Different Lineages of P1A-Expressing Cancer Cells Use Divergent Modes of Immune Evasion for T-Cell Adoptive Therapy

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Abstract

Tumor evasion of T-cell immunity remains a significant obstacle to adoptive T-cell therapy. It is unknown whether the mode of immune evasion is dictated by the cancer cells or by the tumor antigens. Taking advantage of the fact that multiple lineages of tumor cells share the tumor antigen P1A, we adoptively transferred transgenic T cells specific for P1A (P1CTL) into mice with established P1A-expressing tumors, including mastocytoma P815, plasmocytoma J558, and fibrosarcoma Meth A. Although P1CTL conferred partial protection, tumors recurred in almost all mice. Analysis of the status of the tumor antigen revealed that all J558 tumors underwent antigenic drift whereas all P815 tumors experienced antigenic loss. Interestingly, although Meth A cells are capable of both antigenic loss and antigenic drift, the majority of recurrent Meth A tumors retained P1A antigen. The ability of Meth A to induce apoptosis of P1CTL in vivo alleviated the need for antigenic drift and antigenic loss. Our data showed that, in spite of their shared tumor antigen, different lineages of cancer cells use different mechanisms to evade T-cell therapy. (Cancer Res 2006; 66(16): 8241-9)

Introduction

Identification of cancer antigens has inspired a multitude of approaches for cell-based cancer therapy (1). Cancer patients have been immunized with cancer antigenic epitopes, particularly in combination with adjuvants (2–5). Tumor-infiltrating cells have been expanded and transferred into cancer patients. An impressive result was recently reported when the patients’ endogenous immune systems were ablated before adoptive transfer (6). Infusion of T-cell clones represent an appealing sophistication of adoptive therapy (7, 8). A number of reasons account for it: First, the specificity, avidity, and effector functions of infused cells can be precisely defined. Second, many tumor antigens are shared by different lineages of cancer cells from different individuals that have the same MHC restricting allele. Thus, it is possible to rescue the T-cell receptor (TCR) genes from T-cell clones shown to be effective and safe in therapy and to insert it into T cells of other individuals with tumors that expressed the same tumor antigen and MHC allele (9–11). Recently developed techniques, such as the use of peptide-MHC tetramers or a bispecific antibody to capture T cells producing IFN-γ in response to specific stimulation, are greatly improving the efficiency of generating T-cell clones (12, 13). Despite its potential, the outcome of adoptive therapy for large established tumors with CTL is unpredictable. Several mechanisms, such as insufficient T-cell engraftment, activation, clonal expansion, survival, and persistence, could be responsible for the failure of T-cell therapy (1). On the other hand, T cells can be rendered anergic (14). Cancer cells can evade immune recognition by down-regulating MHC class I (15, 16), losing tumor antigen (8, 17), or undergoing antigenic drift (18).

An important issue is whether the above-mentioned mechanisms of immune evasion are dictated by genetic defects associated with malignant transformation of cancer cells or by the interaction between cancer antigen and their specific T cells. The former situation called for better understanding of the cancer cells to come up with new approaches to prevent immune evasion whereas the difficulties with the latter may be overcome by better selection of cancer antigen and cancer-specific T cells. This issue has not been addressed because each model involved unique combination of cancer cells, cancer antigen, and antigen-specific T cells.

P1A antigen was initially identified in the mastocytoma P815 tumor cell line (19). Later studies have shown that the antigen is also expressed in several other lineages of cancer cells including plasmacytoma J558 and fibrosarcoma Meth A (20). The CTL-recognized epitope has previously been identified within P1A 35-43, restricted by H-2Ld (21). Transgenic mice expressing a TCR specific for the tumor antigen H-2Ld:P1A 35-43 complex, designated as P1CTL, have been produced (22). By using the P1A/J558/P1CTL tumor model, we have recently shown that adoptively transferred P1CTL can be efficiently activated in vivo (23) and, under optimal conditions, the transgenic T cells could eliminate established large tumors expressing costimulatory molecules of the B7 family (24–28). However, when using P1CTL to treat mice with unmodified J558 tumors, we found that transgenic T cells selected multiple mutations in the P1A antigenic epitope. These mutations severely diminished T-cell recognition of tumor antigen by a variety of mechanisms, including modulation of MHC-peptide interaction and TCR binding to MHC-peptide complex (18). Taking advantage of transgenic T cells capable of recognizing three different lineages of tumors, we evaluated their mechanism of immune evasion of the same T cells. Our data showed that the mode of immune evasion is dictated by cancer cells rather than by cancer antigen.

Materials and Methods

Experimental animals. Transgenic mice expressing a TCR specific for the tumor rejection antigen H-2Ld:P1A 35-43 complex have been described (22). TCR transgenic mice were backcrossed with BALB/c mice for at least 10 generations before they were used for this study. BALB/c mice with a targeted mutation of the RAG-2 gene were purchased from Taconic Farms (Germantown, NY).

Cell lines, tumorigenicity, and isolation of tumor cells from ex vivo tumors. The fibrosarcoma Meth A, mastocytoma P815, and plasmacytoma
JS58 have been described (24). Meth A or P815 tumor cells (1 × 10⁶) were injected in the flank of each mouse. For JS58 cells, 5 × 10⁶ were injected into each mouse. The tumor size was determined by physical examination every 2 to 3 days. To isolate tumor cells from ex vivo tissues, tumors were surgically removed and single-cell suspensions were prepared by grinding tumor tissues over two frosted glass slides. After removing tumor debris, viable cells were enriched by centrifugation through Ficoll-Hypaque medium. The tumor cells were cultured in RPMI medium containing 5% FCS for 1 week to eliminate contaminating host cells before they were used for flow cytometry and molecular analysis of the P1A gene.

**Peptide synthesis.** The wild-type and mutant P1A peptides, as well as the control 4.6-binding peptide from murine cytomegalovirus (HGPFTMPNL), were synthesized by Genemed Synthesis, Inc. (South San Francisco, CA), dissolved in 40% ethanol, and stored at −70 °C. Adoptive transfer of purified transgenic T cells. Pools of spleen and lymph node cells from PICTL-transgenic mice were incubated with a cocktail of monoclonal antibodies (anti-CD4 mAb GK1.5, anti-FeR mAb 2.4G2, and anti-CD11c mAb N418). After removal of unbound mAbs, viable cells were enriched by centrifugation through Ficoll-Hypaque medium. The tumor cells were cultured in RPMI medium containing 5% FCS and 10% heat-inactivated fetal bovine serum. The tumor cells were used as targets. The effector T cells and the targets were incubated together for 6 hours and the percentages of specific lysis were calculated based on the following formula: specific lysis % = 100 × (cpm_mixture − cpm_monomer) / (cpm_monomer − cpm_control).

**Statistics.** For comparison of mice survival, Kaplan-Meier survival analysis and log-rank test were used (version 10.0, SPSS, Inc., Chicago, IL). For comparison of T-cell persistence in blood between groups, Fisher's protected least significant difference test (StatView 5.0, SAS Institute, Inc., Cary, NC) was used. Pearson's χ² tests were used to determine whether significant difference exists between the three tumor cell lines for their immune evasion modes.

**Results**

**Multiple Lineages of Cancer Cells Share the Same Tumor Rejection Antigen P1A and Restricting Elements**

Our previous studies have established that multiple lineages of cancer cells, including plasmacytoma JS58, fibrosarcoma Meth A, and mastocytoma P815 tumors, share the same tumor rejection antigen P1A, yet these cancer cells were derived from the H-2D background (20). Because a dominant epitope P1A 35-43 was presented by H-2L, we therefore compared the P1A and H-2L expression in these cancer cells. As shown in Fig. 1A, all three cell lines expressed significant levels of H-2L; the expression level of P815 cells was ~8-fold higher compared with JS58 and Meth A. P1A mRNA expression was readily detected in all three cell lines by RT-PCR (data not shown). We further quantitated P1A gene expression in all three cell lines by using real-time RT-PCR. As shown in Fig. 1B, the three tumor cell lines had comparable levels of P1A transcript, which is in agreement with our previous analysis by Northern blot (20).

To determine whether P1A antigen expressed in each of the cell lines could be efficiently presented to T cells in vivo, we injected 20 × 10⁶ irradiated tumor cells i.v. into each RAG-2−/− BALB/c mouse. Twenty-four hours later, 5 × 10⁶ CFSE-labeled P1CTL cells, which were transgenic T cells that specifically recognize H-2L, were injected i.v. into each mouse that had previously received tumor cells. Sixty hours after T-cell injection, we measured the CFSE level in T cells. As shown in Fig. 1C, in mice that received no tumor cell injection (control), T-cell division was minimal; in mice that received tumor cells, vigorous T-cell proliferation was detected. The strongest proliferation of P1CTL cells was detected in mice that received Meth A tumor cells whereas P815 and JS58 tumor cells stimulated similar levels of P1CTL proliferation. Thus, P1A antigen expressed in all three lineages of tumor cells could be efficiently presented to T cells in vivo.

All cell lines could be efficiently recognized by activated P1CTL cells. As shown in Fig. 1D, the same P1CTL cells had higher lytic activity against P815 and JS58 compared with Meth A. Because P1A gene is located on X chromosome (Mouse Genome Informatics, ID 98818), we determined copy numbers of X chromosome in each cell by cytogenetic analysis. Only one X chromosome was detected in all three cell lines whereas a Y chromosome was lacking in all three cell lines (data not shown). Thus, all X-chromosomal genes, including P1A, are hemizygous. The hemizygosity of P1A gene makes it susceptible to genetic inactivation.
P1CTL cells were injected i.v. into each tumor-bearing mouse. In untreated mice, the tumor grew progressively and became moribund by ~3 weeks after tumor cells injection. We observed variable responses in P1CTL-treated mice. Regardless of the responding patterns, P1CTL therapy significantly prolonged life of tumor-bearing mice (Fig. 2B). However, all mice became moribund by 5 weeks after T-cell therapy.

**P815.** P815 tumors are highly metastatic. Thus, tumor cells have already spread to many organs once local tumors are palpable. In RAG-2/−/− mice that received 1 × 10^6 P815 cells s.c., subcutaneous P815 tumors typically grew into a tumor mass of <1 cm before the mouse became moribund. P1CTL therapy significantly prolonged the survival of tumor-bearing mice, with a range of 30 to 90 days (Fig. 2C). We observed early death in some mice (Fig. 2C), presumably due to T-cell destruction of metastatic tumors in organs such as the liver. The majority of P1CTL-treated mice eventually became moribund due to tumor recurrence and metastasis (six of seven). In some cases, tumor metastasis and spreading were accompanied by the disappearance of subcutaneous tumors (not shown).

Thus, P1CTL have some therapeutic effect on all three antigen-bearing tumors. However, no tumors except one P815 tumor were cured as a result of adoptive therapy.

**Growth Retardation and Recurrence of Tumors after Adoptive T-Cell Therapy**

We tested the therapeutic effect of P1CTL cells for three lineages of tumors sharing the same antigen. J558. RAG-2−/− BALB/c mice were first injected s.c. in the left flank with 5 × 10^6 J558 cells per mouse. Mice bearing J558 tumors were treated at a time when tumor size reached ~1.2 cm (2 weeks after tumor cell injection). Purified P1CTL cells (5 × 10^6) were injected i.v. into each mouse. In P1CTL treated-mice, tumors shrank dramatically followed by size stabilization and tumor recurrence. Whereas all untreated mice reached the end point within 20 days, all treated mice survived for another 45 days before they reached early removal criteria (Fig. 2A). Thus, P1CTL adoptive therapy significantly prolonged the life of mice with large established J558 tumors.

**Meth.** Meth A cells, 1 × 10^6, were injected s.c. into each RAG-2−/− mouse; 9 days after tumor cell injection, 5 × 10^6 purified P1CTL cells were injected i.v. into each tumor-bearing mouse. In untreated mice, the tumor grew progressively and became moribund by ~3 weeks after tumor cells injection. We observed variable responses in P1CTL-treated mice. Regardless of the responding patterns, P1CTL therapy significantly prolonged life of tumor-bearing mice (Fig. 2B). However, all mice became moribund by 5 weeks after T-cell therapy.

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Thus, P1CTL have some therapeutic effect on all three antigen-bearing tumors. However, no tumors except one P815 tumor were cured as a result of adoptive therapy.

**Figure 1.** Three lineages of cancer cells share the same tumor rejection antigen P1A and restricting elements. A. H-2Ld expression on J558, Meth A, and P815 cells; Black line, P815 cells; blue line, Meth A cells; red line, P815 cells. Dotted lines, respective controls. B. P1A gene expression in J558, Meth A, and P815 tumor cells. Real-time PCR was used to quantitate P1A gene expression. The experiment was repeated twice with similar results. C, all three cell lines could efficiently stimulate P1A-specific T-cell proliferation in vivo. Irradiated (2,000 rad) tumor cells (20 × 10^6) were injected i.v. into each RAG-2−/− BALB/c mouse. Twenty-four hours later, 5 × 10^6 CFSE-labeled P1CTL transgenic T cells were injected i.v. into each mouse that had received irradiated tumor cells or mice received no tumor cell injection (control). Sixty hours after T-cell injection, splenocytes were stained for Vα and CD8 markers and analyzed by flow cytometry. Data shown were gated on Vα8 and CD8+ markers and represented three independent experiments with similar results. D. recognition of the P1A antigen by activated P1CTL cells. P1A 35-43 activated cells and represented three independent experiments with similar results.

**Figure 2.** Response to P1CTL adoptive transfer therapy. A. P1CTL adoptive transfer therapy of mice with large established J558 tumors. Each mouse received 5 × 10^6 purified P1CTL cells. Five mice were treated for this particular experiment. B, P1CTL adoptive transfer therapy of mice with established Meth A tumors. Meth A tumor cells (1 × 10^6) were injected into the flank of each RAG-2−/− BALB/c mouse. Nine days later, 5 × 10^6 purified P1CTL transgenic T cells were injected i.v. into each recipient mouse. C. P1CTL adoptive transfer therapy of mice with established P815 tumors. P815 tumor cells (1 × 10^6) were injected into the flank of each RAG-2−/− BALB/c mouse. Nine days later, 5 × 10^6 purified P1CTL transgenic T cells were injected i.v. into each recipient mouse. Representative of five independent experiments with similar results.
Distinct Molecular Mechanisms in Tumor Evasion of P1CTL

J558. We have previously documented that P1CTL therapy of large established J558 tumors selected multiple mutations in the P1A antigenic epitope. Those mutations severely diminished T-cell recognition of the tumor antigen by a variety of mechanisms, including modulation of MHC:peptide interaction and TCR binding to MHC:peptide complex; this process is called antigenic drift (18). In this study, we further studied the prevalence of this mode of immune evasion in J558 tumors that received P1CTL adoptive therapy. As summarized in Table 1, by using RT-PCR followed by gene cloning and sequencing, we identified P1A gene mutation in 12 of 12 recurrent tumors. Overall, we detected six different forms of point mutations in the P1A cDNA. Four mutations (P6R, P7P, P8G, and P9L) and the resulted amino acid changes in recurrent J558 tumors after P1CTL therapy were characterized before (18). Two new forms of mutations (Fig. 3A) were detected among recurrent tumors. One is a G>T mutation at N388 relative to the full-length cDNA of P1A. This mutation resulted in a single amino acid change at P5 (G to V) relative to the P1A epitope P1A 35-43. The second new form of mutation was detected in J558 tumors that evaded both monoclonal and polyclonal (P1CTL-α-chain transgenic T cells) P1CTL therapy (RE-4 and RE-11), which is a G>C mutation at N396. This mutation resulted in V>L amino acid change at P8 (Fig. 3A). Tumor cells bearing P5V (RE-8) or P8L (RE-4) had severely impaired or lost recognition by activated P1CTL (Fig. 3B). We synthesized mutated peptides and compared their capacities to stimulate T-cell proliferation and to license cytoxicity to T cells. As shown in Fig. 3C, both mutated peptides (P5V and P8L) completely lost the ability to stimulate T-cell proliferation. P8L completely lost the ability to sensitize targets for CTL killing whereas P5V is ~10-fold less efficient (Fig. 3D). Thus, P5V and P8L mutations may be sufficient to cause cancer evasion. Because antigenic drift mutation was detected in all CTL-escape J558 tumor cells, we conclude that antigenic drift is a generalized mechanism used by J558 cells to evade P1CTL response in vivo.

Table 1. Summary of J558 tumors that evaded CTL therapy

<table>
<thead>
<tr>
<th>Tumor/host</th>
<th>Treatment</th>
<th>cDNA clones sequenced</th>
<th>Mutation distribution</th>
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<tr>
<td>J558 RE-1/RAG-2/-/-</td>
<td>P1CTL</td>
<td>33</td>
<td>27 P6R, 6 P9L</td>
</tr>
<tr>
<td>J558 RE-2/RAG-2/-/-</td>
<td>P1CTL</td>
<td>35</td>
<td>28 P6R, 5 P8G, 2 P7P</td>
</tr>
<tr>
<td>J558 RE-3/RAG-2/-/-</td>
<td>P1CTL</td>
<td>5</td>
<td>2 P6R, 3 WT</td>
</tr>
<tr>
<td>J558 RE-4/RAG-2/-/-</td>
<td>P1CTL</td>
<td>6</td>
<td>5 P8L, 1 P6R</td>
</tr>
<tr>
<td>J558 RE-5/RAG-2/-/-</td>
<td>P1CTL</td>
<td>6</td>
<td>6 P6R</td>
</tr>
<tr>
<td>J558 RE-6/RAG-2/-/-</td>
<td>P1CTL</td>
<td>6</td>
<td>4 P6R, 2 WT</td>
</tr>
<tr>
<td>J558 RE-7/RAG-2/-/-</td>
<td>P1CTL</td>
<td>6</td>
<td>5 P6R, 1 WT</td>
</tr>
<tr>
<td>J558 RE-8/RAG-2/-/-</td>
<td>P1CTL</td>
<td>5</td>
<td>5 P5V, 1 WT</td>
</tr>
<tr>
<td>J558 RE-9/RAG-2/-/-</td>
<td>P1CTL</td>
<td>6</td>
<td>6 P6R</td>
</tr>
<tr>
<td>J558 RE-10/RAG-2/-/-</td>
<td>P1CTL</td>
<td>5</td>
<td>5 P6R</td>
</tr>
<tr>
<td>J558 RE-11/RAG-2/-/-</td>
<td>P1CTLaTG</td>
<td>3</td>
<td>2 P8G, 1 P8L</td>
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<tr>
<td>J558 RE-12/BALB/c</td>
<td>P1CTL</td>
<td>27</td>
<td>26 P9L, 1 WT</td>
</tr>
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</table>

NOTE: J558 cells (5 × 10⁶) were injected s.c. into each mouse. When tumors were established in mice (>1.0 cm), we injected 5 × 10⁶ of purified P1CTL cells i.v. into each tumor-bearing mouse. Tumor growth was followed until mouse became moribund. Recurrent tumors were harvested and single-cell suspensions were prepared. Cells were cultured in RPMI complete medium for at least 1 week before being analyzed for P1A gene expression. P1CTLaTG, P1CTL-α-chain transgenic T cells.

Figure 3. Characterization of two point mutations identified in the tumor antigen P1A after P1CTL therapy. A, chromatograms of sequencing reactions of P1A cDNA. A G>C mutation in the P1A epitope–coding region was detected in J558 RE-4 tumor cells; this mutation resulted in a single amino acid change in P5 (G to V) relative to the P1A epitope P1A 35-43. A G>T mutation in the P1A epitope–coding region was detected in J558 RE-8 tumor cells; this mutation resulted in a single amino acid change in P8 (Fig. 3A). Tumor cells bearing P5V (RE-8) or P8L (RE-4) had severely impaired or lost recognition by activated P1CTL (Fig. 3B). We synthesized mutated peptides and compared their capacities to stimulate T-cell proliferation and to license cytoxicity to T cells. As shown in Fig. 3C, both mutated peptides (P5V and P8L) completely lost the ability to stimulate T-cell proliferation. P8L completely lost the ability to sensitize targets for CTL killing whereas P5V is ~10-fold less efficient (Fig. 3D). Thus, P5V and P8L mutations may be sufficient to cause cancer evasion. Because antigenic drift mutation was detected in all CTL-escape J558 tumor cells, we conclude that antigenic drift is a generalized mechanism used by J558 cells to evade P1CTL response in vivo.
Meth A. We harvested six recurrent Meth A tumors and cultured tumor cells for in vitro analysis. As shown in Fig. 4A, tumor cells from four of six recurrent tumors (Meth A RE-1 to RE-4) were fully susceptible to lysis by activated P1CTL, suggesting these tumor cells retained their antigens. Tumor cells from two recurrent tumors, however, were resistant to P1CTL lysis (Meth A RE-5 and RE-6).

To test whether the tumor cells can stimulate proliferation of P1CTL, we labeled the transgenic T cells with CFSE and adoptively transferred cells into mice that received irradiated Meth A RE-5 or Meth A RE-6 cells 24 hours earlier via i.v. injection. Irradiated parental cells were used as a positive control. As shown in Fig. 4B, in mice that received no tumor cell injection, P1CTL cells had minimal cell division as reflected by CFSE dilution. In mice that received WT Meth A cells (ex vivo tumor cells without receiving P1CTL therapy), P1CTL cells divided up to seven times. In mice that received Meth A RE-5 or RE-6 cells, very few P1CTL cells had experienced division. Thus, Meth A RE-5 and RE-6 became unrecognized by P1CTL. Because cell-surface expression of MHC class I (H-2Ld) on Meth A RE-5 and RE-6 cells was the same as that on untreated Meth A cells (data not shown), down-regulation of MHC class I was not responsible for the loss of recognition by P1CTL. We examined whether cells from recurrent tumors lost P1A expression. Quantitative PCR suggested that Meth A RE-5 tumor had ~100-fold reduction of P1A mRNA whereas Meth A RE-6 retained normal levels of P1A gene expression (Fig. 4C). To further understand whether P1A gene mutation is responsible for the loss of T-cell recognition, we cloned and sequenced the P1A cDNA from these recurrent tumors. As shown in Fig. 4D, in six of six Meth A RE-5 cDNA clones, we detected a T>G mutation at position 590 of P1A cDNA; this single nucleotide mutation resulted in an early stop codon of the P1A gene. Thus, the early stop codon caused dramatic down-regulation of P1A gene expression, which was likely responsible for the loss of recognition by P1CTL in Meth A RE-5 tumor.

In six of six Meth A RE-6 cDNA clones, we detected a T>C single nucleotide mutation at position 389 of P1A cDNA; this mutation resulted in the replacement of tryptophan (W) at position 6 of P1A 35-43 epitope by arginine (R). This mutation (P6R) has been frequently detected in P1CTL-escape J558 tumor variants and has been shown to be sufficient to cause the loss of recognition by P1CTL (18).

P815. Four recurrent P815 tumors from P1CTL-treated mice (designated as P815 RE-1 to RE-4) were further analyzed. We first tested whether these recurrent tumor cells were still recognizable by activated P1CTL. As shown in Fig. 5A, all four tumors have severely impaired or completely lost recognition by activated P1CTL. P815 RE-1 and P815 RE-2, two recurrent tumors that completely lost recognition by P1CTL, were found to have lost P1A mRNA expression (Fig. 5B); thus, antigen loss is responsible for the immune evasion in these two tumors. In two recurrent tumors that have severely impaired recognition by P1CTL (P815 RE-3 and RE-4), their P1A gene expression was still detectable but severely reduced. Real-time PCR revealed that P815 RE-3 and RE-4 had 142-fold and 100-fold reduction in P1A gene expression, respectively (Fig. 5C). To further understand whether the mutation of P1A gene is responsible for the loss of T-cell recognition, we further cloned and sequenced the P1A cDNA in these two recurrent tumors. As shown in Fig. 5D, in six of six cDNA clones of P815 RE-3 tumor, we detected T and A nucleotide insertion at position 386 of P1A cDNA, which resulted in a stop codon at P7 of the P1A epitope. Thus, the P1A T-cell epitope P35-43 was not encoded by this truncated mRNA and, therefore, the tumor cell line lost the expression of the tumor antigen. In six of six cDNA clones of the P815 RE-4 tumor, we detected deletion of nucleotides C and T at positions 511 and 512 of the P1A cDNA, which disrupted the open reading frame of P1A gene, although at a position downstream of the P1A epitope. Because both variants harbor
premature termination codon, it is likely that premature termination codon–mediated RNA decay is responsible for the drastic loss of P1A mRNA.

Taken together, our data showed that the predominant mechanism for immune evasion differs in different tumor cell lines. J558 cells avoid P1CTL by antigenic drift, which resulted in amino acid replacement of the antigenic epitope, whereas P815 cells evade T-cell recognition by antigenic loss. Interestingly, although the majority of Meth A tumors retained P1A antigenic epitope, the cells can undergo both antigenic drift and antigenic loss.

T-Cell Apoptosis as a Mechanism of Tumor Recurrence for Meth A Tumor

Because Meth A and P815 tumor cells had similar levels of P1A gene expression, and most P1CTL-escape Meth A tumor cells (four of six) still retained sensitivity to P1CTL lysis whereas all P1CTL-escape P815 tumors lost or mutated their antigen, we asked whether these two lineages of cells stimulated P1CTL differentially. We first compared P1A-specific T-cell expansion and persistence in the peripheral blood of each mouse after T-cell adoptive transfer. Data from a representative mouse in each group are shown in Fig. 6A and data from each group are summarized in Fig. 6B. Although the same numbers of P1CTL cells were injected into both groups of mice, P1A-specific T-cell numbers remained high in mice bearing P815 tumors throughout the observation period. In contrast, P1A-specific T-cell numbers in mice bearing Meth A tumors were much lower throughout the observation period ($P = 0.0027$, Fisher's protected least significant difference test) and almost completely undetectable at the end of the observation period.

We injected CFSE-labeled P1CTL cells into mice that had established P815 or Meth A tumors at 10 days after s.c. tumor cell inoculation. As shown in Fig. 6C, at 80 hours after P1CTL transfer, few dividing T cells were detected in tumor-free RAG-2−/− mice whereas vigorous T-cell proliferation was detected in tumor-bearing mice. Much faster T-cell proliferation was observed in spleen of P815 tumor–bearing mice compared with Meth A tumor–bearing mice. About 25% of the T cells from tumor-bearing mice down-regulated CD62L and an overwhelming majority up-regulated CD44 in both P815 tumor– and Meth A tumor–bearing mice. However, up-regulation of CD44 is somewhat more pronounced in P815 tumor–bearing mice. Thus, day 10 P815 tumors are more efficient in stimulating proliferation of T cells and acquisition of activation markers compared with day 10 Meth A tumors. Because at the per cell basis, Meth A tumor cells were more efficient in stimulating proliferation of P1CTL in the spleen (Fig. 1C), this data may suggest that more P815 tumor cells metastasize into the spleen than Meth A tumor cells in day 10 tumor–bearing mice.

The disappearance of T cells in Meth A tumor–bearing host could be due to T-cell apoptosis. We tested whether P1A-specific T cells underwent apoptosis in both Meth A tumor– and P815 tumor–bearing host. As shown in Fig. 6D, the death rate of T cells was much higher in Meth A tumor–bearing host compared with that of P815 tumor–bearing host (17.4% versus 4.49%). The significant increase in T-cell apoptosis in the Meth A tumor–bearing mice showed that the tumor caused T-cell death. Taken together, reduced proliferation and apoptosis of activated T cells explains the reduced number of antigen-specific T cells in the Meth A tumor–bearing mice.

Discussion

A major obstacle to cancer immunotherapy is the ability of cancer cells to evade host immunity. A host of mechanisms, including antigenic drift (18), antigenic loss (8, 17), and T-cell tolerance (14), have been described. A key issue is whether the tumor antigens or cancer cells dictate the mechanism of immune evasion. This issue is difficult to address as distinct tumor antigens are identified in different lineages of tumor cells. Here we take advantage of our previous observation that P1A, the first unmutated tumor antigen to be identified, is expressed in multiple
lineages of tumors. Using transgenic T cells specific for the shared tumor antigen, we found that even for the same T cells, different lineages of cancer cells employed different mechanisms to evade T-cell responses.

We have shown that J558 tumor cells replaced their amino acids in the antigenic epitope to evade T-cell recognition. The antigenic drift changes either MHC:peptide interaction or TCR binding to MHC:peptide complex (18). In this study, we showed that antigenic drift occurred in 100% of recurrent J558 tumors that escaped P1CTL therapy in RAG-2\(^{-/-}\) BALB/c mice. These mutations occurred at P5, 6, 7, 8, and 9 positions of the P1A antigenic epitope. Functional tests suggest that each of the mutations was sufficient to abrogate their capacity in stimulating proliferation, sensitizing CTL lysis, or both. Whereas antigenic drift is observed in all recurrent J558 tumors, it was not observed in P815; however, it was observed in one of six recurrent Meth A tumors. The differences in rates of antigenic drift in different tumor lines are highly significant (Table 2).

Tumor antigen loss has been shown as a major mechanism of tumor evasion of CTL response both in animal models (17) and in cancer patients (8, 29) that received CTL targeted therapy. All of four recurrent P815 tumors lost P1A mRNA, with two complete losses and two >100-fold reductions. Antigenic loss was found in 1 of 6 recurrent Meth A tumors and none of 12 J558 tumors (Table 2). Thus, antigenic loss is the primary mechanism by which P815 evades immune recognition. In two cases studied, the loss was caused by early termination codon caused by mutations. The fact that different tumors employed different mechanisms to evade T-cell recognition showed that different types of cancer cells may

<table>
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<th>Tumors</th>
<th>J558</th>
<th>Meth A</th>
<th>P815</th>
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<td>0 of 12</td>
<td>1 of 6</td>
<td>4 of 4</td>
<td>0.00257</td>
</tr>
<tr>
<td>Antigenic retention</td>
<td>0 of 12</td>
<td>4 of 6</td>
<td>0 of 4</td>
<td>0.017</td>
</tr>
</tbody>
</table>

NOTE: Data shown are number of recurrent tumors in each group that employed antigenic loss, antigenic drift, or antigenic retention to survive T-cell therapy. Adjusted \(\chi^2\) tests were used to determine whether significant difference exists between the three tumor cell lines.
have unique mechanisms to actively create mutants of the same gene. The mutations allow cancer cells to overcome the selection pressure imposed by antigen-specific T cells. These tumor cell lines provide valuable models to uncover the underlying mechanisms.

Interestingly, the majority of the Meth A stumor that survived PICTL therapy retained tumor antigen P1A as they are highly susceptible to killing by the T cells. Our analysis showed that despite rapid proliferation of T cells after adoptive transfer, the number of PICTL in the tumor-bearing mice declined after the first 2 weeks. Further studies showed that a large proportion of T cells underwent programmed cell death in Meth A tumor–bearing mice. In contrast, after initial expansion, the levels of PICTL remain constant in the P815 tumor–bearing mice. Thus, although the same T cells and antigens are involved, Meth A tumor caused disappearance of PICTL cells from the host whereas the P815 tumors did not. Although the distinction is largely unknown at this point, our data are consistent with a previous study by Levey et al. (30), who showed that Meth A tumor cells are immunosuppressive.

Levels of antigen expression by cancer cells have been shown to be a critical factor in determining whether tumor antigen–specific T cells are activated or remain tolerant (31) and therefore potentially may affect immune evasion. In this and our previous studies (20), we have observed substantially comparable P1A gene expression among the three tumor cell lines (Fig. 1B). Moreover, we showed that the expression levels of P1A antigen in each lineage of tumor cells were sufficient to activate T cells in vivo (Fig. 1C), although the relative efficacy seemed to vary depending on the route of tumor cell injection and when the T cells were administrated (Figs. 1C and 6C). Thus, T cells in different tumor–bearing mice received sufficient antigenic stimulations.

An interesting issue is whether T cells are stimulated by directly presented or cross-presented P1A antigen. We have reported that unless J558 tumor cells ectopically express costimulatory molecule B7−1, they cannot activate T cells through direct antigen presentation (23). This observation is now being extended into P815 and Meth A (data not shown). Thus, the induction of T cells by the three tumor cell lines is likely via cross-presentation.

The mode of immune evasion could potentially be determined by the differential efficiency of T-cell elimination of tumor cells (Fig. 1D). We think this is unlikely as activated P1CTL killed P815 and J558 at a comparable efficacy in vitro, yet the two cell lines used different mechanisms to evade T cells in vivo. Based on these considerations, it is likely that, in addition to quality and quantity of tumor antigens, the biological properties of cancer cells dictate the mechanism of immune evasion to T-cell therapy.

Taken together, our data presented in this article revealed distinct mechanisms of immune evasion in different cancers. As summarized in Table 2, J558 tumors undergo antigenic drift whereas P815 cells experience antigenic loss. The ability of Meth A tumor to cause T-cell death alleviated the need to down-regulate P1A antigen in cancer cells. The fact that three different tumor cell types employed distinct mechanisms to evade the same T cells has important implications for the challenge of effective immune therapy: because different cancer cells used distinct modes to evade immune recognition, specific mechanisms are likely to exist in different cancer cells. Such mechanisms may involve the metastatic characters of tumor cells, the ability of tumor cells to induce apoptosis of activated T cells, the susceptibility of tumor cells to T-cell destruction, and the expression of genes leading to genetic instability in cancer cells. As such, different methods may be needed to avert immune evasion by cancer cells.

Because we transferred T cells into lymphopenic host, which would mount optimal T-cell response (6, 32, 33), our results showed that even in the most favorable circumstances, monospecific T cells are unlikely effective against established tumors. Therefore, one must use T cells specific for multiple antigens to achieve effective therapy.

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References


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