Therapy with Allogeneic Immune Peritoneal Cells

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SUMMARY

The growth-inhibitory effect in vivo of murine allogeneic immune peritoneal cells (C57BL versus SL2 lymphoma cells) on SL2 lymphoma cells injected i.p. into the syngeneic recipient (DBA/2 mouse) was studied. The peak value of immunity and the peak value of the growth-inhibitory effect in vitro was reached 9 days after immunization.

In 2 of 5 mice (survival time, 37 and 68 days), $2 \times 10^6$ SL2 lymphoma cells were eradicated by 1 i.p. injection of $2 \times 10^6$ C57BL allogeneic immune peritoneal macrophages (plus contaminating lymphocytes) obtained 9 days after immunization. In 4 of 5 DBA/2 mice, $2 \times 10^6$ SL2 cells were eradicated with three successive i.p. injections of $2 \times 10^6$ C57BL allogeneic immune peritoneal macrophages (plus lymphocytes) collected 10 days after immunization (survival time, 200 days; at this time of survival the experiment was stopped).

Immune peritoneal lymphocytes, normally contaminating $2 \times 10^6$ macrophages, did not inhibit growth of lymphoma cells in vivo. However, $2 \times 10^6$ immune peritoneal macrophages, freed of contaminating lymphocytes, caused a slight inhibition of growth of lymphoma cells in vivo.

Peritoneal cells from C57BL mice immunized against DBA/2 liver cells or against CBA-derived TLC5 lymphoma cells were not capable of eradicating SL2 lymphoma cells in DBA/2 mice.

INTRODUCTION

The fact that allogeneic immune peritoneal macrophages are cytotoxic to tumor cells in vitro is well established (7, 13–18, 21). There are also several reports of passive transfer of resistance to allograft mouse tumors by peritoneal cell suspensions, consisting largely or entirely of macrophages obtained from immunized mice. Transfers of this type have been reported by Baker et al. (3) using the A/J tumor Sarcoma I injected into C57BL/6K mice, by Amos (2) using DBA/2 ascites lymphoma and C3H mice, by Old et al. (19) using the C3H sarcoma BP8 and C57BL/6 mice, and by Bennett (4) using Sarcoma I and BP8 and C57BL/6 mice.

In the experiments of Amos and Bennett the peritoneal cell populations were freed of lymphocytes, so that the suppressive effect could be attributed specifically to macrophages. Brunner et al. (5, 6) and Alexander et al. (1) have shown that immune lymph node cells and lymphoid cells from the spleen can also eradicate tumor cells.

This paper describes experiments in which allogeneic immune peritoneal cells are used for the eradication of cancer in the syngeneic tumor-bearing mice. This system has the advantage of being independent from the immunogenicity of the tumor, which may be very weak. Moreover, the cytotoxicity of allogeneic immune peritoneal cells is strong and reproducible (7).

The question of which main cell type (macrophage or lymphocyte) is acting as effector cell in this in vivo system was also investigated. Part of these results were described in a preliminary report (8).

MATERIALS AND METHODS

Mice. Purebred (a) C57BL, (b) DBA/2 mice, and (c) CBA mice, 8 to 10 weeks old, were used (a) as a source of immune peritoneal cells (macrophages and lymphocytes), (b) for growth of the syngeneic ascitic SL2 lymphoma and as SL2-bearing mice to be cured, and (c) as source of TLC5 lymphoma cells.

Lymphomas. The DBA/2 lymphoma SL2, which arose spontaneously as an ascitic tumor, and the chemically induced CBA-derived TLC5 lymphoma were maintained by weekly i.p. passage. Both lymphomas were acquired through Dr. P. Alexander. Doubling time of the SL2 lymphoma in vitro is 12 to 16 hr.

Immunization. A single i.p. injection of $10^7$ SL2 cells was given to allogeneic C57BL mice. Peritoneal cells were harvested from immunized mice at various times after immunization (see “Results”). Control C57BL mice were immunized i.p. with $10^5$ to $10^6$ DBA/2 liver cells or with $10^7$ CBA-derived TLC5 lymphoma cells.

Cell Suspensions. Peritoneal cells were obtained by washing the peritoneal cavity with 5 ml of Fischer's medium (10). There are also several reports of passive transfer of resistance to allograft mouse tumors by peritoneal cell suspensions, consisting largely or entirely of macrophages obtained from immunized mice. Transfers of this type have been reported by Baker et al. (3) using the A/J tumor Sarcoma I injected into C57BL/6K mice, by Amos (2) using DBA/2 ascites lymphoma and C3H mice, by Old et al. (19) using the C3H sarcoma BP8 and C57BL/6 mice, and by Bennett (4) using Sarcoma I and BP8 and C57BL/6 mice.

In the experiments of Amos and Bennett the peritoneal

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exudates usually contain about equal numbers of macrophages and lymphocytes. The concentration was $2 \times 10^6$ macrophages/ml; (c) immune lymphocytes from a peritoneal cell suspension obtained 9 days after immunization. The cells were centrifuged, resuspended at a concentration of $2 \times 10^6$ macrophages/ml, and exposed to glass (Petri dish) for 45 min at $37^\circ$. The lymphocyte suspension was contaminated with 4% macrophages as shown by microscopic examination (This cell suspension is a control of the activity of the lymphocytes contaminating $2 \times 10^6$ immune macrophages.); (d) $2 \times 10^6$ nonimmune peritoneal C57BL macrophages (plus contaminating lymphocytes)/ml; (e) $2 \times 10^6$ peritoneal C57BL macrophages (plus lymphocytes)/ml from mice immunized i.p. with $10^6$ to $10^8$ DBA/2 liver cells, collected 9 days after immunization; (f) $2 \times 10^6$ immune C57BL lymph node cells/ml, obtained from the lymphonodi mandibulares 9 days after immunization; (g) immune (versus SL2) C57BL lymphoid cells from the spleen, collected 9 days after immunization suspended at a concentration of $4 \times 10^6$ cells/ml. The spleen was dissected and the cells were rinsed out from the spleen by injecting 5 ml of Fischer's medium; (h) C57BL 9-day immune peritoneal macrophages (contaminated with lymphocytes) suspended at a concentration of $2 \times 10^6$ cells/ml from C57BL mice immunized i.p. against CBA-derived TLC5 lymphoma cells.

**Antilymphocyte Serum.** Antilymphocyte serum was a horse anti-mouse serum; it was heat-inactivated for 30 min at $56^\circ$, absorbed with (a) C57BL liver cells (1:1, v/v) for 30 min at $4^\circ$ and with (b) normal C57BL peritoneal macrophages by passing the serum over 20 monolayers of $2 \times 10^6$ macrophages in Nuncion Petri dishes (diameter, 3 cm) in 20 min.

**Monolayers.** The method of preparation of monolayers is a modification of the method of Evans and Alexander (9). Peritoneal exudate cells were harvested from C57BL mice by syringing out the cavity with Fischer's medium; $2 \times 10^6$ macrophages seeded into 3.0-cm diameter Nuncion culture dishes formed confluent monolayers. Cultures were incubated for 60 min at $37^\circ$. The lymphocytes present were then removed by intensive washing of the macrophages with jets of tissue culture fluid. At this stage lymphocytes constituted less than 3% of the culture (assessed by microscopic examination). The cultures were washed and 3 ml of Fischer's medium supplemented with 10% heat-inactivated fetal bovine serum were added; the cultures were incubated overnight and challenged with SL2 cells.

**Cytotoxicity.** Immune and normal macrophage monolayers were challenged with SL2 cells. The cytotoxicity of macrophages was assessed 9 hr after challenge of the immune cultures and was expressed as

$$% \ GI = (N - T)/N \times 100$$

where $% \ GI$ is the percentage of growth inhibition; $N$ is the number of lymphoma cells in the controls; and $T$ is the number of lymphoma cells in the test system. Target cells were counted with a hemocytometer after "washing out" from the surface of the monolayers. Experiments were carried out in triplicate.

**RESULTS**

**Optimal Immunization Time.** Immune peritoneal cells were harvested at various times following immunization and $2 \times 10^6$ immune macrophages (contaminated with lymphocytes) were injected i.p. into DBA/2 mice that had received $2 \times 10^6$ SL2 cells 2 hr earlier. Control DBA/2 mice were given injections of $2 \times 10^6$ SL2 cells only or with normal peritoneal cells 2 hr following injection of $2 \times 10^6$ SL2 cells. Chart 1 shows that 9 days is the optimal immunization time and that in this experiment there is no relation between growth inhibition *in vivo* and the number of immune peritoneal lymphocytes per $2 \times 10^6$ immune macrophages.

**The Relative Potency of the Cytotoxic Macrophages *in Vitro.*** Monolayers of immune C57BL (versus SL2) macrophages can inhibit growth of SL2 cells *in vitro* (7). Chart 2 shows that even at a ratio of immune macrophages to SL2 cells of 1:1 there was cell death as measured by reduction of cell counts in immune cultures. Chart 2 shows again that immunity of peritoneal macrophages is strongest 9 days after i.p. immunization. The results were reproduced in 3 consecutive experiments.

**Therapeutic Effect of Immune Peritoneal Cells.** Immune peritoneal macrophages ($2 \times 10^6$) were harvested from C57BL mice 9 days and 20 days following i.p. immunization. These macrophages (contaminated with lymphocytes) were injected i.p. into DBA/2 mice 2 hr following i.p. injection of $2 \times 10^6$ to $2 \times 10^8$ SL2 cells. The results are shown in Table 1. With 20-day immune exudate cells 3 of 5 mice survived a dose of $2 \times 10^6$ SL2 cells for more than 35 days. However, with 9-day immune exudate cells 2 of 5 mice survived a dose of $2 \times 10^6$ SL2 cells for the same period.
Further it was shown that (a) $2 \times 10^6$ nonimmune C57BL peritoneal macrophages (contaminated with lymphocytes), (b) $2 \times 10^6$ C57BL immune (versus SL2) lymph node cells, (c) $4 \times 10^6$ C57BL immune (versus SL2) lymphoid cells of the spleen, (d) $2 \times 10^6$ C57BL immune (versus DBA/2 liver) peritoneal macrophages (contaminated with lymphocytes), and (e) $2 \times 10^6$ peritoneal macrophages (contaminated with lymphocytes) from C57BL mice immunized against TLC5 lymphoma cells, when injected i.p. into DBA/2 mice 2 hr after i.p. injection of $2 \times 10^6$ SL2 cells had no therapeutic effect (Table 2).

**Therapy with Purified Lymphocytes or Macrophages.** A purified suspension of immune peritoneal lymphocytes was prepared as described under "Materials and Methods." The lymphocyte suspension was injected (1 ml) i.p. into DBA/2 mice 2 hr after injection of $2 \times 10^6$ SL2 cells. The mice treated with this suspension died at the same time as the controls ($16.2 \pm 0.3$ days against $15.5 \pm 0.1$ days of survival of the controls).

A purified suspension of immune peritoneal macrophages was prepared as described under “Materials and Methods.” Two $10^6$ of these purified macrophages were injected i.p. into DBA/2 mice 2 hr following challenge with $2 \times 10^6$ SL2. This experiment was repeated 3 times. Two out of 15 mice survived for more than 200 days while the other 13 mice showed only a slight prolongation of survival time compared with the controls ($17.0 \pm 1.8$ days against $14.2 \pm 1.8$ days of survival of the controls).

**Therapeutic Effect of 3 Successive Injections of Immune Peritoneal Cells.** Three successive i.p. injections of $2 \times 10^6$ C57BL immune peritoneal macrophages (contaminated with lymphocytes), collected 10 days after i.p. immunization, were given to DBA/2 mice 2, 24, and 48 hr following i.p. injection of $2 \times 10^6$ to $2 \times 10^8$ SL2 cells.

Table 3 shows that 3 successive i.p. injections were considerably more effective than 1 injection of 9- or 20-day immune peritoneal cells.

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**Table 2**

<table>
<thead>
<tr>
<th>Control cell suspension</th>
<th>No. of survivors at 35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2 \times 10^6$ nonimmune C57BL peritoneal macrophages contaminated with lymphocytes</td>
<td>0/5 ($14.0 \pm 1.1^\text{a}$)</td>
</tr>
<tr>
<td>$2 \times 10^6$ C57BL immune (vs. DBA/2 liver) peritoneal macrophages contaminated with lymphocytes</td>
<td>0/5 ($15.8 \pm 1.1$)</td>
</tr>
<tr>
<td>$2 \times 10^6$ C57BL immune (vs. TLC5) peritoneal macrophages contaminated with lymphocytes</td>
<td>0/5 ($13.0 \pm 1.0$)</td>
</tr>
<tr>
<td>$2 \times 10^6$ C57BL immune (vs. SL2) lymph node cells</td>
<td>0/5 ($13.2 \pm 0.9$)</td>
</tr>
<tr>
<td>$4 \times 10^6$ C57BL immune (vs. SL2) lymphoid cells of the spleen</td>
<td>0/5 ($14.6 \pm 0.4$)</td>
</tr>
<tr>
<td>Medium only</td>
<td>0/5 ($12.0 \pm 0.0$)</td>
</tr>
</tbody>
</table>

^a^ Numbers in parentheses, real survival time.
When $2 \times 10^6$ purified immune macrophages were injected into DBA/2 mice challenged with $2 \times 10^4$ SL2 cells, 2 out of 15 mice survived for more than 200 days. However, the other 13 mice had only a slight prolongation of life-time. These results indicate that both cell types must be present in an immune cell suspension to obtain an optimal therapeutic effect. This does not exclude interactions between macrophages and lymphocytes such as (a) facilitation of antibody production by retaining and processing antigen (11, 12, 22, 23) before presentation of antigen to lymphocytes and (b) the production by challenged lymphocytes of a cytolytic factor that can arm normal macrophages (20).

The reaction described in this paper is specific as treatment of SL2-bearing DBA/2 mice with (a) allogeneic immune (versus SL2) lymphoid cells of the spleen or with (b) peritoneal cells from C57BL mice immunized against DBA/2 liver cells, or else (c) peritoneal cells from C57BL mice immunized against CBA/derived TEC5 lymphoma cells had no therapeutic effect.

**REFERENCES**

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