Differential Effects of Ether Lipids on the Activity and Secretion of Interleukin-1 and Interleukin-2

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Alkyl lysophospholipids (ALP) are synthetic analogues of lysophosphatidylcholine and represent a new generation of antitumor drugs currently being tested in phase-I trials in patients with cancer. The present study reports the differential modulation of human immunocompetent cells in vitro by ALP. Serum-bound ALP effectively blocked the response of growth factor–dependent cells to interleukin-2 (IL-2), inhibited the cellular production and release of IL-2 and suppressed the comitogenic effect of interleukin-1 (IL-1) on mouse thymocytes. In contrast, ALP-primed, monocyte-derived macrophages (MO) lost their ability to release IL-1 in response to stimuli like lipopolysaccharides (LPS) during terminal maturation from monocytes. Supernatants from ALP-primed, LPS-induced MO possessed costimulatory as well as direct mitogenic activity. Neither ALP alone nor ALP-conditioned MO supernatants stimulated mouse thymocytes. Priming of MO by ET-18-OH, an ALP molecule not substituted in the sn-2 position, occurred at concentrations 4- to 16-fold higher than the most active compounds ET-18-OCH₃ and the thioether analogue BM 41.440. ALP also primed MO for subsequent activation of tumor cytotoxicity by LPS and interferon-gamma.

IL-1 has multiple biological activities in common with ALP, and it may mediate antitumor activity and other ALP effects in vivo. The ability of ALP to induce differential immunomodulation, as demonstrated in this study, may make ALP worthy of study for the therapy of both autoimmune and neoplastic disease.

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**MATERIALS AND METHODS**

**Lysophospholipids.** Natural 2-LPC was purchased from Sigma Chemical Co. (St. Louis, MO) and rac-1-octadecyl-2-methoxy-sn-glycero-3-phosphocholine (ET-18-OCH₃) from Medmark Chemicals (Grünwald b. München, FRG). Rac-1-octadecyl-sn-glycero-3-phosphocholine (ET-18-OH) was provided by H. U. Weltzien (Max Planck Institut, Freiburg, FRG); rac-1-mercapto-hexadecyl-2-methoxy-methyl-sn-glycero-3-phosphocholine (BM 41.440) was a gift from W. Pahlke (C. F. Boehringer Mannheim GmbH, Mannheim, FRG).

**Cells.** Peripheral blood leukocytes were separated fromuffy coat preparations of healthy blood donors by density gradient centrifugation on Ficoll-Hypaque. Monocytes were isolated from other mononuclear cells (MNC) by adherence to plastic petri dishes (60 min at 5 X 10⁶ MNC per ml RPMI1640 supplemented with 5 X 10⁻⁴ M 2-mercaptoethanol, antibiotics and 15% fetal calf serum [FCS]), cultured overnight in RPMI1640 plus 10% human AB-group serum and recovered by vigorous pipetting at 4 C. Monocytes were greater than 90% pure as estimated by cytochemistry and antigen analysis (11). They were cultured in suspension at 3 X 10⁶/ml RPMI1640 plus 10% AB-serum on hydrophobic Teflon foils for various time periods (12) and subjected to further experimentation at their sequential stages of differentiation. MO phenotype was determined to judge the stage of maturation (11), which was completed after 10 days in culture, after which the cells were termed mature monocyte-derived MO (TMO).

**MLA144 cells** (a T-cell line of gibbon origin, ref. 13) were provided by H. Rabin (National Cancer Institute, Frederick, MD), maintained in RPMI1640 plus 10% FCS and subcultured every 3-4 days.

**Activation of monocyte MO and production of supernatants.** Monocyte MO were cultured at 5 X 10⁶ per 0.2 ml RPMI1640 plus 30% FCS for 24 hr with and without ALP. The medium was then replaced with fresh RPMI1640 plus 30% FCS, the cells were incubated for 30 min, and 10 μg/ml lipopolysaccharides (LPS, Salmonella abortus equi M6; donated by Dr. Galanos, Max Planck Institut, Freiburg, FRG) was added in RPMI1640 plus 10% FCS. Supernatants were collected after 24 hr, centrifuged at 200 g for 10 min and stored at 4 C.

**Thymocyte proliferation assay (IL-1).** Thymocytes prepared from 3- to 4-week-old C57/HF/J mice (Bonholtgard, 868 Ry, Denmark) were cultured in triplicate at 5 X 10⁶ cells/ml RPMI1640 plus 10% FCS with and without 1:200 diluted phytohemagglutinin (PHA) (Gibco, Grand Island, NY) in a final volume of 0.2 ml in flat-bottomed 96-well microplates containing 1:4 dilutions of test
supernatants. The cultures were incubated for three days
before they were pulsed for 6 hr with 0.2 μCi ³H-thymidine
(sp act 28 Ci/mmol; Amersham and Buchler, Braunschweig, FRG) and subsequently harvested. IL-1 activity
is expressed as counts/min (cpm). In some experiments,
thymocytes were cultured with ALP alone or with and without ALP in the presence of recombinant human interleukin-1-alpha (Hoffman-LaRoche, Nutley, NJ) at a 1:7,500 dilution.

**Bioassay for interleukin-2.** IL-2-dependent mouse cells
(HT-2, gift from Dr. Larrick, Cetus Co., Emeryville, FL)
were passaged three times a week in an IL-2-containing
medium conditioned for 48 hr by human blood MNC
stimulated with 1:50 diluted PHA, 2 μg/ml concanavalin
A and irradiated allogeneic lymphoblastoid cells in a ratio
of 1:1. HT-2 cells were taken for the IL-2 assay after two
days of last passaging, washed twice in serum-free
medium for 30 min each time and subsequently
cultured with the 1:2 diluted test supernatants for 24 hr before the 6-hr ³H-thymidine incorporation
was measured.

**Tumor-cytostasis assay** (14). Monocyte-derived TMO
were seeded at 5 × 10⁵/0.2 ml RPMI1640 plus 30% FCS
and cultured for 24 hr with or without 16 μg/ml
ET-18-OCH₃, MO were then incubated twice with fresh
complete medium for 30 min each time and subsequently
cultured with serum-free RPMI1640 (containing 0.1 mg/ml lactalbuminhydrolysate, 0.2 mg/ml fetuin and 0.15 U/ml swine insulin) in the presence of LPS or recombinant human interferon-gamma (rIFNg, Biogen, Geneva, Switzerland) for 24 hr. MO monolayers were then rinsed twice
with warm serum-free medium before 10⁶ U937 cells (K.
Niellsson, Uppsala, Sweden) were added. After coincubation
of MO and targets for another 48 hr, the cultures were pipetted to bring target cells into suspension, 0.1 ml aliquots were transferred to a new microplate and ³H-thymidine uptake was measured with a 6-hr pulse. Activation indices were calculated by dividing cpm values of target cells after coincubation with control MO vs cpm values of target cells after coculture with activated MO.

**RESULTS**

Based on our earlier findings that ALP interfere with normal lymphocyte transformation and inhibit growth of activated lymphoblasts (10), we started an investigation into the immunomodulatory effects of ALP in vitro by looking at the response of IL-2-dependent cells in the presence of ALP. As shown in Figure 1A, ET-18-OCH₃ inhibited IL-2 response of HT-2 cells at concentrations above 2 μg/ml, whereas lower doses had a minor, reproducible stimulatory effect. The production of IL-2 was also inhibited within a similar dose range of ET-18-OCH₃ (Fig. 1B). Both effects were seen only with those analogues of 2-LPC that are modified in
sn-1 and sn-2, whereas the ether analogue ET-18-OH had no effect. In the presence of ALP, the response of normal lymphoblasts to IL-2-containing supernatants is suppressed similarly, as is the release of IL-2 (data not shown).

Peripheral blood monocytes respond to activation by
LPS with the release of the lymphocyte-activating factor termed IL-1 (15). We measured IL-1 in MO superna-
tant by its ability to act on C3H/HeJ mouse thymocytes
either directly or in the presence of the mitogen PHA. If
monocytes were first incubated for 24 hr with

**FIG. 1. Inhibition of (A) response to and (B) activity of interleukin-2 (IL-2) by alkyl lysophospholipids (ALP). HT-2 cells were cultured with IL-2 standard preparations in the presence of ET-18-OCH₃. After 24 hr, the 6-hr thymidine uptake was measured; results are expressed as percentage of control cultures without ET-18-OCH₃.**

**FIG. 2. Inhibition of the comitogenic (interleukin-1-like) and generation of a mitogenic activity in lipopolysaccharides stimulated blood monocytes after preculture with alkyl lysophospholipids (ALP). Blood monocytes were separated by adherence, cultured overnight and incubated with ALP for 24 hr. Then the medium was changed, cells were rinsed twice and 10 μg/ml LPS was added for another 24 hr before supernatants were collected and tested for their ability to be directly stimulatory to mouse thymocytes or indirectly in the presence of PHA. Results are given as counts/min, mean of triplicate values.**