d and H2-D^d antigens (data not shown), as detected by an indirect immunofluorescence assay using anti-H-2K^d (16-1-11N) and anti-H-2D^d (15-5-5S) Mabs (18). Because of these properties, 10T-1 cells were suitable as recipient cells for transfection studies.

Following cotransfection of 10T-1 cells with DNAs from p216 and pSV2-neo plasmids, the transfecants were selected in G418 medium. The G418-resistant colonies were obtained at a frequency of 200 to 250 per 4 x 10^5 transfected cells. Several G418-resistant colonies were isolated randomly, expanded in culture, and analyzed for cell surface expression of 216 gene-encoded TSTA using Mab CP28. Of 20 clones tested, 4 expressed high levels of 216 gene-encoded TSTA (Table 1). The fluorescence intensities of these 4 clones were equal to or greater than that of UV-1591 tumor cells. These 4 clones, designated 10T-216-1, 10T-216-2, 10T-216-5, and 10T-216-9.
were used in further studies, as were 2 transfected clones (10T-216-3 and 10T-216-4) that were G418 resistant but negative for expression of 216 gene-encoded TSTA. The fluorescence profile of two representative 216-positive transfectants shown in Fig. 1 reveals that both of them strongly reacted with the Mab CP28. As expected, the UV-1591 tumor, from which the 216 gene was cloned, also reacted strongly with Mab CP28. In contrast, as mentioned before, the 10T-1 cell line did not react with Mab CP28. Two clones, 10T-216-1 and 10T-216-5, were subcloned by rosetting, and cells from the rosette-positive colonies were used in subsequent studies.

The antigenicity and tumorigenicity of 10T-1 transfectants were assessed by injecting 2 × 10⁶ cells s.c. into normal C3H and nude mice. The data shown in Table 1 indicate that all 4 transfectants expressing the 216 gene product were rejected by normal C3H mice and yet retained their tumorigenicity as exhibited by 100% tumor incidence in nude mice. As expected, the control UV-1591 cells produced tumors only in nude mice but not in normal C3H mice. In contrast, the 2 transfectants that were negative for 216 gene expression (10T-216-3 and 10T-216-4) were tumorigenic in both normal and nude mice, as was the 10T-1 cell line.

In order to determine whether the antigenic transfectants are cross-reactive with the UV-1591 tumor cell line, an in vitro CTL and an in vivo immunization and challenge assay were performed using two clones that were highly positive for 216 gene-encoded TSTA (10T-216-1 and 10T-216-5). The results shown in Fig. 2 reveal that UV-1591-immune CTLs were able to kill not only UV-1591 target cells, but also 216-positive 10T-1 transfectants. In contrast, UV-1591-specific CTLs were unable to kill 10T-1 target cells and an unrelated syngeneic tumor, MCA-113.

Table 1 In vivo tumorigenicity of 10T-1 transfectants

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Expression of 216 antigen</th>
<th>Tumor incidence* (no. with tumor/no. injected)</th>
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<tbody>
<tr>
<td>10T-216-1</td>
<td>+</td>
<td>0/10 10/10</td>
</tr>
<tr>
<td>10T-216-2</td>
<td>+</td>
<td>0/5  5/5</td>
</tr>
<tr>
<td>10T-216-5</td>
<td>+</td>
<td>0/10 10/10</td>
</tr>
<tr>
<td>10T-216-9</td>
<td>+</td>
<td>0/5  5/5</td>
</tr>
<tr>
<td>10T-216-3</td>
<td>−</td>
<td>0/5  5/5</td>
</tr>
<tr>
<td>10T-216-4</td>
<td>−</td>
<td>0/5  5/5</td>
</tr>
<tr>
<td>10T-1</td>
<td>−</td>
<td>0/10 10/10</td>
</tr>
<tr>
<td>UV-1591</td>
<td>+</td>
<td>0/10</td>
</tr>
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</table>

* Two million cells were injected s.c. Tumor growth was monitored at weekly intervals for 8 wk.

Cross-reactivity between UV-1591 and 10T-216 transfectants was also investigated in a cold-target inhibition assay (15). CTLs against UV-1591 killed about 65% of 51Cr-labeled 10T-216 targets at an effector/target ratio of 50:1. When unlabelled UV-1591 tumor cells were added to the reactive mixture at 1:1, 1:2, and 1:4 ratios of hot to cold targets, the killing of hot 10T-216 targets decreased to 57%, 40%, and 29%, respectively (data not shown). In contrast, addition of unlabelled 10T-1 cells had little (<10%) or no effect on the cytotoxicity of UV-1591-specific CTLs against 10T-216-1 cells (data not shown). Thus, these results indicated that target antigens other than the 216 gene product were not involved in the killing of 10T-216 transfectants by UV-1591 CTLs.

In a reciprocal experiment, CTLs were generated by immunizing mice with one of the 216-positive 10T-1 transfectants (10T-216-1) and used as effectors against various target cells. The results shown in Fig. 3 indicate that CTLs against 10T-216-1 cells were cytotoxic to UV-1591 and the two 216-positive 10T-1 transfectants. Surprisingly, 10T-216-1-specific CTLs also killed 10T-1 target cells very effectively. As before, there was no significant killing of MCA-113 tumor cells by 10T-216-1-specific CTLs.

In order to determine whether the killing of 10T-1 cells by 10T-216-1 CTLs was mediated by CTLs or some other effector cell types, 10T-216-1 CTLs were treated with either anti-mouse

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**Fig. 1.** Cell surface expression of 216 gene-encoded TSTA in 10T-1 transfectants. Single cell suspensions were stained with the monoclonal antibody CP28 plus GAM, respectively, for the various cell lines as described in "Materials and Methods." Fluorescein isothiocyanate-conjugated GAM was used as a second antibody. The percentages of cells reactive with GAM were as follows: UV-1591, 2.7% and 66%; 10T-1, 4% and 4.2%; 10T-216-1, 3% and 67%; 10T-216-5, 8.6% and 99.9%.

**Fig. 2.** Killing of 216-positive 10T-1 transfectants by UV-1591 CTLs. C3H mice were immunized by i.p. injections of 5 × 10⁶ UV-1591 cells. Two wk later, their spleen cells were cocultured with γ-irradiated (10,000 R) UV-1591 cells for 5 days. The lytic activity of these effector cells was tested against various target cells in a 4-h ⁵¹Cr release assay. O, UV-1591; •, 10T-216-1; △, 10T-216-5; ●, 10T-1; □, MCA-113. Bars, SE.

**Fig. 3.** Cytotoxic activity of 10T-216-1 CTLs. CTLs were generated in cultures of spleen cells from mice immunized in vivo and then in vitro with 10T-216-1 cells. Other parameters were identical to experiments described in Fig. 2. The various targets used are: UV-1591 (O), 10T-216-1 (●), 10T-216-5 (△), 10T-1 (□), and MCA-113 (□). Bars, SE.
Thy 1.2 monoclonal antibody (Becton Dickinson, Mountain View, CA) and complement or rabbit anti-asialo-GM1 antibody (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and complement and then used in the CTL assay against 10T-1 targets. The results indicated that treatment with anti-Thy 1.2 antibody and complement but not with anti-asialo-GM1 antibody and complement totally abrogated the cytotoxic activity of 10T-216-1 CTLs (data not shown). This demonstrated that the killing of 10T-1 cells was mediated by CTLs and not natural killer cells.

Cross-reactivity between 216-positive transfectants and UV-1591 was also investigated in an in vivo immunization and challenge assay. As shown in Table 2, immunization with either 10T-216-1 or 10T-216-5 clones generated protective immunity against challenge with the immunizing tumor. In addition, there was complete protection against challenge with UV-1591 tumor and the other 216-positive transfectant clone, but not against challenge with another syngeneic, non-cross-reactive UV-induced tumor, UV-2237. Interestingly, immunization with either 10T-216-1 or 10T-216-5 cells followed by challenge with the 10T-1 cell line also reduced 10T-1 tumor incidence (1/10 and 0/5). As expected, all the mice immunized with RPMI and challenged with various tumor cell lines developed tumors at the challenge site.

In a reciprocal experiment, immunization with UV-1591 tumor and challenge with either UV-1591 or with the two 216-positive transfectants resulted in complete protection against both UV-1591 and the two 216-positive transfectants, but not against challenge with either 10T-1 or UV-2237 (Table 3). As mentioned before, the 10T-1 cell line was neither immunogenic nor cross-protective with any of the tumor cell lines tested.

**DISCUSSION**

The identification and isolation of a gene (216) encoding a novel MHC Class I molecule from UV-1591 tumor that functions as a tumor rejection antigen afford new opportunities to examine tumor-specific antigenicity in terms of a single well-defined antigen. The most conclusive evidence that the 216 gene products correspond to a TSTA came from the transfection studies of UV-1591 progressor variants that had lost the dominant “A” antigen. Reintroduction of the 216 gene into A– progressor variants of UV-1591 resulted in tumor rejection by normal immunocompetent mice and reversion to the parental tumor phenotype (8). Stauss et al. (8) have shown that the 216-encoded antigen can elicit specific transplantation immunity against the parental tumor and is responsible for the regressor phenotype of the UV-1591 parental tumor cell line. Because UV-1591 expressed multiple TSTAs, several of which were shown to be altered MHC Class I antigens, it remained to be determined whether the 216 gene-encoded antigen can also function as a tumor rejection antigen when expressed on unrelated progressor tumors, or whether its function was restricted only to UV-1591 progressor variants.

To answer this question, we introduced the 216 gene into cells of an unrelated (non UV-induced), nonantigenic, progressor tumor line, 10T-1, and determined whether the 216 gene product could also function as a tumor rejection antigen in 10T-1 cells. Cotransfection of 10T-1 cell line with DNAs from p216 and pSV2-neo plasmids followed by selection in medium containing G418 yielded several hundred G418-resistant colonies. Of 20 randomly isolated G418-resistant colonies, 4 expressed the 216 gene-encoded antigen on their cell surface at levels equal to or greater than those of the parental UV-1591 tumor cell line (Fig. 1). The 10T-216-5 clone, in particular, reacted very strongly with the Mab CP28 (99% antigen-positive cells). This high expression could be due to insertion of multiple copies of the 216 gene into the 10T-1 genome or to overexpression of the gene.

All 4 transfectant clones expressing the 216 gene product were completely rejected when transplanted s.c. into normal syngeneic recipients, but grew progressively when injected s.c. into immune-deficient nude mice (Table 1). Thus, the 216-positive transfectants were highly antigenic, and their rejection in normal mice was immunologically mediated. In contrast, the two 10T-1 transfectants that were G418 resistant but did not express the 216 gene product grew progressively in both normal and nude mice. This result suggests that mere acquisition of the pSV2-neo plasmid DNA by 10T-1 transfectants is not sufficient to produce antigenic modification capable of inducing an immune rejection response in normal syngeneic mice. These results demonstrate that a cloned TSTA gene from a UV-induced murine skin cancer can function as a tumor rejection antigen when expressed on unrelated, nonantigenic tumor cells and cause their rejection in normal syngeneic mice.

**Table 2 In vivo cross-reactivity**

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Challenge tumor</th>
<th>Tumor incidence&lt;sup&gt;a&lt;/sup&gt; (No. of mice with tumor/no. of mice challenged)</th>
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<tbody>
<tr>
<td>RPMI</td>
<td>10T-216-1</td>
<td>5/5</td>
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<tr>
<td></td>
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<td>UV-2237</td>
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<sup>a</sup> C3H mice were immunized with a single s.c. injection of RPMI or 2 × 10<sup>6</sup> cells of 10T-216-1 or 10T-216-5. On Day 14, the mice were exposed to 450 R of X-radiation. Two days later, mice were challenged on the contralateral side with 2 × 10<sup>6</sup> cells of the indicated tumor cell lines.

<sup>b</sup> Data are for 4 wk after challenge.

**Table 3 In vivo cross-reactivity**

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Challenge tumor</th>
<th>Tumor incidence&lt;sup&gt;a&lt;/sup&gt; (No. of mice with tumor/no. of mice challenged)</th>
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<td>UV-2237</td>
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<sup>a</sup> Immunization and challenge were performed as described in the legend to Table 2, except that 10T-1 cells were irradiated with 10,000 R of γ-rays prior to immunization.

<sup>b</sup> Data are for 4 wk after challenge.
To determine whether transfectants expressing the donor TSTA were immunologically cross-reactive with the parental UV-1591 tumor, we performed in vitro CTL and in vivo immunization and challenge assays. The results indicated that the 216-positive 10T-1 transfectants were completely cross-reactive, both in vitro (Figs. 2 and 3) and in vivo (Tables 2 and 3), not only with UV-1591 tumor, but also with each other. Furthermore, this cross-reactivity was tumor specific in that immunity induced by UV-1591 tumor or by 216-positive 10T-1 transfectants did not cross-react with unrelated syngeneic tumors, such as MCA-113 and UV-2237. These results suggest that the antigenic molecules expressed on UV-1591 tumor and 216-positive 10T-1 transfectants may be similar or even the same. Interestingly, the recognition of this single antigen on 10T-1 cells appears sufficient to mediate the rejection of the UV-1591 tumor despite the fact that UV-1591 expresses two additional Class I gene products equally capable of generating immunity against this tumor (4–8).

Surprisingly, we also found that 10T-1 transfectants expressing the 216 gene product were also cross-reactive, both in vitro and in vivo, with the recipient, nonantigenic 10T-1 cell line (Fig. 3; Table 2). These results suggest that both 10T-1 cell line and 10T-1 transfectants expressing the 216 gene product may contain a “common” antigen. This “common” antigen seems to be nonimmunogenic by itself, but very immunosensitive because 10T-1 cells, despite their apparent inability to induce a detectable immune response, were nevertheless rejected by an ongoing immune response once elicited by the 216-positive 10T-1 transfectants. The ability of 216-positive 10T-1 transfectants to cross-react with the original 10T-1 tumor is a remarkably similar finding to that of Itaya et al. (19), who found that transfectants of mouse lung carcinoma (3LL) cells expressing an allogeneic MHC Class I antigen induced antitumor transplantation resistance against the original weakly immunogenic tumor. Since the 216 gene-encoded TSTA of UV-1591 tumor represents a true MHC Class I molecule, it is quite possible that this antigen can present processed peptides to generate CTLs cross-reactive with similar antigens restricted by the H-2K\(^+\) products on 10T-1 tumor. This would enhance the intrinsic immunogenicity of a previously nonimmunogenic or weakly immunogenic TSTA present on the original 10T-1 tumor, analogous to the 3LL transfectants expressing an allogeneic MHC Class I antigen (19). Contrary to these results are those of Cole et al. (20) who found that transfection of murine sarcoma cells with a cloned alloantigen gene did not reduce their tumorigenicity in syngeneic mice, suggesting that expression of a MHC Class I alloantigen on the surface of these tumor cells was insufficient for tumor rejection.

One of the main objectives of tumor immunology is to enhance immune responses to tumors that are not very immunogenic. Although the presence of TSTAs have been demonstrated on many virus-, chemical-, and UV-induced tumors, the failure to detect host immune responses against spontaneous tumors led to widespread skepticism that TSTA or any immune mechanism plays a role in controlling the growth of spontaneous tumors (21, 22). Efforts directed towards augmenting the immune response against poorly antigenic tumors by introducing “foreign” antigenic determinants into tumor cells have included viral infection (23–25), somatic cell hybridization (26–31), triinitrophenyl-haptenation of tumor cells (32, 33), and transfection with allogeneic and syngeneic MHC Class I genes (19, 34–36). In addition, recent studies indicate that various chemical (37, 38) and physical (39–41) mutagenic agents, and the nonmutagenic agent 5-azacytidine (42), are able to increase the antigenicity of poorly antigenic tumors, resulting in their immunological rejection in syngeneic hosts. The fact that 10T-1 transfectants expressing the 216 gene product can induce cross-protective immunity against the original nonantigenic 10T-1 cell line suggests that this approach, i.e., transfection with a cloned TSTA gene, could also be used to enhance the immune response against weakly immunogenic tumors. The exact mechanism by which 10T-1 transfectants expressing the 216 gene product induce cross-protective immunity against the original nonantigenic 10T-1 cells is as yet unclear.

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REFERENCES


Generation of Antigenic Variants from a Nonantigenic Murine Tumor Cell Line by Transfection with a Gene Encoding a Novel Tumor-specific Transplantation Antigen

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