Augmentation of Antitumor Cytotoxicity in MOPC-315 Tumor Bearer Spleen Cells by Depletion of Glass-adherent Cells Prior to in Vitro Activation

Margalit B. Mokyr, Donald P. Braun, and Sheldon Dray

Department of Microbiology and Immunology, University of Illinois at the Medical Center, Chicago, Illinois 60612

ABSTRACT

Noncytotoxic, MOPC-315 tumor bearer spleen cells were converted to a cytotoxic state by in vitro activation with MOPC-315 stimulator tumor cells. The level of in vitro cytotoxicity exhibited by activated spleen cells from mice bearing small tumors was similar to that of activated spleen cells from normal mice while that exhibited by activated spleen cells from mice bearing large tumors was much lower. The decrease in the level of in vitro antitumor cytotoxicity observed in activated tumor bearer spleen cells during progressive tumor growth correlated with an increase in the percentage of macrophages in the spleen. Depletion of glass-adherent cells from the spleens of tumor-bearing mice included the removal of most macrophages and resulted in the expression of cytotoxicity upon culture. Depletion of glass-adherent cells from the spleens of tumor-bearing mice included the removal of most macrophages and resulted in the expression of cytotoxicity upon culture and in the augmentation of cytotoxicity upon activation. Still, unactivated or MOPC-315-activated, nonadherent, tumor bearer spleen cells did not lyse allogeneic EL4 leukemia or syngeneic normal BALB/c target cells. The in vitro cytotoxic activity exhibited by activated, nonadherent, tumor bearer spleen cells against MOPC-315 target cells was at least 10-fold greater than that exhibited by activated normal spleen cells or activated, unfractionated, tumor bearer spleen cells. Furthermore, activated nonadherent, tumor bearer spleen cells were also superior to activated, unfractionated, tumor bearer spleen cells in mediating in vivo antitumor activity in the local adoptive transfer assay. Thus, activated, nonadherent, tumor bearer spleen cells might be useful in immunotherapeutic regimes requiring histocompatible cells with augmented antitumor cytotoxicity.

INTRODUCTION

The effectiveness of adoptive cellular immunotherapy of cancer depends on the ability of the infused cells to survive in the recipient host and to implement a potent antitumor response. The ready availability and histocompatibility of the tumor bearer's own lymphoid cells would make them an ideal source of cells for adoptive therapy provided methods can be developed to potentiate their antitumor cytotoxicity. Previous work from this laboratory has demonstrated that at terminal stages, lymphoid cells from MOPC-315 tumor-bearing mice are unresponsive to tumor antigens in the macrophage migration-inhibitory factor assay, while at all stages, their lymphoid cells do not exhibit antitumor cytotoxicity in the ⁵¹Cr release assay and in the Winn assay. However, lymphoid cells from plasmacytomas can be converted by immune RNA to respond to tumor antigens in the macrophage migration-inhibitory factor assay (4) and by in vitro activation with stimulator tumor cells to exhibit antitumor cytotoxicity (19).

The in vitro generation of cytotoxic cells is being widely studied for its possible use in immunotherapy. The advantages of utilizing in vitro activated cells over in vivo-sensitized cells for therapeutic purposes are: (a) the risks involved in immunizing hosts with malignant cells are eliminated; (b) the levels of cell-mediated lysis produced are significantly higher (2, 3); (c) antigens which are weakly immunogenic in vivo can evoke high levels of cell-mediated lysis upon in vitro activation (2); and (d) a more rapid generation of cytotoxicity (5 days) occurs (7, 19).

Augmentation of the level of antitumor cytotoxicity exhibited by activated tumor bearer lymphoid cells would facilitate their usage in immunotherapeutic regimes. Since spleen cells from tumor-bearing animals were reported to contain T-cells which suppress antitumor cytotoxicity (13, 14) and/or macrophages which suppress lymphoproliferative responses to tumor antigens (15), removal of such cells prior to in vitro activation might augment their antitumor cytotoxicity.

In the present study, we evaluated the effect of depleting glass-adherent cells from the spleens of tumor-bearing mice on the expression of antitumor cytotoxicity by the nonadherent cell population. In addition, we depleted glass-adherent cells prior to in vitro activation in an attempt to augment the antitumor cytotoxicity of activated tumor bearer spleen cells.

MATERIALS AND METHODS

Spleen Cell Suspensions. Spleen cell suspensions were prepared from spleens of normal female BALB/c mice (8 to 12 weeks old; Laboratory Supply Co., Indianapolis, Ind.) or from BALB/c mice bearing various sizes of s.c. MOPC-315 tumors. In any individual experiment performed, the spleens used in each group were obtained from at least 5 but usually 10 to 15 mice. Single-cell suspensions were prepared by mechanical disruption between glass slides as previously described (19), and the viability as determined by trypan blue dye exclusion (0.4%) always exceeded 95%.

Target Cells. Target cells used in the ⁵¹Cr release assay included MOPC-315 plasmacytoma, EL4 leukemia, and normal BALB/c spleen cells. MOPC-315 was maintained by serial s.c. inoculation in syngeneic BALB/c mice. Single-cell suspensions were prepared by mechanical disruption
spleen cells (6 x 10^8) suspended in 30 ml warmed Eagle's minimal essential medium (Grand Island Biological Co.) and tumor-bearing mice was determined both by morphological and functional criteria. Enumeration of macrophages by morphological criteria was done by counting, at least 2500 but usually 5000 nucleated cells on Wright-stained smears generously prepared by Dr. Eugene P. Mayer of our department. Enumeration of macrophages by functional criteria was done by determining the percentage of cells that ingested latex beads (Difco Laboratories, Detroit, Mich.) according to the method of Rosenstrech et al. (24). More than 90% of the cells identified as macrophages by morphological criteria phagocytized latex beads.

RESULTS

Development of in Vitro Anti-MOPC-315 Tumor Cytotoxicity by in Vitro Activation of Noncytotoxic Spleen Cells from Mice Bearing MOPC-315 Tumors of Various Sizes. BALB/c mice given s.c. injections with 1 to 3.5 x 10^6 MOPC-315 tumor cells [a dose which leads to progressively growing tumors that kill the mice in 20 ± 3 days (S.E.)] do not exhibit antitumor cytotoxicity at any stage of tumor growth (19). In an attempt to evoke in vitro anti-MOPC-315 cytotoxicity in these noncytotoxic, tumor bearer spleen cells, spleen cells from mice bearing various sizes of tumors were activated in vitro with mitomycin C-treated MOPC-315 stimulator cells at R/S ratio of 30/1 for 5 days and were subsequently tested in the ^51Cr release assay. No significant antitumor cytotoxicity was observed with normal or tumor bearer spleen cells cultured without stimulator cells (less than 3%). In vitro activation led to the generation of antitumor cytotoxicity in both normal and tumor bearer spleen cells (Table 1). The level of antitumor cytotoxicity obtained with activated spleen cells from mice bearing tumors 11 mm in diameter was similar to that obtained with activated spleen cells from normal mice (55% compared to 43%). Activated spleen cells from mice bearing larger tumors exhibited a decreased level of antitumor cytotoxicity, e.g., 6% with activated spleen cells from mice bearing 30-mm-diameter tumors. In our previous studies (19), activation of tumor bearer spleen cells for lesser (4 days) or greater (6 and 7 days) periods of time did not lead to higher levels of antitumor cytotoxicity.

Determination of the Optimal R/S Ratio for the Development of in Vitro Anti-MOPC-315 Tumor Cytotoxicity in Normal or Tumor Bearer Spleen Cells Activated in Vitro with MOPC-315 Stimulator Cells. To investigate whether the reduced capacity of activated spleen cells from mice bearing large MOPC-315 tumors to mediate in vitro antitumor cytotoxicity was due to the use of a suboptimal R/S ratio during in vitro activation, 75 x 10^6 spleen cells from normal mice or mice bearing 27-mm tumors were cocultivated with various numbers of stimulator tumor cells and subsequently tested in the ^51Cr release assay (Table 2). Substantial levels of antitumor cytotoxicity were exhibited by activated spleen cells from normal mice when the R/S ratios used were 30/1 or 90/1 (52 and 50%, respectively) whereas the maximal level of antitumor cytotoxicity exhibited by activated spleen cells from mice bearing large tumors was achieved with an R/S ratio of 30/1 and was at best 9%. R/S ratios of 10/1 and 270/1 were clearly suboptimal for generating antitumor cytotoxicity in both normal and tumor bearer spleen cells. Since an R/S ratio of 30/1
was optimal for the generation of antitumor cytotoxicity in tumor bearer spleens, this R/S ratio was used for further experiments.

Characteristics of Spleens from Normal Mice and from Mice Bearing Progressively Growing MOPC-315 Tumors. Since the reduced capacity of activated spleen cells from mice bearing large MOPC-315 tumors to mediate in vitro antitumor cytotoxicity was not due to the use of an inappropriate R/S ratio during in vitro activation, other spleen characteristics such as splenic weight, nucleated cell number, and macrophage percentage were evaluated during progressive tumor growth (Table 3). Base-line studies demonstrated that the average spleen weight of normal mice was 150 mg, yielding approximately 100 million nucleated cells, 3.6% of which were morphologically identified as macrophages. When spleens of tumor-bearing mice were evaluated, an increase in tumor size was accompanied by the following: an increase in spleen weight (ranging from 26.9 ± 1.9 mm to 28.7 ± 0.6 mm), nucleated cell number (ranging from 160 million for 7.1-mm-diameter tumors to 185 million for 25.5-mm-diameter tumors), and macrophage percentage were evaluated during progressive tumor growth (Table 3). Base-line studies demonstrated that the average spleen weight of normal mice was 150 mg, yielding approximately 100 million nucleated cells, 3.6% of which were morphologically identified as macrophages. When spleens of tumor-bearing mice were evaluated, an increase in tumor size was accompanied by the following: an increase in spleen weight (ranging from 26.9 ± 1.9 mm to 28.7 ± 0.6 mm), nucleated cell number (ranging from 160 million for 7.1-mm-diameter tumors to 185 million for 25.5-mm-diameter tumors), and macrophage percentage were evaluated during progressive tumor growth (Table 3). 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tumors (which is similar to that exhibited by activated spleen cells from normal mice) can also be augmented by removal of glass-adherent cells prior to activation. Nonadherent spleen cells from mice bearing 7-mm tumors were cultured in vitro in the presence or absence of stimulator tumor cells and subsequently tested for their in vitro antitumor cytotoxicity (Table 5). Unfractionated spleen cells cultured in the absence of stimulator cells did not exhibit antitumor cytotoxicity (3%), whereas nonadherent spleen cells did (19%). In vitro activation of unfractionated spleen cells led to a substantial level of antitumor cytotoxicity (53%) which was well within the range of that exhibited by activated normal spleen cells in other experiments (i.e., 43%, Table 1; 47%, Table 7). Depletion of glass-adherent cells combined with in vitro activation augmented the antitumor cytotoxicity to a level which was greater than the sum of the levels of cytotoxicity exhibited by spleen cells subjected to either process alone (96 versus 19 + 53%). These data suggest that spleen cells from mice bearing small tumors or larger tumors contain immune cells the expression of which is suppressed by glass-adherent cells, the removal of which permits the generation of higher levels of antitumor cytotoxicity upon in vitro activation.

In Vitro Cytotoxic Activity of Unfractionated or Glass-nonadherent Normal Spleen Cells Cultured in Vitro in the Presence or Absence of Stimulator Tumor Cells. In an attempt to determine whether the augmented antitumor cytotoxicity of unactivated or activated, nonadherent, tumor bearer spleen cells was due to nonspecific activation by column passage, normal spleen cells were depleted of glass-adherent cells, activated in vitro with MOPC-315 tumor cells, and subsequently tested for their in vitro antitumor cytotoxicity (Table 6). No cytotoxicity was exhibited by either unfractionated or nonadherent normal spleen cells cultured in the absence of stimulator tumor cells (less than 3%). In vitro activation led to the generation of antitumor cytotoxicity in both unfractionated and nonadherent normal spleen cells (Table 7). However, the level of antitumor cytotoxicity exhibited by activated, unfractionated spleen cells was at least 2-fold higher than that exhibited by activated, nonadherent spleen cells at all R/S ratios used. Thus, the removal of glass-adherent cells from spleen cells of normal mice prior to in vitro activation is detrimental to the development of antitumor cytotoxicity under our culture conditions; a finding which is consistent with reports by other investigators (27). Furthermore, since column fractionation of normal spleen cells did not lead to augmented antitumor cytotoxicity upon in vitro activation, the augmented antitumor cytotoxicity exhibited by activated, nonadherent tumor bearer spleen cells (Table 4) cannot be attributed to activation by column passage.

In Vitro Antitumor Cytotoxicity of Unfractionated or Glass-nonadherent Tumor Bearer Spleen Cells That Were Cultured Alone or in Admixture with Normal Spleen Cells in the Presence or Absence of MOPC-315 Stimulator Cells. Unfractionated or nonadherent spleen cells from mice bearing 19-mm tumors were cultured alone or in admixture with normal spleen cells in the presence or absence of MOPC-315 stimulator cells and were subsequently tested for their in vitro antitumor cytotoxicity (Table 7). Culture of normal spleen cells or unfractionated tumor bearer spleen cells in the absence of stimulator cells did not lead to the generation of antitumor cytotoxicity (1.7 and –2.4%, respectively). In vitro activation of normal or tumor bearer spleen cells led to the generation of anti-MOPC-315 cytotoxicity in both cultures, but the level of cytotoxicity exhibited by activated normal spleen cells (47%) was greater than that exhibited by activated tumor bearer spleen cells (30%). Admixing tumor bearer spleen cells with normal spleen cells at ratios of 3/1 and 1/1 led to levels of antitumor cytotoxicity higher (60 and 50%) than those exhibited by unmixed activated tumor bearer spleen cells (30%) and to levels equal to or higher than those exhibited by unmixed activated normal spleen cells (47%). These results suggest that the suppressive effect inherent in spleen cells from mice with large tumors can be overcome by the addition of normal spleen cells.

Nonadherent tumor bearer spleen cells cultured in the absence of stimulator cells exhibited significant levels of antitumor cytotoxicity when cultured alone or in admixture with normal spleen cells. In vitro activation of these nonadherent, tumor bearer spleen cells augmented their antitumor cytotoxicity. This augmentation was substantial since the level of antitumor cytotoxicity exhibited at an effector/target cell ratio of 20/1 by activated, nonadherent, tumor bearer spleen cells (96%) was much higher than was the level exhibited at an effector/target cell ratio of 100/1 by either activated unfractionated tumor-bearer spleen cells (30%) or activated normal spleen cells (47%). Admixing nonadherent tumor bearer spleen cells with normal spleen cells at a 1/1 ratio prior to activation reduced the level of anti-tumor cytotoxicity from 96 to 77% when tested at an effector/target cell ratio of 20/1, but this level of antitumor cytotoxicity was still higher than that exhibited at an effector/target cell ratio of 100/1 by unfractionated activated

<table>
<thead>
<tr>
<th>Source of responder, tumor-bearer spleen cells</th>
<th>Stimulator tumor cells</th>
<th>% of specific ⁵¹Cr releasea/⁵¹Cr releaseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfractionated</td>
<td>None</td>
<td>2.9 ± 0.7/2.9 ± 0.7b</td>
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<tr>
<td>Nonadherent</td>
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<td>19.4 ± 2.2/19.4 ± 2.2</td>
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<td>MOPC-315</td>
<td>52.9 ± 2.2/52.9 ± 2.2</td>
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<td>Nonadherent</td>
<td>MOPC-315</td>
<td>96.0 ± 1.3/96.0 ± 1.3</td>
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</table>

a ⁵¹Cr release assay was performed at an effector/target cell ratio of 50/1.
b Mean ± S.E.
tumor bearer spleen cells (30%) or activated normal spleen cells (47%). Thus, the cytotoxic activity exhibited by activated nonadherent tumor bearer spleen cells was at least 10-fold greater than that exhibited by activated normal spleen cells. These data suggest that removal of glass-adherent cells prior to in vitro activation allows the nonadherent tumor bearer spleen cells to respond to stimulator tumor cells in a manner similar to a secondary response.

**In Vitro Cytotoxic Activity of Unactivated Nonadherent and Activated Nonadherent Tumor Bearer Spleen Cells against MOPC-315, EL4, and Normal BALB/c Target Cells.**

Glass-nonadherent, tumor bearer spleen cells were cultured in vitro in the presence or absence of stimulator tumor cells and subsequently tested for their in vitro antitumor cytotoxicity against MOPC-315, EL4 leukemia of C57Bl origin, and normal BALB/c target cells. The normal BALB/c target cells used were blast cells induced by stimulation with concanavalin A and were susceptible to lysis by C57Bl/6 spleen cells activated with MOPC-315 stimulator cells but resistant to lysis by either C57Bl/6 spleen cells activated with EL4 stimulator cells or BALB/c spleen cells activated with EL4 stimulator cells (data not shown). Unactivated, nonadherent, tumor bearer spleen cells exhibited appreciable levels of cytotoxicity against MOPC-315 (15%) but no cytotoxicity against EL4 or normal BALB/c target cells (less than 3%; Table 8). Activation of nonadherent tumor bearer spleen cells resulted in heightened levels of anti-MOPC-315 cytotoxicity (93%), low levels of anti-EL4 cytotoxicity (5%), and no cytotoxicity against normal target cells (less than 3% Table 8).

**In Vitro (Winn Assay) Antitumor Cytotoxicity of Unfractionated or Glass-nonadherent, Tumor Bearer Spleen Cells That Were Cultured in Vitro in the Presence or Absence of MOPC-315 Stimulator Cells.**

Unfractionated or glass-nonadherent spleen cells from mice bearing tumors with diameters of 25 or 11 mm were cultured in vitro in the presence or absence of stimulator tumor cells and subsequently tested for their antitumor cytotoxicity in vitro in the presence or absence of stimulator cells and subsequently tested for their in vitro antitumor cytotoxicity in vitro and in vivo (mean tumor diameter, 14 mm). Depletion of glass-adherent cells or in vitro activation of unfractionated cells resulted in low levels of in vitro antitumor cytotoxicity (13 and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence. On the other hand, combining depletion of glass-adherent cells with in vitro activation resulted in high levels of in vitro antitumor cytotoxicity (31% and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence. In the second experiment, cultured unfractionated spleen cells from mice with 11-mm tumors did not lyse target cells when tested in vitro (0%) and did not prevent the appearance of tumors when tested in vivo (mean tumor diameter, 14 mm). Depletion of glass-adherent cells or in vitro activation of unfractionated cells resulted in low levels of in vitro antitumor cytotoxicity (13 and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence. On the other hand, combining depletion of glass-adherent cells with in vitro activation resulted in high levels of in vitro antitumor cytotoxicity (31% and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence. In the second experiment, cultured unfractionated spleen cells from mice with 11-mm tumors did not lyse target cells when tested in vitro (0%) and did not prevent the appearance of tumors when tested in vivo (mean tumor diameter, 14 mm). Depletion of glass-adherent cells or in vitro activation of unfractionated cells resulted in low levels of in vitro antitumor cytotoxicity (13 and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence. On the other hand, combining depletion of glass-adherent cells with in vitro activation resulted in high levels of in vitro antitumor cytotoxicity (31% and 4%, respectively), marginal reductions in tumor size (36 and 16%, respectively) and no reductions in tumor incidence.
cytotoxicity (27% reduction in tumor size and no reduction in tumor incidence) while in vitro activation of unfractionated cells resulted in substantial levels of in vitro antitumor cytotoxicity (54%) and in vivo cytotoxicity (81% reduction in tumor size and 62% reduction in tumor incidence). Combining depletion of glass-adherent cells with in vitro activation led to still higher levels of in vitro and in vivo antitumor cytotoxicity as evidenced by 95% Cr release and complete prevention of tumor growth. Thus, in vitro activation of nonadherent tumor bearer spleen cells resulted not only in heightened levels of in vitro antitumor cytotoxicity but also in heightened levels of in vivo antitumor cytotoxicity.

DISCUSSION

We have shown that noncytotoxic MOPC-315 tumor bearer spleen cells could be converted to a cytotoxic state upon in vitro activation with stimulator tumor cells. Activated spleen cells from mice bearing small tumors (11 mm) exhibited a similar level of in vitro cytotoxicity as did activated spleen cells from normal mice. However, activated spleen cells from mice bearing large tumors exhibited a lower level of cytotoxicity; this lower level was not due to suboptimal culture conditions (R/S ratios or time of incubation) and could be heightened by mixing with normal spleen cells. The decrease in the level of in vitro cytotoxicity obtained with activated tumor bearer spleen cells as tumor growth progressed correlated with an increase in the percentage of macrophages in the spleen. Depletion of glass-adherent, tumor bearer spleen cells included the removal of most macrophages and resulted in the expression of in vitro antitumor cytotoxicity. Combining depletion of glass-adherent cells with in vitro activation led to higher levels of in vitro antitumor cytotoxicity than the sum of the levels of cytotoxicity exhibited by tumor bearer spleen cells subjected to either process alone. Still, unactivated or activated nonadherent, tumor bearer spleen cells were unable to lyse allogeneic EL4 leukemia or syngeneic normal BALB/c target cells. The in vitro cytotoxic activity exhibited by activated, nonadherent spleen cells from mice with large tumors was at least 10-fold greater than that exhibited by activated normal spleen cells. Furthermore, activated, nonadherent, tumor bearer spleen cells also proved superior to activated unfractionated tumor bearer spleen cells in mediating in vivo local antitumor activity.

The finding that activated spleen cells from mice bearing large tumors exhibited reduced levels of cytotoxicity compared to the levels exhibited by activated normal cells or activated cells from mice with smaller tumors might be due to changes in the population of cells, e.g., cytotoxic, helper, suppressor, or tumor cells, residing in the spleens of late-stage tumor-bearing mice. A possible role for tumor cell antigens, either alone or in complexes with antibody, should also be considered.

Studies into the regulation of immune responses have revealed that the balance between immunostimulation and immunosuppression can be subject to the action of macrophages (1). The nature of this regulation appears to be dependent upon the ratio of macrophages to immunocompetent lymphocytes; low ratios augment the response of purified lymphocytes while high ratios suppress their response (20, 21, 28). Our results are consistent with these observations in that an increased percentage of macrophages in the spleens of mice bearing large tumors was associated with a decrease in antitumor cytotoxicity upon in vitro activation. Depletion of glass-adherent cells from tumor bearer spleen cells resulted in (a) a reduction in the percentage of splenic macrophages to a percentage approximating that of normal spleen cells, (b) the expression of in vitro cytotoxicity upon culture, and (c) augmented antitumor cytotoxicity upon in vitro activation. These results suggest that the antitumor cytotoxicity exhibited by tumor

Table 9

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Responder tumor bearer spleen cells</th>
<th>Stimulator tumor cells</th>
<th>% of Cr release</th>
<th>Mean tumor diameter (mm)</th>
<th>Tumor incidence</th>
<th>% reduction of tumor</th>
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<tr>
<td>1</td>
<td>Unfractionated</td>
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<td>0 ± 0.3</td>
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<td>4.2 ± 1.7</td>
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<td>12.9 ± 0.7</td>
<td>9.1 ± 2.3</td>
<td>7/7</td>
<td>36</td>
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<td>53.7 ± 3.5</td>
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<td>94.8 ± 0.3</td>
<td>0</td>
<td>0/8</td>
<td>100</td>
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* In Experiment 1, responder spleen cells from mice bearing tumors with diameters of 25.3 ± 2.7 and in Experiment 2 from mice bearing tumors with diameters of 10.3 ± 1.5 mm.

| Mean tumor diameter: sum of tumor sizes within group/total mice within group. |
| Number of mice showing tumors/total mice. |
| Mean ± S.E. |

\[ \text{In vivo (51Cr release) and in vivo (Winn assay) antitumor cytotoxicity of unfractionated or glass-nonadherent, tumor bearer spleen cells that were cultured in vitro in the presence or absence of MOPC-315 stimulator cells} \]
bearer spleen cells is subject to suppression by macrophages. Still, the possibility that the suppression seen is mediated by an adherent cell other than macrophages cannot be ruled out. Folch and Waksman (12) have shown that T-cells which inhibit the response of normal rat spleen cells to supraoptimal doses of mitogen are also removed by glass adherence. In an attempt to identify the nature of the adherent suppressor cell that operates in the MOPC-315 tumor system, experiments are now being performed to evaluate what effect removal of phagocytic cells has on anti-MOPC-315 cytotoxicity. Further, it will be necessary to assess the effect of adding an increasing number of purified macrophages to nonadherent, tumor bearer spleen cells and to normal spleen cells on the ensuing antitumor cytotoxicity.

Other investigators have reported marked increases in macrophages in the spleens of tumor-bearing animals (9-11, 15, 18) and have indicated their involvement in the suppression of the following: (a) mitogenic responsiveness (10, 18); (b) lymphocyte proliferation in mixed leukocyte-tumor cell interactions (15, 18); (c) antibody production to sheep RBC (22); (d) generation of cells cytotoxic to alloantigens (11, 18). Our results extend these findings by suggesting that the in vitro generation of antitumor cytotoxicity in spleen cells of tumor-bearing mice is also subject to negative regulation by macrophages.

The cytotoxic activity exhibited by activated, nonadherent, tumor bearer spleen cells was at least 10-fold greater than that exhibited by activated unfractionated tumor bearer or normal spleen cells. This dramatic increase cannot be attributed to nonspecific activation by column passage, since column fractionation of normal spleen cells did not lead to augmented antitumor cytotoxicity upon in vitro activation. A more likely explanation is that tumor-immune cells within the spleens of tumor-bearing mice are suppressed by glass-adherent cells and that depletion of glass-adherent cells prior to in vitro activation allows the nonadherent tumor bearer spleen cells to respond to stimulator tumor cells in a manner similar to a secondary response.

We have previously used another method to augment the antitumor cytotoxicity of activated spleen cells. Pretreatment of BALB/c mice with Bacillus Calmette-Guérin prior to the activation of their spleen cells with MOPC-315 tumor cells resulted in higher levels of in vitro antitumor cytotoxicity than that exhibited by spleen cells subjected to activation alone (5). Therefore, Bacillus Calmette-Guérin pretreatment of tumor-bearing hosts, followed by depletion of glass-adherent cells, and activation of the nonadherent populations is being evaluated at present as a method to further augment antitumor cytotoxicity of tumor bearer cells. Another method that might be used to further augment antitumor cytotoxicity is activation of tumor bearer spleen cells with immune RNA since immune RNA can convert unresponsive lymphoid cells from MOPC-315 tumor-bearing mice to a responsive state as assessed by the macrophage migration inhibition assay (4) and can confer in vitro antitumor cytotoxicity on normal cells (17).

Several studies have evaluated the ability of in vitro-activated spleen cells from normal (8, 16, 19, 23) or tumor-immune (3) animals to mediate in vivo antitumor activity. The possibility of applying these models to human immunotherapy, however, might be facilitated by the ability to sensitize the lymphoid cells from tumor-bearing individuals. Treves et al. (25, 26) utilized activated spleen cells from mice bearing progressively growing tumors to protect against lethal lung metastases following excision of the primary tumor and obtained 57% survival as compared to 30 to 40% survival in control animals. We have reported that in vitro activation of spleen cells from mice bearing MOPC-315 tumor leads to the generation of cells capable of mediating in vivo local antitumor activity (19). In this report, we were successful in substantially augmenting the in vivo local antitumor activity of tumor bearer spleen cells by depleting glass-adherent cells prior to in vitro activation. If these activated, nonadherent tumor bearer spleen cells also prove to be more effective than activated, unfractionated tumor bearer spleen cells in mediating systemic antitumor immunity, they might become a valuable adjunct to conventional therapeutic modalities requiring histocompatible cells with potent antitumor activity.

ACKNOWLEDGMENTS

We wish to acknowledge the expert technical assistance of Katherine Siessmann whose efforts were invaluable in the performance of this work, as well as the assistance of Mitzi B. Sabato in the preparation of this paper.

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Margalit B. Mokyr, Donald P. Braun and Sheldon Dray

Cancer Res 1979;39:785-792.

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