Inhibition of Murine Lymphokine-Activated Killer (LAK) Cell Activity by Adherent Cells

ROSS E. LONGLEY,* DEDRA STEWART,* KIMBERLY G. ROE,† AND ROBERT A. GOOD‡

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institute, Ft. Pierce, Florida 34946; †Department of Biological Sciences, University of Central Florida, Orlando, Florida 32816; and ‡Department of Pediatrics, All Childrens Hospital, St. Petersburg, Florida 33701

The effects of adherent cell depletion, indomethacin, and prostaglandin E_2 (PGE_2) on murine LAK cell activity were investigated. Removal of plastic adherent cells from splenocyte suspensions either prior to 5-day culture with 1000 U/ml of recombinant human IL-2 (rIL-2) or prior to assay resulted in an enhanced LAK cell cytotoxicity compared to that of whole spleen cell suspensions. Indomethacin enhanced LAK cell cytotoxicity of whole splenocyte suspensions if present during the culture period, but had no effect on whole splenocyte or adherent cell-depleted cell suspensions if added just prior to assay. PGE_2 suppressed LAK cell activity of nonadherent splenocyte but not whole splenocyte suspensions when present during the culture period. *In vivo* treatment of mice with indomethacin enhanced cytotoxicity directed toward both LAK sensitive, natural killer (NK) resistant (P-815) and LAK, NK sensitive (YAC-1) tumor cell targets. Splenocytes from indomethacin-treated mice cultured with additional indomethacin and rIL-2 exhibited highest LAK cell activity. The results from this study indicate that LAK cells are regulated by adherent cells which suppress LAK cell activity. This suppression can be reversed both *in vitro* and *in vivo* by indomethacin. This study has important implications for the possible clinical use of indomethacin in the potentiation of *in vivo* and *in vitro* LAK cell activity for immunotherapeutic protocols.

INTRODUCTION

One of the most promising areas in the treatment of human malignancies has been the use of LAK cells in adoptive immunotherapy regimens (1, 2). However, a number of undesirable side effects occur as a consequence of toxicity associated with high-dose interleukin-2 (IL-2) maintenance therapy (3, 4). Termination of IL-2 treatment results in complete reduction of these side effects, but with a corresponding reduction in the effectiveness and absolute number of LAK cells available to kill malignant cells. While anti-inflammatory agents such as corticosteroids could possibly be used to control such side effects, similar studies in laboratory animals have demonstrated that such treatments serve to decrease *in vitro* antitumor activity of LAK cells, and thus abrogate any positive effects of LAK cell therapy (5).

In studies which were designed to optimize LAK cell generation and activities in C567BL/6J mice, we report here that adherent cell-depleted splenocyte preparations demonstrate greater LAK cell activity than that observed with whole, unseparated

spleen cell preparations. We show that a similar *in vitro* suppressive effect may be mediated, in part, by the addition of prostaglandin E_2, which is known to be produced by adherent cells (macrophages) and which has been shown previously to suppress cytotoxic activity of NK (6, 7) and cytotoxic T-lymphocytes (CTL) (8–12).

We also report that indomethacin, a known anti-inflammatory agent and inhibitor of prostaglandin synthesis, serves to enhance both *in vitro* and *in vivo* LAK cell-mediated cytotoxicity. These results suggest that current clinical protocols which include the administration of indomethacin to control inflammatory effects of IL-2/LAK cell therapy may also serve to enhance *in vivo* LAK cell activity. In addition, clinical *in vitro* protocols for preparation of LAK cells for adoptive immunotherapy might include removal of adherent cells and addition of indomethacin prior to culture with IL-2 in order to further maximize LAK cell activity.

MATERIALS AND METHODS

Mice. C57BL/6J (B6) mice were obtained from Jackson Laboratories (Bar Harbor, ME). Experiments were performed with male mice, 6 to 10 weeks of age.

Cell lines. P-815 and YAC-1 tumor cell lines (LAK and NK cell targets, respectively) were obtained from American Type Culture Collection (Rockville, MD) and were maintained in culture by *in vitro* passage with RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 60 μg/ml L-glutamine, and 5×10^{-3} *M* 2-mercaptoethanol (Grand Island Biologicals Co., Grand Island, NY). Cells in log-phase growth were used for NK and LAK cell assays.

Preparation of splenocyte suspensions. Whole splenocyte suspensions were prepared as previously described (13). Briefly, spleens of two to six mice were aseptically removed and disassociated in cold (4°C) RPMI 1640 medium. The resulting cell suspension was incubated at 0°C for 5 min to allow large debris to settle. The cells remaining in the supernatant were removed and washed once with RPMI 1640 medium. Viable cell counts were determined by trypan blue exclusion and cells adjusted to the appropriate concentration in RPMI 1640 medium.

Adherent cell-depleted splenocyte cultures were prepared as follows. Aliquots of 15–20 ml of whole splenocyte suspensions were added to 100 × 20-mm plastic tissue culture dishes (Falcon, Becton–Dickinson, Oxnard, CA) and incubated for 1 hr at 37°C. Plates were then washed vigorously with warm (37°C) RPMI 1640 medium, and the resulting nonadherent cells collected by decanting the warm wash medium to a tube.

IL-2. A highly purified recombinant human IL-2 preparation cloned in *Escherichia coli* (rIL-2) was generously provided by the Cetus Corp. (Emeryville, CA) (14, 15). Units of IL-2 activity were calculated from the total specific activity reported by the manufacturer. Activity was verified by comparing the activity of a standard preparation of human IL-2 (Biological Response Modifiers Program, Bethesda, MD) with that of the manufacturer's material, using the IL-2-dependent cell line HT-2 (16) in the standard microassay (17). One unit of activity is defined as the reciprocal of the dilution of IL-2 which results in one-half of the maximal proliferation of HT-2 cells.

Generation of LAK cells. LAK cells were generated by culturing whole or adherent cell-depleted splenocytes in 25-cm^2 tissue culture flasks (Corning Glassware, Corning,

NY) containing 5–10 ml of RPMI 1640 medium. The final concentration of cells was adjusted to 5×10^6 cells/ml. The rIL-2 preparation was added to these cells at various concentrations in initial studies to determine an optimal concentration (1000 U/ml) for generation of LAK cells with maximal activity. Culture flasks were incubated upright for 5–8 days at 37°C in 5% CO_2–95% air atmosphere. After incubation, LAK cells were removed from the flask and were used for *in vitro* cytotoxicity studies. Whole splenocyte LAK cell suspensions were obtained by removal of the LAK culture flask contents, followed by addition of cold (4°C) RPMI 1640 medium to the empty flask, and incubation for 2 hr at 4°C. Following this incubation, the cold medium containing the previously adherent cells was removed and combined with the initial contents of the flask. This low-temperature incubation step allowed recovery of cells which had adhered to the plastic surface of the tissue culture flask during LAK cell generation. This adherent population represented approximately 10% of the total number of cells recovered. Adherent cell-depleted LAK cells derived from rIL-2 cultures of whole splenocytes were obtained by collecting the contents of a LAK culture flask with warm, RPMI 1640 medium only, leaving behind the flask-adherent cells. Both cell populations were then utilized as effector cells in LAK and/or NK cell assays.

LAK and NK cell assays. LAK and NK cell assays were performed in similar fashions, using the murine P-815 mastocytoma cells as targets for LAK cells and YAC-1 cells as targets for NK cells, using the standard chromium release assay (18). Briefly, target cells $3–5 \times 10^6$ cells/ml were incubated with 100 μCi of ^{51}Cr (NEZ-030S, New England Nuclear, Boston, MA) for 2 hr in a 37°C water bath. Cells were washed three times in RPMI 1640 medium. A volume of 100 μl containing 5×10^4 target cells was added to wells of a microtiter plate. Splenocyte preparations were harvested, washed once in RPMI 1640 medium, and added to triplicate wells at various dilutions resulting in effector(LAK or NK):target (P-815 or YAC-1) ratios of 100:1, 50:1, and 25:1. The microtiter plates were centrifuged at 30*g* for 3 min and then incubated at 37°C for 4 hr. Following incubation, the plates were centrifuged at 400*g* for 5 min to pellet the cells. The amount of radioactivity released into 0.150 ml of the supernatant collected from each microtiter well was determined. Spontaneous release controls were obtained from supernatants of target cells incubated in medium alone. Maximum release controls were determined from cultures in which target cells were incubated with a 0.1% (v/v) solution of Nonidet-P40 detergent (Bethesda Research Laboratories, Rockville, MD). The percentage ^{51}Cr release (percentage cytotoxicity) was determined from the following formula:

$$\text{percentage cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100.$$

Prostaglandin and indomethacin. Prostaglandin E_2 (PGE_2) and indomethacin were purchased from Sigma Chemical Co. (St. Louis, MO). PGE_2 was dissolved in absolute ethanol to a concentration of 2×10^{-3} *M*, and stored at −20°C. This stock solution was later diluted in RPMI 1640 medium and added to cultures at concentrations of 10^{-7} to 10^{-5} *M*. Controls containing equivalent concentrations of ethanol were included for each dilution of PGE_2 tested. Indomethacin was dissolved in absolute ethanol at a concentration of 10.0 mg/ml and diluted to the appropriate concentration with RPMI 1640 medium.

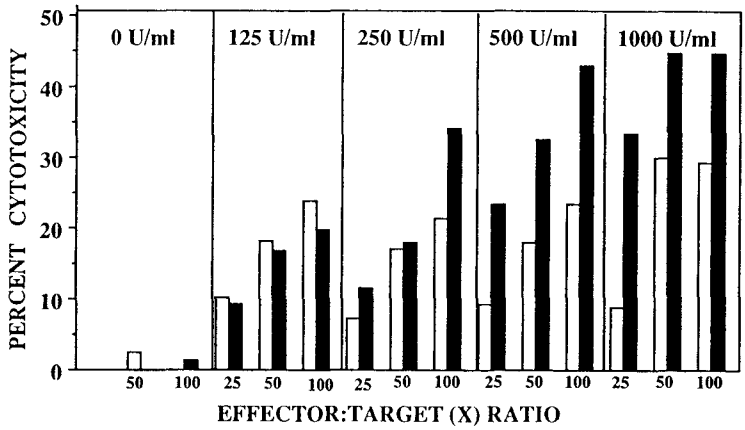

FIG. 1. Effect of adherent cell depletion on LAK cell activity. Whole splenocytes were cultured for 5 days with various concentrations of rIL-2. Some splenocyte cultures were depleted of adherent cells. Both populations were assayed for LAK activity. (□) Whole splenocytes; (■) adherent cell-depleted splenocytes.

In vivo studies with indomethacin. Groups of six mice each were provided with 28, 14, and 7 μg/ml indomethacin dissolved in 0.16% ethanol in drinking water for 14–21 days, *ad libitum.* Drinking water was changed twice weekly. Control animals received ethanol in their drinking water at a concentration equivalent to that present in the indomethacin preparations.

Statistics. Statistical differences in responses to various treatment protocols were determined using χ^2 analysis. In some cases, the mean LAK cell and NK cell cytotoxicity was calculated from triplicate well determinations and standard error of the mean determined.

RESULTS

Effect of adherent cell depletion on LAK cell activity. Whole splenocytes were cultured with various concentrations of rIL-2 for 5 days. Following incubation, whole splenocyte and adherent cell-depleted splenocyte suspensions were prepared from LAK cell culture flasks and assayed for LAK cell cytotoxicity. Figure 1 shows that increasing concentrations of rIL-2 (>125 U/ml) resulted in the generation of splenocyte preparations which exhibited increasing LAK cell activity, regardless of cell type tested. Whole splenocyte LAK cell cultures which were depleted of adherent cells prior to the assay consistently demonstrated greater LAK cell cytotoxicity at optimal rIL-2 concentrations (500 and 1000 U/ml) and at all effector:target cell ratios compared to that of whole splenocyte LAK cell cultures. A higher proportion of LAK cells in the adherent cell-depleted cultures could not fully account for this increased cytotoxicity in that even 25:1 E:T ratios expressed higher levels of cytotoxicity compared to 100:1 ratios of whole splenocyte preparations. These results indicate that removal of adherent cells from IL-2-activated cultures results in increased LAK cell activity.

Effect of adherent cell depletion prior to culture. Whole splenocytes and adherent cell-depleted splenocyte suspensions were prepared and were cultured with 1000 U/ml of rIL-2 for 5 and 8 days. Some whole splenocyte LAK cell cultures were depleted

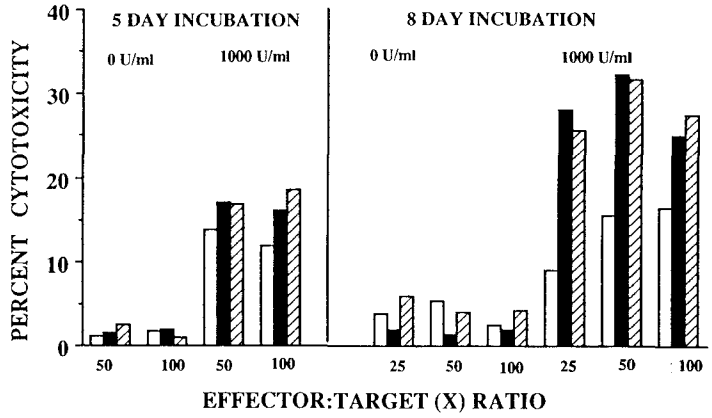

FIG. 2. Effect of adherent cell depletion prior to culture. Whole splenocytes and adherent cell-depleted splenocytes were cultured with 1000 U/ml of rIL-2 for 5 and 8 days. Some whole splenocyte cultures were depleted of adherent cells after the 5- or 8-day culture. LAK activity of (□) whole splenocytes; (■) adherent cell depleted prior to LAK cell assay, and (▨) adherent cell depleted prior to culture ($P < 0.005$ for depletion prior to or following LAK cell assay compared to whole splenocytes).

of adherent cells immediately prior to the LAK cell assay. Figure 2 shows that LAK cell cytotoxicity was greatest in all adherent cell-depleted cultures which had been cultured with rIL-2 compared to whole splenocyte preparations at all E:T ratios tested. The mean percentage enhancement of cytotoxicity was 40.5% for those depleted of adherent cells prior to culture with rIL-2 and 29.7% for suspensions depleted of adherent cells after culture with rIL-2 and immediately prior to LAK cell assay (5-day incubation). This enhancement of cytotoxicity was even greater for splenocytes cultured for 8 days (124% for cultures depleted of adherent cells prior to assay and 119% in those depleted prior to IL-2 culture). Adherent cell depletion, either before or after culture with rIL-2, resulted in a significant enhancement of cytotoxicity compared to nondepleted (whole) splenocytes ($P < 0.005$ for depletion prior to or following LAK cell generation compared to whole splenocytes). These results indicate that adherent cells serve to suppress LAK cell activity, since their physical removal either before IL-2 culture or immediately before LAK cell assay results in enhancements of LAK cell activity.

Effect of indomethacin on LAK cell activity of whole splenocytes. In order to determine if the suppression of LAK cell activity by adherent cells could possibly be mediated by prostaglandins, whole splenocytes were cultured with rIL-2 and indomethacin, an inhibitor of prostaglandin synthesis. Controls consisted of splenocytes cultured with rIL-2 and medium or ethanol. Splenocytes were collected after 5 days incubation and LAK cell cytotoxic activity was determined. Figure 3 shows that LAK cells generated in cultures of whole splenocytes which contained 5.0 and 10.0 μg/ml of indomethacin demonstrated an increased level of cytotoxic activity compared to ethanol or medium controls ($P < 0.05$). These results show that indomethacin potentiates LAK cell cytotoxicity when present during the culture of whole splenocytes with rIL-2.

Effect of indomethacin addition immediately prior to LAK cell assay. To determine if the potentiating effects of indomethacin on LAK cell activity could be immediately

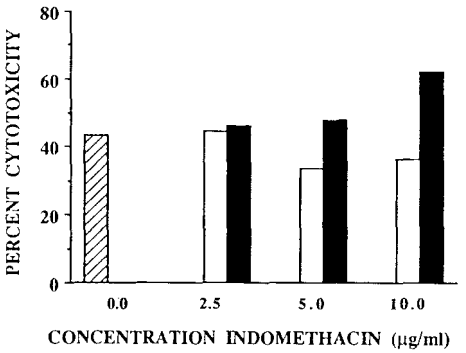

FIG. 3. LAK cell activity of whole splenocytes cultured for 5 days with 1000 U/ml of rIL-2 in the presence of indomethacin (■), ethanol (□), or medium (▨). E:T ratio = 100:1. Differences in LAK cell activities of indomethacin-treated splenocytes compared to ethanol or medium were significant ($P < 0.05$).

induced, whole splenocyte and adherent cell-depleted splenocytes were cultured with rIL-2 for 5 days without indomethacin. Some whole splenocyte suspensions were additionally depleted of adherent cells prior to LAK cell assay. Indomethacin was then added to effector/target combinations immediately prior to the assay. The results as illustrated by Fig. 4 again confirm that the removal of adherent cells, either prior to LAK cell culture or prior to LAK cell assay, results in increased LAK cell activity, compared to that exhibited by whole splenocyte suspensions. However, the relatively short-term incubation of these cell preparations with indomethacin did not significantly enhance LAK cell activity of any cell preparation tested ($P = 0.50$ for whole splenocytes, 0.70 for adherent cell depletion prior to assay, and 0.30 for adherent cell depletion prior to culture). These results suggest that the pharmacological action of indomethacin on adherent cells in whole splenocyte preparations requires an incubation time of greater than 5 hr in order for the potentiation of LAK cell activity to be observed.

Effect of PGE_2 on LAK cell activity of adherent cell-depleted and whole splenocytes. To directly determine if PGE_2 could suppress LAK cell cytotoxicity, adherent cell-

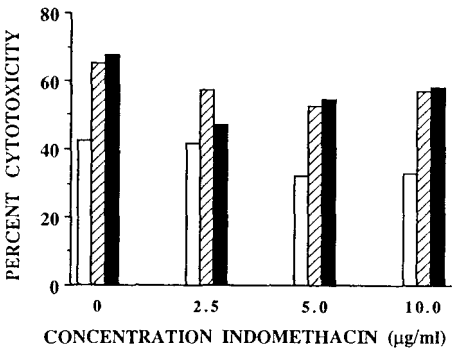

FIG. 4. Effect of indomethacin on LAK cell activity when added prior to assay. Whole splenocytes (□) and adherent cell-depleted splenocytes (■) were cultured with 1000 U/ml of rIL-2 for 5 days. Some whole splenocytes were depleted of adherent cells prior to LAK cell assay (▨). E:T ratio = 100:1. Differences in LAK cell activities between the two populations were not significant.

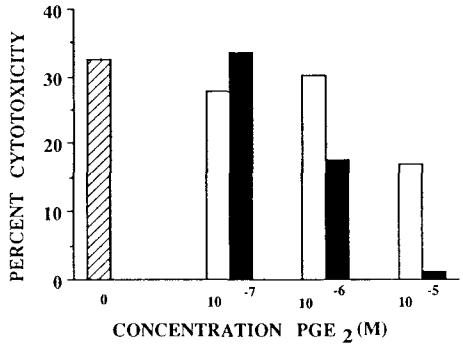

FIG. 5. LAK cell activity of adherent cell-depleted splenocytes cultured with 1000 U/ml of rIL-2 for 5 days in the presence of PGE_2 (■), ethanol (□), or medium (▨). E:T ratio = 100:1. Mean of two separate experiments, $P < 0.005$ for differences in LAK cell activity of PGE_2-cultured splenocytes compared to ethanol- or medium-cultured splenocytes.

depleted or whole splenocytes were cultured with rIL-2 and PGE_2 for 5 days, and LAK cell activity was determined. Figure 5 shows that higher concentrations of PGE_2 significantly inhibited LAK cell cytotoxicity of adherent cell-depleted splenocyte cultures. This inhibition was 28 and 88% at 10^{-6} and 10^{-5} *M* PGE_2, respectively, compared to the corresponding alcohol control (mean of two separate experiments, $P < 0.005$). PGE_2 suppressed LAK cell cytotoxicity of whole splenocytes only at the highest concentration tested (10^{-5} *M*) (Fig. 6). This suppression was probably due to cytotoxicity of the PGE_2 itself. These results indicate that PGE_2 directly suppresses LAK cell activities of adherent cell-depleted splenocyte populations.

Effect of in vivo treatment of mice with indomethacin on LAK cell and IL-2-induced NK cell activities. Mice were provided with 28, 14, and 7 μg/ml of indomethacin dissolved in ethanol–water for 14 days. All mice receiving 28 μg/ml of indomethacin died by Day 7 due to gastrointestinal toxicity of the indomethacin preparation (data not shown). Whole splenocyte suspensions were pooled from each treatment group and cultured with 1000 U/ml of rIL-2 for 5 days. LAK cell and IL-2-induced NK

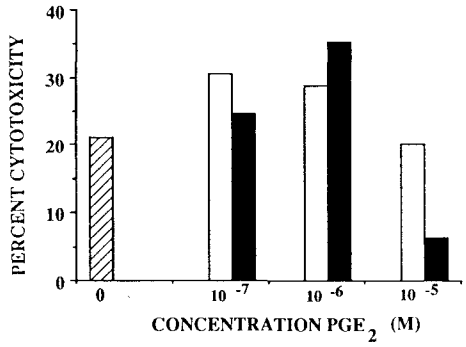

FIG. 6. LAK cell activity of whole splenocytes cultured with 1000 U/ml of rIL-2 in the presence of PGE_2 (■), ethanol (□), or medium (▨). E:T ratio = 100:1. Differences in LAK cell activity for PGE_2-cultured splenocytes and ethanol- or medium-cultured splenocytes were not significant.

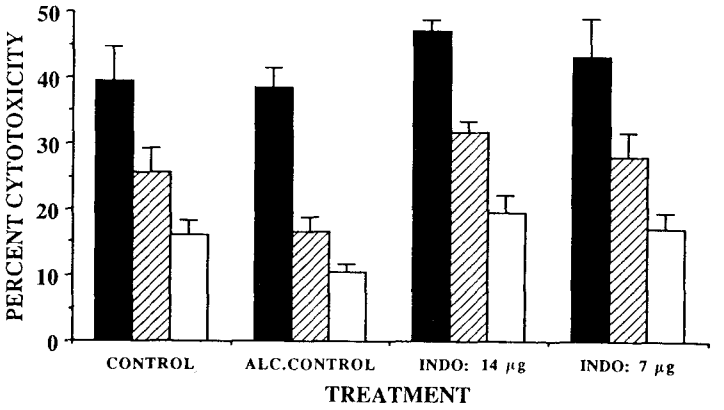

FIG. 7. LAK cell activity of whole splenocytes from indomethacin- and ethanol-treated mice cultured with 1000 U/ml of rIL-2 and assayed at E:T ratios of 100:1 (■), 50:1 (▨), and 25:1 (□). Pooled splenocyte cultures, six animals per group, $P < 0.001$ for differences in LAK cell activity of indomethacin-treated mice compared to ethanol–water- or water-treated mice. Mean LAK cell cytotoxicity, ± standard error of the mean.

cell cytotoxicity was determined. Both LAK cell cytotoxicity (Fig. 7) and NK cell cytotoxicity (Fig. 8) were significantly greater ($P < 0.001$) in mice treated with 14 and 7 μg/ml of indomethacin at all effector:target ratios tested than in controls treated with ethanol–water or water-only. These results indicate that *in vivo* treatment of mice with indomethacin results in an increased ability of whole splenocyte populations to generate LAK and NK cytotoxic effector cells *in vitro*.

Effect of in vivo and additional in vitro treatment of mice with indomethacin on LAK cell activity. Mice were provided with 14 μg/ml of indomethacin dissolved in ethanol–water for 21 days. Controls received an equivalent amount of ethanol–water

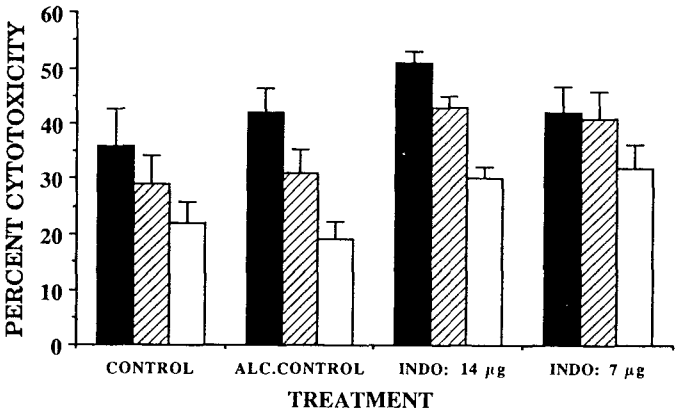

FIG. 8. NK cell activity of whole splenocytes from indomethacin-treated mice cultured with 1000 U/ml of rIL-2 for 5 days and assayed at E:T ratios of 100:1 (■), 50:1 (▨), and 25:1 (□). Pooled splenocyte cultures, six animals per group, $P < 0.001$ for differences in NK cell activity of indomethacin-treated mice compared to ethanol–water- or water-treated mice. Mean NK cell cytotoxicity, ± standard error of the mean.

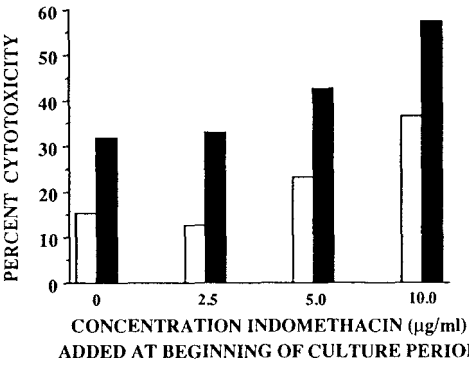

FIG. 9. LAK cell activity of whole splenocytes from indomethacin-treated mice (14 μg/ml) (■) or ethanol-treated mice (□) cultured with 1000 U/ml of rIL-2 for 5 days in the presence or absence of additional indomethacin. Pooled splenocyte cultures, six animals per group. E:T ratio = 100:1.

only. Whole splenocyte suspensions were pooled from each treatment group and cultured with 1000 U/ml of rIL-2 for 5 days. Indomethacin was added to some splenocyte cultures from both treatment groups. Figure 9 demonstrates that indomethacin treatment of mice increased their ability to generate LAK cell activity compared to that of alcohol-treated controls. However, the greatest amount of LAK cell activity was generated by indomethacin-treated mice whose splenocytes were additionally cultured with indomethacin. These results indicate that a combination of *in vivo* indomethacin treatment of mice followed by *in vitro* culture of their splenocytes with rIL-2 and indomethacin generates LAK cells with maximum cytotoxic activity toward P-815 tumor cell targets.

DISCUSSION

The present study demonstrates a significant enhancement of murine LAK cell cytotoxicity if adherent cells are depleted either before or after culture with rIL-2. LAK cell precursors have been reported to be nonadherent (19–21); however, routine depletion of adherent cells prior to murine LAK cell assay is usually not indicated (22–26). While methods for the isolation of highly active LAK cells based on IL-2-induced adherence (A-LAK cells) have recently been reported for mice (27), rats (28), and humans (29), our study emphasizes the importance of adherent cell depletion *prior to* the exposure of potential LAK cell precursors to rIL-2 culture as an additional procedure to maximize LAK cell cytotoxicity for *in vitro* studies or immunotherapy. Our findings are consistent with several reports of a similar suppressive action of adherent cells/monocytes on human LAK cell generation (30–32). The identity of the adherent cell which was responsible for LAK cell suppression when removed *prior to* IL-2 culture is probably the macrophage. This is based on our repeated observations of large, tightly adherent cells which withstand repeated washings with warm (37°C) but not cold (4°C) medium, and the typical morphological staining criteria (nonspecific esterase positive) for the splenic macrophage. Further studies, including the direct addition of graded numbers of purified macrophages to nonadherent splenocyte cultures prior to IL-2 culture, will have to be undertaken in order to confirm identity of this adherent cell.

Adherent cell-depleted cultures, which show maximum LAK cell cytotoxicity after rIL-2 culture, could possibly be enriched for nonadherent LAK effector cells compared to cultures of whole splenocytes. However, this higher proportion of LAK cells could not fully account for the enhancement of their activity reported here. Even at the lowest E:T ratio tested (25:1), adherent cell-depleted splenocytes demonstrated significantly greater cytotoxicity than that demonstrated by the highest E:T ratios (100:1) of whole splenocytes. Even when the number of whole splenocyte effector cells is increased four-fold (from 25:1 to 100:1 E:T ratios), adherent cell-depleted cultures continue to express levels of cytotoxicity substantially higher than those of whole splenocyte–target cell combinations. These results therefore indicate that the increases in LAK cell activity that we observed for adherent cell-depleted, rIL-2 cultures cannot be due solely to simple enrichment of numbers of nonadherent LAK effector cells.

The enhancement of LAK cell activity following culture with indomethacin, an inhibitor of prostaglandin synthesis, is indirect evidence that products of the cyclooxygenase pathway serve to modulate LAK cell activity. Darrow and Tomar (8) reported that indomethacin enhanced the proliferation and induction of cytotoxicity of cytotoxic T-lymphocytes generated from mixed lymphocyte cultures. Furthermore, they showed that indomethacin had no effect if added just prior to the chromium release assay. Our results parallel their observations in that indomethacin is effective only when present during the period of LAK cell culture and not if added just prior to the LAK cell assay.

Prostaglandins of the E series suppress cytotoxic activity of NK cells and CTLs *in vitro* at concentrations greater than 10^{-7} *M* (6, 8, 10, 33). Suppression of murine LAK cell cytotoxicity by PGE_2 has not been reported to date. Our study describes inhibition of LAK cell cytotoxicity when PGE_2 is added to adherent cell-depleted cultures at similar concentrations. The low cytotoxic response observed at the highest concentration of PGE_2 tested (10^{-5} *M*) may be due to the direct toxic effects of this compound on the LAK cell effectors themselves. Prostaglandin E_2 was less effective on the inhibition of LAK cell cytotoxicity of whole splenocytes. This may indicate that endogenous levels of prostaglandins necessitate the addition of much higher levels of exogenous PGE_2 to mediate the same degree of inhibition.

Parha and Lala (34) have recently described regression of experimental metastases after *in vivo* treatment of mice with indomethacin and rIL-2. This combination therapy also increased *in vitro* cytotoxic responses directed against various tumor targets. Our studies described herein show that *in vivo* treatment of mice with indomethacin alone was sufficient to increase subsequent *in vitro* cytotoxicity against LAK cell sensitive targets (P-815) and the NK and LAK sensitive YAC-1 target. Cytotoxic activity of LAK cells could be further increased by culturing splenocytes from indomethacin-treated animals with additional indomethacin. This represents a novel method for maximizing LAK cell cytotoxicity by a combination of both *in vivo* and *in vitro* treatment with indomethacin. Further studies utilizing our method of LAK cell potentiation and an appropriate immunotherapeutic tumor model would be a good test for the effectiveness of such treatment.

The standard clinical protocol for human adoptive immunotherapy with LAK cells includes the *in vitro* generation of autologous LAK cells and infusion of these cells along with relatively high doses of rIL-2 (26). This approach has been somewhat limited due to the toxicity associated with high-dose rIL-2, but not the LAK cells them-

selves (4, 26). Consequently, patients have been treated with indomethacin in order to reduce these side effects (35, 36). Our studies reported here for the mouse coupled with the *in vitro* studies of Ibayashi *et al.* (32) for the human indicate that the effects of indomethacin treatment on subsequent *in vitro* generation and/or activity of human LAK cells, or the potentiation of such LAK cell activity, should be further explored.

Higher doses of indomethacin have been associated with a variety of adverse side effects in humans, including gastrointestinal complications and bone marrow depression, resulting in anemia and leukopenia (37). In our study, indomethacin was toxic only at the highest dose tested (21 μg/ml in drinking water). Mice which were treated with the two lower doses were examined daily and were found to be healthy with no evidence of gastrointestinal complications, hair loss, or weight loss. Hematological depression was not observed in this study.

Our studies presented here identify important parameters concerning the optimization and regulation of LAK cell activity in mice. The data support the hypothesis that adherent cells inhibit LAK cell activity and that LAK cell activity can be potentiated by removal of these adherent cells and by treatment with indomethacin. In addition, we present evidence that PGE_2 may be responsible, in part, for the suppression of LAK cell activity. However, direct measurements of the production of PGE_2 by adherent cells were not done. Our studies have important implications for application of our procedures in human LAK cell immunotherapy protocols. However, further studies will have to be undertaken to determine if sufficient levels of indomethacin which would potentiate LAK cell cytotoxicity could be safely achieved in humans, before such treatment is contemplated.

Future studies from our laboratory will be aimed at further investigation of the kinetics of suppression of LAK cell activity by adherent cells. In addition, we will determine if *in vivo* treatment of mice with a combination of indomethacin and rIL-2 will serve to increase LAK cell cytotoxicity with a concurrent reduction in IL-2-associated toxicity.

REFERENCES

1. Rosenberg, S. A., *In* "Important Advances in Oncology" (V. T. Devita, S. Hellman, and S. A. Rosenberg, Eds.), Lippincott, Philadelphia, 1986.
2. Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., Seipp, C. A., Simpson, C., and Reichert, C. M., *N. Engl. J. Med.* **313,** 1485, 1985.
3. Lotze, M. T., Matory, Y. L., Ettinghausen, S. E., Rayner, A. A., Sharrow, S. O., Seipp, C. A., Custer, M. C., and Rosenberg, S. A., *J. Immunol.* **135,** 2865, 1985.
4. Lotze, M. T., Matory, Y. L., Rayner, A. A., Ettinghausen, S. E., Vetto, J. T., Seipp, C. A., and Rosenberg, S. A., *Cancer* **58,** 2764, 1986.
5. Pape, M. Z., Vetto, J. T., Ettinghausen, S. E., Mule, J. J., and Rosenberg, S. A., *Cancer Res.* **46,** 5618, 1986.
6. Brunda, M. J., Herberman, R. B., and Holden, H. T., *J. Immunol.* **124,** 2862, 1980.
7. Roder, J. C., and Klein, M., *J. Immunol.* **123,** 2785, 1979.
8. Darrow, T. L., and Tomar, R. H., *Cell. Immunol.* **56,** 172, 1980.
9. Leung, K. H., and Mihich, E., *Nature (London)* **288,** 597, 1980.
10. Plaut, M., *J. Immunol.* **123,** 692, 1979.
11. Strom, T. B., Carpenter, C. B., Garovoy, M. R., Austen, K. F., Merril, J. P., and Kaliner, M., *J. Exp. Med.* **138,** 381, 1973.
12. Wolf, M., and Droege, W., *Cell. Immunol.* **72,** 286, 1982.

13. Longley, R. E., and Good, R. A., *Cell. Immunol.* **101,** 476, 1986.
14. Wang, A., Lu, S. D., and Mark, D., *Science* **224,** 1431, 1984.
15. Rosenberg, S. A., Grim, E. A., McGrogan, M., Doyle, M., Kawasaki, E., Koths, K., and Mark, D. F., *Science* **223,** 1412, 1984.
16. Watson, J., *J. Exp. Med.* **150,** 1510, 1979.
17. Gillis, S., Ferm, M. M., Ou, W., and Smith, K. A., *J. Immunol.* **120,** 2027, 1978.
18. Merluzzi, V. J., Savage, D. M., Mertelsmann, R., and Welte, K., *Cell. Immunol.* **84,** 74, 1984.
19. Rosenstein, M., Yron, I., Kaufmann, Y., and Rosenberg, S. A., *Cancer Res.* **44,** 1946, 1984.
20. Grimm, E. A., Ramsey, K. M., Mazumder, A., Wilson, D. J., Djeu, J. V., and Rosenberg, S. A., *J. Exp. Med.* **157,** 884, 1983.
21. Owen-Shaub, L. B., Abraham, S. R., and Hemstreet, G. P., *Cell. Immunol.* **103,** 272, 1986.
22. Rayner, A. A., Grimm, E. A., Lotze, M. T., Wilson, D. J., and Rosenberg, S. A., *J. Natl. Cancer Inst.* **75,** 67, 1985.
23. Merluzzi, V. J., *Cell. Immunol.* **95,** 95, 1985.
24. Thompson, J. A., Peace, D. J., Klarnet, J. P., Kern, D. E., Greenberg, P. D., and Cheever, M. A., *J. Immunol.* **137,** 3675, 1986.
25. Jacobs, S. K., Wilson, D. J., Kornblith, P. L., and Grimm, E. A., *Cancer Res.* **46,** 2101, 1986.
26. Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., Seipp, C. A., Simpson, C. and Reichert, C. M., *N. Engl. J. Med.* **313,** 1485, 1985.
27. Gunji, Y., Vujanovic, N. L., Hiserodt, J. C., Herberman, R. B., and Gorelik, E., *J. Immunol.* **142,** 1748, 1989.
28. Vujanovic, N. L., Herberman, R. B., Maghazachi, A. A., and Hiserodt, J. C., *J. Exp. Med.* **167,** 15, 1988.
29. Melder, R. J., Whiteside, T. L., Vujanovic, N. L., Hiserodt, J. C., and Herberman, R. B., *Cancer Res.* **48,** 3461, 1988.
30. Hoyer, M., Meineke, T., Lewis, W., Zwilling, B., and Rinehart, J., *Cancer Res.* **46,** 2834, 1986.
31. Froelich, C. J., and Guiffaut, S., *J. Immunol. Methods* **86,** 205, 1986.
32. Ibayashi, Y., Hoon, D. S., and Golub, S. H., *Cell. Immunol.* **110,** 365, 1987.
33. Baker, P. E., Fahey, J. V., and Munck, A., *Cell. Immunol.* **61,** 52, 1981.
34. Parha, R. S., and Lala, P. K., *J. Exp. Med.* **165,** 14, 1987.
35. Rosenberg, S. A., Lotze, M. T., Muul, L. M., Chang, A. E., Avis, F. P., Leitman, S., Linehan, W. M., Robertson, C. N., Lee, R. E., Rubin, J. T., Seipp, C. A., Simpson, C. G., and White, D. E., *N. Engl. J. Med.* **316,** 889, 1988.
36. West, W. H., Tauer, K. W., Yanelli, J. R., Marshall, G. D., Orr, D. W., Thurman, G. B., and Oldham, R. K., *N. Engl. J. Med.* **316,** 898, 1988.
37. Barbara B. Huff (Ed.), "Physicians Desk Reference," Medical Economics Co. Oradell, NJ, 1986.