Advantages of Adoptive Chemoimmunotherapy with Polyethylene Glycol-cultured, Antigen-activated, Tumor-infiltrated Spleen Cells for the Complete Eradication of Lethal MOPC-315 Plasmacytomas

Maribeth Laude, Katherine L. Siessmann, Margalit B. Mokyr, and Sheldon Dray

Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, Illinois 60680

ABSTRACT

The incorporation of polyethylene glycol-6000 (PEG) into the culture media of tumor-infiltrated spleen cells (TISpC) and MOPC-315 stimulator tumor cells at a responder to stimulator cell ratio of 30/1 had been shown to lead to the appearance of CD8+ T-cells that were effective in adoptive chemoimmunotherapy (ACT) of mice bearing a barely palpable MOPC-315 tumor (J. A. Wise, M. B. Mokyr, and S. Dray, Cancer Res., 49: 3613–3619, 1989). Here we show that in the presence of substantially fewer added stimulator tumor cells (responder to stimulator cell ratio, 100/1), the inclusion of PEG in the cultures of TISpC also enhanced the appearance of cells that were highly effective in curing such mice by ACT. Moreover, these PEG-cultured TISpC were more effective in ACT than TISpC cultured in the presence of an optimal concentration of recombinant interleukin-2 (60 IU/ml). The potency of the tumor-eradicating activity of the PEG-cultured TISpC in ACT was further illustrated by their ability to cause the complete regression of a large (20–22 mm) s.c. MOPC-315 tumor in conjunction with a dose of drug that by itself did not cause tumor regression. PEG-cultured TISpC that were effective in ACT were capable of exerting a potent, direct, in vitro lytic activity against MOPC-315 tumor cells in an antigen-specific manner. In fact, PEG-cultured TISpC were more effective than recombinant interleukin-2-cultured TISpC, not only in ACT, but also in their ability to lyse MOPC-315 tumor cells in vitro. Thus, a direct specific lytic activity against the tumor by cytotoxic T-lymphocytes is the apparent mechanism through which the complete regression of the large tumor burden is brought about by the PEG-cultured TISpC. Finally, we suggest that the incorporation of PEG to render ineffective lymphoid cells effective in ACT may offer some advantages compared with the incorporation of recombinant interleukin-2 and may be suitable for protocols to generate human cytotoxic cells for cancer therapy when there are relatively low numbers of available tumor cells.

INTRODUCTION

Many investigators have been using various methods to enhance the antitumor activity of lymphoid cell populations derived from tumor-bearing hosts for use in cancer therapy (1, 2). The source of these tumor-reactive cells has varied, e.g., peripheral blood (3) or the tumor site itself (4, 5). Most investigators have used rIL-2 to induce the activation and proliferation of the tumor-reactive lymphocytes. Lymphoid cells that were cultured in the presence of relatively high concentrations of rIL-2 and in the absence of added tumor antigen resulted in a population of lymphoid cells that exhibited potent specific CTL-type lytic activity in vitro (8–10). Tumor-infiltrating lymphocytes cultured in the presence of relatively low concentrations of rIL-2 and added tumor antigen exhibited CTL-type lytic activity and have been reported to be much more effective in vivo for cancer therapy than lymphokine-activated killer cells generated with relatively high concentrations of rIL-2, without added tumor antigen (4).

In our laboratory, we have used an alternative approach to enhance the lytic activity of TISpC as well as their effectiveness in ACT. Specifically, the addition of the hydrophilic polymer PEG to cultures of TISpC and MOPC-315 stimulator tumor cells (R/S = 30/1), (i.e., mitomycin C-inactivated tumor cells as the source of added tumor antigen), has been shown to enhance the effectiveness of lymphocytes from untreated tumor-bearing mice to lyse MOPC-315 tumor cells (11, 12) and to prevent the establishment of a barely palpable tumor by ACT (9, 13). In our system, although TISpC cultured with PEG showed some effectiveness in ACT, the addition of both PEG and added tumor antigen resulted in much greater effectiveness. In comparing the addition of PEG with the addition of rIL-2 to cultures of TISpC and added tumor antigen, the addition of PEG was at least as effective as rIL-2 in generating lymphocytes for the cure of tumor-bearing mice by ACT (9).

Since in some circumstances relatively few tumor cells may be available, an aim of this study was to determine if PEG and/or rIL-2 could enhance the effectiveness in ACT of TISpC cultured with MOPC-315 stimulator tumor cells at a higher R/S ratio of 100/1 than that of 30/1 used in previous in previous work (9). Specifically, we determined whether these cultured TISpC could prevent the progression of a small (barely palpable) s.c. MOPC-315 tumor in mice upon ACIT. Because the PEG-cultured TISpC were found to be more effective than the rIL-2-cultured TISpC, we then determined whether the PEG-cultured TISpC could cause the complete regression of a very large, late stage MOPC-315 tumor in mice treated with a subcurative dose of CY. Finally, we determined whether the in vitro cytotoxic responses of cultured TISpC correlated with their in vivo effectiveness in causing tumor regression.

MATERIALS AND METHODS

Animals and Tumors. The MOPC-315 plasmacytoma was maintained in 7–10-week-old female BALB/c mice (Charles River Breeding Laboratories, Wilmington, MA) as described previously (14). MOPC-315-bearing mice were prepared by s.c. injection of 1 × 10⁶ MOPC-315 tumor cells, a dose that is at least 300 times the minimal lethal tumor dose and kills the mice in 15 ± 1 (SE) days. After MOPC-315 inoculation, the tumor was barely palpable by day 4 and progressed to 20–22 mm by day 10. Mice bearing day 10 MOPC-315 tumors were used as a source of MOPC-315 tumor cells. Athymic nude mice used in ACIT were nu/nu outbred, primarily of BALB/c background, 7–9 weeks old (Harlan Sprague Dawley, Indianapolis, IN).
Chemotherapy. A stock solution of CY (Mead Johnson and Co., Evansville, IN) was dissolved in sterile distilled water to a concentration of 20 mg/ml. This solution was further diluted in 0.9% NaCl. For chemotherapy, CY was given as a single i.p. injection of 10 mg/kg to day 4 MOPC-315 tumor-bearing BALB/c mice or 50 mg/kg to day 8 MOPC-315 tumor-bearing athymic nude mice.

Spleen Cell Suspensions. For ACIT, TISpC or fresh “immune” spleen cells were used. TISpC were obtained from day 10, MOPC-315 tumor-bearing mice that had received no chemotherapy. The spleens from such mice contained metastatic tumor cells comprising approximately 6–10% of the spleen cell population. The fresh “immune” spleen cells were obtained from BALB/c mice that had completed the regression of a large MOPC-315 tumor as a consequence of a low dose of L-phenylalanine mustard (2.5 mg/kg melphalan; Burroughs Wellcome Co., Research Triangle Park, NC) (16). Single cell suspensions from the spleens were prepared by mechanical disruption between glass slides. The spleen cell viability as determined by trypan blue dye exclusion (0.4%) always exceeded 95%. Spleen cells from at least 10 mice were pooled for use in each experiment.

In Vitro Immunization of TISpC by Short-Term Culture. The in vitro immunization of TISpC was done according to the methods we have described previously for the generation of an antitumor response (11, 14). Briefly, 80 × 10⁶ TISpC were cultured in the presence of 0.8 × 10⁴ mitomycin C-inactivated (50 µg/ml, at 37°C for 30 min; Sigma Chemical Co., St. Louis, MO) MOPC-315 stimulator tumor cells in 75-cm² tissue culture flasks (Corning Plastics, Corning, NY) at 37°C for 5 days in 50 ml of RPMI 1640 supplemented with 5% heat-inactivated fetal bovine serum, 1% nonessential amino acids, 50 units/ml penicillin, 50 µg/ml streptomycin (Grand Island Biological Co., Grand Island, NY), and 5 × 10⁻⁴ M 2-mercaptoethanol. To some cultures, 2% w/v PEG (BDH Chemicals, Poole, England) and/or 60 IU/ml rIL-2 (1 IU of rIL-2 corresponds to 1/6 Cetus units; Cetus Corp., Emeryville, CA) were added at the time of culture initiation. In control experiments, the stimulator tumor cells were omitted.

ACIT. BALB/c recipient mice were given s.c. injections of 1 × 10⁴ MOPC-315 tumor cells on day 0, and 4 days later, when the tumor was small (i.e., barely palpable), they were given i.p. injections of a subcurative dose of CY (10 mg/kg). Donor spleen cells (3.13 × 10⁶ to 25 × 10⁶) were administered i.v. to these mice 1 day later. Mice were monitored every other day for tumor progression and regression, and mice that survived an observation period of 60 days and were tumor-free were considered “cured.” In each experiment, one control group of mice bearing a small tumor was treated with the same dose of CY (i.e., 10 mg/kg) alone. In the MOPC-315 tumor model, a low dose of CY that was not curative at an early stage of tumor growth due to the absence of sufficient antitumor immunity (17) was curative at an advanced stage of tumor growth due to the immunomodulatory activity of the drug that leads to the appearance of potent antitumor immunity in the hitherto immunosuppressed tumor bearer (17, 18). To establish a model for adoptive immunotherapy of a large tumor, athymic nude mice were used to minimize the development of host T-cell antitumor immunity after administration of the drug and thereby maximize the importance of the adoptively transferred cells to the therapeutic outcome. Accordingly, athymic nude recipient mice were given s.c. injections of 1 × 10⁴ MOPC-315 tumor cells on day 0, and 8 days later, when the tumor was large (i.e., about 20 mm), they were given i.p. injections of 50 mg/kg CY (16). Donor spleen cells (100 × 10⁶) were administered i.v. to these mice 2 h after CY therapy. The mice were monitored daily for 38 days after ACIT.

In Vitro Antitumor Cytotoxicity Assay. The lytic activity of cultured spleen cells was determined by the 3.5-h ⁵¹Cr-release assay, as described previously (14). Briefly, 2.5 × 10⁶ ⁵¹Cr-labeled MOPC-315 target cells were incubated with various numbers of spleen cells in 12 × 75-mm plastic tubes. At the end of the incubation period, the cells were pelleted and both supernatants (Sup) and pellets (P) were counted in an Auto-Gamma scintillation counter. The percentage of ⁵¹Cr release for each sample was calculated as follows:

\[
\text{% ⁵¹Cr release} = \frac{(\text{Sup} - \text{R/S})}{(\text{Sup} + \text{Cr} + \text{C})} \times 100
\]

where ⁵¹Cr is measured in cpm. Subsequently, the percentage of specific ⁵¹Cr release was calculated by the following formula:

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

where T is the percentage release with test lymphocytes, C is the mean of 3 replicates of the percentage of spontaneous release (which ranged 25–30%), and M is the mean of 3 replicates of the percentage of maximal release obtained by detergent lysis in 2% Nonidet P-40 (Sigma) (which ranged 85–92%). We (14) have observed, as have others (19), that the level of antitumor cytotoxicity obtained with spleen cells of individual mice cultured under the same conditions may vary from one experiment to another. Still, the pattern of results remains consistent. The level of antitumor cytotoxicity is presented as the mean percentage-specific ⁵¹Cr release of triplicate samples ± SE. Variations in the percentage of ⁵¹Cr released between individual samples rarely exceeded 5%.

Statistical Analyses. To determine the significance of the differences in the fraction of mice surviving after different treatments, the G test of independence was used (20). For all other statistical analyses, the Student’s t test was used. A P value of <0.05 was considered significant in both tests.

**RESULTS**

Ability of TISpC Cultured in the Presence of PEG at a R/S Ratio of 100/1 to Cure Mice Bearing a Barely Palpable MOPC-315 Tumor upon ACIT. We had shown previously that the inclusion of PEG into 5-day cultures of TISpC with MOPC-315 stimulator tumor cells at a R/S ratio of 30/1 led to the appearance of CD8+ T-cells capable of preventing the establishment of a barely palpable lethal MOPC-315 tumor upon ACIT (13). An aim of this work was to determine if PEG can also lead to the acquisition of an enhanced effectiveness in ACIT in the presence of substantially fewer added stimulator tumor cells in the cultures of TISpC. Accordingly, TISpC were cultured with MOPC-315 stimulator tumor cells at a R/S ratio of 100/1 in the presence or absence of PEG and subsequently, various numbers of these cells were tested for their effectiveness in preventing the establishment of a barely palpable MOPC-315 tumor upon ACIT (Fig. 1). For all cell numbers tested (3.13 × 10⁶ to 25 × 10⁶), many more mice were cured by TISpC cultured in the presence of PEG than in the absence of PEG. For example, whereas 12.5 × 10⁶ TISpC cultured in the presence of PEG cured 100% of the mice, only 25% of the mice were cured with 12.5 × 10⁶ TISpC cultured in the absence of PEG. Moreover, 3.13 × 10⁶ TISpC cultured in the presence of PEG were superior in their therapeutic effectiveness to 12.5 × 10⁶ or even 25 × 10⁶ TISpC cultured in the absence of PEG.

Comparison of TISpC Cultured in the Presence of PEG and/or rIL-2 for Their Ability to Cure Mice Bearing a Barely Palpable MOPC-315 Tumor by ACIT. We had also shown previously that the inclusion of PEG in the 5-day cultures of TISpC and MOPC-315 stimulator tumor cells at a R/S of 30/1 was at least as effective as the inclusion of an optimal concentration of rIL-2 (60 IU/ml) in enhancing the generation of TISpC effective in curing mice upon ACIT (9). This comparison of rIL-2 and PEG was re-examined at a R/S ratio of 100/1. As with the R/S ratio of 30/1, the optimal concentration of rIL-2 in cultures of TISpC and stimulator tumor cells at a R/S ratio of 100/1 was found to be 60 IU/ml (e.g., for 12.5 × 10⁶ cells transferred, 7 of 7 mice were cured by ACIT when TISpC were cultured with 60 IU/ml rIL-2). Therefore, in subsequent experiments comparing PEG and rIL-2, TISpC were cultured for 5 days in the presence...
of 60 IU/ml rIL-2 and MOPC-315 stimulator tumor cells at a R/S ratio of 100/1 and assessed for their ability to cure mice by ACT (Fig. 1). At all cell numbers transferred, except 25 × 10⁶, TISpC cultured in the presence of PEG were significantly more effective in ACT than TISpC cultured in the presence of rIL-2. For example, when 12.5 × 10⁴ cells were transferred into recipient mice, only 54% of the mice were cured by TISpC cultured in the presence of rIL-2 as compared with 100% of the mice cured by TISpC cultured in the presence of PEG. In fact, 6.25 × 10⁶ PEG-cultured TISpC were at least as effective in ACIT as 25 × 10⁴ rIL-2-cultured TISpC.

Finally, we determined whether the inclusion of rIL-2 in addition to PEG in the culture medium could further enhance the generation of TISpC, which upon ACT could prevent the establishment of a barely palpable MOPC-315 tumor (Fig. 1). The incorporation of rIL-2 in cultures of TISpC containing PEG did not further increase their effectiveness in ACIT relative to TISpC cultured in the presence of PEG alone (e.g., for 6.25 × 10⁶ TISpC transferred, 57% were cured with both PEG and rIL-2 in the culture versus 71% with PEG alone).

Exertion of Enhanced in Vitro CTL-type Lytic Activity by PEG-cultured TISpC. Previously it had been shown that MOPC-315 tumors that were in the process of host immune-mediated regression contained CD8⁺ T-cells capable of exerting a CTL-type lytic activity in vitro (21). Initially, we determined whether TISpC that had generated enhanced lytic activity against MOPC-315 upon culture in the presence of PEG and MOPC-315 stimulator tumor cells at a R/S ratio of 30/1 could also generate enhanced lytic activity upon culture with PEG and stimulator tumor cells at a R/S ratio of 100/1. For this purpose, TISpC were cultured with MOPC-315 stimulator tumor cells in the presence or absence of PEG and assayed for their in vitro lytic activity against MOPC-315 target cells. TISpC cultured in the presence of both stimulator tumor cells and PEG exhibited a potent, direct anti-MOPC-315 lytic activity that was much greater than that exhibited by TISpC cultured in the presence of either stimulator tumor cells or PEG alone (Fig. 2). Thus, the inclusion of PEG in the culture medium that rendered TISpC more effective in ACIT also rendered the TISpC more cytotoxic in vitro for the MOPC-315 tumor cells.

Next, we determined the specificity of the in vitro lytic activity of TISpC cultured in the presence of MOPC-315 stimulator tumor cells and PEG by measuring their lytic activity against a panel of tumor targets. TISpC, which exhibited potent in vitro lytic activity against the "autochthonous" tumor, exhibited much less lytic activity against the antigenically related syngeneic plasmacytomas, MOPC-104E and RPC-5. Virtually no lytic activity was exhibited against 2 syngeneic thymomas, WEHI-22.1 and WEHI-7.1, an allogeneic thymoma, EL4, and a natural killer cell-susceptible lymphoma, YAC-1. Thus, TISpC cultured in the presence of PEG, which were effective in ACT, exhibited potent, direct, in vitro lytic activity in an antigen-specific manner.

Comparison of TISpC Cultured in the Presence of PEG and/or rIL-2 for Their Ability to Lyse MOPC-315 Tumor Cells in Vitro. We determined the cytotoxic activity exhibited by TISpC cultured in the presence of rIL-2 or with both rIL-2 and PEG and then compared their cytotoxicity with TISpC cultured in the presence of PEG. Specifically, cells from the same pool of cultures that were used in ACIT (Fig. 1) were assessed for their in vitro cytotoxic activity against MOPC-315 target cells (Fig. 3). At all effector to target cell ratios tested, TISpC cultured in rIL-2 exhibited an enhanced level of cytotoxicity, in accordance with their increased effectiveness in ACIT, as compared with TISpC cultured in the absence of rIL-2. As was the situation with effectiveness in ACIT, TISpC cultured in the presence of PEG were more effective than TISpC cultured in the presence of rIL-2, also in their ability to lyse MOPC-315 tumor cells in vitro, and the addition of rIL-2 to cultures of TISpC and PEG did not increase their lytic activity. Thus, the in vitro cytotoxicity observed with TISpC cultured with PEG and/or rIL-2 correlated with their in vivo effectiveness in ACIT.
In this preliminary experiment, the tumor partially regressed and the therapy extended the survival of the mice by a few days (from 8 days for mice receiving CY therapy alone to 12 days for mice receiving $10^6$ PEG-cultured TISpC in addition to CY; $P < 0.1$). In an attempt to amplify the therapeutic effect of ACIT for athymic nude mice bearing a large tumor, the dose of CY used was increased to 50 mg/kg to reduce the tumor burden that the PEG-cultured TISpC would have to eradicate in order to cure the mice. This dose of CY did not cure any of the mice, nor did it cause any regression of the tumor, although tumor progression was somewhat slower. When $10^6$ PEG-cultured TISpC were administered about 2 h after this dose of CY, complete regression of the 20-mm tumor was observed in all of the mice (10 of 10) within about 14 days after the therapy, and these mice remained tumor-free for about 3 more weeks when the experiment was terminated (Fig. 4). In fact, PEG-cultured TISpC were at least as effective as fresh spleen cells obtained from tumor-immune BALB/c mice (“immune” spleen cells) in causing the complete regression of a large MOPC-315 tumor upon ACIT. Interestingly, the onset of regression due to the fresh “immune” spleen cells occurred about 2–3 days after the onset of regression caused by PEG-cultured TISpC ($P < 0.001$). Moreover, tumor regrowth occurred in 3 of the 10 mice treated with the fresh “immune” spleen cells, but in none of the mice treated with PEG-cultured TISpC. Thus, TISpC cultured with PEG not only prevented the establishment of a barely palpable MOPC-315 tumor in BALB/c mice, but also caused the complete regression of a large MOPC-315 tumor in athymic nude mice in conjunction with a low subcutaneous dose of CY.

In Vitro Cytolytic Potential of Fresh Spleen Cells Obtained from Immune Mice. Since cytotoxic activity correlated with the effectiveness of TISpC in ACIT, we determined whether the short but statistically significant delay in tumor regression correlated with the transient inability of the fresh “immune” spleen cells to exert potent CTL-type lytic activity. To evaluate this possibility, the fresh “immune” spleen cells were tested for their in vitro cytotoxic activity against MOPC-315 tumor cells at the time they were used for ACIT or after they were cultured for 1, 2, or 3 days in the presence or absence of stimulator tumor cells (Fig. 5). The fresh “immune” spleen cells were poorly cytotoxic for MOPC-315 target cells (i.e., 9% specific $^{51}$Cr release at an effector-to-target cell ratio of 100/1). However, when these fresh “immune” spleen cells were cultured in the presence of MOPC-315 stimulator tumor cells for a few days, they acquired potent anti-MOPC-315 lytic activity so that by day 3 after culture initiation they were essentially as cytotoxic for MOPC-315 tumor cells as were TISpC cultured with PEG for 5 days (i.e., 70% specific $^{51}$Cr release at a R/S ratio of 100/1). Thus, the fresh spleen cells from immune mice that were only marginally cytotoxic for MOPC-315 tumor cells could acquire a potent direct anti-MOPC-315 cytotoxicity within 2–3 days after exposure to the tumor cells, a period that is comparable with the lag period observed for the regression of the tumor.

DISCUSSION

Herein we have shown that the inclusion of PEG is more effective than the inclusion of rIL-2 in short term cultures of TISpC and MOPC-315 stimulator tumor cells at a R/S ratio of 100/1 in generating cells effective in curing BALB/c mice bearing a small (barely palpable) s.c. MOPC-315 tumor upon...
ACIT. Such PEG-cultured TISPc were also effective in causing the complete regression of a large (20-22 mm) s.c. MOPC-315 tumor in athymic nude mice treated with a dose of CY, which by itself did not cause significant tumor regression. Furthermore, we demonstrated that TISPc cultured with stimulator tumor cells at a R/S ratio of 100/1 in the presence of PEG exhibited potent, direct, specific lytic activity against MOPC-315 target cells. Thus, a CTL-type lytic response is likely to be the most important mechanism through which PEG-cultured TISPc bring about the complete regression of a large tumor burden upon ACIT.

We have reported previously that TISPc cultured with stimulator tumor cells at a R/S ratio of 30/1 in the presence of PEG are at least as effective as TISPc cultured in the presence of an optimal concentration of rIL-2 (60 IU/ml) in curing mice bearing a barely palpable tumor upon ACIT (9). Here we show that under conditions of fewer added stimulator tumor cells (R/S ratio of 100/1), PEG-cultured TISPc are actually more effective than RIL-2-cultured TISPc in ACIT. In fact, such PEG-cultured TISPc were found to be 4 times more effective than TISPc cultured in the presence of RIL-2 in preventing the progression of lethal tumors. Since the generation of cells effective for ACIT among TISPc is greatly amplified if tumor cells are added during the short term culture with PEG or RIL-2 (13), and since the number of tumor cells available for in vitro stimulation may be limiting, the superiority of PEG over RIL-2 in rendering TISPc curative in ACIT under these conditions represents an attractive feature for the inclusion of PEG in protocols aimed at generating tumor bearer lymphocytes effective in ACIT. Some additional attractive features for the use of PEG include its stability and low cost relative to RIL-2.

The fact that inactivated stimulator tumor cells must be present in the culture for splenic cells to become effective in ACIT indicates that the metastatic tumor cells are not an optimal source of antigen during culture. Accordingly, the metastatic tumor cells may not be sufficiently immunogenic (e.g., as a result of too few cells or loss of some surface antigens (23)) or may exert some immunosuppressive activity (24-26).

To evaluate the role of splenic metastases in rendering MOPC-315 TISPc effective in ACIT, a subline of the tumor that does not metastasize to the spleen would have to be found. Still, from our findings, it is clear that even in the presence of the potential immunosuppressive activity of MOPC-315 tumor metastases, culture of these spleen cells with mitomycin C-treated MOPC-315 stimulator cells and PEG renders the TISPc highly effective in ACIT.

The mechanism through which PEG leads to the acquisition of enhanced curative effectiveness of TISPc is not known at present. However, in light of reports by other investigators, it is plausible that PEG may be promoting cellular interactions, including the facilitation of antigen presentation. Specifically, PEG has been shown to: (a) promote cell fusion (27) and consequently has been used to generate cell hybridomas for monoclonal antibody production; (b) mediate viral fusion with erythrocyte membranes (28); and (c) enhance autologous mixed lymphocyte reactions (29, 30). In the latter study, la antigens expressed by the stimulator cells were essential for the enhancement caused by PEG because when anti-la antibodies were added to the reaction, no enhanced response was observed.

We have previously shown that the inclusion of rIL-2 in short term cultures containing PEG, TISPc, and MOPC-315 stimulator tumor cells at the lower R/S ratio of 30/1 enhanced the therapeutic effectiveness of the TISPc in ACIT (9). Here we show that the addition of rIL-2 to PEG-containing cultures of TISPc and MOPC-315 stimulator tumor cells at a R/S ratio of 100/1 did not further enhance the therapeutic effectiveness of the TISPc in ACIT. Thus, the ability of rIL-2 to facilitate the generation of cells effective in ACIT appears to depend on the relative number of added stimulator tumor cells in the culture. We are currently considering the possibility that at the R/S ratio of 30/1, an earlier stimulation and expansion of cells specific for the tumor occurs and that under these conditions the presence of rIL-2 may disproportionately further expand or activate this population of T-cells. At the lower antigen concentration that exists at the R/S ratio of 100/1, fewer specific T-cells may initially be stimulated by the tumor cells, and rIL-2 may cause less activation and proliferation of the T-cell population specific for the tumor.

We have previously shown that CD8+ T-cells are responsible for the curative effectiveness in ACIT of TISPc cultured in the presence of PEG with MOPC-315 tumor cells at a R/S ratio of 30/1 (13). Although the phenotype of the cells responsible for the therapeutic effectiveness of TISPc cultured in the presence of PEG at a R/S ratio of 100/1 has not been established, it is quite likely that also under these culture conditions, CD8+ T-cells are essential for the therapeutic effect. Since: (a) the cells responsible for the therapeutic effectiveness of PEG-cultured TISPc are CD8+; (b) the CD8+ T-cells are classically CTL (31, 32); and (c) the CTL-type lytic mechanism was shown to be an important mechanism through which host CD8+ T-cells mediate their tumor-eradicating activity, we determined whether PEG-cultured TISPc can exert a CTL-type lytic activity in vitro. Our results illustrated that PEG-cultured TISPc exerted a potent, direct, specific in vitro lytic activity against MOPC-315 tumor cells. The likelihood that the transferred cells exert a potent in vivo CTL-type lytic activity against MOPC-315 tumor cells is supported by our current observations that: (a) the potency of the in vivo lytic activity correlated with the in vitro effectiveness for tumor eradication in ACIT; (b) the lag period for eradication of the large tumor in athymic nude mice was shorter when donor cells exhibited more potent cytolysis at the time of ACIT; and (c) the lag period observed in the regression of the large tumor correlated with the transient inability of the fresh “immune” spleen cells to exert potent CTL-type lytic activity. Moreover, the potential role of a CTL-type lytic mechanism in MOPC-315 tumor eradication after ACIT is supported by our earlier observations.
that culture of TISpC with PEG and rIL-2 for 27 days, which led to more than a 2-fold increase in the percentage of CD8+ T cells, i.e., from 36 to 83%, as well as a great increase in the in vitro cytotoxic activity, was accompanied by a 4-fold increase in the curative effectiveness of the cultured TISpC in ACIT (9). Although CTL-type lytic activity is most likely the primary antitumor mechanism exerted by the PEG-cultured TISpC upon ACIT, it may not be the only mechanism involved in tumor eradication. The possibility remains that the donor lymphocytes may secrete lymphokines in response to in vivo exposure to the tumor and that these lymphokines in turn may recruit and activate other cells, such as macrophages (33-35) to aid in tumor eradication. The possibility also exists that T-cells derived from the athymic nude mice are recruited and activated (36) to aid in the complete eradication of the tumor.

Athymic nude mice were used instead of normal BALB/c mice to establish a model for ACIT of mice bearing a large tumor because, as we have shown previously (37), a low dose of CY (10 mg/kg) is not a subcurative dose in these BALB/c mice, i.e., all of the mice are cured by the cooperation between the toxic activity of the drug and the potent host T-cell-dependent antitumor immunity that emerges shortly after the chemotherapy (20). The use of the athymic nude mice not only facilitated the development of an ACIT model for a large tumor by minimizing the contribution of host T-cells to the therapy, but also allowed for an ACIT model in which the adaptively transferred lymphocytes are critical for the curative effectiveness of the therapy. Using the athymic nude mouse tumor model, we illustrated here the potency of the in vivo tumor eradicating activity of the PEG-cultured TISpC by demonstrating their ability to cause the complete regression of a large MOPC-315 tumor under conditions in which the drug itself did not cause any tumor regression.

PEG-cultured TISpC were apparently superior to fresh “immune” spleen cells in causing the complete regression of a large tumor by ACIT. Accordingly, although spleen cells from both sources caused the complete regression of the large tumor within 14 days, the tumor reappeared in 30% of the athymic nude mice treated with fresh “immune” spleen cells, but in none of the athymic nude mice treated with PEG-cultured TISpC during the 38 days of observation. Currently we are considering the possibility that the tumor regrowth in these athymic nude mice may be the result of a failure by the fresh “immune” spleen cells to maintain their potent tumor-eradicating activity. As another possibility, the emergence of a tumor clone that is not recognized by CTL within the fresh “immune” spleen cell population may result in tumor regrowth in these mice (10). Regardless of why tumors that had apparently regressed completely started to regrow in mice that had received fresh “immune” spleen cells but not in mice that had received PEG-cultured TISpC, our observations point out another potential advantage for the use of PEG in preparing tumor bearer lymphoid cells to be used in ACIT.

The use of short term cultures of tumor-bearer lymphocytes, added tumor antigen, and PEG appears to be a useful method for consistently enhancing the generation of highly specific cytotoxic lymphocytes from a heterogeneous population of lymphocytes. It remains to be determined for other tumor systems whether PEG will retain its advantage over rIL-2 for generating cells effective in ACIT.

REFERENCES


ADOPTIVE CHEMOIMMUNOTHERAPY


Advantages of Adoptive Chemoimmunotherapy with Polyethylene Glycol-cultured, Antigen-activated, Tumor-infiltrated Spleen Cells for the Complete Eradication of Lethal MOPC-315 Plasmacytomas

Maribeth Laude, Katherine L. Siessmann, Margalit B. Mokyr, et al.


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