Broadening of Epitope Recognition During Immune Rejection of ErbB-2-Positive Tumor Prevents Growth of ErbB-2-Negative Tumor

Shari A. Pilon, Carmen Kelly and Wei-Zen Wei

*J Immunol* 2003; 170:1202-1208; doi: 10.4049/jimmunol.170.3.1202

http://www.jimmunol.org/content/170/3/1202

**References**

This article cites 39 articles, 26 of which you can access for free at:
http://www.jimmunol.org/content/170/3/1202.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Broadening of Epitope Recognition During Immune Rejection of ErbB-2-Positive Tumor Prevents Growth of ErbB-2-Negative Tumor

Shari A. Pilon,* Carmen Kelly, † and Wei-Zen Wei2*†

Tumor heterogeneity is a limiting factor in Ag-specific vaccination. Ag-negative variants may arise after tumor cells bearing the immunizing Ags are destroyed. In situ priming to tumor-associated epitopes distinct from and not cross-reactive with the immunizing Ags may be crucial to the ultimate success of cancer vaccination. Immunization of BALB/c mice with DNA encoding wild-type human ErbB-2 (Her-2/neu, E2) or cytoplasmic ErbB-2 (cytE2), activated primarily CD4 or CD8 T cells, respectively, and both vaccines protected against ErbB-2-positive D2F2/E2 tumors. In ≥50% of protected mice, a second challenge of ErbB-2-negative D2F2 tumor cells was rejected. Recognition of non-ErbB-2, tumor-associated Ags was demonstrated by immune cell proliferation upon stimulation with irradiated D2F2 cells. This broadening of epitope recognition was abolished if CD4 T cells were depleted before D2F2/E2 tumor challenge, demonstrating their critical role in Ag priming. Similarly, mice that rejected D2F2/cytE2 tumor cells, which express only MHC I epitopes of ErbB-2, were not protected from a second challenge with D2F2 cells. Depletion of CD8 T cells abolished protection against D2F2, indicating the activation of D2F2-specific CTL. Therefore, long term protection may be achieved by immunization with dominant Ag(s), followed by a general enhancement of CD4 T cell activity to promote priming to multiple tumor-associated Ags. The Journal of Immunology, 2003, 170: 1202–1208.

Cancer vaccines can induce significant immunity against a particular Ag or group of Ags on tumor cells, leading to tumor destruction. Ag-negative tumors, however, often arise and cannot be eliminated unless the immune system learns to recognize tumor-associated Ags not included in the original vaccines, i.e., epitope spreading or broadening of epitope recognition (1–3).

Epitope spreading was more extensively described in murine experimental autoimmune diseases, including relapsing experimental autoimmune encephalomyelitis (EAE), a model for the human disease multiple sclerosis (4, 5). EAE can be induced by vaccination with a single MHC class II-restricted peptide derived from a myelin protein such as proteolipid protein (PLP). After the original episode subsides, a relapse of the disease occurs, and CD4 T cell responses against other CNS proteins, such as myelin basic protein, in addition to the original PLP peptide can be detected (6, 7). A proposed model for relapsing EAE is that CD4 T cells recognizing self-PLP are activated by peptide vaccination to induce an inflammatory cascade that results in the destruction of myelin. As myelin debris is taken up by macrophages, B cells, or other professional APC in the CNS, secondary epitopes from myelin basic protein or PLP are presented on their MHC class II mol-
In this study we demonstrate broadened epitope recognition induced during immune rejection of an ErbB-2-positive tumor that was mediated by CD4 T cells.

Materials and Methods

Animals and cell lines

BALB/c and C57BL/6 mice (6–8 wk old) were purchased from Charles River Laboratory (Frederick, MD). All animal procedures were performed in accordance with the regulations of Wayne State University Division of Laboratory Animal Resources, following the protocols approved by the animal investigation committee.

D2F2 is a mouse mammary tumor cell line derived from a spontaneous mammary tumor that arose in BALB/c hyperplastic alveolar nodule line D2 (21). EL-4 is a C57BL/6 thymoma line. B16 is a C57BL/6 melanoma. Cell lines were maintained in vitro in DMEM supplemented with 5% heat-inactivated cosmic calf serum (HyClone, Logan, UT), 2 mM l-glutamine, 50 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. Stable clones of D2F2 cells expressing wild-type or cytoplasmic ErbB-2 have been previously described (22). EL-4 cells were cotransfected with pRSV2/neo and pCMV/E2, which encodes wild-type ErbB-2 (E2). Stable clones of EL-4/E2 were established, and ErbB-2 expression was verified on the cell surface by flow cytometry (not shown). Transfected cell lines were maintained in medium containing 0.8 mg/ml G418 (Geneticin; Sigma-Aldrich). All tissue culture reagents were purchased from Life Technologies (Gaithersburg, MD) unless otherwise specified.

Surgical resection

Naïve mice were injected s.c. with 2 × 10^5 D2F2/E2 cells in 100 μl of PBS. Tumors were allowed to grow for ~7–10 days until the tumor volume reached 10 mm^3. Mice were anesthetized, and tumors were surgically removed. Sham surgery was performed on control mice. The incision site was closed with wound clips, which were removed 1 wk after surgery. At 2 wk after surgery, mice were challenged with 2 × 10^5 D2F2/E2 or D2F2/cytE2 tumor cells. C57BL/6 mice were challenged s.c. with 2 × 10^3 EL-4/E2 cells. At 6–10 wk after the initial tumor challenge, tumor-free mice were rechallenged with 2 × 10^2 D2F2 (BALB/c) or EL-4 (C57BL/6) cells in the opposite flank. Naïve mice were also injected with 2 × 10^3 D2F2 or EL-4 cells to verify tumor growth. Tumors were measured weekly, and tumor volume was calculated. Animals were sacrificed when the tumor volume reached 600 mm^3. The percentage of tumor-free mice was analyzed by Kaplan-Meier methods, and statistical significance was determined by the log-rank test.

T cell depletion

mAb GK1.5 (American Type Culture Collection, Manassas, VA) and 2.43 (American Type Culture Collection) were used to deplete CD4 and CD8 T cells, respectively. Each mouse was injected i.p. on days 5, 4, and 3 before tumor challenge with 500 μg of mAb GK1.5 or 2.43. Thereafter, depletion was maintained by i.p. injection with mAb GK1.5 or 2.43 every 3 days.

Proliferation assay

Lymph nodes or spleens were collected, and mononuclear cells were isolated by Ficol gradient. Effector cells were plated at 1 × 10^5 cells/well in 2 ml HEPES-buffered RPMI 1640 medium supplemented with 5% FCS, 2 mM l-glutamine, 50 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 96-well, flat-bottom plate. Stimulator cells (3T3, 3T3/E2, D2F2, or D2F2/E2) were irradiated at 25,000 rad and plated at 1 × 10^4 cells/well. Each sample was performed in triplicate. The cells were incubated at 37°C in 5% CO₂ for 4–5 days and pulsed with [3H]thymidine at 1 μCi/well for 18 h. Thymidine incorporation was measured with a Trilux Beta Scintillation Counter (Wallac, Turku, Finland). Control wells were cultured with medium alone to determine background proliferation. Positive proliferation was determined by culturing effector cells with 1 μg/ml Con A. Results were reported as the stimulation index (SI; mean counts per minute of stimulated cells/mean counts per minute of medium-cultured cells).

Generation of CTL and CTL assay

Splenocytes from immunized mice were isolated 2 wk after the second DNA vaccination by Ficol gradient and were incubated for 7 days with irradiated 3T3 cells transfected with ErbB-2, K^b, and B7.1 as previously described. Mice were palpated weekly to detect tumor growth.

Table I. Rejection of D2F2/E2 cells resulted in priming to D2F2 tumor associated Ags in ErbB-2 immune mice

<table>
<thead>
<tr>
<th>Vaccinea</th>
<th>Expt. no.</th>
<th>Mice Protected from D2F2/E2 (%)b</th>
<th>Mice Protected from D2F2 after D2F2/E2 Rejectionc</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (2×)</td>
<td>2</td>
<td>8/8 (100)d</td>
<td>1/8 (13)d</td>
</tr>
<tr>
<td>E2 (3×)</td>
<td>2</td>
<td>7/8 (88)d</td>
<td>6/7 (86)d</td>
</tr>
<tr>
<td>E2 + GM-CSF (2×)</td>
<td>1</td>
<td>8/8 (100)d</td>
<td>5/8 (63)d</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4/4 (100)d</td>
<td>3/4 (75)d</td>
</tr>
<tr>
<td>Vector control</td>
<td>1</td>
<td>0/8 (0)e</td>
<td>0/4 (0)e</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0/8 (0)e</td>
<td>0/4 (0)e</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0/4 (0)e</td>
<td>0/4 (0)e</td>
</tr>
</tbody>
</table>

a BALB/c mice were immunized two or three times at 2-wk intervals, each time by i.m. injection of pCMV/E2 or control vector pCMV in 0.1 ml of PBS. Some mice also received pEFBos/GM-CSF in the same injection.

b Two weeks following the last DNA injection, mice were challenged s.c. with 2 × 10^5 D2F2/E2 tumor cells. Tumors were palpated weekly, and tumor incidence is reported as the number of mice protected per the number of mice challenged at 6 wk after tumor cell injection.

c Mice that rejected D2F2/E2 tumor were rechallenged in the opposite flank 7–10 wk later with 2 × 10^5 ErbB-2-negative D2F2 cells. Mice were palpated weekly to detect tumor growth.

^p < 0.05 compared with vector control or naïve mice, as analyzed by log-rank test.

d Naïve mice were injected with D2F2 tumor cells as tumor growth controls.
described (23). On day 7 cells were restimulated. On day 14 cytotoxic activity was analyzed using $[^{3}H]$chromate-labeled D2F2 and D2F2/E2 cells as targets. The percentage of specific lysis was calculated as 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release). Spontaneous and maximum release were determined in the presence of medium and 1/6 N HCl, respectively.

Results

Priming to D2F2 tumor-associated Ags during D2F2/E2 tumor rejection in ErbB-2-immunized mice

Priming to ErbB-2-independent Ags was tested as outlined in the scheme of Table I. BALB/c mice were immunized two or three times, at 2-wk intervals, by i.m. injection with 100 μg of each plasmid DNA. Two weeks after the last immunization, mice were challenged with 2 × 10^5 D2F2/E2 cells. Mice that rejected D2F2/E2 cells were rechallenged in 7–10 wk with 2 × 10^5 ErbB-2-negative D2F2 cells. The results of three independent experiments are summarized in Table I. Consistent with our previous reports, two or three vaccinations with pCMV/ErbB-2 with or without pEFBos/GM-CSF induced strong protection, and 88–100% of immunized mice rejected D2F2/E2 tumors (24). Mice that received control pCMV vector all developed tumor from the same challenge. Rejection of D2F2/E2 cells was a result of anti-ErbB-2 immunity, as DNA immunized mice all developed tumors when they were challenged with ErbB-2-negative D2F2 tumor cells (not shown). To determine whether priming to epitopes besides ErbB-2 was induced during D2F2/E2 rejection, tumor-free mice were rechallenged with D2F2 cells. Significant protection against D2F2 tumor was observed in mice previously immunized three times with pCMV/E2 or twice with a combination of pCMV/E2 and pEFBos/GM-CSF, but not in mice immunized twice with pCMV/E2, although all primary D2F2/E2 tumors were rejected in this group. Therefore, priming to D2F2 tumor-associated Ags was induced during immune rejection of D2F2/E2 tumor rejection and prevented D2F2 tumor growth. This priming was enhanced in mice that received repeated immunizations of ErbB-2 or coimmunization with GM-CSF DNA, suggesting a positive correlation between strong immune reactivity to ErbB-2 and priming to D2F2 Ags.

Poor priming to tumor-associated Ags without prior vaccination

It is possible that the mere exposure to D2F2/E2 cells primed the immune system to D2F2 tumor-associated Ags and contributed to D2F2 tumor rejection. To test this possibility, BALB/c mice were injected s.c. with D2F2/E2 cells. The tumors were surgically removed when they reached the volume of ~10 mm^3. After a recovery period of 2 wk, mice were challenged in the opposite flank with 2 × 10^5 D2F2 cells. Compared with sham-operated mice, which developed tumors with an average volume of 500 mm^3 in 19 ± 5 days (Fig. 1A), mice which experienced D2F2/E2 tumor growth and surgical resection developed the same size D2F2 tumor in 24 ± 5 days ($p > 0.05$; Fig. 1B). There was no significant difference between the two groups. Therefore, D2F2 tumor growth, followed by surgical resection, did not induce significant immunity to D2F2. Rather, priming against D2F2 tumor-associated Ags was initiated during active immune rejection of D2F2/E2 cells (Table I).

To measure immune reactivity to ErbB-2 and D2F2 tumor-associated Ags, lymphocytes were isolated from mice that were immunized three times with pCMV/E2 and pEFBos/GM-CSF DNA and had rejected D2F2/E2 tumor cells. Immune lymphocytes were incubated with irradiated D2F2, D2F2/E2, 3T3, or 3T3/E2 cells for 4–5 days at an effector to stimulator ratio of 10:1. Cell proliferation was measured by $[^{3}H]$thymidine incorporation, and the SI was calculated using lymphocytes incubated in medium alone (Fig. 2).

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Priming against D2F2 tumor-specific Ags was not induced during D2F2/E2 tumor growth. Mice were injected s.c. with 2 × 10^6 D2F2/E2 cells, and tumors were surgically removed when their volume reached ~10 mm^3 (B). Sham surgery was performed on control mice that did not receive tumor cell injection (A). At 2 wk after surgery, mice were challenged s.c. in the opposite flank with 2 × 10^5 D2F2 cells. Tumor size was measured weekly with a caliper. Each line represents the tumor volume of an individual mouse. There were four mice in each group.

Cell proliferation was induced in the presence of D2F2/E2 (SI = 14.2; $p < 0.001$) or 3T3/E2 cells (SI = 6.6; $p < 0.001$). Significant proliferation was also induced in the presence of D2F2 cells (SI = 5; $p < 0.001$). Incubation of immune lymphocytes with 3T3 cells did not result in detectable cell proliferation. Lymphocytes from mice that received control vector pCMV and developed tumor from injected D2F2/E2 cells did not proliferate when incubated with any of the stimulator cells. These results suggested that mice that rejected D2F2/E2 tumor developed CD4 T cell reactivity to D2F2-associated Ags.

Immune effector cells mediating D2F2/E2 tumor rejection

Induction of CD4 T cells reactive to D2F2 Ag may be critical to the recognition of new epitopes. To test the role of CD4 T cells in tumor rejection, BALB/c mice were immunized three times with pCMV/E2 and pEFBos/GM-CSF and challenged s.c. with 2 × 10^5 D2F2/E2 cells. CD4, CD8 or both T cell populations were depleted by i.p. injection with specific mAb, starting 1 wk before tumor challenge and continuing for 4 wk (Fig. 3A). By flow cytometry, <0.1% of the treated lymph node cells were CD4 or CD8 positive (not shown). D2F2/E2 tumor was rejected in all immunized mice.

![FIGURE 2](http://www.jimmunol.org/)

**FIGURE 2.** Ag-specific proliferation of T cells from mice that rejected D2F2/E2 tumor. Mice were immunized three times with pCMV/E2 and pEFBos/GM-CSF and challenged with D2F2/E2 tumor as described in Table I. Lymph node cells were isolated from tumor-free mice at 6 wk after D2F2/E2 tumor injection (□) or from control vector injected mice that developed D2F2/E2 tumor (●). Lymphocytes were cultured with irradiated stimulator cells at a 10:1 ratio. On day 5, 1 μCi of $[^{3}H]$thymidine was added, and thymidine incorporation was measured after overnight incubation. The SI was calculated as counts per minute of lymphocytes cultured with stimulators/counts per minute of lymphocytes in medium. There were ~<2000 cpm in wells containing lymphocytes and medium alone. *, $p < 0.001$ when the SI of cells cultured with test stimulators is compared with the SI of cells cultured with 3T3 cells as measured by Student’s $t$ test.
mice rejected the challenge. Depletion of both CD4 and CD8 T cell populations abolished the protection, and all mice developed tumors. Therefore, in ErbB-2- and GM-CSF DNA-immunized mice, CD4 T cells were the primary effectors that mediated D2F2/E2 tumor rejection. Although excellent protection against D2F2 cells was observed in such vaccinated mice (Table I), it was difficult to further analyze the role of CD4 T cells, because most mice did not survive the first tumor without CD4 T cells.

**Priming to new Ags during D2F2/E2 tumor rejection**

The observed broadening of epitope recognition was further tested in mice whose primary D2F2/E2 tumors were rejected by CD8, but not CD4, T cells. Mice were immunized with pCMV/cytE2 encoding full-length ErbB-2 that is released into the cytoplasm upon synthesis and promptly degraded by the proteasome (22). We reported previously that CD8 T cells mediated D2F2/E2 tumor rejection following immunization with cytE2 and GM-CSF DNA and that CD4 T cells were not required for this tumor rejection (24). Immunized BALB/c mice were challenged s.c. with $2 \times 10^5$ D2F2/E2 cells. As shown in Fig. 4A, six of eight mice rejected D2F2/E2 tumor following immunization with cytE2 and GM-CSF DNA. Tumors that were not rejected were analyzed by flow cytometry for expression of ErbB-2 (not shown). In control vaccinated mice, ErbB-2 expression was maintained on D2F2/E2 cells. In cytE2- plus GM-CSF-vaccinated mice, which developed tumor, ErbB-2 expression was decreased or absent, indicating selection against ErbB-2-bearing tumor cells and an outgrowth of ErbB-2-negative tumor cells. To assess epitope broadening, the six tumor-free mice were rechallenged with D2F2 cells at wk 11, and four mice were protected (Fig. 4B). This is significantly different from D2F2 tumor growth in naive mice.

Similar results were observed in C57BL/6 mice immunized three times with cytE2 and GM-CSF DNA and challenged with...
FIGURE 5. Rejection of D2F2/cytE2 in cytE2- and GM-CSF DNA-immunized mice did not induce priming to D2F2-associated Ags. BALB/c mice were immunized i.m. three times at 2-wk intervals with 100 μg each of pCMV or pCMV/cytE2 and pEFBos/GM-CSF. A, At 2 wk after the last immunization, mice were challenged s.c. with 2 × 10^6 D2F2/cytE2 cells. B, At 7 wk after the first tumor challenge, tumor-free and naive mice were injected s.c. with 2 × 10^6 D2F2 cells in the opposite flank. Tumor growth was monitored.

EL-4/E2 tumor cells that expressed ErbB-2. All immunized mice rejected the initial tumor challenge (Fig. 4C). These tumor-free mice received a second challenge of EL-4 cells at wk 8, and half of the mice rejected EL-4 challenge (Fig. 4D; p < 0.05). In a repeated experiment, 10 immunized mice were each challenged with EL-4 and B16 tumor cells on the opposite flanks after they had rejected EL-4/E2 tumor cells. All mice developed B16 tumor, but only four mice also developed EL-4 tumor (Fig. 4E). Therefore, priming against EL-4 tumor-associated Ags was induced during immune rejection of EL-4/E2 tumor.

Induction of anti-ErbB-2 CTL by cytE2 DNA was verified by the 51Cr release assay. Splenocytes were isolated from mice immunized twice with cytE2 and GM-CSF DNA without tumor challenge and were stimulated twice in vitro with irradiated APC. 3T3/munized twice with cytE2 and GM-CSF DNA without tumor challenge. At 7 wk after the first tumor challenge, tumor-free and naive mice were injected s.c. with 2 × 10^6 D2F2 cells in the opposite flank. Tumor growth was monitored.

Membrane-associated ErbB-2 on D2F2/E2 tumor contributed to the broadening of epitope recognition

To measure epitope priming without CD4 T cell reactivity to ErbB-2, cytE2- and GM-CSF DNA-immunized mice were challenged with D2F2/cytE2 cells. MHC class I-associated ErbB-2 epitopes, but not intact ErbB-2, were expressed on these cells (22). As shown in Fig. 5A, all mice immunized with cytE2 and GM-CSF DNA rejected D2F2/cytE2 tumor cells, demonstrating that CTL alone was sufficient for primary tumor rejection. When these mice received another challenge with the parental D2F2 tumor, all of them succumbed to the tumor within 3 wk, demonstrating the absence of in vivo priming to ErbB-2-independent, D2F2-associated Ags (Fig. 5A). Compared with the results shown in Fig. 4B, the surface expression of ErbB-2 on D2F2/E2 cells was critical for in vivo Ag priming.

Effectors cells mediating epitope broadening and tumor rejection

To test directly the activity of CD4 T cells in priming against D2F2 tumor Ags, mice were immunized with cytE2 plus GM-CSF DNA, and CD4 T cells were depleted starting 1 wk before D2F2/E2 tumor challenge. The depleted state was maintained for 4 wk, as shown in the schematic drawing in Fig. 6A. Ten of 14 mice immunized with cytE2 plus GM-CSF rejected D2F2/E2 tumor cells. Likewise, 10 of 14 similarly vaccinated mice that had been depleted of CD4 T cells also rejected D2F2/E2 tumor cells (Fig. 6B). By wk 7 CD4 T cells had recovered (not shown), and mice were injected s.c. with D2F2 cells. As shown in Fig. 6C, seven of 10 mice that had intact CD4 T cells were protected from D2F2 cells. In contrast, depletion of CD4 T cells during D2F2/E2 challenge abolished protection against D2F2 cells. Therefore, CD4 T cells were required for priming to non-ErbB-2 Ags on D2F2 tumor cells during D2F2/E2 rejection.

To determine the effector cells responsible for the ultimate rejection of D2F2, 12 BALB/c mice were immunized three times with cytE2 and GM-CSF DNA and challenged with D2F2/E2 tumor. In this experiment all immunized mice rejected D2F2/E2 cells (not shown) and were divided into two groups. In one group of six mice, CD8 T cells were depleted before D2F2 challenge. The other group was left untreated (Fig. 7A). While four of six untreated mice rejected a second challenge of D2F2 cells, all CD8 T cell-depleted mice developed D2F2 tumor, demonstrating that anti-D2F2 CD8 T cells were induced during D2F2/E2 tumor rejection and mediated the rejection of ErbB-2-negative D2F2 cells (Fig. 7B).

Discussion

Treatment of cancer with chemotherapy, radiation, or hormone therapy often results in the development of resistant tumors and more aggressive disease (26–29). The same can be expected in cancer vaccination. Ag-negative variants may arise after tumor cells bearing the immunizing Ags are destroyed. Only if priming
against nonimmunizing Ags associated with the primary tumors is induced will long term immune surveillance be achieved. Broadening of epitope recognition has been observed in a few experimental and clinical tumor vaccine studies (10, 12, 30, 31), but the mechanism of this important event has been poorly defined. To induce and amplify epitope broadening, improved understanding of the basic mechanisms is essential.

Here, we demonstrate priming to ErbB-2-independent, tumor-associated Ags in E2- or cytE2 DNA-immunized mice that rejected D2F2/E2 tumor. Vaccination with E2 induced primarily CD4 effectors, while vaccination with cytE2 induced exclusively a CTL response. After either vaccination, ErbB-2-positive D2F2/E2 cells, but not parental ErbB-2-negative D2F2 cells, were rejected. Rejection of D2F2/E2 tumor resulted in protection against a second challenge with D2F2 tumor cells. This was contingent upon CD4 T cells by i.p. injection of mAb 2.43. Depletion was maintained by i.p. injection of mAb 2.43 every 3 days. At 1 wk after initiation of 2.43 treatment, mice were not depleted.

The presence of pre-existing antitumor CD4 T cells or the activation of CD4 T cells at the tumor site by a strong Ag triggers cytokine production, APC maturation, and activation of additional CD4 and CD8 T cells to recognize non-ErbB-2 epitopes. This results in the destruction of ErbB-2-negative tumor at the second encounter. Therefore, cancer vaccines that activate CD4 T cells may induce broader epitope recognition, as observed in mice that received two or more immunizations with E2 (63–88%) vs cytE2 (~50%). Without a pre-existing CD4 T cell response, priming to new Ags can take place only if the tumor cells express an Ag that can, by itself, trigger a significant CD4 T cell response. This response may initiate the inflammatory cascade necessary to prime against other tumor-associated Ags. These results also indicate that CD4 T cell-reactive Ags, including membrane-associated and secreted molecules, may be more efficacious vaccination targets. Long term protection against tumor may be achieved by inducing strong immunity to these Ags and enhancing CD4 T cell activity during tumor rejection to augment epitope broadening.

Acknowledgments

We thank Amy Rogowski and John Zielinski for their contributions to this study.

References

33. Kotera, Y., K. Shimizu, and J. J. Mule. 2001. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immu-
nization. Cancer Res. 61:1015.
34. Shimizu, K., E. K. Thomas, M. Giedlin, and J. J. Mule. 2001. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immu-
nization. Cancer Res. 61:1015.
stimulation induction, T cell activation, and experimental allergic encephalomy-
37. Kotera, Y., K. Shimizu, and J. J. Mule. 2001. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immu-
nization. Cancer Res. 61:1015.
38. Shimizu, K., E. K. Thomas, M. Giedlin, and J. J. Mule. 2001. Comparative analysis of necrotic and apoptotic tumor cells as a source of antigen(s) in dendritic cell-based immu-
nization. Cancer Res. 61:1015.