T-Cells for Tumor Therapy Can Be Obtained from Antigen-loaded Sponge Implants

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ABSTRACT

The aim of the current study was to evaluate the therapeutic use of tumor-specific T-cells obtained from s.c. Ag (antigen)-loaded sponge implants. Naive C57BL/6 mice were implanted with small, polyurethane sponges containing irradiated FBL-3 tumor cells. On day 10, cells that had accumulated in the sponges were harvested and tested for specific cytolytic and proliferative function in vitro. The results demonstrated that CD8+ and CD4+ T-cells immune to FBL-3 could both be primed in and obtained from the Ag-loaded sponge implants. Limiting dilution analysis demonstrated that the frequency of FBL-3-specific cytotoxic T-lymphocytes elicited from Ag-loaded sponges was four times greater than the frequency from draining lymph nodes and at least 50 times greater than from spleen and peripheral blood. Tumor-specific T-cells could similarly be obtained from Ag-loaded sponges implanted into previously primed mice and into mice bearing disseminated FBL-3. In both circumstances, elicited T-cells exhibited much higher cytolytic activity than T-cells from sponges implanted in naive mice, indicating an in situ accumulation and expansion of primed T-cells in response to the antigen stimulation. Tumor-specific T-cells obtained from Ag-loaded sponge implants could be grown long-term in vitro by periodic restimulation with irradiated FBL-3 and low concentrations of Interleukin 2. Adoptive transfer of the resultant expanded T-cell lines into mice with disseminated FBL-3 revealed that cultured sponge-infiltrating T-cells could mediate effective antitumor therapy. Thus, in vivo immunization with Ag-loaded sponges provides a potentially useful technique for procuring primed, Ag-specific T-cells for tumor therapy.

INTRODUCTION

A major problem for Ag-specific T-cell therapy in humans is procuring a primed T-cell population for expansion in vitro. Most published attempts to treat autologous malignancy with autologous Ag-specific T-cells have used T-cells infiltrating sites of tumor or T-cells from lymph nodes (1-3). Procuring T-cells from tumor is difficult, and the amount recoverable is finite. Regional lymph nodes as a source of primed T-cells have the same limitation. Peripheral blood represents the only other currently available source. However, often times Ag-specific T-cells present in vivo will localize at sites of antigen deposition and be difficult to detect in peripheral blood, especially shortly after priming. Moreover, cells infiltrating tissue may have important properties not enriched for in peripheral blood. Therefore, studies were initiated to evaluate the potential utility of injecting tumor antigens into s.c. implanted sponges to procure immune T-cells for therapy.

The sponge matrix implantation model was first described by Leighton for studying tumor growth (4). Several studies have employed sponge implants for induction of immune responses as indicated in vivo by secondary stimulation with irradiated FBL-3 tumor cells for 5 days and tested in a standard 4-h 51Cr release assay. Briefly, sponge-infiltrating lymphocytes were plated at 2 x 104 cells/well in 96-well U-bottom microtiter plates with 51Cr-labeled target cells at effector:target ratios ranging from 80:1 to 1:1. Plates were incubated at 37°C for 4 h, and the amount of 51Cr released into 100 ml of the supernatant from each well was determined. The percentage of specific lysis was calculated as described (18). All determinations of cytotoxicity were carried out in triplicate and at a minimum of three effector:target ratios. LUs were calculated using the exponential fit equation as described by Press et al. (19). One LU is defined as the number of effector cells required to cause 33% lysis of target cells. LUs are expressed as the number of LU/107 lymphocytes.

T-Cell Cytotoxicity Assay. Sponge-infiltrating lymphocytes were activated in vitro by secondary stimulation with irradiated FBL-3 tumor cells for 5 days and tested in a standard 4-h 51Cr release assay. Briefly, sponge-infiltrating lymphocytes were plated in 96-well U-bottom microtiter plates with 51Cr-labeled target cells at effector:target ratios ranging from 80:1 to 1:1. Plates were incubated at 37°C for 4 h, and the amount of 51Cr released into 100 ml of the supernatant from each well was determined. The percentage of specific lysis was calculated as described (18). All determinations of cytotoxicity were carried out in triplicate and at a minimum of three effector:target ratios. LUs were calculated using the exponential fit equation as described by Press et al. (19). One LU is defined as the number of effector cells required to cause 33% lysis of target cells. LUs are expressed as the number of LU/107 lymphocytes.

T-Cell Proliferation Assay. Sponge-infiltrating lymphocytes were cultured at 2 x 104 cells/well in 96-well U-bottom microtiter plates in triplicate with varying doses of irradiated (12,000-rad γ-radiation) FBL-3 cells or purified inactivated F-MuLV proteins as stimulators plus 5 x 105 irradiated (3,000-rad...
γ-radioactivity) B6 spleen cells/well as accessory cells. Plates were incubated in a humidified atmosphere under 5% CO2 tension at 37°C for 72 h and then incubated for 18 h with 1 μCi of [3H]thymidine per well before harvesting.

Limiting Dilution Analysis. Tumor-reactive CTL frequency was enumerated under conditions similar to those described by others (20, 21). Varying dilutions of effector cells (1:1 series dilutions from 5 × 10^4 to 1.6 × 10^3) were cultured in round-bottomed 96-well plates with 2 × 10^4 irradiated FBL-3 tumor cells and 5 × 10^3 irradiated syngeneic spleen cells/well. Each effector cell dilution was carried out in 24 replicated wells. Culture media consisted of a 1:1 mixture of RPMI 1640 (Gibco Laboratories, Inc. Grand Island, NY) and EHEAA medium (Biofluids, Inc., Rockville, MD) with 5 × 10^-5 M 2-mercaptoethanol, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 10 mM-glutamine, and 10% fetal calf serum and contained 10 units/ml IL-2. The plates were cultured in a humidified atmosphere under 5% CO2 tension at 37°C. Cytolytic activity was assessed after 7 days in a standard 4-h 51Cr release assay. Microwells were scored as positive if the mean chromium release was more than three standard deviations above control wells. The frequency of tumor-reactive CTL was calibrated by using a computer program kindly provided by Dr. Orosz and described in detail elsewhere (21-24). Frequency was estimated according to Poisson distribution of responder cells/microwell related to the percentage of microwells that scored positive or negative for cytolytic activity. The minimum frequency of CTL was estimated with 95% confidence limits. Significance was assessed by a χ^2 test.

Generation of Cultured Tumor-Specific T-Cells. Lymphocytes derived from sponge implants of B6 mice were grown under conditions for generating FBL-3 tumor-specific T-cells as described before (25, 26). Briefly, 5 × 10^6 spleen-infiltrating lymphocytes were specifically activated by culture for 5 days with 2.5 × 10^5 irradiated (12,000-rad γ-radiation) FBL-3 cells and 5 × 10^6 irradiated (3,000-rad γ-radiation) B6 spleen cells as accessory cells in wells of a 24-well plate. Specific activation was confirmed by examining the ability of cell lines to specifically lysiss FBL-3 in a standard 4-h 51Cr release assay as described above. For in vitro generation of long-term cultured FBL-3 specific T-cells, spleen-infiltrating cells were first passed through nylon wool, and collected, nonadherent cells (T-cell enriched) were stimulated with irradiated FBL-3 cells under conditions as described above. Derived FBL-3 specific T-cells were maintained by periodic restimulation with irradiated FBL-3 cells plus irradiated syngeneic spleen cells followed by expansion with recombinant IL-2 (10 units/ml) twice every week. For determining the phenotype, T-cells were stained with fluorescein-conjugated anti-Thy 1.2 (1%), fluorescein-conjugated anti-Lyt2 (2%), or phycoerythrin-conjugated anti-L3T4 (3%) monoclonal antibodies (Becton Dickinson Immunocytochemistry Systems, Mountain View, CA) at 4°C for 30 min and analyzed by FACS. Cultured FBL-reactive T-cells were used in adoptive transfer experiments following 45 days in culture.

Therapy Model: Adoptive Chemoimmunotherapy. This assay, previously described in detail (27-29), consists of treating mice bearing established disseminated FBL-3 leukemia with a combination of chemotherapy and adoptively transferred immune cells. On day 0, host mice are inoculated i.p. with 5 × 10^6 viable, FBL-3 leukemia cells. By day 5, the tumor is disseminated and mice are treated with CY at a dose of 180 mg/kg followed in 5 h by adoptively transferred donor T-cells. Without therapy, mice die of disseminated leukemia in 2 weeks. CY reduces the tumor burden, potentially ablates host suppressor T-cells, and prolongs survival to 4 weeks. Treatment with immune T-cells alone and curative with survival directly proportional to the number of tumor-reactive T-cells transferred.

RESULTS

Tumor-specific T-Cells Can Be Primed and Obtained from s.c. Ag-Loaded Sponges Implanted into Naïve Hosts. To study whether Ag-loaded sponge implants can be used to prime mice to tumor, naive C57BL/6 mice were implanted s.c. with polyurethane sponges bearing irradiated FBL-3 tumor cells. In the control groups, naive C57BL/6 mice were implanted with sponges containing either HBSS alone or irradiated BALB/c spleen cells. After 10 days, cells that had accumulated in the sponge implants were harvested and activated by in vitro stimulation with the corresponding antigen. The ability to prime and to elicit class I MHC restricted CD8+ CTL was evaluated by assessing lytic activity against FBL-3 tumor. The ability to prime and elicit class II MHC restricted CD4+ T-cell responses was evaluated by assessing the ability to proliferate in response to FBL-3 and F-MuLV. Results of the cytolytic assay demonstrated that lymphocytes from sponges loaded with FBL-3 tumor specifically lysed FBL-3 (Table 1), but not EL-4 or LSTRA tumor cells. Control lymphocytes from sponges loaded with allogeneic BALB/c spleen cells (H-2*) specifically lysed LSTRA (H-2b) but not FBL-3 (H-2b) or EL-4 (H-2b). Results of proliferative assays (Fig. 1) demonstrated that sponge-infiltrating lymphocytes exhibited specific proliferative activity to irradiated FBL-3 tumor cells or F-MuLV antigen presented by irradiated syngeneic antigen-presenting cells. Thus, the results demonstrate that both CD8+ and CD4+ T-cells primed to FBL-3 tumor cells could be elicited and obtained within 10 days from Ag-loaded sponges implanted in naive mice.

Tumor-specific T-Cells Can Be Obtained from Ag-Loaded Sponges Implanted into Previously Immunized Hosts and Tumor-bearing Hosts. C57BL/6 mice were immunized in vivo with irradiated FBL-3 tumor cells twice (on days 0 and 14). Ten days later, mice immune to FBL-3 were implanted s.c. with polyurethane sponges either with HBSS alone or with irradiated FBL-3 tumor cells. After an additional 10 days, cells that had accumulated in the sponge implants from each group of mice were harvested, activated by in vitro stimulation with antigen, and tested for specific cytotoxic activity against FBL-3 tumor cells. The sponge-infiltrating lymphocytes from both groups could specifically lyse FBL-3 (Table 2). The cells elicited from sponges in immune mice exhibited greater specific lytic activity than from sponges in naïve mice. Specific CTL could be obtained from sponges implanted in immune mice and loaded with HBSS alone, but substantially more activity was elicited from sponges loaded with irradiated FBL-3 (Fig. 2), demonstrating the importance of antigen stimulation.

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Table 1. Specific cytolytic activity of lymphocytes obtained from Ag-loaded sponges implanted into naïve hosts

<table>
<thead>
<tr>
<th>Effect cell origin</th>
<th>% Lysis of FBL-3</th>
<th>LU_51Cr/10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80:1</td>
<td>20:1</td>
</tr>
<tr>
<td>HBSS sponges</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>FBL-3 sponges</td>
<td>33.9</td>
<td>26.5</td>
</tr>
<tr>
<td>BALB/c sponges</td>
<td>6.0</td>
<td>4.9</td>
</tr>
<tr>
<td>B6 or BALB/c</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>BALB/c x B6</td>
<td>55.5</td>
<td>34.6</td>
</tr>
</tbody>
</table>

*In this representative of two experiments, three naïve C57BL/6 mice of each group received implants of polyurethane sponges containing HBSS alone, irradiated FBL-3 tumor cells, or irradiated BALB/c spleen cells. Ten days after sponge implantation, lymphocytes infiltrating into the sponge implants of each mouse were harvested, pooled, and tested for specificity and cytolytic activity to FBL-3 tumor cells.

**The effector cells from each group of sponges were secondarily activated in vitro by stimulation with irradiated FBL-3 tumor cells for 5 days as described in “Materials and Methods.” Allotreactive T-cells were used as positive control effector cells and were generated by standard 5-day mixed lymphocyte culture.

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TUMOR THERAPY WITH T-CELLS FROM AG-LOADED SPONGES

Fig. 1. Tumor-reactive CD4<sup>+</sup> T-cells can be obtained from Ag-loaded sponge implants. Naive C57BL/6 mice received s.c. implants of sponges loaded with irradiated FBL-3 tumor cells. After 10 days, cells that had accumulated in the sponge implants were harvested, and CD4<sup>+</sup> T-cell responses were evaluated by culture with irradiated syngeneic spleen cells as antigen-presenting cells plus medium only or irradiated FBL-3 tumor cells (effector:stimulator ratio of 2:1, 4:1, and 8:1) or purified inactivated F-MuLV antigen (at 1/50,000, 1/100,000, 1/200,000 protein dilution). Proliferative responses of CD4<sup>+</sup> T-cells were assayed after 96 h in culture by [3H]thymidine incorporation.

Previous studies have shown that T-cells immune to FBL-3 coexist with FBL-3 tumor in mice bearing disseminated FBL-3 and destined to die of FBL-3 (30). To determine whether such primed T-cells could be elicited from FBL-3 loaded sponges implanted into mice bearing leukemia, C57BL/6 mice received i.p. injections on day 0 with 10<sup>6</sup> viable FBL-3 leukemia cells. By day 5, the tumors were disseminated. The mice then received s.c. implants of sponges loaded with either HBSS or irradiated FBL-3 tumor cells. After 10 days, cells that had accumulated in these sponge implants from each group of mice were harvested, activated by in vitro stimulation with antigen, and tested for specific cytotoxic activity against FBL-3 tumor. For hosts bearing disseminated leukemia, the lymphocytes derived from Ag-loaded sponges could specifically lyse FBL-3 tumor cells (Table 2). No viable FBL-3 cells were noted to exist in the sponge-infiltrating lymphocytes, despite the existence of disseminated FBL-3 in blood, spleen and lymph nodes.

Frequency of Tumor-reactive CTL Elicited from Sponge Implants Is Substantially Higher than that from Draining Lymph Nodes, Spleens, and Peripheral Blood. Sponges containing irradiated FBL-3 were implanted s.c. into naive C57BL/6 mice. After 10 days, peripheral blood, draining lymph nodes, spleen and sponges were harvested and tested for specific cytotoxic activity (Table 3) and for frequency of FBL-3 tumor-specific CTL (Table 4) as determined by limiting dilution analysis. Primed CTL elicited by immunizations with FBL-loaded sponges localized to the sponges with minimal systemic priming detectable (Table 3). The frequency of FBL-reactive CTL frequency in sponge tumor grafts was approximately 4 times higher than in draining lymph nodes and more than 50 times greater than in spleen and peripheral blood (Table 4). Whether primed T-cells infiltrated into sponges possess biological properties different from primed T-cells present in lymph nodes, spleen, and blood were not evaluated.

Tumor-reactive T-Cells Obtained from Sponge Matrix Grafts Can Be Grown to Large Numbers in vitro with Maintenance of Specific Function. Efficacy in tumor therapy with immune T-cells is in general related to the number of T-cells utilized. To determine whether sponge-infiltrating T-cells could be expanded to the extent necessary for therapy, cells derived from sponge implants were passed through nylon wool to enrich for T-cells and then periodically re-stimulated with irradiated FBL-3 cells plus rIL-2 (10 units/ml) every 2 weeks. Sponge-infiltrating cells contained approximately 20-30% T-cells, of which the majority were CD8<sup>+</sup> T cells. The total cell number increased approximately 20 times after 45 days in culture. The culture conditions used were expanded and enriched for CD8<sup>+</sup> T cells in preference to CD4<sup>+</sup> T cells. By day 45, the cultured cell population consisted of greater than 99% Thy-1.2<sup>+</sup> T-cells and 98% CD8<sup>+</sup> T-cells (Fig. 3). Functional studies showed that sponge-infiltrating T-cells expanded by intermittent in vitro restimulation with antigen plus low-dose IL-2 retained CTL specificity (Fig. 4).

Cultured Tumor-reactive T-Cells Obtained from Sponge Implants Can Mediate Antitumor Therapy in Vivo. Cultured FBL-3-reactive sponge-infiltrating T-cells were used in adoptive transfer experiments following 45 days in culture. The bulk cultured cells contained greater than 95% T-cells and were tested in an adoptive transfer model termed adoptive chemoimmunotherapy. In this model, tumor is disseminated at the time of therapy, and antitumor efficacy requires that transferred T-cells be specifically immune to FBL-3 and capable of persisting long term in vivo. The results of therapy (Fig. 5) showed that B6 mice receiving no treatment had a median survival time of 12 days. Therapy on day 5 with CY alone prolonged the median survival time to 24 days, but all mice died by day 27. By contrast, therapy on day 5 with CY plus 10<sup>7</sup> cultured, immune T-cells from sponge implants cured 100% of mice. As a positive control, therapy on day 5 with CY plus 10<sup>7</sup> cultured T-cells derived from spleens of mice immunized in the standard fashion by i.p. injection of irradiated FBL-3 cells cured 75% of mice.

DISCUSSION

Successful immunotherapy of cancer with adoptively transferred tumor-specific T-cells requires large numbers of functional T-cells. The limited therapeutic efficacy of a small number of tumor-specific T-cells can be greatly augmented by growing the T-cells in vitro and treating with the increased numbers (18). A major problem for developing specific T-cell therapy of human malignancy is procuring a
primed population for expansion in vitro. The current studies showed that primed, tumor-specific T-cells could be obtained from s.c. Ag-loaded sponge implants and grown to large numbers in vitro for antitumor therapy. T-cells immune to FBL-3 tumor cells could be primed and obtained within 10 days from Ag-loaded, small, polyurethane sponges implanted into naive or immune C57BL/6 mice as well as into tumor-bearing mice. Both primed CD8+ and CD4+ T-cells could be recovered. The recovered Ag-specific T-cells could be grown to large numbers in vitro and mediate the eradication of disseminated leukemia in vivo.

Procuring primed T-cells from humans is problematic. T-cells primed to tumor may be present and enriched at sites of tumor deposition, but procuring tumor-infiltrating T-cells is difficult, and the amount recoverable is finite. Possibly more problematic, tumor specific T-cells residing long term at sites of tumor may be anergic or otherwise tolerant to tumor tissue. Regional lymph nodes as a source of primed T-cells have similar limitations. T-cells derived shortly following immunization or T-cells not residing long term at sites of tumor deposition might be most able to grow in vitro and mediate tumor therapy. Following in vivo immunization, primed T-cells might be procured from peripheral blood. However, cells infiltrating tissue may have important properties not enriched for in peripheral blood, and often times Ag-specific T-cells present in vivo will localize at sites of antigen deposition and be difficult to detect in peripheral blood. The sponge implantation model avoids many of the technical problems encountered when tumor tissue is used as a source of tumor-infiltrating cells. Viable tumor in small, polyurethane sponges implanted s.c. into recipients have been used to induce immunological responses indicative of tumor rejection (5-8). More than 90% of the infiltrated

Table 3 Specific cytolytic activity of T-cells derived from tumor-loaded sponges, regional lymph nodes, spleen, and blood

<table>
<thead>
<tr>
<th>Effector cell origin</th>
<th>LU33/107</th>
<th>LSTRA</th>
<th>EL-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBL-3-loaded sponges</td>
<td>27.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Regional lymph nodes</td>
<td>9.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blood</td>
<td>&lt;1</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*In this representative of two experiments, three C57BL/6 mice of each group received implants of polyurethane sponges containing irradiated FBL-3 tumor cells. Ten days after sponge implantation, lymphocytes infiltrating into the sponge implants of each mouse were harvested, pooled, and tested for specific cytolytic activity to FBL-3.

*The effector cells from each group of sponges were secondarily activated in vitro by stimulation with irradiated FBL-3 tumor cells for 5 days.

*Cytoxicity assays were assessed at effector:target ratios of 80:1, 20:1, and 5:1 in a standard 4-h 51Cr release assay. LU were calculated as described in "Materials and Methods."

*ND, not done.

Table 4 Frequency of FBL-reactive CTL present in sponges, lymph node, spleen, and blood from mice primed with Ag-loaded sponges

<table>
<thead>
<tr>
<th>Responder cell origin</th>
<th>Mouse no.</th>
<th>Frequency</th>
<th>CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sponge infiltrating lymphocytes</td>
<td>1</td>
<td>7962</td>
<td>7934-7990</td>
<td>0.8537</td>
</tr>
<tr>
<td>Regional lymph nodes</td>
<td>2</td>
<td>4515</td>
<td>3953-5573</td>
<td>0.2122</td>
</tr>
<tr>
<td>3</td>
<td>7084</td>
<td>5840-9003</td>
<td>0.1621</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
<td>&lt;385,529</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>&lt;385,529</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&lt;385,529</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>&lt;385,529</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2</td>
<td>&lt;385,529</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>&lt;385,529</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*In this representative of two experiments, three C57BL/6 mice received implants of polyurethane sponges containing irradiated FBL-3 tumor cells. Ten days after sponge implantation, sponges as well as relevant tissues of each mouse were harvested and enumerated for FBL reactive CTL by limiting dilution analysis.

*The minimal CTL frequency detectable in this analysis was 1 CTL/385,529 cells.

*CI, 95% confidence interval of frequency estimation.

*Draining brachial lymph nodes.

*ND, not significant.
recombinant IL-2 (10 units/ml) every 2 weeks. On days 0, 20, and 45, cultured sponge-obtained from sponge implants were placed in culture and periodically restimulated with expansion in infiltrating lymphocytes were harvested and stained with fluorescein-conjugated anti-Thy 1.2, anti-CD8, or anti-CD4 monoclonal antibodies and analyzed by FACS.

Thus, use of implanted sponges not only provides easy accessibility to a primed population of tumor-reactive T-cells for a greater number of T-cells could be elicited. But with the advantage of easy procurement of reacting cells. Other advantages of immunization with Ag-loaded sponges are that immunization is rapid, can be rapidly detected, and tends to remain localized. Tumor-reactive CTL could be detected by the 7th day after implantation with optimal priming by the 10th day. Primed T-cells could be selectively isolated directly from Ag-loaded sponges rather than from spleen, lymph nodes, or peripheral blood lymphocytes. The frequency of FBL-3 tumor-specific CTL acquired in Ag-loaded sponges was approximately 4 times higher than that in draining lymph nodes and at least 50 times higher than that in spleen and peripheral blood, as determined by limiting dilution analysis. Our presumption is that the number of CTL in regional lymphocytes will be a function of time and that, at a later point in time, the same primed CTL will be recoverable from distant lymphoid organs. In mice immune to or bearing FBL-3 tumor, a greater number of T-cells could be elicited. The rapid priming and/or enrichment of previously primed responses provides the advantage of rapid elicitation and detection of primed responses. The selective localization of priming to sponges will allow concurrent priming to several different antigens, each in a different sponge implant, without worries about immunodominance of one antigen preventing priming to the others.

Over the last few years, T-cell recognition of antigens presented in association with MHC molecules has become better understood. It is now generally accepted that MHC class I molecules present peptides derived from proteins that are produced endogenously in the cells to CD8+ T-cells, whereas MHC class II molecules present peptides derived from exogenous antigens to CD4+ T-cells (33). Peptides naturally presented by class I MHC molecules have a specific motif defined by each respective MHC allele (34, 35). Synthetic peptides that correspond to the identified naturally presented antigens exhibited remarkable activity in inducing T-cell responses (36, 37). However, not every peptide with the appropriate motif can induce T-cell responses. Elicitation of T-cell responses to peptides still has an undetermined empirical aspect. Methods for in vitro priming of T-cells to peptides are not yet ideal. Individual, peptide-loaded sponges might...
provide a system for testing the immunogenicity of sets of different peptides to determine the response of an individual to each peptide. Sponges loaded with synthetic peptides might provide a rapid means of procuring a primed population of T-cells for analysis or therapy. In preliminary studies, we have demonstrated that peptide-loaded sponges can elicit CD4+ T-helper cells specific to the corresponding immunizing peptides. Conditions for eliciting peptide-specific CTL are under investigation.

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REFERENCES


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