Antigen Expressed on Tumor Cells Fails to Elicit an Immune Response, Even in the Presence of Increased Numbers of Tumor-specific Cytotoxic T Lymphocyte Precursors

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ABSTRACT

We have used T-cell receptor (TCR) transgenic mice to analyze the interaction of tumors with the immune system. We show that the tumor cell line Lewis lung-lymphocytic choriomeningitis virus (LL-LCMV), genetically manipulated to express an H-2 Dp-restricted epitope of the lymphocytic choriomeningitis virus glycoprotein (LCMV33-41), can grow progressively in TCR transgenic mice, where ~50% of CD8+ T cells are specific for LCMV33-41. TCR transgenic T cells were not deleted in tumor-bearing mice, and tumor-reactive T cells were not deleted. Tumor resistance correlated with the switch of TCR transgenic T cells from a CD44+ phenotype to a CD44+ phenotype and increased capacity to produce IFNγ in vitro. Results similar to those obtained in TCR transgenic mice were also obtained using an adoptive transfer system, wherein normal C57BL/6 hosts. These data indicate that even large tumors may not induce specific immunization, tolerance, or anergy to tumor antigens, and that high numbers of tumor-specific CTL precursors are not sufficient to provide tumor resistance.

INTRODUCTION

Despite the application of increasingly intense chemotherapeutic and radiotherapeutic regimens, overall cancer cure rates have remained essentially unchanged for the past 30 years (1). As a result, increasing attention is now being directed at immunotherapeutic approaches for the treatment of cancer. The development of rational immunotherapy is dependent upon a detailed understanding of the interaction between host immune responses and the tumor. It is believed that the most important effector cells involved in antitumor immunity are CD8+ CTLs, which recognize tumor-specific or tumor-associated antigens in the context of tumor MHC class I molecules (2). Tumor-associated antigens include mutated self antigens, re-expressed embryonic antigens, tissue-specific differentiation antigens, or viral antigens (3). Despite the obvious presence of novel antigens, however, few murine or human tumors induce clinically significant antitumor CTL responses (4).

It is unclear why tumors fail to activate antitumor immune responses. Tumors may actively inhibit immune responses by releasing immunosuppressive cytokines (5, 6) or may simply be unable to provide the appropriate costimulatory signals for optimum T cell activation (7, 8). In fact, the majority of tumors do not express costimulatory molecules, nor do they express many of the adhesion molecules required for efficient activation of naive T cells. It is, therefore, unlikely that tumors themselves function as effective APCs. This has important implications with respect to the induction of an antitumor response: (a) presentation of antigen without attendant costimulation may induce anergy in specific T cells (9); and (b) a T-cell response may only be induced if tumor antigen is transferred from tumor cells to host APCs (10), which have strong costimulatory capacity and the ability to locate to the appropriate areas of secondary lymphoid organs. Presentation of tumor antigen by host APCs may also allow concomitant activation of CD4+ T helper cells via antigen presented on MHC class II molecules, which are not expressed by most tumors. However, transfer of tumor antigen to APCs in situ is possibly an inefficient process, especially given that the antigen will have to gain access to the endogenous MHC class I presentation pathway of the APC to activate CTLs (11). Lack of immunity in tumor-bearing hosts may therefore reflect an inefficiency in "cross priming" of CTLs.

To compound the problem of inefficient cross-priming, tumor-associated antigens may be expressed at low levels or be poorly recognized by the T-cell repertoire. If the number of potentially tumor-reactive T cells is very low, T cells may quickly become tolerized as a result of inappropriate antigen presentation by the tumor. In contrast, if the number of specific T cells is not limiting, an effective response may be initiated. Such a situation has been described for CD4+ T cells recognizing self antigen expressed in pancreatic β cells (12). It is also possible that progressive expansion of the tumor may simply exhaust that of the reactive T-cell population, or that rapid expansion and subsequent activation-induced cell death of all available tumor-reactive CTLs may exhaust the effector response, a situation shown to occur when mice have been infected with high doses of noncytopathic virus (13). It could then be argued that the number of tumor-reactive T cells in the host repertoire may have a significant impact on the ability of the host to mount effective antitumor responses.

In this report, we have determined whether antitumor responses can develop in a situation where the number of tumor-specific CTL precursors is not limiting. The murine tumor model we used is LL-LCMV, a Lewis lung carcinoma expressing moderate levels of MHC class I molecules, which was transfected with a minigene encoding for the H-2Dp-restricted peptide 33-41 of the LCMV glycoprotein, LCMV33-41 (14). We show that LL-LCMV tumors grow unhindered in mice transgenic for a Dp+ LCMV33-41-specific TCR (15), in which greater than 50% of circulating CD8+ T cells are specific for the tumor. However, when LCMV33-41 peptide-loaded DCs are used as APCs, TCR transgenic T cells are activated, and an antitumor immune response is initiated.

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MATERIALS AND METHODS

Mice. C57BL/6 mice were from breeding pairs originally obtained from The Jackson Laboratory (Bar Harbor, ME) and were maintained at the Bio-

medical Research Unit of the Wellington School of Medicine by brother × sis-
ter mating. The "318" mice (15), transgenic for a TCR specific for the LCMV
glycoprotein (amino acids 33–41), were kindly provided by Dr. Hanspeter
Fricher (Institute of Medical Microbiology, University of Freiburg, Freiburg,
Germany) and similarly maintained. All in vivo experiments were performed
according to procedures approved by the Wellington School of Medicine
Animal Ethics Committee.

Tumor Cell Lines. The Lewis lung carcinoma cell line LLTC (C57BL/6,
H-2b; Ref. 16) was kindly provided by Dr. Graeme Finlay, Cancer Research
Institute of Medical Microbiology, University of Freiburg, Germany) and similarly maintained. All in vivo experiments were performed
according to procedures approved by the Wellington School of Medicine
Animal Ethics Committee.

FACS Staining and Reagents. Cells were stained in Dulbecco's PBS (Life
Technologies, Inc., Auckland, New Zealand). LL-LCMV was maintained
in complete medium containing 0.5 mg/ml G418 (Life Technologies, Inc.).
The peptide LCMV33-41 (KAVYNFATM) was prepared by in vitro chemical
synthesis (Chiron Mimotopes, Clayton, Australia).

RESULTS

In Vivo TCR Transgenic T-Cell Proliferation Assays. Single-cell sus-
pensions were prepared from murine spleens or lymph nodes by gently
mashing tissue through nylon gauze with a syringe plunger. The cell sus-
pension was then treated with 0.14 M NH4Cl and 17 mM Tris-HCl for 5 min at
37°C to lyse RBCs. The cells were washed, resuspended in complete medium,
and plated in triplicate at 5 × 10^6 cells/well in flat-bottomed 96-well micro-
plates (Falcon, Oxnard, CA) along with various concentrations of LCMV33-41
peptide. Plates were incubated for 48 h at 37°C, pulsed with 1 µCi/well
[^3]H]thymidine (Amersham International, Buckinghamshire, UK), and then
incubated overnight. The cells were harvested, and [^3]H]thymidine uptake was
measured using a 1450 Microbeta Plus ß-counter (Wallac Oy, Turku, Finland).

For each sample, at least six 2-fold dilutions of culture supernatant were
measured and the cell concentration was adjusted to 6 × 10^6 cells/ml to inhibit
nonspecific staining. Live cells, identified on the basis of forward scatter/side
scatter profile and/or propidium iodide exclusion, were analyzed using a
FACSort (Becton Dickinson, Mountain View, CA) and the CellQuest software
(Becton Dickinson). Instrument compensation was set in each experiment
using single color stained samples.

Adoptive Transfer Experiments. A single-cell suspension was prepared
from the spleens of several TCR transgenic mice and resuspended in IMDM.
The proportion of T cells within the preparation was determined
by FACSort analysis with anti-TCR Vα2 and anti-TCR Vβ8.1. 8.2
monoclonal antibodies, and the cell concentration was adjusted to 6 × 10^6
transgenic T cells/ml. Groups of C57BL/6 recipient mice were injected i.v. in
the lateral tail vein with 50 µl of the transgenic T-cell preparation (3 × 10^6
transgenic T cells/mouse). One day later, recipients were challenged with
LL-LCMV tumor cells or immunized once with DCs, as described above. For
each experiment, a group of adoptively transferred recipients was left
unmanipulated to serve as a control. The difference between treatment groups
and control groups was analyzed by unpaired t test on log (percentage of TCR
transgenic T cells per spleen) and log (mean fluorescence intensity, CD44),
respectively.

In Vitro Tumor Protection Assay. Groups of C57BL/6 or TCR transgenic
318 mice (n = 7–10) were challenged with 1 × 10^6 LL-LCMV tumor cells
injected s.c. into the left flank. Mice were monitored every 3–4 days, and mean
tumor size for each group was calculated as the mean of the products of
the largest and the smallest diameters. Data were recorded until the first animal in
each group reached a threshold tumor size of 200 mm^2, after which time they were
euthanized. In some experiments, mice were immunized twice with DCs at
7-day intervals as described above and challenged with tumor cells 7 days after
the last immunization.

RESULTS

LL-LCMV Tumors Grow in Mice Expressing Transgenic
LCMV33-41-specific TCRs. Mice of the 318 strain express a trans-
gene encoding for a TCR specific for amino acids 33–41 of the
LCMV glycoprotein (LCMV33-41) in association with H-2D^d. The
transgenic TCR is expressed in >50% of circulating CD8+ T cells,
whereas the remaining CD8+ and CD4+ T cells express endogenous
TCRs and TCRβ rearrangements and have a normal repertoire. To
test the hypothesis that tumors develop because of insufficient num-
bers of tumor-specific CTL precursors, we challenged TCR transgenic
mice with LL-LCMV, a transfectant of the Lewis lung carcinoma cell
line LLTC that expresses a minigenic encoding for the LCMV33-41
epitope. These cells express H-2D^d molecules, and in the presence of
IL-2, they can stimulate the proliferation of TCR transgenic T cells in
vitro (14). We show in Fig. 1 that s.c. challenge of TCR transgenic
cells with LL-LCMV tumor cells resulted in outgrowth of solid
tumors, and that the rate of growth of these tumors was identical to the
rate in C57BL/6 mice. Similar growth rates of LL-LCMV tumors in
TCR transgenic and C57BL/6 mice were observed also when mice were
challenged with as few as 10^4 or 10^3 LL-LCMV cells; the
appearance of tumors was delayed in both mouse strains in these
conditions (data not shown). To control for possible variations in
tumor growth unrelated to the expression of the transgenic TCR in the
two mouse strains, we challenged both strains with the parental tumor

96-well microplate (Falcon) and then 5 × 10^5 LCMV-specific TCR transgenic
spleen cells in complete medium containing 20 units/ml recombinant IL-2
(Roche, Nutley, NJ) were added to each well. Plates were incubated for 48 h
at 37°C, pulsed with 1 µCi/well[^3]H]thymidine, and incubated overnight.
The cells were then harvested, and[^3]H]thymidine uptake was measured as
described above.

Bone Marrow-derived DCs and Immunizations. Bone marrow cells were
cultured in six-well plates (Falcon) at 4 × 10^5 cells/ml in complete
medium containing 20 ng/ml IL-4 and 20 ng/ml granulocyte/macrophage-
coloncy-stimulating factor as described (14). Cultures were provided with fresh
complete medium and cytokines every 2-3 days and incubated at 37°C until
the time of assay (6–8 days). Cultures typically contained 75–90% N418-
positive cells as determined by FACS staining. DCs (1 × 10^5 cells/ml) were
loaded with peptide by incubation in complete medium containing 10 µM
LCMV33-41 peptide for 2 h and then washed three times with IMDM to
remove excess peptide. Mice were injected s.c. in the right flank with 10^5
peptide-loaded or untreated DCs in IMDM medium. Injection was repeated
after 7 days.

Antigen Presentation and CTL Precursors

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Fig. 1. LL-LCMV tumor cells presenting the MHC class I-restricted peptide LCMV33-41 grow progressively in transgenic mice expressing a LCMV33-41-specific TCR. Groups of 5–10 TCR transgenic mice (•) or control C57BL/6 mice (○) were challenged with either 10⁶ LL-LCMV tumor cells or 10⁶ LLTC tumor cells not expressing the LCMV33-41 epitope. Mice were monitored every 3–4 days, and mean tumor size for each group was calculated as the mean of the products of bisecting tumor diameters; bars, SE. Measurements for each group were terminated when the first animal developed a tumor in excess of 200 mm².

Fig. 2. Growth of LL-LCMV tumors in TCR transgenic mice is not due to loss of presentation of the LCMV33-41 peptide on the developing tumor tissue. Single-cell suspensions of ex vivo LL-LCMV tumor tissue from C57BL/6 (○) or TCR transgenic hosts (•) were treated with mitomycin C, washed, and incubated with splenocytes from TCR transgenic mice in the presence of 10 units/ml IL-2 for 60 h. Proliferation was measured as [³H]thymidine incorporation over the final 12 h.

cell line LLTC, which does not express the LCMV33-41 epitope. No strain-specific difference in tumor growth was observed, although the LL-LCMV tumor grew with slightly slower kinetics in both mouse strains relative to LLTC.

It was possible that the tumors observed in the TCR transgenic mice represented an epitope-negative cell population selected in the face of an LCMV-specific immune response, either through loss of the antigen or down-regulation of antigen presentation. FACS analysis of ex vivo LL-LCMV tumors did not indicate any variation in surface expression of MHC class I molecules relative to cultured cells (not shown). To establish whether the tumors still presented the LCMV33-41 epitope, we examined the ability of ex vivo tumor tissue to stimulate proliferation of TCR transgenic T cells in vitro in the presence of IL-2. This assay was chosen because, in our hands, it was more sensitive than in vitro cytotoxicity assays. The stimulatory capacity of LL-LCMV tumor cells from TCR transgenic animals was compared to that of tumor cells from C57BL/6 animals (Fig. 2) or tumor cells from in vitro cultures (not shown). No difference in T-cell proliferation was observed, indicating that an epitope-negative tumor population had not been selected in the TCR transgenic mice. The similar growth kinetics of LL-LCMV tumors observed in TCR transgenic and control mouse strains, therefore, suggest that tumors in the transgenic mice were not subjected to an effective LCMV-specific immune response.

TCR Transgenic T Cells Are Not Activated in Response to LL-LCMV Tumor Challenge. TCR transgenic T cells in 318 mice can be identified by staining with anti-TCR Va2 and anti-TCR Vβ8.1, 8.2 monoclonal antibodies and FACS analysis. We therefore determined whether challenge with LL-LCMV tumor cells induced expansion or deletion of transgenic T cells in TCR transgenic hosts. No deviation from the levels in nonmanipulated transgenic mice was observed in spleen or lymph node at any stage after tumor challenge (not shown). In addition, histological examination of the tumor did not indicate any evidence of lymphoid infiltration, suggesting that the T cells had not homed to the tumor. The activation state of TCR transgenic T cells in tumor-bearing mice was evaluated by fluorescent antibody staining and FACS analysis of various activation markers. No increased expression of the CD44 activation marker on splenic TCR transgenic T cells could be demonstrated at any time after tumor challenge (days 6–40). A representative staining for CD44 is shown.
in Fig. 3. Similar results were obtained when TCR transgenic cells from the tumor-draining inguinal lymph node were analyzed. Lymph node cell analysis could be performed only until about day 25 after tumor challenge, due to subsequent tumor growth, which made the retrieval of the lymph node unreliable. Analysis of the expression of other activation markers such as CD62L and CD25 on TCR transgenic T cells also failed to reveal any difference between tumor-bearing and nontumor-challenged transgenic mice (not shown).

The Response of TCR Transgenic T Cells from Tumor-bearing Mice to Antigen in Vitro Is Similar to the Response of Naive T Cells. Activated CD8+ T cells produce increased amounts of IFN-γ in response to antigen. To further address whether the TCR transgenic T cells had been sensitized to antigen in tumor-bearing hosts, we compared the levels of IFN-γ produced in splenic cultures from tumor-bearing animals with those from nonchallenged controls. No IFN-γ production was detected in either cultures in the absence of added LCMV33-41 peptide (not shown). In the presence of 1 μM LCMV33-41 peptide, a significant amount of IFN-γ production could be demonstrated in both cultures, but this amount did not differ between tumor-bearing and nonchallenged animals (Fig. 3c). The concentration of LCMV33-41 peptide used was chosen on the basis of a full peptide dose-response titration. These results suggest that TCR transgenic T cells had not been activated by tumor challenge.

It has been suggested that tumors may anergize specific T cells in vivo by directly engaging the TCR of naive cells without appropriate costimulation (23). To assess whether exposure to LL-LCMV tumors in vivo had an effect on the proliferative capability of transgenic T cells, splenocytes were isolated from tumor-laden and nontumor-bearing TCR transgenic mice and restimulated in vitro with LCMV33-41 peptide. No difference in the proliferative capacity of cells from these different hosts was observed (Fig. 4), suggesting that the TCR transgenic T cells were not anergized in tumor-laden hosts.

**TCR Transgenic T Cells Are Activated in Vivo by Immunization with Peptide-loaded DCs.** The data presented above suggest that TCR transgenic T cells are neither activated nor anergized in response to LL-LCMV tumor challenge. These tumors may therefore be immunologically ignored, possibly because the tumor antigen is not...
Hosts Do Not Respond to Tumor Challenge but Are Activated by DC Immunization. It was possible that subtle changes in the activation status of TCR transgenic T cells in transgenic mice were confounded by the constant renewal of transgenic T cells through thymic emigration. We therefore chose to examine the effect of tumor challenge or DC immunization using a more sensitive readout system. C57BL/6 mice were adoptively transferred by i.v. injection of 3 × 10^6 splenic TCR transgenic T cells from nonmanipulated donors. s.c. challenge with 1 × 10^6 LL-LCMV cells 1 day after adoptive transfer resulted in the appearance and progressive growth of tumors. FACS analysis on day 13 postchallenge indicated that the number of TCR transgenic T cells in the spleens of tumor-bearing recipients was identical to the number in adoptive hosts that had not been challenged (Fig. 6a). The activation status of TCR transgenic T cells in tumor-bearing mice was evaluated by triple staining with fluorescent antibodies for Vα2, Vß8.1, 8.2, and CD44. As was shown with TCR transgenic hosts, expression of CD44 was not affected by tumor challenge, suggesting that the transgenic T cells had remained in a naive state. Ability to produce IFN-γ in vitro was similarly unaffected by tumor challenge (not shown). No change in numbers of TCR transgenic cells or in their expression of activation markers could be demonstrated in the spleens of these immunized mice.

After in vitro restimulation with LCMV33-41 peptide, splenocytes from TCR transgenic mice immunized with peptide-loaded DCs produced significantly more IFN-γ than splenocytes from nonimmunized transgenics or from transgenics that had been treated with DCs not loaded with peptide (Fig. 5c). Little or no IFN-γ could be demonstrated in cultures not restimulated with peptide or in cultures from non-TCR transgenic mice that had been immunized with peptide-loaded DC (not shown). These data indicate that a significant proportion of the TCR transgenic T-cell population was activated by immunization with peptide-loaded DCs.

TCR Transgenic T Cells Adoptively Transferred into C57BL/6 Hosts Do Not Respond to Tumor Challenge but Are Activated by DC Immunization. It was possible that subtle changes in the activation status of TCR transgenic T cells in transgenic mice were confounded by the constant renewal of transgenic T cells through thymic emigration. We therefore chose to examine the effect of tumor challenge or DC immunization using a more sensitive readout system. C57BL/6 mice were adoptively transferred by i.v. injection of 3 × 10^6 splenic TCR transgenic T cells from nonmanipulated donors. s.c. challenge with 1 × 10^6 LL-LCMV cells 1 day after adoptive transfer resulted in the appearance and progressive growth of tumors. FACS analysis on day 13 postchallenge indicated that the number of TCR transgenic T cells in the spleens of tumor-bearing recipients was identical to the number in adoptive hosts that had not been challenged (Fig. 6a). The activation status of TCR transgenic T cells in tumor-bearing mice was evaluated by triple staining with fluorescent antibodies for Vα2, Vß8.1, 8.2, and CD44. As was shown with TCR transgenic hosts, expression of CD44 was not affected by tumor challenge, suggesting that the transgenic T cells had remained in a naive state. Ability to produce IFN-γ in vitro was similarly unaffected by tumor challenge (not shown). No change in numbers of TCR transgenic cells or in their expression of activation markers could be demonstrated in the spleens of these immunized mice.

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Fig. 6. TCR transgenic T cells adoptively transferred into C57BL/6 hosts retain a naive phenotype in response to tumor challenge but are activated after DC immunization. In a, animals from two groups of C57BL/6 mice (n = 4) were adoptively transferred with 3 × 10⁶ TCR transgenic T cells. One group was challenged with 10⁶ LL-LCMV cells 1 day after transfer. Once tumors were established (>5 mm²), splenocyte suspensions were prepared from animals of both groups and analyzed by FACS. Triple staining was performed using antibodies for the transgenic TCR (Va2 and Vß8.1, 8.2) in combination with the activation marker CD44. A representative Va2 versus Vß8.1, 8.2 dot plot and CD44 histogram for gated TCR+ cells from one animal in each group is shown. No significant expansion of TCR transgenic T cells (P = 0.82) or increase in expression of CD44 (P = 0.55) was observed.

In b, groups of C57BL/6 were adoptively transferred as above, and then one group was injected s.c. with 10⁶ peptide-loaded DCs 1 day after transfer. Splenocyte suspensions were prepared and analyzed by FACS as above at different intervals after immunization (days 3, 5, 7, and 13). A significant expansion of TCR transgenic T cells (P = 0.009) and increase in CD44 expression (P = 0.024) were observed, which peaked at day 7 after immunization (example shown). Nonimmunized recipients and recipients injected with DCs without peptide gave indistinguishable results in this assay.
DISCUSSION

The appearance and progressive growth of antigenic tumors in immunocompetent hosts is a phenomenon that has been recognized for a number of years (4, 24, 25) but for which there is still no sound explanation. It has been proposed that tumor cells may either specifically inactivate tumor-specific T cells through their failure to express costimulatory molecules (7, 8) or may have a generalized immune suppressive activity through the production of immunoregulatory cytokines (5, 6). In either instance, immunotherapeutic approaches would have variable success dependent upon the period of exposure of the immune system to the tumor. Success would depend upon the ability of these procedures to either activate residual T cells that have not yet been disabled by contact with the tumor or to overcome low level suppression of these effector cells. An alternative explanation for the lack of immunogenicity of antigenic tumors may be that the tumors are simply "ignored" by the immune system. Tumor-specific T-cell responses may not be induced because T-cell activation is dependent upon the capture and presentation of tumor antigen by host APCs, a process that may be inefficient. Furthermore, because naive T cells are not thought to circulate through nonlymphoid tissues like tumors (26) the direct induction of T cell anergy or deletion by the tumor is unlikely. In this latter case, the activation of antitumor immune responses through the use of immunotherapy is likely to be useful, and may lead to measurable effects on the tumor itself. Clearly, how much effort we decide to invest in immunotherapy may depend on which of the above scenarios holds true.

In this report, we have used TCR transgenic mice to analyze the interaction of tumors with the immune system. We have used a mouse strain transgenic for a TCR recognizing an epitope of the LCMV glycoprotein, LCMV33-41, and a tumor cell line, LL-LCMV, which has been genetically manipulated to express the same epitope. The LCMV33-41 epitope is expressed on LL-LCMV tumor cells at sufficient levels to induce the proliferation of TCR transgenic T cells in vitro. In addition, activated TCR transgenic T cells have the capacity to induce tumor protection upon adoptive transfer into naive hosts,4 confirming that activated TCR transgenic T cells have sufficient affinity to specifically attack and kill LL-LCMV tumor cells in vivo. Nonetheless, LL-LCMV tumors can grow progressively in TCR transgenic mice in which 50% of peripheral CD8+ T cells are tumor specific, and the kinetics of tumor growth are identical to those observed in nontransgenic mice. Tumor-specific transgenic T cells shown). No evidence of activation was seen when animals were immunized with DCs not loaded with LCMV33-41 peptide.

Activation of TCR Transgenic T Cells Correlates with Resistance to LL-LCMV Tumor Challenge. We wished to establish whether TCR transgenic mice immunized with peptide-loaded DCs also became resistant to a challenge with LL-LCMV tumor. Groups of mice (n = 5–10) were injected twice s.c. with either 105 or 106 DCs only (O), with an interval of 7 days between injections. Animals were challenged with 106 LL-LCMV tumor cells 7 days after the last immunization. Mice were monitored for tumor growth every 3–4 days, and mean tumor size for each group was calculated as the mean of the products of bisecting tumor diameters; bars, SE. Measurements for each group were terminated when the first animal developed a tumor in excess of 200 mm2.

Fig. 7. Presentation of LCMV33-41 peptide on bone marrow-derived DCs injected s.c. induces protection against tumor challenge in TCR transgenic mice and C57BL/6 mice. Groups of mice (n = 5–10) were injected twice s.c. with either 105 peptide-loaded DCs (■) or 106 DCs only (O), with an interval of 7 days between injections. Animals were challenged with 106 LL-LCMV tumor cells 7 days after the last immunization. Mice were monitored for tumor growth every 3–4 days, and mean tumor size for each group was calculated as the mean of the products of bisecting tumor diameters; bars, SE. Measurements for each group were terminated when the first animal developed a tumor in excess of 200 mm2.

4 J. F. Hermans, unpublished observation.
(either transgenic or adoptively transferred) are not deleted in tumor-bearing mice and their surface phenotype and cytokine secretion patterns remain typical of naive T cells from nonmanipulated transgenic mice. Tumor-specific transgenic T cells remained functional in tumor-bearing animals, even if exposed to tumor in vivo for long periods, as is demonstrated by their undiminished capacity to proliferate to antigen in vitro. Together, these results suggest that the appearance of transformed cells expressing novel antigens may go completely unnoticed by the immune system, inducing neither an active antitumor response nor anergy or deletion.

The results described are similar to those reported by Ohashi et al. (27), who showed that mice expressing the LCMV glycoprotein in β islet cells of the pancreas do not develop autoimmune diabetes unless infected with LCMV. No specific sensitization could be demonstrated in animals that had not been infected with LCMV, indicating that expression of a novel antigen on a cell type without strong antigen-presenting capabilities may result in ignorance rather than response or anergy. Immune ignorance in our tumor model may be particularly surprising, as the progressive growth of tumor cells leads to increasingly larger tumor antigen load, and the necrosis commonly observed in later stages of tumor progression should favor the shedding of tumor antigen and consequent uptake and presentation by host APC. Despite these considerations, we failed to observe any effect on tumor-specific T cells (activation or anergy), even at very advanced tumor stages. Essentially identical conclusions were reached also in a recent study, where solid pancreatic tumors expressing the LCMV glycoprotein grew progressively in immunocompetent hosts and failed to induce any functionally detectable immune response (28).

Recent reports making use of mice transgenic for a membrane form of chicken ovalbumin expressed on pancreas β islet cells and kidney proximal tubular cells have indicated that antigen produced in these peripheral tissues can be taken up by host APCs and can induce specific activation and subsequent deletion of adoptively transferred ovalbumin-specific CD8+ T cells (29, 30). The authors suggest that this may be a specific mechanism used to tolerate T cells to peripheral antigens that are not expressed in the thymus. Our results differ from that report in that we could not observe any activation, or deletion, of tumor-specific T cells in the presence of tumors. There are several possible explanations for this difference. One is the quantity or subcellular localization of the antigens being examined. Expression of LCMV33-41 epitope in tumor cells was achieved by using a minigene encoding the minimal T cell epitope in the absence of any signal sequence (14). Presumably, the peptide epitope would be expressed in the cytosol and then transferred into the endoplasmic reticulum for association with MHC class I. The levels of expression of the epitope on LL-LCMV tumor cells, as evaluated by their ability to induce specific T-cell proliferation in vitro, were relatively low. In these circumstances, the amount of antigen shed by tumor cells and taken up by host APCs may be insufficient to induce T-cell activation. As was described previously for some self antigens, expression at low levels may be insufficient to reach the threshold required to induce tolerance (31). In our case, however, expression is not sufficiently low to also prevent recognition by effector T cells that have been activated in the context of DCs. Immunological ignorance in our model may otherwise reflect poor colonization of the tumor mass by DCs, thereby lowering the transport of tumor antigen to the lymph node and hence the possibility of T-cell activation. This mechanism has been well documented in the case of tumors; it is reported that tumors often have poor infiltration of DCs or may use active mechanisms to inhibit the migration of DCs to the lymph node (32). We could not observe any inhibitory effect of LL-LCMV tumors on the stimulatory function of DCs, because DCs isolated from tumor laden mice did not show reduced stimulatory capacity in vitro. In addition, DC immunization was effective at inducing tumor protection in tumor-laden animals.

Induction of effective CD8+ immune responses may require the participation of CD4+ T helper cells that can provide “help” (33, 34), perhaps by activating APCs through CD40L/CD40 interaction (35). It is therefore possible that the lack of activation of TCR transgenic T cells we observed in tumor-laden hosts was due to the insufficient presentation of MHC class II-restricted tumor antigen in vivo. However, we have observed that immunization with MHC class II-deficient, LCMV33-41 peptide-loaded DCs can induce some activation of TCR transgenic T cells to the production of “memory” cytokines. In contrast, no such increased production could be demonstrated in tumor-laden mice. These data imply that it is not simply the lack of CD4+ T cell help that prevents the activation of TCR transgenic T cells in tumor-bearing hosts.

We show that protective immune responses can be induced against otherwise nonimmunogenic tumors by appropriate immunization with DCs. DC immunization induced delayed tumor growth, as well as the switch of TCR transgenic T cells from a CD44low to a CD44high phenotype and also induced the capacity to produce high amounts of IFN-γ in response to specific antigen in vitro. Although we observed no better protection in mice expressing large numbers of tumor-specific T cells, this may be due to the limited amount of T-cell help elicited in these mice, which is not sufficient to sustain the full activation of large numbers of CD8+ T cells. Experiments to test this possibility are presently in progress.

The data discussed above, together with the finding that T cells from nonimmunized tumor-bearing animals have undiminished capacity to proliferate to antigen in vitro, suggest that even large tumor masses may not induce specific tolerance or anergy to tumor antigens. This represents an ideal premise for the use of vaccination procedures to elicit tumor-specific immune responses, with the only limitations to the success of this approach being the capacity of vaccination to induce a powerful and sustained immune response and of activated T cells to access the tumor mass in sufficient numbers.

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