Effect of Polyethylene Glycol 6000 on the Generation of Antitumor Cytotoxicity in MOPC-315 Tumor Bearer Spleen Cells Cultured in the Presence or Absence of Inactivated Stimulator Tumor Cells

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ABSTRACT

Noncytotoxic spleen cells from BALB/c mice bearing 15- to 26-mm (but not 29-mm) s.c. MOPC-315 tumors that were cultured in medium containing 2% polyethylene glycol 6000 (PEG) developed substantial levels of anti-MOPC-315 cytotoxicity as assayed by 51Cr release. The level of cytotoxicity obtained increased with progression of tumor growth. Addition of mitomycin C-treated stimulator tumor cells and PEG to the culture of tumor bearer spleen cells resulted in augmentation of antitumor cytotoxicity to a level that was greater than the sum of the levels of cytotoxicity exhibited by spleen cells cultured in the presence of either mitomycin C-treated tumor cells or PEG. Maximal levels developed when the spleen cells were cultured for 5 to 6 days in 2% PEG at a responder/stimulator cell ratio of 15/1 or 30/1. Tumor bearer spleen cells that were cultured in PEG with or without added MOPC-315 stimulator cells exhibited strong anti-MOPC-315 cytotoxicity but were virtually noncytotoxic to allogeneic EL4 and syngeneic blast cells. Furthermore, these spleen cells were far superior to spleen cells cultured without PEG in mediating in vivo antitumor activity in the local adoptive transfer assay. Thus, tumor bearer spleen cells cultured in the presence of PEG might be useful in immunotherapeutic regimens requiring histocompatible cells with augmented antitumor cytotoxicity but devoid of reactivity against normal cells.

INTRODUCTION

The effectiveness of adoptive immunotherapy of cancer depends on the ability of the infused cells to survive in the recipient host and to mediate a potent antitumor response (11). An ideal source of histocompatible cells is the tumor bearer's own cells, provided that methods can be developed to potentiate their antitumor cytotoxicity. One method used for this purpose is the in vitro immunization of spleen cells by cocultivation with stimulator tumor cells (16, 18, 27, 28). However, antitumor cytotoxicity was not generated when spleen cells from animals at advanced stages of tumor growth were used (16, 19, 27) unless, prior to in vitro immunization, suppressor elements were removed (18, 20, 23, 24, 26). For example, in the MOPC-315 tumor system, the inability of spleen cells from terminal tumor-bearing mice to mediate in vitro antitumor cytotoxicity following in vitro immunization was due to the presence of metastatic tumor cells as well as to an increased percentage of macrophages and possibly suppressor T-cells (20). Removal of these suppressor cells from spleen cell suspensions of terminal tumor bearers was achieved by depletion of glass-adherent cells (20). In vitro immunization of the glass-nonadherent cells resulted in levels of antitumor cytotoxicity that were as much as 10-fold greater than were those exhibited by unfractionated, in vitro immunized spleen cells from normal mice or mice at early stages of tumor growth (18).

Although depletion of suppressor cells effectively facilitated in vitro immunization, the method has the disadvantage of low cell recovery (15 to 25%) (20). For this reason, an alternative method was sought. For normal spleen cells, PEG has been shown to augment the antitumor cytotoxicity generated by in vitro immunization (14) and to enhance the proliferation of T-lymphocytes in response to tumor antigens (1, 22). The present study was designed to determine whether the addition of PEG to the in vitro immunization culture of tumor bearer spleen cells would potentiate antitumor cytotoxicity in the MOPC-315 tumor system.

MATERIALS AND METHODS

Spleen Cell Suspensions. Spleen cell suspensions were prepared from spleens of normal female BALB/c mice (6 to 12 weeks old; Laboratory Supply Co., Indianapolis, Ind.) or from BALB/c mice bearing s.c. MOPC-315 tumors. In any individual experiment, the spleens used in each group were obtained from at least 3 but usually 5 to 8 mice. Single-cell suspensions were prepared by mechanical disruption between glass slides as described previously (19), and the viability as determined by trypan blue exclusion (0.4%) always exceeded 95%.

MOPC-315 Tumor Cells. The MOPC-315 plasmacytoma was maintained by serial s.c. inoculation of $3.5 \times 10^6$ tumor cells into syngeneic BALB/c mice. Using this method, the tumor diameter (the average of perpendicular measurements with calipers) increased linearly with time between 10 days (10 mm) and 18 days (25 mm) after injection. All animals died by Day 24. Single-cell suspensions were prepared by mechanical disruption between glass slides as described previously (19), and the viability as determined by trypan blue exclusion (0.4%) always exceeded 85%.

Target Cells. The EL4 leukemia was maintained in ascitic form by weekly i.p. injection of $10^7$ cells into syngeneic C57BL/6 mice (6 to 12 weeks old; Laboratory Supply Co.). Normal BALB/c target cells were blast cells induced by stimulation with Con A (5 μg/ml) for 48 to 72 hr and were susceptible to lysis by C57BL/6 spleen cells immunized against MOPC-315.

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cells but resistant to lysis by either C57BL/6 or BALB/c spleen cells immunized against EL4 cells (18).

In Vitro Immunization. The in vitro method for generating antitumor cytotoxicity in lymphoid cells was described previously (7, 19). Briefly, responder spleen cells (75 × 10⁶) were cultured with mitomycin C-treated (50 µg/ml for 30 min) MOPC-315 stimulator cells (2.5 × 10⁶ unless otherwise stated) in Roswell Park Memorial Institute Medium 1640 supplemented with 5% fetal calf serum, 1% nonessential amino acids, 50 units penicillin per ml, and 50 µg streptomycin per ml (Grand Island Biological Co., Grand Island, N. Y.). The cultures were incubated at 37° in 5% CO₂ in air for 2 to 7 days.

Addition of PEG to Cultures. PEG (Carbowax PEG 6000; Fisher Scientific Co., Fair Lawn, N. J.) was dissolved at 37° in Roswell Park Memorial Institute Medium 1640 [20% (w/v) stock solution], sterilized by filtration, and added to culture medium at a final concentration of 0.5 to 4.0% (w/v).

In Vitro Antitumor Cytotoxicity Assay. The cytotoxic activity of unimmunized or in vitro immunized spleen cells was determined as described previously (19) using the 3.5-hr in vitro ⁵¹Cr release assay (6). The percentage of specific ⁵¹Cr release was calculated by the following formula

\[
\% \text{ of specific } ⁵¹\text{Cr release} = \frac{T - C}{M - C} \times 100
\]

where T is the percentage of release with test spleen cells; C is the percentage of spontaneous release by target cells alone, which ranged between 10 and 18%; and M is the percentage of maximum ⁵¹Cr release obtained by 3 cycles of freezing and thawing, which ranged between 78 and 81%. The percentage of specific ⁵¹Cr release in the text represents the mean ± S.E. of triplicate samples. When the difference between values was 10 or greater, it was statistically significant by Student’s t test (p < 0.05). The data shown are representative of the data obtained in 3 to 5 individual experiments. We have observed (17), as have others (7), that the level of antitumor cytotoxicity obtained by spleen cells cultured under the same conditions may vary substantially from one experiment to another. Still, the effect of PEG and/or mitomycin C-treated tumor cells added to cultures was consistent.

In Vivo Antitumor Activity. The in vivo antitumor activity of spleen cells was evaluated by the local adoptive transfer assay [Winn assay (29)]. Viable MOPC-315 cells (2 or 5 × 10⁶/ml) were mixed with an equal volume of cultured spleen cells (40 or 100 × 10⁶/ml), respectively, and 0.2 ml of the mixture was immediately injected s.c. into the flank of each of a number of normal BALB/c mice. The mice were examined at 2- to 3-day intervals over a 60-day period. Tumor incidence and survival time were recorded.

RESULTS

Augmentation of in Vitro Antitumor Cytotoxicity of Normal or Tumor Bearer Spleen Cells by the Addition of 2% PEG to the Culture Medium. Spleen cells were obtained from mice bearing large (24-mm) s.c. tumors. When these spleen cells were cultured either with mitomycin C-treated MOPC-315 tumor cells or in 2% PEG for 5 days without added stimulator cells, they were cytotoxic for MOPC-315 target cells (Table 1). When the stimulator cells and PEG were both present, much higher levels of cytotoxicity developed (95 or 67% at E/T ratios of 100/1 or 20/1, respectively). A similar phenomenon was observed using spleen cells from normal mice. Thus, it appeared that mitomycin C-treated stimulator cells and PEG exerted a synergistic effect on the generation of cytotoxicity in normal or tumor bearer spleen cells. In addition, the lymphoid cell recovery from cultures containing PEG was approximately twice that from cultures without PEG (data not shown).

Tumor bearer spleen cells were immunized in vitro against MOPC-315 stimulator cells without PEG or with PEG added at the initiation of culture or on Day 4 (Table 2). The cells from the immunization culture containing PEG from Day 0 were cytotoxic (54%) for MOPC-315 target cells, whereas the cells from the immunization culture containing PEG only during the last 24 hr were essentially no more cytotoxic than were cells immunized in vitro without PEG. Therefore, the augmentation of cytotoxicity that occurred when in vitro immunization was performed with PEG (Tables 1 and 2) was not due simply to the presence of a small amount of PEG carried over into the ⁵¹Cr release assay.

Determination of the Optimal Culture Conditions for the Augmentation of in Vitro Antitumor Cytotoxicity of Tumor Bearer Spleen Cells Using PEG. Tumor bearer spleen cells were cultured for 5 days with or without mitomycin C-treated MOPC-315 stimulator tumor cells in various concentrations of PEG [0 to 4% (w/v)] (Table 3). Spleen cells cultured with 2% PEG exhibited maximal cytotoxicity (25%). At all concentrations of PEG, cultures containing mitomycin C-treated tumor cells exhibited augmented levels of antitumor cytotoxicity; the maximal level obtained (67%) was with 2% PEG.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Augmentation of in vitro anti-MOPC-315 cytotoxicity of normal or tumor bearer spleen cells cultured with or without added stimulator tumor cells by the addition of 2% PEG to the culture medium</td>
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<table>
<thead>
<tr>
<th>Responder spleen cells</th>
<th>Cultured with</th>
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<tr>
<td>Normal</td>
<td>Control</td>
<td>1 ± 0.8b</td>
<td>-2 ± 0.5</td>
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<tr>
<td>Normal</td>
<td>Tumor cellsc</td>
<td>48 ± 2.6</td>
<td>20 ± 0.5</td>
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<tr>
<td>Normal</td>
<td>PEG</td>
<td>41 ± 0.8</td>
<td>18 ± 2.0</td>
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<tr>
<td>Normal</td>
<td>Tumor cells and PEG</td>
<td>101 ± 0.3</td>
<td>66 ± 0.9</td>
</tr>
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<td>Tumor bearerd</td>
<td>Control</td>
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<td>-4 ± 0.4</td>
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<tr>
<td>Tumor bearer</td>
<td>Tumor cells</td>
<td>16 ± 0.3</td>
<td>11 ± 0.2</td>
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<td>PEG</td>
<td>43 ± 0.6</td>
<td>23 ± 0.9</td>
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<td>Tumor bearer</td>
<td>Tumor cells and PEG</td>
<td>95 ± 0.5</td>
<td>67 ± 3.6</td>
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</table>

*a Percentage of specific ⁵¹Cr release using MOPC-315 target cells.

b Mean ± S.E.

c Added mitomycin C-treated MOPC-315 stimulator tumor cells.

d Mice bearing MOPC-315 tumors 24 ± 0.3 mm in diameter.

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Inability of PEG, when present only during the last 24 hr of the in vitro immunization, to augment the in vitro anti-MOPC-315 cytotoxicity of spleen cells from mice bearing large tumors</td>
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</table>

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<tr>
<th>Addition of PEG</th>
<th>100/1</th>
<th>20/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2 ± 0.2b</td>
<td>7 ± 1.6</td>
</tr>
<tr>
<td>Day 4</td>
<td>3 ± 0.8</td>
<td>3 ± 0.9</td>
</tr>
<tr>
<td>Day 0</td>
<td>54 ± 0.8</td>
<td>31 ± 0.3</td>
</tr>
</tbody>
</table>

*a Percentage of specific ⁵¹Cr release using MOPC-315 target cells.

b Mean ± S.E.
Tumor bearer spleen cells were cultured in the presence or absence of 2% PEG with varying numbers of mitomycin C-treated MOPC-315 stimulator tumor cells; the R/S ratios were 5/1 to 300/1 (Table 4). Spleen cells that were immunized in vitro in the presence or absence of PEG exhibited maximal levels of anti-MOPC-315 cytotoxicity at R/S ratios of 15/1 or 30/1; e.g., at a R/S ratio of 30/1 and an E/T ratio of 100/1, the specific 51Cr release was 68% without PEG and 99% with PEG. At all R/S ratios tested, spleen cells that were immunized in vitro in the presence of PEG exhibited higher levels of cytotoxicity than did those immunized without PEG.

Tumor bearer spleen cells were cultured for 2 to 7 days in medium containing 2% PEG and/or mitomycin C-treated MOPC-315 stimulator tumor cells (Table 5). Maximal levels of anti-MOPC-315 cytotoxicity (~100%) were obtained on the fifth to sixth day of culture for tumor bearer spleen cells cultured with both 2% PEG and added stimulator cells. Lower levels of cytotoxicity were obtained with a shorter (3 days) or longer (7 days) duration of culture.

For further experiments, tumor bearer spleen cells were cultured for 5 days with added stimulator cells at a R/S ratio of 30/1 in 2% PEG.

Cytotoxic Activity against Various Target Cells Mediated by Cytotoxic Cells Generated in the Presence of PEG. The cytotoxic activity of normal and tumor bearer spleen cells incubated for 5 days in 2% PEG with or without mitomycin C-treated MOPC-315 stimulator tumor cells was evaluated by performing the 51Cr release assay against MOPC-315 cells, allogeneic EL4 cells, or syngeneic BALB/c blast cells (Table 6). Normal spleen cells incubated in 2% PEG were cytotoxic for all targets tested. Normal cells incubated with both PEG and added MOPC-315 stimulator cells exhibited an even greater level of cytotoxicity against the MOPC-315 target cells (~100% with versus 48% without added stimulator cells), but the activities against the EL4 and blast cells were only minimally augmented (31 versus 38% and 14 versus 15%, respectively). In contrast, tumor bearer spleen cells incubated in PEG with or without added MOPC-315 stimulator cells were cytotoxic for the MOPC-315 target cells (60 and 44%, respectively) but exhibited essentially no activity against the EL4 or blast cells (<4%).

Comparison of the Effect of PEG on Cultures of Tumor Cells from Mice Bearing Tumors of Various Diameters. The ability of PEG to augment the antitumor cytotoxicity of spleen cells from mice bearing various sizes of tumors was investigated (Table 7). With an increase in tumor diameter to greater than 15 mm, there was a substantial decrease in the ability to generate anti-MOPC-315 cytotoxicity by in vitro immunization of the spleen cells. Addition of PEG to the cultures of spleen cells from mice bearing 15- to 26-mm tumors led to the development of increasing levels of cytotoxicity with progression of tumor growth but had no effect when spleen cells from mice bearing 29-mm tumors were used. Addition of mitomycin C-treated tumor cells to cultures of spleen cells from mice bearing 15- to 26-mm (but not 29-mm) tumors in the presence of PEG augmented the antitumor activity obtained. However, the levels of cytotoxicity obtained (89 to 92%) were similar regardless of tumor size within the range of 15 to 26 mm.

In Vivo Antitumor Activity of Cytotoxic Cells Generated in Vitro in Medium Containing 2% PEG. Tumor bearer spleen cells were cultured with or without PEG in the presence or absence of mitomycin C-treated MOPC-315 stimulator cells. On Day 5 of culture, the spleen cells were tested for anti-MOPC-315 cytotoxicity in vivo in the 51Cr release assay and in vivo in the Winn assay (29) (Table 8). Spleen cells that were cultured with both PEG and stimulator cells exhibited high levels of in vivo antitumor cytotoxicity (100, 84, and 68%) and prevented tumor growth in vivo. Spleen cells cultured with PEG alone exhibited low levels of in vivo cytotoxicity (24 and 25%) but also prevented tumor growth in vivo. Spleen cells cultured without PEG exhibited low levels of in vivo cytotoxicity and were ineffective in inhibiting tumor growth in vivo. Thus, in the in vivo assay, tumor bearer spleen cells cultured with or without stimulator cells in medium containing PEG were far superior to spleen cells cultured without PEG, i.e., cumulative tumor incidence of 1 of 27 compared to 34 of 34.

DISCUSSION

We show in this paper that noncytotoxic spleen cells from normal BALB/c mice or BALB/c mice bearing 15- to 26-mm (but not 29-mm) s.c. MOPC-315 tumors exhibited substantial levels of in vitro anti-MOPC-315 cytotoxicity after they were cultured for 5 days in medium containing 2% PEG. Cocultivation of the spleen cells for 5 days with both PEG and mitomycin C-treated stimulator cells augmented the level of cytotoxicity obtained. Maximal levels of the cytotoxic activity of tumor
b l e a r s p l e e n c e l l s w e r e o b t a i n e d w h e n t h e c e l l s w e r e c u l t u r e d f o r 5 t o 6 d a y s i n 2% P E G a t r a t i o o f 1 5 / 1 o r 3 0 / 1 . W h e r e a s t h e c y t o t o x i c c e l l s g e n e r a t e d f r o m n o r m a l s p l e e n c e l l s c u l t u r e d w i t h o r w i t h o u t M O P C - 3 1 5 s t i m u l a t o r c e l l s i n m e d i u m c o n t a i n i n g 2% P E G w e r e c y t o t o x i c n o t o n l y t o M O P C - 3 1 5 t g e n a l b o t t o a l o g e l e c L E 4 a n d s y n g e n e i c n o r m a l b l a s t c e l l s , t h e c y t o t o x i c c e l l s g e n e r a t e d f r o m t u m o r b a r e r s p l e e n c e l l s w e r e c y t o t o x i c o n l y t o M O P C - 3 1 5 . I n a d d i t i o n , t u m o r b a r e r s p l e e n c e l l s c u l t u r e d w i t h P E G w e r e f a r m o r e e f f e c t i v e i n v i v o i n t h e W i n n a s a y ( 2 9 ) t h a n w e r e s p l e e n c e l l s c u l t u r e d w i t h o u t P E G .

W e h a v e d e t e r m i n e d t h a t t h e m a x i m a l a u g m e n t a t i o n o f a n t i - t u m o r c y t o t o x i c i t y o f t u m o r b a r e r s p l e e n c e l l s o c c u r r e d w h e n P E G w a s u s e d a t a c e n t r a t i o n o f 2% i n t h e i n v i t r o i m m u n i z a t i o n c u l t u r e i n a g r e e m e n t w i t h K e d a r e t a l . ( 1 4 ) . H o w e v e r , o t h e r s h a v e s h o w n t h a t 4% P E G w a s t h e o p t i m u m f o r t h e e n h a n c e m e n t o f l y m p h o c y t e p r o l i f e r a t i o n i n r e s p o n s e t o m i t o g e n s ( 2 ) , s y n g e n e i c t u m o r c e l l s ( 1 , 2 2 ) , a n d a u t o l o g o u s l y m p h o c y t e s ( 2 2 ) . I t a p p e a r s , t h e n , t h a t t h e o p t i m a l c o n c e n t r a t i o n o f P E G f o r e n h a n c e m e n t o f c y t o t o x i c o r p r o l i f e r a t i v e a c t i v i t y m a y d i f f e r .

W e r e p o r t e d p r e v i o u s l y t h a t p r o g r e s s i o n o f s . c . M O P C - 3 1 5 t u m o r g r o w t h i s a s s o c i a t e d w i t h a d e c r e a s e i n t h e a b i l i t y o f t h e s p l e e n c e l l s t o m e d i a t e i n v i t r o a n t i - M O P C - 3 1 5 c y t o t o x i c i t y u p o n c u l t u r e w i t h m i t o m y c i n C - t r e a t e d M O P C - 3 1 5 c e l l s ( 1 8 , 1 9 ) . T h i s d e c r e a s e w a s a t t r i b u t e d t o p r o g r e s s i v e c h a n g e s i n t h e s p l e e n c e l l c o m p o s i t i o n ; e . g . , s p l e e n s o f m i c e b e a r i n g 2 5 - m m t u m o r s c o n t a i n e d a n i n c r e a s e i n t h e p e r c e n t a g e o f m a c-

We have determined that the maximal augmentation of anti-tumor cytotoxicity of tumor bearer spleen cells occurred when PEG was used at a concentration of 2% in the in vitro immunization culture in agreement with Kedar et al. (14). However, others have shown that 4% PEG was the optimum for the enhancement of lymphocyte proliferation in response to mitogens (2), syngeneic tumor cells (1, 22), and autologous lymphocytes (22). It appears, then, that the optimal concentration of PEG for enhancement of cytotoxic or proliferative activity may differ.

We reported previously that progression of s. c. MOPC-315 tumor growth is associated with a decrease in the ability of the spleen cells to mediate in vitro anti-MOPC-315 cytotoxicity upon culture with mitomycin C-treated MOPC-315 cells (18, 19). This decrease was attributed to progressive changes in the spleen cell composition; e.g., spleens of mice bearing 25-mm tumors contained an increase in the percentage of macrophages (reaching as high as 23%) and metastatic tumor cells (reaching as high as 12% dinitrophenol-rosettable cells) (18, 20). Progression of s. c. MOPC-315 tumor growth is associated with an increase in the antitumor potential of the spleen cells (18), but this appears to be blocked by increasing concentrations of suppressor cells. In this paper, we demonstrated that progression of tumor growth is associated with an increase in the ability of the spleen cells to mediate in vitro anti-MOPC-315 cytotoxicity when cultured with PEG. Thus, progression of tumor growth is associated with opposite effects on the level of anti-MOPC-315 cytotoxicity obtained when the spleen cells are cultured with mitomycin C-treated tumor cells as opposed to when they are cultured with PEG. It appears that the presence of PEG leads to a differential effect on suppressor and effector cells. Moreover, metastatic tumor cells, which are responsible in part for the reduced ability of tumor bearer spleen cells to mediate in vitro antitumor cytotoxicity upon culture with mitomycin C-treated tumor cells, might actually be required for the generation of anti-MOPC-315 cytotoxicity when cultured with PEG when mitomycin C-treated tumor cells are not added. It is also possible that PEG enhances the proliferation of lymphocytes in response to stimulator cells (1, 22) in a manner which dilutes the suppressor cells, thereby allowing the expression of the existing antitumor cytotoxicity and the generation of additional cytotoxic activity in response to the metastatic tumor cells.

Although substantial levels of anti-MOPC-315 cytotoxicity developed when spleen cells from normal mice were cultured with added MOPC-315 stimulator cells or in the presence of PEG, the cytotoxic activity induced by each of the methods differed in its specificity. Both our group (19) and others (9)
have shown that the culture of normal spleen cells with stimulator cells resulted in specific cytotoxicity. Similar to the recent observations by Kedar et al. (14) in the EL4 and YAC tumor systems, we observed in this paper that culture of normal spleen cells in the presence of PEG with or without stimulator tumor cells resulted in nonspecific cytotoxic activity against syngeneic tumor cells as well as against allogeneic tumor cells and syngeneic normal cells. Thus, although PEG potentiated the cytotoxicity of normal spleen cells against syngeneic tumor cells, the use of such cytotoxic cells in immunotherapy (8, 15) is that the primary site and those obtained from metastatic deposits (10, 12, 21, 25). The importance of this concept was nicely illustrated in the 3LL system (12). Lymphoid cells immunized in vitro against L-3LL were more cytotoxic in vitro to L-3LL than they were to M-3LL, while lymphoid cells immunized against M-3LL were more cytotoxic to M-3LL than they were to L-3LL.

Table 8

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tumor bearer spleen cells cultured with</th>
<th>Antitumor cytotoxicity in vitro</th>
<th>Tumor incidence</th>
<th>MST (days)</th>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>7 ± 1.1</td>
<td>5/5</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Tumor cells</td>
<td>23 ± 0.7</td>
<td>5/5</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>PEG</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Tumor cells and PEG</td>
<td>100 ± 0.4</td>
<td>0/5</td>
<td>&gt;60</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>10 ± 1.1</td>
<td>5/5</td>
<td>24</td>
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<tr>
<td></td>
<td>Tumor cells</td>
<td>8 ± 0.1</td>
<td>5/5</td>
<td>24</td>
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<tr>
<td></td>
<td>PEG</td>
<td>24 ± 0.7</td>
<td>0/5</td>
<td>&gt;60</td>
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<td></td>
<td>Tumor cells and PEG</td>
<td>84 ± 1.1</td>
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<td>1 ± 0.3</td>
<td>7/7</td>
<td>24</td>
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<td></td>
<td>Tumor cells</td>
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<td>7/7</td>
<td>28</td>
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<tr>
<td></td>
<td>PEG</td>
<td>25 ± 0.5</td>
<td>0/5</td>
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<tr>
<td></td>
<td>Tumor cells and PEG</td>
<td>68 ± 1.4</td>
<td>1/7</td>
<td>&gt;60</td>
</tr>
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</table>

* Mice bearing MOPC-315 tumors 22 ± 0.3, 25 ± 1.0, and 22 ± 0.6 mm in diameter in Experiments 1, 2, and 3, respectively.
* Percentage of specific *51Cr release at an E/T ratio of 20/1 using MOPC-315 target cells.
* Determined by the Winn assay at an E/T ratio of 20/1. Each mouse was given an injection of 5 × 10⁶ MOPC-315 cells and 1 × 10² cultured spleen cells in Experiment 1, or 2 × 10⁶ MOPC-315 cells and 4 × 10⁶ cultured spleen cells in Experiments 2 and 3, as described in "Materials and Methods."
* Number of mice developing a tumor within 60 days/total number of mice per group.
* MST, Median survival time. The MST of mice given injections of tumor cells alone was 18 days in Experiment 1 and 24 days in Experiments 2 and 3.
* Mean ± S.E.
* Added mitomycin C-treated MOPC-315 stimulator cells.
* ND, not done.

There is a striking similarity between the effect of PEG on tumor bearer spleen cells reported here and the effect of Con A on spleen cells from animals immunized *in vivo* to allogeneic tumor cells as reported by Bonavida (3). The addition of Con A to cultures of tumor-immune spleen cells led to the development of antitumor cytotoxicity with specificity for the priming antigen. The addition of both Con A and stimulator tumor cells to the cultures of tumor-immune cells increased the level of activity even further. Thus far, the effect of Con A on the antitumor response of tumor bearer spleen cells has not been evaluated. However, since tumor bearer spleen cells have been primed *in vivo*, we are investigating the possibility that Con A might trigger a secondary response in the absence of stimulator tumor cells.

Potentiation of antitumor cytotoxicity of MOPC-315 tumor bearer spleen cells was also accomplished by treating the donors of spleen cells with BCG prior to *in vitro* immunization of the spleen cells (4). However, different strains of BCG, and even different batches of the same strain, might exert opposite effects on the *in vitro* generation of antitumor cytotoxicity (17). This unpredictable effect of BCG might limit its usefulness as a method to potentiate the generation of antitumor cytotoxicity in vitro immunization of tumor bearer spleen cells even though cell recovery is quite high. On the other hand, the effectiveness of PEG, a well-defined chemical substance, in augmenting cytotoxicity is apparently independent of the lot used.

Partial recovery of the antitumor potential of MOPC-315 tumor bearer spleen cells was achieved by depletion of dinitrophenol-adherent cells prior to *in vitro* immunization (20). Such a depletion included the removal of most tumor cells, with a minimal reduction in the percentage of macrophages. Depletion of glass-adherent cells from tumor bearer spleen cells prior to *in vitro* immunization resulted in much greater augmentation in the level of antitumor cytotoxicity than did that obtained with the same spleen cells which had been depleted of dinitrophenol-adherent cells prior to immunization. Depletion of glass-adherent cells included not only the removal of most tumor cells, as did depletion of dinitrophenol-adherent cells, but also the removal of most macrophages (20). Although these methods led to augmentation of antitumor cytotoxicity, such an approach is limited by the low cell recovery (15 to 25%) (20). The use of PEG in the immunization cultures of tumor bearer spleen cells obviates the need for prior column fractionation to remove suppressor cells. Furthermore, the cell recovery was as much as 200% (presumably due to the better survival and/or the proliferation of the splenic lymphocytes) compared to cultures without PEG, and substantial antitumor cytotoxicity developed even in the absence of added stimulator tumor cells.

One explanation of the limited success of *in vitro* immunized lymphoid cells in immunotherapy (8, 15) is that the primary tumor site may not be the optimal source of stimulator cells for the *in vitro* generation of antitumor cytotoxicity, since antigenic differences may exist between tumor cells obtained from the primary site and those obtained from metastatic deposits (10, 12, 21, 25). The importance of this concept was nicely illustrated in the 3LL system (12). Lymphoid cells immunized in *vitro* against L-3LL were more cytotoxic in *vitro* to L-3LL than they were to M-3LL, while lymphoid cells immunized against M-3LL were more cytotoxic to M-3LL than they were to L-3LL.
More importantly, only lymphoid cells immunized against M-3LL were able to inhibit lung metastases following inoculation of either L-3LL or M-3LL in a local adoptive transfer assay (12). Thus far, it is not known whether there are antigenic differences of either L-3LL or M-3LL in a local adoptive transfer assay (12).

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REFERENCES


Effect of Polyethylene Glycol 6000 on the Generation of Antitumor Cytotoxicity in MOPC-315 Tumor Bearer Spleen Cells Cultured in the Presence or Absence of Inactivated Stimulator Tumor Cells

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