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*J Immunol* 2002; 169:5522-5530; doi: 10.4049/jimmunol.169.10.5522
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Lack of Effector Cell Function and Altered Tetramer Binding of Tumor-Infiltrating Lymphocytes

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Tumor-specific CD8 T cell responses to MCA102 fibrosarcoma cells expressing the cytotoxic T cell epitope gp33 from lymphocytic choriomeningitis virus were studied. MCA102gp33 tumors grew progressively in C57BL/6 mice, despite induction of peripheral gp33-tetramer+ T cells that were capable of mediating antiviral protection, specific cell rejection, and concomitant tumor immunity. MCA102gp33 tumors were infiltrated with a high number (~20%) of CD11b+CD11c+ macrophage-phenotype cells that were able to cross-present the gp33 epitope to T cells. Tumor-infiltrating CD8 T cells exhibited a highly activated phenotype but lacked effector cell function. Strikingly, a significant portion of tumor-infiltrating lymphocytes expressed TCRs specific for gp33 with 18 U.S.C. Section 1734 solely to indicate this fact.

Received for publication June 14, 2002. Accepted for publication September 24, 2002.

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3 Abbreviations used in this paper: LCMV, lymphocytic choriomeningitis virus; tg, transgenic; TIL, tumor-infiltrating lymphocyte; NP, nucleoprotein.

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Peptides and virus

LCMV gp33 (KAVYNFATM), nucleoprotein (NP)396 (FQPQNGQFI), and adenovirus E1A234-243 (SGPSNTTPEI) peptides were purchased from NeoSystem (Strasbourg, France). The original cysteine at the anchor position 41 in the LCMV gp33 peptide was replaced by methionine. The LCMV-WE isolate used in this study was originally obtained from R. Zinkernagel (University Hospital, Zurich, Switzerland). Mice were infected i.v. with 200 pfu and viral titers were determined in a virus plaque assay as described (27).

In vivo analysis of cell rejection

Spleen cells from H8-tg mice were incubated at 5 × 10⁶ cells/ml in ice-cold PBS containing 0.5 mM CFSE (Molecular Probes, Eugene, OR) for 10 min at 37°C. The cells were washed once in PBS containing 1% FCS and 10⁶ cells were injected i.v. into recipient mice. The percentage of CFSE⁻ donor cells among recipient PBL was determined by flow cytometry.

Immunohistochemistry

Tumor sections (5–7 μm) were cut on a cryostat microtome, air dried, fixed in acetone, and blocked with TBS containing 5% mouse serum. Anti-CD8, CD44 (clone IM7), CD45 (clone 30-F11), CD62L (clone 7D4), CD11b, and CD11c were purchased from BD Pharmingen. H-2D b MHC class I tetramers complexed to P14 cell-derived TCR were a kind gift of Dr. J. Altman (Emory University, Atlanta, GA). Tetramers were stained with FITC conjugated anti-CD8 mAb for a further 20 min. TILs or spleen cells were stained with tetramers either directly after cell purification or after preculture (~10⁶ cells/ml) in tissue culture medium (IMDM plus 10% FCS) for 24 h at 37°C. Addition of IL-2 to the culture medium did not influence tetramer staining results. For direct ex vivo functional analysis of PBL, RBCs were removed by nonfixating Pharam Lyse buffer (BD Pharmingen) according to the instructions of the manufacturer. For intracellular IFN-γ staining, purified TILs or PBL (10⁵) were stimulated for 5 h in 96-well flat-bottom microtiter plates with gp33 peptide-loaded bone marrow-derived dendritic cells (28) (10⁵) in the presence of 1 μg/ml brefeldin A (Golgistop; BD Pharmingen). Cells were then surface stained with anti-CD8-FITC, washed, permeabilized, and restained with PE-conjugated rat anti-mouse IFN-γ mAb (clone XMG1.2; BD Pharmingen). Cells were analyzed on a FACSort flow cytometer (BD Biosciences, Mountain View,

Cell isolation

For isolation of tumor-infiltrating cells, tumors were cut into small pieces and digested in PBS containing 0.1% collagenase (Sigma, Taufelich, Germany), 0.01% hyaluronidase (Sigma), and 0.002% DNase I (Sigma) for 2 h at 37°C. Undigested material was allowed to settle and released cells were recovered and washed. CD8 T cells were isolated using anti-CD8-conjugated beads (MACS; Miltenyi Biotec, Bergisch-Gladbach, Germany) and MS columns. For isolation of macrophages, anti-CD11b-conjugated beads (MACS; Miltenyi Biotec) were used.

T cell cultures and cytotoxicity assay

To test APC function, spleen cells (2 × 10⁵) from P14 TCR-tg or LCMV-immune B6 mice were stimulated with the indicated numbers of CD11b⁺ cells (purity > 95% CD11b⁻, ~0.1% CD11c⁻), 2 × 10⁶ irradiated (10,000 rad) MCA102gp33⁺ tumor cells, or 2 × 10⁵ B6 spleen cells loaded with 10⁻⁶ M gp33 peptide (1 h at 37°C). The cultures were performed in 24-well plates in 1 ml IMDM supplemented with 10% FCS, penicillin/streptomycin, and 0.001 M 2-ME. To induce a CTL response in vitro, 2 × 10⁶ cells/ml, followed by incubation at 4°C for 20 min with 100 μl of properly diluted mAb. For PBL stimulation 10 U/ml heparin was added to the staining buffer. The following mAb were used: CD8 (clone 53-6.7), CD11b (clone M1/70), CD11c (clone HL3), CD25 (clone 7D4), CD44 (clone IM7), CD45 (clone 30-F11), CD62L (clone MEL-14), CD69 (clone H1.2F3), CD90.1 (clone OX-7), TCR Vα2 (clone B20.1), TCR-Vβ (clone H57-597), IA⁻ (clone AF6-120.1). All mAb were purchased from BD Pharmingen. H-2D b MHC class I tetramers complexed with gp33-primed B6 LCMV memory mice. To test for Ag-specific cell proliferation, 2 × 10⁵ purified TILs or 2 × 10⁶ spleen cells from P14 TCR-tg mice were labeled with CFSE and stimulated with 2 × 10⁵ gp33-peptide loaded B6 spleen cells in 3-well plates in 1 ml tissue culture medium. P14 TCR-tg spleen cells (2 × 10⁵) were stimulated similarly for 3 days. Cytolytic activity was tested in ⁵¹Cr release assays using EL-4 target cells loaded with gp33 peptide (10⁻¹⁰ M for 2 h at 37°C) or with the control D b -binding adenovirus peptide aa 234–243. To test for Ag-specific cell proliferation, 2 × 10⁵ purified TILs or 2 × 10⁵ spleen cells from P14 TCR-tg mice were labeled with CFSE and stimulated with 2 × 10⁵ gp33-peptide loaded B6 spleen cells for 3 days in 24-well plates in 1 ml tissue culture medium.

Flow cytometry

Lymphocytes were resuspended in PBS containing 2% FCS and 0.1% NaN₃ at a concentration of 10⁵–10⁶ cells/ml, followed by incubation at 4°C for 20 min with 100 μl of properly diluted mAb. For PBL staining 10 U/ml heparin was added to the staining buffer. The following mAb were used: CD8 (clone 53-6.7), CD11b (clone M1/70), CD11c (clone HL3), CD25 (clone 7D4), CD44 (clone IM7), CD45 (clone 30-F11), CD62L (clone MEL-14), CD69 (clone H1.2F3), CD90.1 (clone OX-7), TCR Vα2 (clone B20.1), TCR-Vβ (clone H57-597), IA⁻ (clone AF6-120.1). All mAb were purchased from BD Pharmingen. H-2D b MHC class I tetramers complexed with gp33-primed B6 LCMV memory mice. To test for Ag-specific cell proliferation, 2 × 10⁵ purified TILs or 2 × 10⁵ spleen cells from P14 TCR-tg mice were labeled with CFSE and stimulated with 2 × 10⁵ gp33-peptide loaded B6 spleen cells for 3 days in 24-well plates in 1 ml tissue culture medium.
CA) using CellQuest software. Before analysis of PBL, RBCs were lysed using FACS-Lysing Solution (BD PharMingen).

**Results**

**Growth of MCA102_{gp33} tumors**

To examine the immunogenicity of the gp33 CTL epitope as a tumor-associated Ag, parental and gp33-transfected MCA102 fibrosarcoma cells (10⁶) were transplanted s.c. into unprimed B6 mice. As shown in Fig. 1A, injection of both types of tumor cells resulted in a similar, progressive tumor growth. To exclude the possibility that MCA102gp33 tumor development was due to the selection of gp33 Ag loss variants, tumors were isolated and tested in ⁵¹Cr release assays. MCA102gp33 cells isolated from tumors were lysed by gp33-specific CTL as efficiently as MCA102_{gp33} cells kept in vitro culture, indicating that the gp33 epitope was still expressed in MCA102_{gp33} tumors growing in B6 mice (Fig. 1B). In LCMV-immune B6 mice possessing gp33-specific memory T cells, MCA102_{gp33} but not parental MCA102 tumors were rejected (Fig. 1C). LCMV infection of MCA102_{gp33} tumor-bearing B6 mice 10 days after tumor cell injection led to a significant decrease in tumor size after day 15 (Fig. 1D). However, tumor regression was transient and after day 25 progressive tumor growth was observed in all mice. All tumors isolated after this time point represented gp33 Ag loss variants (data not shown). Thus, the gp33 epitope on MCA102 tumor cells serves as a tumor rejection Ag in gp33-primed but not unprimed B6 mice.

**gp33-specific T cell immunity in MCA102_{gp33} tumor-bearing B6 mice**

MHC class I tetramers containing the gp33 epitope (Dᵇ-gp33) were used to examine whether inoculation of B6 mice with MCA102_{gp33} tumor cells could induce gp33-specific T cells. Strikingly, a high number (0.5–8% of CD8) of gp33-tetramer⁺ cells was detected in PBL of MCA102_{gp33} tumor-bearing mice (Fig. 2A). To investigate whether this response represented gp33-specific T cell immunity, three functional assays were used.

First, mice bearing MCA102_{gp33} tumors were challenged with LCMV 2 wk after tumor cell injection, and viral titers were determined. High viral titers were found in mice bearing parental MCA102 tumors, whereas mice bearing MCA102_{gp33} tumors had low titers already on day 4 after infection (Fig. 2B). To demonstrate that the observed antiviral protection was due to gp33-specific T cells, the experiment was repeated using H8-tg mice (25) that ubiquitously express the gp33 epitope as a transgene. These mice have a normal T cell repertoire, but due to central tolerance induction they lack gp33-specific T cells. Fig. 2B, column 3, shows that injection of MCA102_{gp33} tumor cells did not reduce viral titers in H8-tg mice, indicating that the observed antiviral protection in MCA102_{gp33} tumor-bearing B6 mice was due to the gp33-specific

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**FIGURE 2.** gp33-specific T cell immunity in MCA102_{gp33} tumor-bearing B6 mice. A, gp33-tetramer staining of PBL and percentages of gp33-tetramer⁺ cells of CD8 T cells from individual MCA102_{gp33} tumor-bearing mice at the indicated time after tumor cell injection. B, Antiviral protection induced by injection of 10⁶ viable (columns 1–3), 10⁷ irradiated (columns 4 and 5), and 10⁷ freeze/thawed (columns 6 and 7) tumor cells. Mice were challenged with LCMV 2 wk after tumor cell injection. Dots represent viral titers in the spleen 4 days after infection in individual mice and the dotted line corresponds to the detection limit of the virus plaque assay. C, Kinetics of adoptively transferred CFSE⁺ H8-tg donor cells in PBL of the indicated tumor-bearing mice. D, gp33-specific CTL activity of in vitro stimulated lymphocytes from PBL, spleen, and draining lymph nodes (LN) from B6 mice on day 10 (top) and day 20 (bottom) after tumor cell injection. Lymphocytes were stimulated in vitro with gp33 peptide-loaded B6 spleen cells. After 5 days, CTL activity of the cultures was determined in 5-h ⁵¹Cr release assays using EL-4 target cells loaded with gp33 (filled symbols) or control adenovirus E1A peptide (open symbols).
T cell response. Efficient antiviral protection was also obtained when irradiated MCA102gp33 tumor cells were used for immunization (Fig. 2B, columns 4 and 5), whereas injection of nonviable tumor cells (freeze/thawed) failed to induce a gp33-specific response.

Second, the ability of MCA102gp33 tumor-bearing mice to reject gp33 Ag-expressing spleen cells was examined. For these experiments, spleen cells from H8-tg mice, labeled with the vital dye CFSE, were transferred into tumor-bearing mice 2 wk after tumor cell injection, and the kinetics of the transferred donor cells was followed. As shown in Fig. 2C, shortly after adoptive transfer, CFSE+ H8 spleen cells represented 3–5% of host PBL. Afterward, the number of CFSE+ cells decreased over the course of 3 days in mice bearing MCA102gp33 tumors, whereas they persisted in mice bearing parental tumors.

Third, we compared gp33-specific CTL activity of in vitro stimulated lymphocytes from PBL, spleen, and draining lymph nodes of MCA102gp33 tumor-bearing mice. In mice bearing day 10 tumors, gp33-specific CTL activity was predominantly observed in cultures of PBL (Fig. 2D, upper panel). At a later stage (day 20), strong gp33-specific CTL activity was also detected in cultures of spleen and draining lymph nodes (Fig. 2D, lower panel), gp33-specific CTL activity was not detected in T cell cultures from normal mice or mice bearing parental MCA102 tumors (data not shown). Taken together, these data demonstrate that growth of MCA102gp33 tumors induced a strong gp33-specific CTL response capable of mediating antiviral protection and specific rejection of gp33-expressing spleen cells in vivo.

Resistance of MCA102gp33 tumor-bearing mice to a second tumor challenge

The unrestricted growth of MCA102gp33 tumors in the presence of a potent gp33-specific T cell response was unexpected. A kinetic disparity between tumor growth and induction of functional gp33-specific immunity may have occurred, and the tumor has already grown beyond the critical mass that can still be eliminated by T cells (29, 30). To provide T cells with a kinetic advantage, B6 mice bearing 5-day (group 1) or 10-day (group 2) MCA102gp33 tumors on the left flank were challenged with 106 MCA102gp33 tumor cells in the right flank. As shown in Fig. 3A, both groups of tumor-bearing mice were resistant to a second tumor challenge. Concomitant tumor immunity was gp33 Ag-specific because mice bearing MCA102gp33 tumors were not protected against a second tumor challenge with parental MCA102 tumor cells (Fig. 3B). These results demonstrate that gp33-specific T cells induced by the first tumor cell injection could mediate rejection of newly transplanted tumor cells. Thus, the kinetic balance between tumor growth and induction of gp33-specific immunity plays an important role in this model.

Cross-presentation of gp33 by CD11b+ cells isolated from the tumor mass

MCA102gp33 tumors that grew for 2–3 wk in B6 mice were infiltrated with CD4 and CD8 T cells (2–5%), a high number (~20%) of CD11b+ cells, and a few (<1%) CD11c+ cells (Fig. 4A). The CD11b+ cells expressed CD45 and MHC class II molecules, but not the dendritic cell marker CD11c, and thus represented most likely tumor-infiltrating macrophages (Fig. 4B). To test whether these cells could present the gp33 epitope to T cells, CD11b+ cells were isolated from the tumor mass (purity >95% CD11b+, <0.1% CD11c+) and were used as APC for in vitro stimulation of responder spleen cells from P14 TCR-tg mice. Irradiated MCA102gp33 tumor cells were included in the assay to judge the direct priming capacity of the tumor cells themselves. As shown in
Kinetic analysis showed that the ability of gp33-tetramers to stain purified TILs from MCA102 gp33 tumors gradually increased with the duration of in vitro culture (Fig. 5C). However, the intensity of gp33-tetramer staining of TILs was lower than the intensity of PBL from tumor-bearing mice (Fig. 2A). The reduced ability to stain freshly isolated TILs with tetramers was not due to TCR down-regulation, because the cells expressed TCRs at normal levels (Fig. 5A). Thus, a significant portion of the T cells infiltrating MCA102 gp33 tumors were specific for the gp33 epitope. However, TILs bound gp33-tetramers only after 24 h preculture in the absence of gp33-expressing/presenting cells.

Altered tetramer binding behavior of gp33-specific T cells isolated ex vivo from a gp33-expressing environment

The altered gp33-tetramer binding of TILs could be due to specific factors present in the MCA102 gp33 tumor mass or, alternatively, could represent a more general phenomenon where T cells analyzed ex vivo from an Ag-expressing environment cannot efficiently be stained with tetramers. To address this issue, T cells from P14 TCR-tg mice specific for gp33 were adoptively transferred into H8-tg mice that express the gp33 epitope on all MHC
class I + cells (25). As a control, P14 T cells were also transferred into B6 mice. Immediately after cell transfer, both types of recipient mice were infected with LCMV to induce expansion of the transferred donor cells. P14 T cells (Thy1.1+) were traced in the recipient mice (Thy1.2+) using Thy1.1-specific mAb. In B6 mice, P14 T cells could be stained with both mAb specific for the tg TCR Vα2 chain and with gp33-tetramers (Fig. 6A, left panels). In striking contrast, a large fraction of P14 T cells isolated from H8-tg mice could not be stained with gp33-tetramers, despite positive staining with TCR Vα2-specific mAb (Fig. 6A, right panels). Similar to TILs from MCA102 gp33 tumors, the ability of gp33-tetramers to stain P14 T cells from H8-tg mice increased significantly after overnight in vitro culture at 37°C but not at 4°C (Fig. 6B).

**Tetramer staining was specific because P14 T cells were not stained with NP396-tetramers.**

**TILs in MCA102 gp33 tumors lack effector cell functions**

The paradox of progressive tumor growth despite the high percentage of gp33-tetramer + cells within the CD8 subset led us to examine CTL activity, IFN-γ secretion, and proliferative capacity of purified TILs from MCA102 gp33 tumors. As shown in Fig. 7, PBL from MCA102 gp33 tumor-bearing mice directly used in 5- or 18-h 51Cr release assays efficiently lysed gp33 peptide-loaded target cells. Ex vivo CTL activity of spleen cells was also detected in 18-h 51Cr release assays (~30% specific lysis at a CD8 to target ratio of 60:1). However, the activity was considerably lower than that of PBL. CTL activity of TILs was <15% in 5-h assays, even after the purified cells had been precultured for 24 h. Under these conditions, most of the cells could be stained with gp33-tetramers (Fig. 5B). In 18-h assays (Fig. 7, right panels), gp33-specific CTL activity of TILs was detectable; however, this activity was ~10-fold lower on a CD8 cell to target ratio when compared with PBL. IFN-γ secretion of TILs after gp33 Ag stimulation was determined by intracellular cytokine staining. As shown in Fig. 8A, TILs isolated from MCA102 gp33 tumors were unable to produce notable amounts of IFN-γ after gp33 Ag stimulation when assayed ex vivo or after a 24-h preculture. In contrast, most of gp33-tetramer + cells in PBL of MCA102 gp33 tumor-bearing mice produced high levels of IFN-γ after gp33 Ag stimulation (Fig. 8B). Finally, the proliferative capacity of TILs after gp33 Ag stimulation was tested. TILs purified from MCA102 gp33 tumors were labeled with the vital dye CFSE and were stimulated for 4 days in vitro using gp33 peptide-loaded spleen cells. Under these conditions P14 T cells proliferated vigorously, as indicated by a decrease in their CFSE content. In contrast, TILs failed to divide at all (Fig. 8C), even when the culture medium was supplemented with 40 U/ml IL-2 (data not shown). Furthermore, gp33 stimulation of TILs for 5 days in vitro failed to induce gp33-specific CTL activity, whereas spleen cells
from the same tumor-bearing mice specifically lysed target cells when stimulated under the same conditions (Fig. 8D). In short, these data demonstrate that TILs in MCA102_{gp33} tumor-bearing mice were functionally impaired.

### Discussion

Tumor-specific CTLs are frequently detected among PBL of patients with progressive malignancies (21–23). The MCA102_{gp33} tumor model mimics this situation and provides a striking example of progressive tumor growth despite induction of abundant numbers of tumor-specific CD8 T cells. The functional dichotomy of gp33/tumor-specific T cells in the periphery and inside the tumor mass demonstrated here helps to explain this paradox.

The frequency of gp33-specific T cells in tumor-bearing mice was determined by using MHC class I tetramers containing the gp33 epitope. The analysis revealed a high proportion (0.5–8% of CD8) of gp33-tetramer^- cells in PBL. In naive B6 mice, the precursor frequency of cells specific for the gp33 epitope has been estimated to be in the range of 1:10^7/10^8 in CD8 T cells (13). Thus, gp33-specific T cells expanded ~10^2-fold within 2–3 wk after tumor cell challenge. Clonal expansion of these cells was accompanied by acquisition of effector cell function because MCA102_{gp33} tumor-bearing mice were protected against LCMV infection and were able to reject gp33-expressing spleen cells in vivo. In this respect, the MCA102_{gp33} model exhibits similarities to the allogeneic H-2K\(^{16}\) tumor model in which mice bearing K\(^{16}\)-positive tumors were able to reject K\(^{16}\)-positive skin transplants (31). However, it differs from most other models with defined tumor-associated Ags showing T cell priming (14, 15), tumor regression (16), T cell ignorance (17–19), or tolerance induction (2, 20, 32).

In a murine colon carcinoma model, peripheral T cells from late stage tumor-bearing mice expressed Ag receptors that contained low amounts of CD3-\(\gamma\) and completely lacked CD3-\(\zeta\) (33). The model described here differs because peripheral T cells in MCA102_{gp33} tumor-bearing mice were functional and immune dysfunction was restricted to T cells inside the tumor mass. Interestingly, in patients with colorectal carcinomas CD3-\(\gamma\) levels were low in TILs but only slightly reduced in PBL (34).

The potent induction of a gp33-specific T cell response in tumor-bearing mice raises the question of the mechanism of gp33 Ag presentation. MCA102_{gp33} tumors were infiltrated with a high number of CD11b^- macrophage phenotype cells. This could be due to soluble factors produced by the tumor cells, specific cell-cell interactions, and/or tumor cell necrosis. Cell debris and possibly also heat shock protein-associated peptides (35) could be taken up by these cells and subsequently cross-presented to CD8 T cells, either inside the tumor mass or after migration to lymphoid organs. Our experiments further showed that tumor-infiltrating CD11b^- cells, but not MCA102_{gp33} tumor cells themselves, were able to activate gp33-specific T cells in vitro. However, it is doubtful that the systemic gp33-specific T cell immunity observed in tumor-bearing mice was due to Ag presentation by macrophages inside the tumor mass. CD11c^- cells, most likely dendritic cells, were also detected in MCA102_{gp33} tumors, albeit at a lower frequency. In addition, metastasizing MCA102_{gp33} cells were found by PCR in local lymph nodes and spleens of tumor-bearing mice with (triangles) or without (circles) exogenously added IL-2. After 5 days, CTL activity of the cultures was determined in 5-h \(^51\)Cr release assays using EL-4 target cells loaded with gp33 (filled symbols) or control adenovirus E1A peptide (open symbols). CTL activity of cultures using responder spleen cells from the same MCA102_{gp33} tumor-bearing mice is included for comparison (right).
(data not shown). Thus, it is more likely that gp33-specific T cell immunity was induced in lymphoid organs of tumor-bearing mice.

In contrast to unprimed B6 mice, LCMV-immune mice or mice already bearing a tumor on the opposite flank rejected MCA102gp33 tumor cells. How can these findings be explained? It has been demonstrated that tumors grow more effectively when transplanted as solid fragments compared with cell suspensions, even though the latter contain more cells (5, 18). Thus, T cells are likely to be more effective against isolated tumor cells compared with tumor cell elimination from a solid tumor mass. In unprimed B6 mice, gp33-tetramer+ T cells were below the detection limit on day 10 after tumor cell injection (Fig. 2A). This time period would allow the transplanted tumor cells to develop a solid tumor mass that is more difficult to control. In mice with an increased frequency of gp33-specific T cells, this kinetic balance is shifted toward protective antitumor immunity.

LCMV infection of B6 mice bearing day-10 MCA102gp33 tumors induced transient tumor regression, followed by outgrowth of gp33 loss variants (Fig. 1D). Due to the low level of viral replication in these mice (Fig. 2B), the number of gp33-tetramer+ T cells in PBL was 10-fold reduced when compared with LCMV-infected control mice (data not shown). Nevertheless, the tumor regression observed indicates that gp33-specific CTL induced by LCMV infection were able to eliminate gp33-expressing tumor cells, even at a later stage of tumor development. It is therefore possible that additional factors triggered by the viral infection, such as induced CD4 T cells and activated APC, help to improve and sustain gp33-specific T cell activity.

The peculiar gp33-tetramer binding behavior of TILs from tumor-bearing mice deserves discussion. Most of the TILs could only be stained with gp33-tetramers after cell purification and a resting period. The impaired tetramer staining of TILs was not due to Ag-induced TCR down-regulation, because ex vivo isolated cells expressed TCRs at normal levels. In contrast, gp33-tetramer+ T cells in PBL could be immediately stained by conventional methods. These circulating T cells probably had less direct contact with gp33-expressing/presenting cells than T cells from the tumor mass. Our results further revealed that “tetramer-negative T cells” were not restricted to TILs in MCA102gp33 tumors, but were also found among gp33-specific P14 T cells isolated from gp33-expressing H8-tg mice. Thus, our data suggest that the inability to stain Ag-specific T cells with tetramers represents a phenomenon that is observed when T cells derived from an environment with high Ag load are analyzed ex vivo.

Failure to stain Ag-specific CD8 T cells with MHC tetramers is not an unprecedented observation. Tetramer-negative Ag-specific CD8 T cells have been described in newborn mice infected with oncogenic polyoma virus (36) and in chronic hepatitis B virus patients (37). Discrepancies between cytokine release or cytolytic activity and tetramer binding have also been found in melanoma patients (38) and have been reported for cultured T cells (39). Furthermore, triggering T cell clones under certain in vitro stimulation conditions has been demonstrated to lead to a loss of tetramer labeling (40). Moreover, tetramer binding has been shown to be an active cellular process requiring cytoskeletal rearrangements (41), and efficient binding also depends on the integrity of lipid rafts on the surface of T cells (42). Thus, interaction of tetrameric MHC molecules with TCRs appears to be influenced by the activation state of the T cell and follows different rules when compared with binding of Abs to cell surface molecules.

The lack of effector cell function of tumor-specific T cells inside the MCA102gp33 tumor provides an explanation for the tumor outgrowth despite potent systemic gp33-specific T cell immunity. It is possible that soluble factors such as TGF-β (8–10) produced by the tumor cells themselves or by infiltrating cells inhibited the response of the TILs at the tumor site. However, supernatant of MCA102gp33 tumor cells contained only low levels of TGF-β1 or -β2 (~20 pg/ml, data not shown). Alternatively, permanent stimulation of gp33-specific T cells by gp33-expressing tumor cells and by gp33 cross-presenting macrophages in the absence of costimulatory signals may lead to the observed loss of effector cell function of the TILs. In this respect, it is noteworthy that neither MCA102gp33 tumor cells nor tumor-infiltrating macrophages express costimulatory molecules such as CD80, CD86, or ICAM-1 (data not shown). The activated phenotype and the lack of effector function of TILs in MCA102gp33 tumors are reminiscent of virus-specific “Sisyphean” CD8 T cells found in persistently infected hosts (43). Similarly, P14 T cells isolated from LCMV-infected H8-tg mice ubiquitously expressing gp33 have been shown to exhibit an activated phenotype and to be anergic (25). As demonstrated in this study, these T cells also display an altered gp33-tetramer staining behavior similar to TILs from MCA102gp33 tumors (Fig. 6). Thus, we favor the view that gp33-specific T cells activated in lymphoid organs of MCA102gp33 tumor-bearing mice “exhaust” inside the tumor mass due to the high Ag load present mainly on nonprofessional APC.

In conclusion, the present study provides a striking in vivo example of how a tumor can grow progressively despite potent systemic tumor-specific T cell immunity. The model helps us to understand failures in tumor regression in patients despite successful induction of tumor-specific CTLs in the periphery. Our study emphasizes the importance of understanding the local mechanisms that prevent destruction of an established tumor by T cells.

Acknowledgments

We thank Drs. S. Batsford and S. Ehl for comments on the manuscript, Dr. S. Rosenberg for MCA102 tumor cells, Dr. M. Weller for CCL-64 cells, and Dr. J. Altman for modified H-2Db and β2-microglobulin constructs. We also thank Oliver Schweier for generation of MHC tetramers, Marlies Rawiel for expert technical assistance, and Theresa Treuer, Rainer Bronner, and Thomas Imhof for animal husbandry.

References


