Successful In Vitro Graft-Versus-Tumor Effect Against an Ia-Bearing Tumor Using Cyclosporine-Induced Syngeneic Graft-Versus-Host Disease in the Rat


Lethally irradiated LouM rats reconstituted with syngeneic bone marrow and then treated with cyclosporine (CsA) for 40 consecutive days following transplant developed a graft-versus-host disease (GVHD)-like syndrome after CsA cessation. This model of GVHD was used to define and characterize a graft-versus-tumor (GVT) effect against a syngeneic plasmacytoma CRL1662 cell line which expresses class II major histocompatibility (MHC) antigen (Ia). Nylon wool-nonadherent spleen cells from animals who developed syngeneic GVHD were capable of significant lysis against chromium-labeled tumor target cells in a four-hour chromium released cell mediated lympholysis assay; maximum lysis occurred five days following cessation of CsA when clinical signs first appeared. Cytolytic activity declined to baseline as GVHD symptoms resolved. Fractionation of splenocytes into lymphocyte subsets demonstrated that cytolytic lymphocytes (CTLs) of the OX8 phenotype (non-helper T) were capable of significant lysis against tumor target cells. Lysis of tumor cells was blocked by preincubation with monoclonal antibodies (MoAb) specific for the rat anti-class II MHC antigen but not with MoAb against class I. Incubation of tumor cells with gamma-interferon increased expression of tumor class II MHC antigens and significantly increased their susceptibility to lysis by nylon wool-nonadherent splenocytes from animals with syngeneic GVHD. These studies have demonstrated an in vitro GVT of syngeneic GVHD against an Ia-bearing tumor; the effector cell is a CTL of the OX8 phenotype specific for the class II MHC antigen.

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MATERIALS AND METHODS

Plasmacytoma. The plasmacytoma tumor cell line CRL1662 initially isolated from (Lou X AO) F hybrid rats was purchased from American Type Culture Collection, Rockville, MD. The cells were cultured in tissue culture medium (RPMI 1640 with 10% fetal calf serum and .05 mg/mL of gentamicin sulfate).

Bone marrow transplantation. Female LouM rats, aged 4 to 6 weeks (Coronavirus-free) were purchased from the National Institute of Health Animal Resources, Bethesda, MD. LouM recipient rats were irradiated (1,050 rad) at 108 rad/min from a dual-source 

Cs small animal irradiator (Atomic Energy of Canada Ltd, Kanata, Ontario). LouM donor animals were asphyxiated by CO2 and marrow was obtained from femurs, tibias, and humeri. Marrow cells were suspended in RPMI 1640 and adjusted to a concentration of 6 x 10^6 nucleated cells/mL and 1 mL was infused into recipient animals by intravenous (IV) injection through the tail vein one day

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following irradiation (day 0). Following transplant, rats were kept four to six animals per cage in virally free isolation and were given medicated drinking water supplemented with bacitracin, tetracycline, and neomycin.7

Cyclosporin. CsA was the generous gift of Sandoz, Ltd, Basel, Switzerland. The powdered CsA was dissolved in 95% ethanol and diluted in a 4% Emulphor (GAF Corp, Wayne, NJ) solution in deionized water. Rats received 1 mL of 1.25 mg/mL of CsA subcutaneously from the day of marrow infusion for 40 consecutive days. The total dose of CsA per animal per day was 12.5 mg/kg.

Assessment of GVHD. Rats were examined daily for signs of clinical GVHD, such as red ears or dermatitis. Skin biopsies were taken at predetermined intervals (7, 14, 21, and 28 days) following cessation of CsA. Previously described criteria were used for histological documentation of GVHD.8 Grade 2 acute GVHD was defined by the presence of lymphocytic exocytosis, epidermal destruction with vascular changes of the basal layer, dyskeratotic cells, and/or lymphocytic dermal and/or epidermal infiltration. Animals were killed at various times after discontinuation of CsA (days 5, 12, and 22) for assessment of cellular immune reactivity and autopsies were performed; the skin, tongue, liver, intestine, and spleen were histologically examined for the presence of GVHD.

Cell-mediated lympholysis assay. Animals were divided into four groups: (1) rats that underwent syngeneic BMT and were not treated with CsA (transplant control); (2) transplanted rats not treated with CsA, but received 5 x 10⁶ CRL 1662 tumor cells on day 40 following transplant (tumor control); (3) rats that underwent transplant, treated with CsA for 40 consecutive days following transplant (syngeneic GVHD control), and (4) rats that underwent transplant, received CsA for 40 days, and were then injected with 5 x 10⁶ tumor cells on day 40 when CsA was discontinued (syngeneic GVHD-tumor).

Animals from each group were killed at various times (days 5, 12, and 22) following discontinuation of CsA and the cell-mediated lympholysis (CML) assay was performed using standard techniques.9,11 Briefly, spleens were removed from test animals, passed over a screen mesh into a cellular suspension, monocyte/macrophage depleted by iron-carboxyl ingestion, then enriched for T lymphocytes by passage over nylon wool, and used as effector cells in a four-hour chromium release CML assay. Target cells were CRL1662 cells or CRL1662 cells incubated with rat gamma-interferon (IFN) for 48 hours (10⁴/10⁶ CRL1662 cells in 30 mL medium) labeled with 100 to 200 mCi of ⁵¹Cr for one hour. For the cold target inhibition experiments, cold target cells were three-day-old phytohemagglutinin (PHA)-induced blast cells or four-day-old concanavalin A (Con A)-induced blast cells from LouM splenocytes enriched for T lymphocytes. For each experiment assays were performed in sextuplicate. Maximum and spontaneous ⁵¹Cr release were obtained by adding 0.1 mL 6N HCL or 0.1 mL of complete medium, respectively, to cells containing 10⁴ target cells. Percent specific ⁵¹Cr release was calculated according to the following formula: % specific ⁵¹Cr release = (cpm experimental – cpm spontaneous) / (cpm maximum – cpm spontaneous).

Natural killer cell assays. Natural killer (NK) assays in test animals were performed as previously described.13 Briefly, cells from a murine tumor cell line (YAC), were labeled with ⁵¹Cr and then served as targets for assessment of NK cell activity. This assay was performed in a similar manner as the four-hour chromium release CML assay described above.

Cell separation by panning. Nylon wool-nonadherent spleen cells from animals with syngeneic GVHD were separated into lymphocyte subsets by panning, as previously described.14 Briefly, 10⁴ lymphocytes, incubated (one hour at 4°C) with 0.2 mL of 1:10 dilution of monoclonal antibodies (MoAb) (in phosphate-buffered saline) were placed in Petri dishes isolated with affinity-purified goat anti-mouse IgG (Tago Ind, Burlingame, CA), the plates were lightly centrifuged (200 g for two minutes) and incubated for one hour at 4°C. The nonadherent fraction was aspirated and used for further analysis. To confirm the purity of the selected cell population, MoAb-labeled cells were washed twice, incubated with fluorescein-conjugated goat anti-mouse Ig (Tago), washed two more times, then detected on a flow cytometer (FACS I, Becton Dickinson, Mountainview, CA; or EPICS 752; Coulter Electronic, Hialeah, FL).

The murine MoAb directed against rat lymphocyte determinants used in this analysis consisted of W3/13 (pan-specific for rat T lymphocytes), W3/25 (specific for rat T-helper lymphocytes), and OK8 (identifies the rat non-T helper lymphocytes). All were purchased from Sera Labs (Accurate Chemical and Scientific Corp, Westburg, NY).

Cell separation with fluorescence activated cell sorting. Nylon wool-nonadherent spleen cells from animals with syngeneic GVHD were stained with MoAb and incubated with fluorescein-conjugated goat anti-mouse Ig (Tago), as described above. They were then separated into antigen-positive and antigen-negative cell fractions by flow cytometry with a fluorescence activated cell sorter (EPICS 752; Coulter Electronics) and used in further analysis. Murine MoAb used in this analysis included W3/25 (specific for rat helper-T lymphocytes) and OK8 (rat non-T helper lymphocytes).

Identification of target antigen. CRL1662 plasmacytoma tumor cells labeled with ⁵¹Cr were incubated with normal mouse serum or MoAb (0.1 mL of a 1:10 dilution per 10⁶ cells) for one hour at 4°C. The cells were then washed twice in cold RPMI 1640 and used as targets in the CML assay. Nylon wool-nonadherent spleen cells from the syngeneic GVHD animals were used as effectors. Effector/target ratio was 100:1.

Murine MoAb directed against class II rat lymphocyte determinants were purchased from Sera Labs. The specific MoAb (from ascites fluid) used in this evaluation included F17-23-2 (MHC II, polymorphic) and OK8 (MHC I, monomorphic RT-1A).

Gamma-IFN incubation of CRL1662 cells. In microtiter tissue culture plates, CRL1662 cells (10⁴ cells) were cultured at 37°C with increasing concentrations of gamma-IFN (Amergen Biologicals, Thousand Oaks, CA) for 24, 48, and 72 hours. The concentrations of gamma-IFN per 10⁴ cells studied were 5, 10, 100, 1,000, and 10,000 U. Following incubation, the cells were stained with MoAb against the class II MHC determinant (F17-23-2), counterstained with fluoresceinated goat anti-mouse IgG, and analyzed by flow cytometry.

CML assays as previously described were performed using nylon wool-nonadherent spleen cells from test animals as effector cells and CRL1662 cells incubated with gamma-IFN (10³U/5 x 10⁶ cells in 30 mL culture medium for 48 hours) and then labeled with ⁵¹Cr were used as target cells.

Statistical methods. To compare results from the four different treatment groups, an analysis of variance was used to test for overall group differences. To test for differences between groups within each experiment, t tests were used to compare mean counts.

RESULTS

CRL 1662 plasmacytoma characteristics and kinetics. Rat CRL1662 tumor cells were chosen because of their expression of class II MHC antigen by both immunoperoxidase staining and fluorescence staining with flow cytometric analysis using the monoclonal antibody F17-23-2 against class II MHC antigens.

Kinetics of the tumor were evaluated in the LouM rat (unpublished observation, July 1987). Intramuscular injec-
tion of $5 \times 10^6$ cells into the right hind leg produced palpable tumors in test animals in 2 to 3 weeks. When tumor cells were injected IV or intraperitoneally, reproducible kinetics were not obtained.

**Induction of CsA-induced syngeneic GVHD.** Approximately 70% to 80% of LouM rats treated for 40 consecutive days with CsA developed clinical and histological grade 2 acute GVHD. This syndrome occurred three to ten days following cessation of CsA therapy; there were no deaths related to acute GVHD. In most of the rats who developed acute GVHD, the clinical syndrome and histological changes were present for five to ten days following the initial presentation and resolved spontaneously without further treatment. Approximately 25% of these rats who developed acute GVHD evolved into a mild chronic form of GVHD with weight loss and partial alopecia and histological changes consistent with this diagnosis; no additional treatment was necessary for these rats since the chronic GVHD either stabilized or resolved.

**CML assay to assess graft-v-tumor effect.** Studies were performed to evaluate whether animals treated with CsA who developed syngeneic GVHD had increased cytolytic activity against tumor target cells. Figure 1 summarizes the in vitro cytolytic activity on days 5, 12, and 22 following discontinuation of CsA (equivalent to days 45, 52, and 62 following transplantation) of nylon wool-nonadherent spleen cells from test animals against the tumor target cells. These results illustrate that on days 5 and 12 following CsA cessation, nylon wool-nonadherent spleen cells from CsA-treated syngeneic transplant recipients who developed syngeneic GVHD were capable of mediating significantly more lysis of CRL1662 tumor targets compared with syngeneic transplant recipients who did not receive CsA (transplant control) or to those that received only the injected tumor (tumor control) ($P = .0026$ and $P = .0017$, for days 5 and 12, respectively). CsA-treated syngeneic transplants without evidence of GVHD were also assayed on day 12 following CsA discontinuation and were incapable of lysing tumor cells. Figure 1 also demonstrates the time course for CTL activity against the target tumor cells. Maximum cell lysis was seen in the CsA-treated syngeneic GVHD five days after cessation of CsA therapy when clinical signs of grade 2 GVHD first appeared, manifested by erythroderma of the ears and dermatitis. Appreciable lysis continued to occur on day 12 after CsA cessation in animals who had histologically confirmed GVHD on days 5 and 12. However, cytolytic activity was significantly decreased in animals assayed on day 22 following CsA discontinuation when the clinical signs had resolved and histology demonstrated only minimal GVHD. These test animals had confirmed grade 2 GVHD on days 5 and 12 with appropriate clinical signs. It is also interesting to note that splenocytes from animals who received tumor injected at the time the CsA was discontinued and developed GVHD were significantly more effective in lysing tumor cells compared with animals with GVHD, but without the previous exposure to tumor injection ($P = .002, P = .024$, and $P = .0001$ for days 5, 12, and 22, respectively).

**Identification of the effector cell(s) mediating lysis.** Nylon wool-nonadherent spleen cells from test animals were separated into lymphocyte subsets by panning and by FACS using appropriate MoAb, as previously described.

In the panning experiments, the nonadherent (negatively selected) cell fractions were used in CML assays. Figure 2 shows the results of a representative experiment in which nylon wool-nonadherent spleen cells from a rat with syngeneic GVHD were fractionated into distinct subpopulations recognized by MoAb for rat T lymphocytes subsets. These results demonstrate that the subset depleted of W3/25 (helper T cells) and enriched for the OX8 subpopulation was responsible for significantly more lysis than the other two subpopulations ($P = .0001$). Depletion of T-cell subsets with OX8 (non-helper T lymphocytes) or W3/13 (pan T) removed cell-mediated cytolytic activity against CRL1662 target cells. When nylon wool-nonadherent spleen cells from tumor control animals and animals who developed syngeneic

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**Fig 1.** Nylon wool-nonadherent spleen cells from syngeneic marrow recipients in four treatment groups were assessed for their ability to lyse $^{51}$Cr-labeled CRL1662 tumor target cells in a four-hour chromium release assay 5, 12, and 22 days following discontinuation of CsA (equivalent to 45, 52, and 62 days after transplant). Treatment groups consisted of (1) no CsA, no tumor; (2) no CsA, tumor injected day 40 following transplant; (3) CsA treatment for 40 consecutive days following transplant with clinical and histological acute GVHD, no tumor; and (4) CsA treatment for 40 consecutive days with acute GVHD and tumor injected on day 40 at time CsA was discontinued. Test animals who received CsA therapy but did not develop clinical GVHD were also assayed on day 12 following CsA cessation. Effector:target ratio was 100:1. Bars indicate mean values obtained from assays performed in sextuplicate.
GVHD and had tumor injected at the time CsA was discontinued were fractionated into lymphocyte subsets by panning (data not shown), the population of cells depleted of W3/13-labeled cells (pan T) demonstrated lysis against both CRL1662 and YAC targets. The population of cells depleted of W3/13 lymphocytes demonstrated 29.1% and 68.2% lysis against YAC cells for tumor control and syngeneic GVHD with tumor injected animals, respectively, compared with only 10.6% for animals with syngeneic GVHD alone.

Table 1 shows the results of two representative experiments where nylon wool-nonadherent spleen cells from CsA-treated syngeneic marrow transplant recipients exhibiting clinical evidence of syngeneic GVHD and confirmed by histology were stained with MoAb and sorted by FACS. In the first experiment those cells labeling with OX8 (rat non-T helper lymphocytes) exhibited significant lysis against tumor cells where the negative fraction showed only minimal lysis. In the second experiment, splenocytes were stained with OX8 and W3/25 (helper T-lymphocytes); as in the previous experiment, the fraction labeling with OX8 demonstrated significant lysis as did the subpopulation that did not label with W3/25.

Results from both the panning experiments and the cell sorting using FACS demonstrate that the subpopulation of lymphocytes with the OX8 phenotype in rats with syngeneic GVHD is responsible for in vitro tumor lysis. That fraction labeling with W3/25 or depleted of OX8 lymphocytes is incapable of in vitro tumor lysis.

Identification of target antigen. To demonstrate that the class II antigen on the tumor cells was the target antigen recognized by the effector cell in these series of experiments, CRL1662 cells were preincubated with MoAbs specific for rat class I and class II antigens. Table 2 summarizes the results of representative experiments where the preincubated tumor cells are used as targets in a CML assay using effector cells from animals with syngeneic GVHD (not exposed to tumor). Preincubation of target tumor cells with the rat anti-Ia MoAb polymorphic determinant (F17-23-2) significantly reduced lysis by effector cells from animals exhibiting syngeneic GVHD. Preincubation of target cells with either normal mouse serum or anti-class I (OX18) antibody did not result in reduction of lysis.

The data presented above illustrate that splenic T cells from animals with syngeneic GVHD were capable of lysing Ia-bearing tumor cells. Lysis appears to be mediated by a T lymphocyte of the OX8 phenotype recognizing class II or Ia determinants. To further document that the effector cell recognizing self-class II MHC antigens was identical to the effector cell recognizing the syngeneic CRL1662 tumor cell, cold target inhibition experiments were performed using PHA or Con A stimulated self-lymphoblasts. Previous results have shown that after PHA and Con A stimulation, 30% to 40% of the rat T lymphoblasts express class II antigens. Figure 3 gives the results of an experiment where LouM cold targets (blast cells) were added at increasing concentrations to lytic assays using CRL1662 51Cr labeled targets. These results demonstrate that the cold LouM lymphoblasts are effective at inhibiting lysis of labeled Ia-bearing CRL1662 target cells.

Effect of gamma-IFN incubation of tumor target cells. As shown in Fig 4, incubating CRL1662 cells in culture with escalating concentrations of gamma-IFN increases both the number of cells expressing the class II antigen and the mean fluorescence. Without incubation of

Table 1. Cell Sorting by FACS

<table>
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<tr>
<th>Experiment</th>
<th>MoAb</th>
<th>Cell Lysis (%)</th>
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</thead>
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<tr>
<td>1</td>
<td>OX8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>34.3 ± 0.5*</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9.4 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>OX8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>29.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>W3/25 (helper T)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>63.1 ± 0.2</td>
</tr>
</tbody>
</table>

*Mean percent specific 51Cr release ± SE.

Table 2. Effect of Preincubation of CRL1662 Target Cells With MoAb

<table>
<thead>
<tr>
<th>Animal</th>
<th>Effector:Target Ratio</th>
<th>Normal Mouse Serum</th>
<th>OX 18</th>
<th>Anti-Class I</th>
<th>F17-23-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100:1</td>
<td>54.3 ± 0.1*</td>
<td>52.9 ± 0.2</td>
<td>4.9 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50:1</td>
<td>32.5 ± 0.1</td>
<td>36.0 ± 0.1</td>
<td>10.9 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

*Mean percent specific 51Cr release ± SE.
advantage was the rat to determine whether the effector cells associated target antigen animals who developed syngeneic GVHD. No in those was injected mia associated with CTLs capable of lysing target cells express-
(unpublished data, January 1987), which appears to be the target antigen for these effector cells.

In this report, a series of experiments were performed in the rat to determine whether the effector cells associated with syngeneic GVHD can provide an anti-tumor effect against tumors expressing class II or Ia antigens. Experiments were designed to answer four questions: first, to determine whether splenocytes from animals with syngeneic GVHD have significant lysis against Ia-bearing tumors and to characterize the time course of this effect. Second, studies were performed to identify the effector cell mediating in vitro lysis of tumor cells, and third, to determine the target antigen recognized by these effectors. Last, gamma-IFN, a well recognized immune modulator capable of increasing expression of class II antigen, was studied to determine its effect on class II expression on tumor cells, and ultimately, its effect on increasing the susceptibility of these tumor cells to lysis.

Nylon wool-nonadherent splenocytes from rats with documented syngeneic GVHD were capable of in vitro lysis of Ia-bearing tumor cells. The ability to lyse tumor was temporally associated with the clinical course of the syndrome—once the GVHD resolved T cell-enriched splenocytes were incapable of tumor lysis. In addition, nylon wool-nonadherent splenocytes from animals who received CsA but did not develop the syndrome could not lyse tumor, demonstrating that the GVHD syndrome was required to generate effective CTLs. It was also interesting to note that splenocytes from animals who developed syngeneic GVHD and were exposed

gamma-IFN, 63% of cells expressed Ia; incubation with 10 U/10^6 cells for 48 hours increases the total number to 81% with a mean fluorescence of 72. Escalating the concentration to 1,000 and 10,000 U/10^6 cells increases the total number of cells expressing Ia to 88% and 99%, respectively, with a mean fluorescence of 72 and 160, respectively.

Cytolytic activity was compared between CRL1662 targets and CRL1662 cells incubated with gamma-IFN targets using nylon wool-nonadherent spleen cells from animals with CsA-mediated GVHD as effectors (Fig 5). In the experiment shown, incubating the targets with 10,000 U of gamma-IFN increased the number of cells expressing Ia from 63% to 99% (Fig 4) and increased percent lysis of targets from 25% to 55%.

**DISCUSSION**

The major complication of autologous or syngeneic BMT continues to be relapsing disease despite aggressive preparative regimens and the use of tumor purging. Since the majority of hematopoietic malignancies express class II MHC antigens, these tumors may be effectively targeted by autoreactive T lymphocytes against class II antigens. Previous experiments in the rat have demonstrated that syngeneic recipients treated with daily injections of CsA develop a clinical syndrome similar to acute GVHD that is associated with CTLs capable of lysing target cells expressing class II or Ia antigens. In another series of experiments, Lewis Brown Norway (LBN) rats were treated with CsA following autologous transplant; Brown Norway (BN) leukemia was injected at various times following the discontinuation of CsA, attempting to demonstrate a survival advantage in those animals who developed syngeneic GVHD. No advantage was seen; however, further investigation revealed that the BN leukemia did not express the class II antigen (unpublished data, January 1987), which appears to be the target antigen for these effector cells.

In this report, a series of experiments were performed in the rat to determine whether the effector cells associated target antigen animals who developed syngeneic GVHD.
to tumor at the time CsA was discontinued were capable of significantly more tumor lysis than animals with syngeneic GVHD alone. Results from the panning experiments suggest that this increased lysis may be due to enhanced NK activity.

Despite the fact that spleen cells from animals with syngeneic GVHD and challenged with tumor were significantly more effective in lysing tumor target cells, an in vivo anti-tumor benefit was not observed in these studies. Since this was a solid tumor requiring an injection of 5 x 10^6 cells for a mass to develop, a subtle immune response may not be recognized against a large number of tumor cells. It has long been recognized that patients who develop GVHD following allogeneic BMT for hematopoietic malignancies have a decreased incidence of relapsing disease and improved long-term survival; to date, a similar beneficial graft-vs-tumor effect has not been seen following allogeneic BMT for solid tumors. If a survival advantage is to be documented with immune modulation in the animal following transplantation, one may expect to observe it in a model of minimal residual disease, as what is seen with leukemia following BMT. We are currently investigating such a model in the mouse.

Cells mediating lysis against target tumor cells in vitro appear to be CTLs of the OX8 phenotype targeted against the class II MHC antigen. Lysis was blocked by MoAb against class II, but not class I. Lysis against labeled tumor target cells was effectively inhibited by cold self-lymphoblasts exhibiting class II antigen. In addition, incubating targets with gamma-IFN increased expression of the class II antigen and then, when these targets were used in a CML assay, increased lysis against tumor was observed. The results of these experiments support the observation, first reported by Hess et al, that in this autologous system, CTLs are targeted against class II MHC antigens. CTLs are generally thought to recognize alloantigen presented by class I MHC molecules. However, other studies have exhibited responses involving both CD8+ and CD4+ CTLs with class II restriction. Recently, Shinohara et al reported on the stimulation of mouse lymphocytes with antigen capable of inducing CD8+ and CD4+ CTLs with class II restriction. In the system presented here, OX8+ CTLs specific for the class II antigen are capable of lysing autologous tumor.

Recently a phase I/II study was completed where patients with refractory lymphomas underwent autologous BMT and were treated with low-dose CsA attempting to induce syngeneic GVHD. All the patients developed a cutaneous skin rash consistent with acute GVHD; there was no additional toxicity associated with the CsA treatment. Because of the number of relapses seen with autologous BMT one may speculate that the addition of an immune purge may be of benefit in this setting. The results of these rat experiments support the cytolytic properties of syngeneic GVHD effectors against Ia-bearing cells. The additional benefit of other immune modulators such as gamma-IFN is of considerable interest and will require further investigation.

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Successful in vitro graft-versus-tumor effect against an Ia-bearing tumor using cyclosporine-induced syngeneic graft-versus-host disease in the rat

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