ABSTRACT

Human and murine cancers can express multiple independent antigens as targets for cytolytic T-cells (CTLs). The immune response against one such tumor was studied at the cellular and molecular level by analyzing the T-cell receptor β chains (CD8+) of CTLs isolated in vivo to a murine UV light-induced cancer. A 3.5-10-fold enhancement of CD8+CTLs above background (naïve spleen, 4-5% CD8+ among CD8+ T-cells) was demonstrated in the responses of ten individual animals to this tumor. The dominance of CD8+ usage was exclusively limited to the CD8+ compartment and correlated with recognition of the A but not the B and C antigens on the tumor. In addition, the amino acid sequences of the putative third complementarity determining regions of the T-cell receptor β chains of CTLs isolated in vivo were remarkably similar to each other suggesting restriction also at the clonal level. Cells responding to four other syngeneic UV-induced tumors, each expressing different unique antigens, or to a variant of the same tumor that had selectively lost the A but retained the independent B and C antigens, induced a 2-10-fold or less enhancement of CD8+ CD8+ cells above background. Thus, the host responds to only one of the several possible target antigens and with relatively few CTL clonotypes.

INTRODUCTION

TCRαβ is a plasma membrane-bound heterodimeric glycoprotein structurally similar to the Fab fragment of immunoglobulins in having variable (V) and constant (C) domains (Ref. 1; for review see Ref. 2). As with immunoglobulin genes, functional TCR V-region genes are created by rearrangement of distinct variable (V), diversity (D), and joining (J) gene segments with additional diversity generated by variable splicing of the gene segments as well as the random addition of nucleotides between these segments. The V-(D)-J junctional site, termed the CDR3, is thought to play a major role in determining fine specificity for antigens (3-7). Dominant usage of a V-segment gene occurs in CD4+ and CD8+ T-cell responses to numerous antigens including tumor antigens (3-5, 8-14). Previous experiments have identified common Vα use by tumor-infiltrating lymphocytes from different human uveal melanomas (12). The melanoma samples from which lymphocytes were isolated for this analysis were from an unselected patient population presumably with differing class I molecules. It has been suggested that a given TCR Vα region can bind to nonpolymorphic regions of the MHC and therefore belong to different T-cell clonotypes (14, 15). As a recent example, using sequence analysis of the CDR3s, it has been shown that the TCRs of CTLs responding to a single foreign nonapeptide can be clonotypically highly diverse, despite a dominant usage of a certain V-region segment (14). In contrast, the TCRs of CTLs responding to an antigenic peptide homologous to self were found to be very restricted in the V-region segment usage as well as in the composition of the CDR3s (10). It was suggested that the natural tolerance to self peptides presented by class I MHC molecules may substantially reduce the size of the TCR repertoire of CTLs that can respond to antigenic peptides homologous to self (10). While antigens on virally induced tumors are encoded by viral genes which may or may not be homologous to self, antigens on tumors induced by physical or chemical carcinogens probably represent antigenic peptides homologous to self since they are encoded by cellular genes of the host. Therefore, on one hand, we might expect the TCRs of the CTLs responding to such antigens to be restricted; on the other, we might predict the response to be diverse since a single malignant cell may express multiple, independent tumor antigens (19, 20).

Using an UV-induced murine tumor expressing multiple antigens, we have analyzed at the molecular level the TCR repertoire of CTLs responding in vivo. Our experiments are the first to show that the host responds in a remarkably restricted manner to a nonvirally induced tumor; CTLs of relatively few clonotypes recognize only one of several possible target antigens on the tumor.

MATERIALS AND METHODS

Mice. Five-10-week-old germ-free-derived C3H/HeN/MTV mice were purchased from the National Cancer Institute-Frederick Cancer Research Facility (Bethesda, MD) and were maintained as described previously (19). Tumor Cell Lines. Derivation of the UV-induced and spontaneous tumors in C3H/HeN mice has been described (19, 20). The tumors used are strongly immunogenic in that these tumors when transplanted into young syngeneic mice grow during the first 10 days and then regress. These tumors grow progressively in nude mice and eventually kill by infiltrative growth without macroscopic evidence of distant metastases. All cell lines were cultured in minimal essential medium (410-1100; Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum.

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The abbreviations used are: TCR, T-cell receptor; CDR3, third complementarity determining region; MHC, major histocompatibility complex; CTL, cytolytic T-cell; PEL, peritoneal exudate lymphocyte; FITC, fluorescein isothiocyanate; PEl, phycoerythrin; cDNA, complementary DNA; PCR, polymerase chain reaction; MAb, monoclonal antibody.
Induction of PELs. Tumor cells (3 \times 10^2 - 1 \times 10^7) were injected four times at 3-day intervals. On day 2 after the final injection, the mice were sacrificed by cervical dislocation. Five ml of phosphate-buffered saline/1% fetal calf serum were injected i.p. and cells were removed by peritoneal lavage.

Generation and Maintenance of CTL Clones. Methods for generating and maintaining CTL lines and clones have been described (19). Briefly, mice were primed with 1 \times 10^7 tumor cells i.p. Splenectomies from these immune mice were used as responder cells in mixed lymphocyte tumor cell culture with the immunizing tumor cells as the stimulators. The CTLs were tested by \(^{31}\)Cr release assay for cytotoxicity and specificity. For passage of CTLs, 1 \times 10^5 CTLs, 5 \times 10^4 irradiated (2000 R) syngeneic spleen cells, and 2.5 \times 10^4 mitomycin C-treated tumor cells were mixed and plated in 1.5 ml of medium [RPMI 1640 (Gibco 430-1800), 33% (v/v) of a secondary mixed lymphocyte culture as a source of T-cell growth factor (21), 10% non-heat-inactivated fetal calf serum, 100 units/ml penicillin/streptomycin (Gibco 600-5140AG), 50 \mu g/ml gentamicin (Gibco 600-5750AD), and 5 \times 10^{-5} M 2-mercaptoethanol] in each well of a 24-well tissue culture plate [Costar 3424, Cambridge, MA]. Similar scaled down conditions were used for passage in V-bottomed 96-well non-tissue culture-treated plates (Linbro 76-223-05; McLean, VA).

\(^{31}\)Cr Assay. The cytotoxicity of CTLs was determined by a 4.5-h \(^{31}\)Cr release assay, as described previously (22). The percentage of specific lysis was calculated as:

\[
\frac{(\text{Experimental release} - \text{spontaneous release})}{(\text{Maximum release} - \text{spontaneous release})} \times 100
\]

Spontaneous release was <15% of maximum.

Staining and Cell Sorting. FITC-conjugated rat anti-mouse L3T4 (anti-CD4; RM-45), anti-mouse L3.4 (anti-CD8; 53-6.7), and PE-conjugated mouse anti-mouse Vß13 (MR-12-4) were purchased from Pharmingen (San Diego, CA). PELs or CTL clones (5 \times 10^4 - 1 \times 10^5) were incubated with FITC-anti-L3.4 and PE-anti-Vß13 or FITC-anti-L3T4 and PE-anti-Vß13 for 30 min on ice. Cells were washed 3 times with phosphate-buffered saline containing 10% bovine serum albumin and 0.1% sodium azide. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson). PELs were sorted on Coulter Epics 753 (model 2566; argon laser run at 488 nm; Coulter Corporation, Hialeah, FL). Sorted cells were expanded for 16 h in 24-well plates (No. 3424; Costar, Cambridge, MA), each well containing 1 \times 10^5 sorted cells, 5 \times 10^4 irradiated (2000 R) syngeneic spleen cells, and 2.5 \times 10^4 mitomycin C-treated tumor cells in 1.5 ml of CTL passage medium.

Preparation of cDNA. PELs or CTL clones (1 \times 10^5 - 2 \times 10^6) were lysed in RNAzol B (Tel-Test, Inc., Friendswood, TX) and total RNA was prepared as prescribed by the manufacturer. First strand cDNA was synthesized using the cDNA Synthesis System Plus (Amersham, Arlington Heights, IL) following the accompanied protocol.

PCR Conditions and Primers Used. Vß primers specific for the following gene segments were used (nomenclature according to Ref. 23): 1, 2, 3, 4, 5, 1.6, 8.1, 8.2, 8.3, 9, 10a, 11, 12, 13, and 17a. One of two primers were used for Vß13. Sequences for each oligonucleotide (except Vß8.1, 8.2, and 8.3 and one of the two for Vß13) were as follows: 5'-GAGCTAGCGAATTC-(25 Vß-specific sense nucleotides from the 5' terminus of the V gene segment)-3'. The other Vß primers were identical except the Vß-specific sequences were from approximately the middle of the V gene segment. The Cß primer sequence was as follows: 5'-GAGCTAGCGAATTC-(AGCCTTTTGTTTGTTTGCAATC-3'). Underlining indicates an EcoRI restriction site. \(\beta\) chain amplifications were performed using 20 pmol of each \(\beta\) primer, 20 pmol of Cß primer, 25 pmol of deoxynucleotide triphosphates (Pharmacia, Piscataway, NJ), 5% of the cDNA reaction, and 0.5 unit Taq polymerase and buffer (Boehringer Mannheim, Indianapolis, IN) in a total volume of 100 \mu l (24). All PCR reactions were subjected to denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 30-60 cycles on a Perkin Elmer Thermal Cycler 480 (Norwalk, CT).

Sequencing. PCR products were gel isolated, digested with EcoRI (New England Biolabs, Beverly, MA), and ligated into the sequencing vector M13 mp19. Sequencing was performed using the dyeode chain termination method (25) with the Sequenase DNA sequencing kit (U. S. Biochemical, Cleveland, OH).

Results

Induction of Cytolytic T Cells with Unique Tumor Specificity in Vivo. Long term cultures can bias the analysis of the TCR repertoire. Therefore, a new protocol was developed in our laboratory4 to generate sufficient PELs for simultaneous cytolytic assays, staining analyses, and RNA extractions. Tumor cells (3 \times 10^2 - 1 \times 10^7) were given i.p. 4 times at 3-day intervals. On day 2 after the fourth injection, PELs were harvested. Using this approach, we could generate highly specific CTL for the 6130 tumor, as shown in Fig. 1A, as well as CTL directed against other UV-induced tumors (Fig. 1, B and C). Previous studies (19, 20) have shown that the unique tumor antigens expressed by these tumors were not likely to represent germline mutations since autochthonous normal control cells from each mouse of tumor origin, including 6130, did not express these antigens. This was confirmed for the 6130 tumor (data not shown). CTL activity of PELs was completely abrogated by pretreatment with an anti-CD8 MAb and complement, as shown in Fig. 1D for anti-6130 effector cells. The parental 6130 tumor has at least three antigens defined by CTL clones designated as A, B, and C (20). Using antigen loss variants of 6130 [A' B' C' VAR4 and A' B' C' C' VAR3 (20)], we determined that the PEL response is directed against the A antigen (Fig. 1E).

Selective Stimulation of the Vß13 Subgroup in the CTL Response to 6130 Tumor Cells. To examine the diversity of Vß genes used by tumor-specific PELs, we analyzed reverse-transcribed cDNA from the mRNA of anti-6130 PELs using PCR. We used 15 different Vß-specific oligonucleotides as \(\beta\) primers and a Cß-specific oligonucleotide for the \(\beta\) primer as described in "Materials and Methods." The most striking and consistent amplification was obtained with the Vß13-Cß pair of primers tested in 5 individual mice (data not shown). In confirmation of these findings, cytofluorometry of 6130-reactive CTLs using an anti-Vß13 MAb demonstrated that Vß13+ cells comprised 25-65% of all CD8+ cells for each of 10 individual mice tested, as compared with 4.4% background level for CD8+ spleen cells from nonimmunized animals (Fig. 2; Table I). This expansion of Vß13+ T-cells was seen only in the CD8+ compartment. Vß13 usage among CD8+ cells in mice immunized with 4102, 6132A, 6138, 6139B, and the A' B' C' 6130 variant VAR4 ranged from 1 to 12% (Fig. 2; Table I).

Analysis of Vß13+ TCR Isolated from CTL Responding to 6130 Tumor Cells. We analyzed the Vß13+ cells responding to the 6130 tumor by generating cDNA from the tumor-specific PELs. PCR was performed using the midregion Vß13 and Cß primers, and the amplified product was subcloned into the sequencing vector M13 mp19. Individual plaques representing the TCR \(\beta\) chains from single clones were picked and sequenced. The results (Figs. 3 and 4; "A" isolates, two mice pooled) demonstrated a strong conservation of TCR structure in several respects: (a) 8 of the 14 sequences were identical, indicating the possibility of a single dominant CTL clone; (b) 13 of the 14 sequences utilized the Jß8.1 gene segment, which is thought to be infrequently used by mature T-cells (26, 27); (c) all 13 of these clones encoded CDR3s of identical length (9 amino acids) as defined by Candeias et al. (26) [CAS<CDR3>GxG)]. The 9-residue length is rare among Vß13+ sequences reported in literature (5, 14, 28); (d) 12 of the 14 sequences encoded an arginine (R) residue in the first or second amino acid position of the CDR3. Most saliently, this residue is encoded by an N-region addition whereas the germline

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4 P. Monach, personal communication.
encodes a serine (S) (5). In 4 of the sequences, a conserved glycine (G) and glutamine (Q) pair was also present. Interestingly, glutamine was also present in all of the clones, but at different positions. DJ1.1, and not DJ2.1, encodes a glutamine in 1 of the 3 reading frames. The finding of DJ1.1 in all of the 14 sequences suggests that there was perhaps a selection for the glutamine.

To verify the above findings, another mouse was immunized and cDNA was generated from the PELs as before (Figs. 3 and 4, "B" isolates, one mouse). To rule out PCR contamination, a second Vß13 primer, which binds to a region approximately 150 base pairs upstream from the region bound by the previously used Vß13 primer, was used to perform PCR and the amplified products were subsequently sequenced. The sequences from this mouse demonstrated a less uniform TCR primary structure than in the first group of sequences. Nonetheless, Jß1.1 was used in 5 of 12 cases, and arginine (encoded by N nucleotide addition) and glutamine were present in 9 of 12 cases. Furthermore, the 2 “dominant” subsets, B1-B5 and B7-B10, had CDR3 lengths identical to that of the clones in the previous batch of PELs which used Jß1.1.

We compared the CDR3 sequences derived from the PELs to that of an anti-A specific Vß13+ CTL clone BS1 derived from a long term 6130 A antigen-specific CTL line, generously provided by B. Starr of this laboratory (Fig. 5A). cDNA was generated and PCR performed using the upstream Vß13 primer. Sequence analysis revealed that BS1 also utilized DJ1.1 and Jß1.1 (Fig. 3). At the amino acid level of the CDR3, BS1 most resembled PCR isolates A1-A8, differing by only one amino acid at position 2 of the CDR3 (Fig. 4). Since the conserved characteristics found among anti-6130 PEL CDR3s appear to correlate to A antigen specificity, we sought to demonstrate more directly that the Vß13+ CTLs generated in vivo were anti-A specific. Vß13+ CD8+ cells were sorted to 99% purity (data not shown) and were used in a 51Cr release assay. Although the sorted cells killed slightly less efficiently (Fig. 5B) than the BS1 clone (Fig. 5A) at the same effector:target ratio, indicating that some Vß13+ cells may be noncytolytic, these sorted cells were clearly very specific for the A antigen. We have attempted cloning out the Vß13+ PELs in vitro to see if some Vß13+ CD8+ cells are nonlytic against 6130. However, to date we have not been successful maintaining PELs in long term cultures.

Analysis of Vß13+ TCR Isolated from CTL Responding to a Different Tumor, 6138. One of the mice given an injection of 6138 tumor cells showed a notably elevated percentage (12.1% (Table I)) of Vß13+ cells among the CD8+ PELs. The PELs from this mouse specifically killed 6138 tumor cells (data not shown). We then generated cDNA from these PELs to determine the degree of similarity between the CDR3s of Vß13+ PELs from mice immunized with 6130 and 6138 tumor cells. The result revealed a clear difference in the Jß usage, CDR3 length, and amino acid content of the CDR3s between the two groups (Fig. 4). The CDR3s of 6138-induced Vß13+ PELs demonstrated a weaker conservation in the TCR primary structure. This probably reflects the weaker Vß13 response seen among anti-6138 PELs. The comparisons of CDR3s of 6130- and 6138-induced
Table 1 Significant increase of Vß13+ CD8+ T-lymphocytes induced by 6130 but not other tumor cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% Vß13+ of CD8+ n</th>
<th>% Vß13+ of CD8+ n</th>
</tr>
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<tbody>
<tr>
<td>6130</td>
<td>42.3 ± 4.7b</td>
<td>10</td>
</tr>
<tr>
<td>VAR4c</td>
<td>8.2 ± 0.4</td>
<td>3</td>
</tr>
<tr>
<td>6132</td>
<td>6.4 ± 1.3</td>
<td>3</td>
</tr>
<tr>
<td>6138d</td>
<td>6.9 ± 2.8</td>
<td>3</td>
</tr>
<tr>
<td>6139B</td>
<td>3.1 ± 1.0</td>
<td>5</td>
</tr>
<tr>
<td>4102</td>
<td>7.9 ± 1.2</td>
<td>3</td>
</tr>
<tr>
<td>Normal spleen</td>
<td>4.4 ± 0.2</td>
<td>3</td>
</tr>
</tbody>
</table>

n a number of mice tested. The values are the mean ± SEM. Staining was performed as described in the legend to Fig. 2. 

Several years ago, it was suggested that a single malignant cell can express multiple independent CTL-recognized target antigens (29, 30) and that simultaneous therapeutic attack against the multiple antigens would destroy the cancers effectively because escape variants which had lost all the antigens would be extremely rare. Unfortunately, some of the multiple antigens on the tumors were found to be alloantigens (31) or normal differentiation molecules (32). However, a recent study (19) using tumor cell lines derived from primary tumors (thereby avoiding serial transplantations) confirmed the original notion that a single tumor (including the 6130 tumor used in this study) expressed multiple unique tumor-specific antigens. These antigens were absent from autologous normal control cells and were absent from other syngeneic tumor cells of the same differentiation lineage indicating that they were neither allo- nor differentiation antigens. It has been suggested that human tumors also express multiple independent target antigens (33–36), although cells of the same differentiation lineage and same MHC class I expression have not yet been tested to indicate

**DISCUSSION**

This study shows that a tumor expressing multiple antigens elicits CTLs directed against only one antigen and that the responding CTLs are clonotypically restricted. This suggests that the dominant CTLs recognize a single or a very limited number of determinants. DNA sequence analysis of 26 Vß13+ PCR isolates derived from 6130-reactive PELs in 2 separate experiments (Figs. 3 and 4) demonstrated a remarkable degree of conservation of the Vß-J junctional regions of these genes for the following reasons. A high percentage of isolates in each experiment were identical at the nucleotide level (Fig. 3); 17 of the 26 isolates (A1–8, B1–5, B7–10) represented only 3 unique sequences. This implies that the Vß13+ response to 6130 tumor cells may be dominated by the expansion of relatively few CTL progenitor clones. Alternatively, a few clones may be more active than others, resulting in higher RNA levels and subsequent preferential PCR amplification. In either case, there are likely dominant subpopulations defined either by their higher numbers or their activity. In addition, there was a notable dominance in the use of Dß1.1 and Jß1.1 gene segments, conservation of a CDR3 length of 9 amino acids, and remarkable selection for arginine and glutamine among the anti-6130 CDR3s. Finally, the CDR3 of the anti-A CTL clone BS1 was also remarkably similar to those of the anti-6130 Vß13+ PELs, thus corroborating the anti-A specificity of the PEL responses. Taken together, the results indicate a high degree of restriction in the CDR3 of the β chain suggesting that the response in vivo was dominated by rather few CTL clonotypes. In addition, the CDR3 conservation makes it unlikely that a superantigen effect is alone responsible for the Vß13 predominance among T-cells responding to 6130. Although the TCR profiles of the Vß13+ CD8+ population have not been determined, the loss of anti-A response in 2 of 4 Vß13-depleted mice suggest that the anti-A Vß13+ CD8+ population may also be clonotypically few in number since one-half of the mice could not compensate for the loss of the dominant anti-A responding Vß13+ CD8+ cells. Direct confirmation of this hypothesis will, of course, depend upon the molecular analysis of isolated Vß13+ CTL clones.

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Table 2 Significant increase of Vß13+ CD8+ T-lymphocytes induced by 6130 but not other tumor cells

<table>
<thead>
<tr>
<th>Antigen</th>
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<td>6138d</td>
<td>6.9 ± 2.8</td>
<td>3</td>
</tr>
<tr>
<td>6139B</td>
<td>3.1 ± 1.0</td>
<td>5</td>
</tr>
<tr>
<td>4102</td>
<td>7.9 ± 1.2</td>
<td>3</td>
</tr>
<tr>
<td>Normal spleen</td>
<td>4.4 ± 0.2</td>
<td>3</td>
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**Oligoclonal T-cell response to a tumor**

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**In vivo Depletion of Vß13+ Cells.** In order to test for the in vivo relevance of Vß13 expansion upon 6130 tumor challenge, Vß13+ cells were depleted by i.p. injection of MR12-4 (anti-Vß13) ascites. This treatment resulted in the loss of Vß13 expansion in 4 of 4 mice (Fig. 2) and the complete loss of the anti-A response in 2 of 4 mice (Fig. 1F). The retention of an anti-A response in 2 of 4 mice implies that, although Vß13 may be a dominant participant, some of the Vß13-depleted mice can compensate with other Vß subgroups.

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**Fig. 3.** Nucleotide sequences encoding the CDR3 region of 6130 tumor-induced anti-A Vß13+ T-cells. The portions of the Vß13, DJ1.1, and JB1.1 gene segments are indicated by the dots that are aligned with the overlying gene segments; underlined dots indicate identity to the DJ2.1 gene segment. Nucleotides flanking the D gene segments are shown as N-region insertions. The sequence of the 3' end of Vß13 was based on identity between previously published Vß13+ DNAAs (5, 28). DJ1.1 and DJ2.1 sequences are from Ref. 40 and the JB1.1 sequence is from Ref. 41. 5' boundaries for JB2 gene segments were determined by comparison with the borders of the germline sequence (40). Flanking V and J sequences found up- and downstream of those shown here were identical to those published previously (40, 42). For the A isolates, a mid-Vß13 region primer was used for PCR. For the B isolates and BS1 clone, the upstream Vß13 primer was used to rule out contamination from the previous experiment.

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**Anti-6130 T-cell responses.** Several years ago, it was suggested that a single malignant cell can express multiple independent CTL-recognized target antigens (29, 30) and that simultaneous therapeutic attack against the multiple antigens would destroy the cancers effectively because escape variants which had lost all the antigens would be extremely rare. Unfortunately, some of the multiple antigens on the tumors were found to be alloantigens (31) or normal differentiation molecules (32). However, a recent study (19) using tumor cell lines derived from primary tumors (thereby avoiding serial transplantations) confirmed the original notion that a single tumor (including the 6130 tumor used in this study) expressed multiple unique tumor-specific antigens. These antigens were absent from autologous normal control cells and were absent from other syngeneic tumor cells of the same differentiation lineage indicating that they were neither allo- nor differentiation antigens. It has been suggested that human tumors also express multiple independent target antigens (33–36), although cells of the same differentiation lineage and same MHC class I expression have not yet been tested to indicate...
tumor specificity. In the present study, we have analyzed the response to the 6130 tumor which expresses at least three CTL-defined antigen designated as A, B, and C (20). Results using defined antigenic variants of 6130 tumor (Fig. 1E) and sorted Vß13⁺ CD8⁺ cells (Fig. 5B) demonstrate that the Vß13⁺ cells are directed against the CTL-recognized A antigen even though each of the three antigens serve as equally effective targets for lysis by CTL clones (20). These results show the existence of an immunodominance in the immune response to multiple antigens expressed by the same tumor, as has been suggested previously by the analysis of immune responses to allogeneic normal (37) or malignant cells (38).

The limited clonal diversity of the CTL response to tumors expressing multiple antigens may favor immune evasion by allowing sequential selection of antigen loss variants during continued tumor growth (39). The limited clonal diversity of the CTLs may also make the antitumor response more susceptible to suppressive mechanisms in the host. Possibly, protocols can be developed to overcome the hierarchy and limited plasticity of the antitumor response. For example, the limited plasticity may be overcome by stimulating secondary CTL populations that are not part of the dominant tumor response. Such clones were observed in this study by eliciting CTL with immunizations in mice depleted of the dominant clonotypes. While depletion of Vß13⁺ cells in vivo resulted in the loss of the Vß13 expansion in 4 of 4 mice tested (Fig. 2), complete abrogation of anti-A CTL response occurred in only one-half of these individuals (Fig. 1F). We are currently in the process of identifying the CTL clonotypes of secondary responders that retained an anti-A specificity.

The hierarchy in the response to multiple tumor antigens might be due to competition of multiple antigenic peptides for presentation by the MHC class I molecule. In this case, hierarchy may be overcome by selectively immunizing with a high concentration of the recessive peptides alone. Unfortunately, the structural nature of the CTL-recognized unique tumor antigens on UV or chemically induced tumors remains unknown; thus, we are unable to directly link the selected Vß13⁺ TCR usage to a tumor-specific antigen seen in this study may reflect the reduced size of the T cell repertoire responding to antigens homologous to self. By similar reasoning it would follow that some mutant peptides may not be recognized by the immune system since they may not be significantly different from self. Alternatively, the mutation may be such that the mutant peptides cannot be bound by the MHC molecule. We find that relatively few clonotypes of CTLs respond to one of several possible target antigens on a tumor. Such severe restrictions in the host response may enable the tumor to escape and destroy its host.

ACKNOWLEDGMENTS

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DNA Sequence Analysis of T-Cell Receptor Genes Reveals an Oligoclonal T-Cell Response to a Tumor with Multiple Target Antigens

Steven Seung, James L. Urban and Hans Schreiber


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