The Cytotoxic T-Lymphocyte Response against a Poorly Immunogenic Mammary Adenocarcinoma Is Focused on a Single Immunodominant Class I Epitope Derived from the gp70 Env Product of an Endogenous Retrovirus

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ABSTRACT

The TS/A mouse mammary adenocarcinoma is a poorly immunogenic tumor widely used in preclinical models of cancer immunotherapy. CTLs have often been indicated as important in TS/A tumor destruction, but their generation in this model has been rarely studied, nor have their precise target(s) been identified. We hypothesized that the gp70 Env product of an endogenous murine leukemia virus could be a target antigen for TS/A-specific CTLs and investigated this possibility in four different TS/A cell lines engineered with the genes that encode IFN-α, IFN-γ, interleukin-4, and B7.1, respectively. All tumor cell lines expressed gp70, albeit at different levels, as demonstrated by reverse transcription-PCR analysis. Transfected tumor cells exhibited a delayed growth in vivo, and partial tumor regression. Spleen cells from mice that displayed tumor regression had high percentages of CD8+ T cells that were specifically stained with L^1 tetramers loaded with gp70_{423–431}, the antigenic epitope of gp70 protein. Mixed leukocyte-peptide and mixed leukocyte-tumor cultures, set up by stimulating splenocytes with the immunogenic peptide and with transfected TS/A tumor cells, respectively, resulted in significant large increases in tetramer-reactive CD8+ T cells and showed high lytic activity specific for gp70_{423–431}. Finally, in a Cold Target Inhibition assay, lytic activity of a mixed leukocyte-tumor culture was inhibited in an overlapping fashion by both the TS/A line used for restimulation and 293L^4 cells loaded with gp70_{423–431} peptide, but not by 293L^4 cells pulsed with an irrelevant H-2 L^1 epitope, thus demonstrating that all or most of the cytotoxic activity was directed exclusively against this antigenic epitope.

INTRODUCTION

Transfer and expression of cytokine or costimulatory genes into tumor cells has been regarded as a powerful tool to trigger a potent antitumor reaction in both experimental models and clinical trials. Although these studies have been conducted in a variety of different experimental models, the use of TS/A mouse mammary adenocarcinoma has become a sort of “gold standard” for this approach. In fact, this tumor cell line represents one of the few available models for mammary neoplasia, and attempts to increase its immunogenicity relied on transfecion with a wide variety of genes encoding both Th1 and Th2 cytokines, chemokines, and costimulatory molecules (1–13). Previous studies showed that the immune mechanisms operating in rejection of transfected TS/A tumors depends on the type of cytokine or molecule introduced and involves different host-dependent effector mechanisms and cells belonging to both the innate and adaptive immune response (1–3). Although the results of in vivo depletion experiments in these studies have frequently inferred a role for ThS/A-specific CTLs in tumor destruction, a precise molecular target(s) has never been described. To characterize the antigenic epitope(s) recognized by CTLs during TS/A rejection, in this study we used TS/A transfecants that expressed IFN-γ (TS/A-IFN-γ; Ref. 4) and IL-4 (TS/A-IL-4; Ref. 14), prototypic Th1 and Th2 cytokines, respectively; IFN-α, a cytokine involved in the innate immune response (TS/A-IFN-α; Ref. 6); and the B7.1 costimulatory molecule (TS/A-B7.1; Ref. 7).

It has been previously reported that retrovirally encoded proteins are potential immunoreactive antigens expressed by a variety of mouse tumor cell lines. These observations have been made for viral antigens coded by the Friend-Moloney-Rauscher MuLV complex (15, 16) and, more recently, for products of endogenous MuLV (17–19). Here we report that regression of genetically engineered TS/A, irrespective of the cytokine or costimulatory molecule introduced, is associated with generation and expansion of tumor-specific CTLs that recognize a single antigenic peptide (gp70_{423–431}; AH1) derived from the envelope protein gp70 of an endogenous MuLV, which has already been demonstrated to behave as the CTL-immunodominant epitope of CT26 colon carcinoma (19). In addition, the AH1-specific CTL response elicited comprises most, if not all, of the lytic activity inducible against TS/A tumors undergoing rejection, as demonstrated by cold-target inhibition experiments, thus indicating that gp70_{423–431} can be considered the immunodominant tumor antigen of anti-TS/A CTL response.

MATERIALS AND METHODS

Mice. We purchased 6–8-week-old female BALB/c mice (H-2^d) from Charles River Laboratories (Calco, Como, Italy) and housed them in a specific pathogen-free animal facility. Procedures involving animals and their care were in conformity with institutional guidelines that complied with national and international laws and policies.

Cell Lines and CTL Bulk Culture and Clone. The TS/A parental cell line TS/A-pc (20) and its transfected derivatives TS/A-psVneo, which contains a mock plasmid (5); TS/A-IFN-γ, which secretes 6000 IU/ml IFN-γ (4); TS/A-IL-4, which secretes 1000 IU/ml IFN-α (6); TS/A-IL-4, which secretes 40 IU/ml IL-4 (4); and TS/A-B7.1, which expresses B7.1 molecule (7), have been described previously. The 293L^4 cell line is a human embryonal kidney cell line stably transfected with pL4.444 plasmid (kindly provided by Dr. U. D’Oro, NIH, Bethesda, MD), which expresses the H-2 L^4 class-I molecule. This cell line does not express gp70 (data not shown). The CT26 colon adenocarcinoma and the J558 myeloma were both from BALB/c mice.

All tumor cell lines were cultured in DMEM (Life Technologies, Inc., Paisley, United Kingdom) supplemented with 2 mM L-glutamine (Life Technologies), 10 mM HEPES (PAA Laboratories, Linz, Austria), 50 μM
2-mercaptoethanol (Sigma, St. Louis, MO), 150 units/ml streptomycin (Bristol-Myers Squibb, Sermoneta, Latina, Italy), 200 units/ml penicillin (Pharmacia & Upjohn, Milan, Italy), and 10% heat-inactivated FCS (Life Technologies). Transfected TS/A and 293Ld cells were cultivated in the presence of 500 μg/ml G418 (Geneticin; Life Technologies, Inc., Grand Island, NY) calculated to give 100% antibiotic activity. Bulk-AHI was obtained as described in the “Results” section. P1A_{35-45}-specific LDA5 CTL clone was obtained from spleen cells of a BALB/c mouse immunized with the previously described pBKCMV-P1A plasmid (21) by several in vitro restimulations with syngeneic splenocytes pulsed with P1A_{35-45} peptide and after limiting-dilution cloning. Bulk-AHI and the LDA5 CTL clone were cultured in flat-bottomed 24-well plates (Falcon, Becton Dickinson, Lincoln Park, NJ) in the presence of 3 × 10^6 syngeneic splenocytes pulsed with gp70_{423-431} and P1A_{35-45} peptides (1 μM), respectively, in 2 ml of DMEM containing 10% fetal bovine serum and supplemented with 20 μl/ml recombinant IL-2 (courtesy of Euro Cetus-Chiron, Milan, Italy).

**gp70 Expression Analysis.** Total RNA was extracted by Trizol (Life Technologies) from cultured cells; its concentration and quality were assessed by absorbance (A_{260nm}) and the electrophoretic pattern. We reverse-transcribed 1 μg of RNA in a final volume of 30 μl with use of M-MLV reverse transcriptase (Life Technologies) in the presence of random examers as primers (Applied Biosystems) and deoxynucleotide triphosphates. We used 1 μl of cDNA for RT-PCR in a final volume of 25 μl. RT-PCR reactions were set up with primer pairs for gp70 (19) and the housekeeping gene product GAPDH (Clontech, Palo Alto, CA). PCR was carried out by PLATINUM Taq DNA Polymerase (Life Technologies) with cycles consisting of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 1 min. PCR was terminated by elongation at 72°C for 7 min. For both PCR products, a range of 0–43 peptide and after limiting-dilution cloning.

**Bimolecular MHC-Peptide Tetrameric Complexes.** DNA coding for Ld , the monomeric MHC-peptide complexes were formed by combining the MHC Ld , the monomeric MHC-peptide complexes were formed by combining the MHC Ld and a 3′ Ii 35–43 peptide with the BirA enzyme (Avidity, Denver, CO), according to the manufacturer’s instructions, and purified from free biotin by gel-filtration chromatography. Extravidin-R-PE-conjugate (Sigma) was mixed with the biotinylated proteins at a molar ratio of 1:4 to form the peptide-MHC tetramer-PE complex.

**Cell Staining and Flow Cytometry Analysis.** Fresh splenocytes (3 × 10^6/sample) were resuspended in 100 μl of FACS buffer (0.9% NaCl solution containing 2% BSA and 0.02% NaN_3; both from Sigma) with antimouse IgG receptor 2.4G2 mAb ascites (ATCC HB-197) for 10 min at room temperature to reduce the nonspecific staining. After washing, cells were resuspended in FACS buffer and labeled with Ld-gp70_{423-431} tetramer-PE (5 μg/ml) for 20 min at room temperature. Each sample was then stained at 4°C with rat antimouse CD8-Tri-color (0.1 μg/10^6 cells; clone CTCD8α; Caltag, Burlingame, CA) and with hamster antimouse CD3-FITC (1 μg/10^6 cells; clone 145–2C11; Caltag). In vitro-restimulated spleen cells (10^6/sample) were stained with tetramer-PE and rat antimouse CD8-FITC (0.1 μg/10^6 cells; clone CTCD8α; Caltag). Before analysis, cells were washed twice, resuspended in FACS buffer, and analyzed with a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Every sample was also stained with the control P1A_{35-45} tetramer-PE according to the described protocols. In addition, untreated mice were used as negative controls for experimental samples. Tetramer specificity was analyzed by cross-staining of Bulk-AHI and LDA5 CTL clone. Data analysis was carried out using Cell Quest software (Becton Dickinson).

**RESULTS**

**Analysis of gp70 Expression in Parental and Transfected TS/A Mammary Adenocarcinoma Cells.** It has previously been reported that many murine tumor cell lines express the genes of MuLV, which may behave as target of the CTL response (17–19, 26). We therefore sought to test whether gp70 was also expressed by parental TS/A and derivative sublines engineered to express cytokines or costimulatory molecules. As shown in Fig. 1, semi-quantitative RT-PCR conducted in nonsaturating conditions revealed the presence of a gp70 transcript that was quantitatively comparable to that of the GAPDH housekeeping gene. The gene expression profile of TS/A cells (data not shown), assessed with the Affymetrix technology, confirmed very high expression of sequences of the envelope of the...
endogenous MuLV (GenBank accession no. D10049; probes covering the region from 2155 to 2593), whereas no expression was detected for murine endogenous mammary tumor virus (GenBank accession no. AF263910; probes covering the region from 2688 to 2829). Notably, the HER-2/neu transgenic mammary tumor cell line TUBO, derived from the BALB/c strain (27), also showed the gp70 expression, with RT-PCR products observable after 30 amplification cycles.

The gp70-derived AH1 Epitope Is a Target of CTL Recognition in Parental and Transfected TS/A. On the basis of these observations, we presumed that gp70 could behave as a TAA in TS/A adenocarcinoma cells and stimulate the generation of gp70-specific CTLs. To test this hypothesis, we inoculated a group of syngeneic BALB/c mice with TS/A-IFN-α, whose primary challenge rejection has previously been associated with a CD8+ T-cell line and a panel of control cell lines. As expected, this CTL line was assayed against both parental and transfected TS/A cells, whereas J558, which harbors P1A endogenously, and 293Ld cells loaded with P1A35-43 peptide were used for LDAS. Shown in the upper left corner of each plot is the expression of class I H-2 Ld molecule, as determined by flow cytometric analysis, with the corresponding mean fluorescence intensity. The data shown are representative of at least two independent experiments.

Bulk-AH1, which likely reflects the extent of Ld class I molecule expression. The poor killing observed against TS/A-IL-4 likely depended on the extinction of a clone that exhibited both low class I expression and reduced susceptibility to lysis, as demonstrated by parallel experiments conducted with an anti-H-2d mixed leukocyte culture (data not shown). As a control, a P1A-specific CTL clone did not lyse TS/A tumor cells but killed P1A peptide-pulsed 293Ld cells and J558 plasmacytoma cells endogenously harboring the P1A antigen. J558 cells were found to express low levels of gp70 transcript (10–20-fold less than TS/A cells) by RT-PCR analysis (data not shown); accordingly, the extent of recognition by Bulk-AH1 was also very low.

On the whole, these data demonstrate that gp70 is expressed by TS/A tumor cells and that the AH1 antigenic epitope is correctly processed and presented in the context of MHC class I Ld molecule.

Fig. 2. The gp70-derived AH1 antigenic epitope is recognized by specific CTLs in parental (TS/A pc) and transfected TS/A cells. Each plot shows the cytotoxicity of Bulk-AH1 and the P1A-specific CTL clone LDAS (3) against the indicated cell lines. Positive controls for Bulk-AH1 were represented by CT26 and gp70123-431-pulsed 293Ld cells, whereas J558, which harbors P1A endogenously, and 293Ld cells loaded with P1A35-43 peptide were used for LDAS. Shown in the upper left corner of each plot is the expression of class I H-2 Ld molecule, as determined by flow cytometric analysis, with the corresponding mean fluorescence intensity. The data shown are representative of at least two independent experiments.

The AH1 Epitope Is the Immunodominant MHC Class I-restricted Antigen of TS/A Cells. To test whether gp70-specific CTL generation is induced in mice rejecting a TS/A primary tumor challenge, 1 week after tumor rejection spleen cells were directly stained with gp70-specific tetramers. As shown in Fig. 4A, rejection of TS/A tumors was accompanied by gp70-specific CD8+ T-lymphocyte expansion that appeared more pronounced in mice rejecting TS/A that secreted IFN-γ or expressed the B7.1 costimulatory molecule. Lower in vivo expansion was observed in mice receiving injections of TS/A-IFN-α, whereas animals inoculated with TS/A-IL-4 were almost negative. Staining with an unrelated tetramer (tet-P1A) always gave background results (data not shown). Control noninjected animals showed no appreciable reactivity with both tet-gp70 and tet-P1A (data not shown).

In vitro restimulation of splenocytes with the AH1 peptide led to dramatic expansion of tet-gp70+ CD8+ T lymphocytes, which was also very evident in cultures from mice inoculated with IL-4-secreting BALB/c mice receiving injections of replicating TS/A that secreted IFN-α, IFN-γ, or IL-4 or expressed B7.1 partially rejected the primary challenge, and tumors that were not rejected displayed a retarded rate of growth compared with TS/A parental cells or cells transfected with a mock plasmid that grew in all inoculated animals with rapid kinetics (Fig. 3).

Fig. 3. Kinetics of tumor growth in BALB/c mice inoculated with parental (TS/A pc) or transfected TS/A cells. Mice received s.c. injections of 105 replicating TS/A cells at day 0, and tumor growth was monitored by caliper measurements and reported as mean volume ± SD (bars). TS/A transduced with genes encoding IFN-α, IFN-γ, IL-4, or B7.1 underwent partial regression and tumors that were not rejected had delayed growth. Control mice receiving injections of parental cells or TS/A transfected with a mock plasmid (psVneo) had rapidly growing tumors. The cumulative numbers of mice used in the experiments are indicated in each plot.
Moreover, MLPCs from mice that had rejected a primary tumor challenge exhibited very high lytic activity against both gp70-pulsed 293Ld cells and the corresponding engineered TS/A used for challenge (data not shown), an exception being TS/A-IL-4 cells, which were not recognized by AH1-restimulated CTLs, in agreement with the low recognition by Bulk-AH1 (Fig. 2). The experiments described above demonstrated that rejection of a TS/A primary challenge brought about the generation of gp70-specific CTLs. To study whether the AH1 peptide represents the immunodominant epitope of anti-TS/A CTL response, we restimulated spleen cells from tumor-rejecting mice in vitro in a MLTC with the corresponding engineered TS/A used for challenge as stimulator, thus providing the culture with the entire antigenic array of TS/A. After 5 days of culture, MLTCs were tested for gp70-specific CD8⁺ T-lymphocyte expansion by tetramer staining and for cytotoxicity in a 4-h ⁵¹Cr-release assay. As shown in Fig. 4B, in vitro restimulation with tumor cells caused a gp70-specific CD8⁺ T-lymphocyte expansion; indeed, whereas TS/A-IL-4 did not almost restimulated spleen cells, likely due to the immunosuppressive activity of the secreted cytokine and the poor antigenicity of the cell line, TS/A-B7.1 induced extensive expansion of tet-gp70⁺ CD8⁺ T lymphocytes, probably because B7.1 expressed by the tumor cells provided a direct important costimulatory activity to antigen-specific CTLs. Accordingly, strong cytotoxicity was detected in MLTCs exhibiting high amounts of antigen-specific CTLs, whereas TS/A-IL-4-restimulated cultures had no lytic activity (Fig. 4C).

DISCUSSION

In this study we characterized the CTL response induced in vivo against TS/A mouse adenocarcinoma cells genetically engineered to express different cytokine or costimulatory molecules, i.e., IFN-α, IFN-γ, IL-4, and B7.1. Independent of the molecule introduced, the CTL response elicited was constantly directed against a single immunodominant epitope represented by the AH1 peptide derived from the gp70 product of an endogenous MuLV and corresponding to amino acids 423–431 of the protein (19). Indeed, tumor rejection was accompanied by increases in a CD8⁺ T-cell population that was stainable with gp70-specific tetramers; moreover, independent in vitro restimulation of splenocytes from mice that had rejected a primary

To evaluate the contribution of gp70-specific CTLs induced in MLTCs to anti-TS/A cytotoxicity, we carried out cold-target inhibition assays using peptide-loaded 293Ld cells and TS/A cells as cold cells to compete for lysis of ⁵¹Cr-labeled TS/A tumor cells. Increasing numbers of AH1 peptide-coated 293Ld cells, but not 293Ld cells loaded with the irrelevant H-2 Ld P1A epitope, were capable of inhibiting lysis of ⁵¹Cr-labeled TS/A tumor cells (Fig. 5). Interestingly, the cytotoxicity inhibition curve obtained with AH1 peptide-coated 293Ld cells almost completely overlapped that observed with cold TS/A, indicating that the gp70-specific CTLs were responsible for all or most of the lytic activity of MLTCs.

Fig. 4. gp70-specific tetramer staining and analysis of lytic activity of fresh splenocytes and MLTCs from mice rejecting a primary TS/A tumor challenge. One week after tumor rejection, spleen cells were costained with a Tri-color-conjugated anti-CD8 mAb, a FITC-conjugated anti-CD3 mAb, and PE-conjugated Ld-gp70 423–431 tetramer and were analyzed by FACSCalibur (A). After 5 days of culture, MLTCs were costained with FITC-conjugated anti-CD8 mAb and PE-conjugated Ld-gp70 423–431 tetramer (B) and assayed for lytic activity (C) against gp70 423–431-pulsed 293Ld cells (○), P1A 35–43-loaded 293Ld cells (□), and the corresponding engineered TS/A used for challenge (■). Data are means of triplicates of the percentages of specific ⁵¹Cr release at the indicated E:T ratios. The data shown are from one representative experiment of three that gave similar results.

TS/A (data not shown). Moreover, MLPCs from mice that had rejected a primary tumor challenge exhibited very high lytic activity against both gp70-pulsed 293Ld cells and the corresponding engineered TS/A used for challenge (data not shown), an exception being TS/A-IL-4 cells, which were not recognized by AH1-restimulated CTLs, in agreement with the low recognition by Bulk-AH1 (Fig. 2).

The experiments described above demonstrated that rejection of a TS/A primary challenge brought about the generation of gp70-specific CTLs. To study whether the AH1 peptide represents the immunodominant epitope of anti-TS/A CTL response, we restimulated spleen cells from tumor-rejecting mice in vitro in a MLTC with the corresponding engineered TS/A used for challenge as stimulator, thus providing the culture with the entire antigenic array of TS/A. After 5 days of culture, MLTCs were tested for gp70-specific CD8⁺ T-lymphocyte expansion by tetramer staining and for cytotoxicity in a 4-h ⁵¹Cr-release assay. As shown in Fig. 4B, in vitro restimulation with tumor cells caused a gp70-specific CD8⁺ T-lymphocyte expansion; indeed, whereas TS/A-IL-4 did not almost restimulated spleen cells, likely due to the immunosuppressive activity of the secreted cytokine and the poor antigenicity of the cell line, TS/A-B7.1 induced extensive expansion of tet-gp70⁺ CD8⁺ T lymphocytes, probably because B7.1 expressed by the tumor cells provided a direct important costimulatory activity to antigen-specific CTLs. Accordingly, strong cytotoxicity was detected in MLTCs exhibiting high amounts of antigen-specific CTLs, whereas TS/A-IL-4-restimulated cultures had no lytic activity (Fig. 4C).

Fig. 5. Cold target inhibition assay of MLTC. Titrated numbers of cold gp70-pulsed 293Ld cells (○), P1A 35–43-loaded 293Ld cells (□), and transfected TS/A (■) were admixed to hot-engineered TS/A cells and tested in a standard cytotoxicity assay with MLTCs from mice that had rejected a primary tumor challenge. Values are expressed as means of triplicates of the percentages of inhibition of the lysis of TS/A at an effector:hot target (T) ratio of 50. The data shown are representative of at least three independent experiments.
endogenous retroviral gene products are expressed in a variety of human tumors, which may provide the entire antigenic array of the tumor cells. Thus, a similar large increase in CTLs specifically recognizing the AH1 antigenic epitope. Finally, MLTC lytic activity could be inhibited in an overlapping fashion by both TS/A and gp70-negative AH1-pulsed cold target cells, indicating recognition of a common identical molecular target. It is therefore very likely that the AH1 antigenic epitope becomes the tumor immunodominant antigen of TS/A cells when they are made more immunogenic by the introduction of cytokines or immunostimulating molecules.

Previous studies have shown that, depending on the molecule transduced, different effector cells have been indicated to play crucial roles in the antitumor response elicited after s.c. injection of modified TS/A. In particular, the relevant host cells responsible for tumor inhibition appeared to be activated neutrophils for TS/A-IL-4 (14), macrophages for TS/A-IFN-γ (4), and CD8+ T lymphocytes in the case of TS/A-IFN-α (6) and TS/A-B7.1 (7). These conclusions were drawn mainly from histological findings at the site of s.c. tumor injection; on the other hand, in vivo CD8+ T-cell depletion experiments demonstrated the critical role of this T-lymphocyte subset in tumor rejection. Accordingly, we detected the generation of gp70-specific CTLs in all mice rejecting the primary challenge, irrespective of the cell line inoculated. It is questionable whether these CTLs play a direct killing role in vivo in tumor destruction, although this might be the case for TS/A-IFN-α and TS/A-B7.1, as tumor regression is associated with infiltration of CD8+ T cells and direct in vivo expansion of tet-gp70+ CD8+ lymphocytes. For TS/A-IL-4 and TS/A-IFN-γ, this scenario appears less likely because rejection of the former tumor primes but does not cause expansion of gp70-specific CTLs in vivo, whereas in the second case, despite the highest increase in tet-gp70+ CD8+ T cells, tumor development was shown to be hampered primarily by macrophages (4). Indeed, AH1-specific CTLs might play an important role in controlling metastases and secondary challenge with parental untransfected TS/A tumor cells: in all reported cases, primary tumor rejection led to establishment of long-term memory responses that depended by CD8+ T cells. It is not clear at present whether this control takes place through direct recognition and killing of neoplastic cells or through indirect mechanisms involving recruitment of other accessory effector cells. Notably, the IFN-γ-secreting TS/A cell line has been associated with a higher curative efficacy compared with TS/A cells transduced with different cytokine genes (5), and this likely reflects the high expression of class I molecules and IFN-γ-mediated activation of tumor-infiltrating macrophages that can enhance both direct and indirect presentation of tumor antigens. However, these aspects may be strongly dependent on the cell line tested because a previous report showed that in the CT26 model, IFN-γ-promoted tumors escape from the immune system by down-regulating gp70 expression (28).

Endogenous retroviral gene products are expressed in a variety of murine tumor cell lines of different haplotype and may behave as TAAs recognized by both CD4+ and CD8+ T cells (19, 26). In particular, the gp70-derived AH1 peptide is a target of CTL response in the tumor models represented by CT26 colon adenocarcinoma (19), CSM4 sarcoma (18), and TS/A mammary adenocarcinoma, as presented here. Moreover, CTLs specific for a Kb-restricted antigenic epitope contained in the transmembrane component of the retroviral env protein p55E have been found in tumor-infiltrating lymphocytes from MC-38 fibrosarcoma and B16 melanoma (17, 29).

We previously reported that the high immunogenicity of virus-induced or -infected tumors is likely determined by the expression of immunodominant virus-encoded antigen (15, 16). This might also be true for TS/A and other tumors that harbor endogenous retroviral sequences in their genome; therefore, results obtained in these experimental systems should be considered cautiously for their clinical relevance. On the other hand, HERV elements have been identified in the human genome (30, 31), where they may play a role both in autoimmunity (32, 33) and tumorigenesis (34). In particular, HERV-derived gag and pol gene transcripts and products have been detected in tumors and biological fluids of cancer patients (35–38). Interestingly, it has recently been reported that HERV-K transcripts with coding potential for the envelope region are frequently expressed in human breast neoplasia (39). More importantly, an antigenic peptide encoded by a very short open reading frame present in the env region of a spliced HERV-K-related transcript has been shown to be target of a specific CTL response in melanoma patients (40). These data, along with recent preclinical demonstrations that gp70 can be targeted by adoptively transferred CTLs (18) or even by procedures of active immunization (26), indicate that HERV-derived TAAs may constitute a valuable target for immune intervention and that TS/A mammary adenocarcinoma might represent a reliable model to preclinically validate immunological antitumor strategies potentially applicable to clinical settings.

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