Contribution of Lysophosphatidylcholine-Treated Nonadherent Cells to Mechanism of Macrophage Activation (43011)

BENJAMIN Z. NGWENYA AND NOBUTO YAMAMOTO
Department of Microbiology and Immunology, Hahnemann University School of Medicine, Philadelphia, Pennsylvania 19102-1192

Abstract. **Lysophosphatidylcholine (lyso-PC), a product of inflammation, stimulates (*in vivo*) mouse peritoneal macrophages to ingest target cells via Fc receptors. *In vitro* treatment of macrophages with lyso-Pc was unable to enhance ingestion activity. When a mixture of macrophages and nonadherent (B and T) cells was treated with 20 μg of lyso-Pc/ml for 30 min, a greatly enhanced Fc-mediated ingestion was observed at about 3 hr after treatment, suggesting that nonadherent cells contributed to activation mechanism of macrophages. The accumulated evidence suggests that treated B cells collaborated with untreated T cells in a stepwise fashion for the exchange of a signaling factor(s) for macrophage activation. When conditioned medium prepared by stepwise cultivation from treated B cells to untreated T cells was used for cultivation of untreated macrophages, a markedly enhanced Fc-mediated ingestion was observed. However, cultivation of macrophages with stepwise conditioned medium of treated T cells and untreated B cells produced no significant enhancement of phagocytic activity. Therefore, we concluded that lyso-Pc-treated B cells initiated the macrophage activation process by releasing and transmitting a signaling factor to T cells, and, in turn, the T cells modified the factor or supplied a new factor capable of the ultimate activation of macrophages for ingestion capacity. This lyso-Pc-induced factor(s) appears to be distinct from the established interleukins 1 and 2.** [P.S.E.B.M. 1990, Vol 193]

Microbial infection induces inflammation regardless of microorganisms incapability of production of endotoxin (lipopolysaccharide). The inflamed lesion releases cell membrane degradation products and appears to attract monocytes and macrophages which have the ability to ingest invading agents and dead cells. We previously reported that administration of various lysophospholipids, degradation products of membraneous phospholipids, to mice activate macrophages for Fc-mediated ingestion activity (1–3). *In vitro* treatment of macrophages alone with lysophosphatidylcholine (lyso-Pc) was unable to enhance ingestion activity (1). Thus, involvement of nonadherent cells in the activation of macrophages was suspected. In fact, when a mixture of macrophages and nonadherent cells (B and T) was treated with 20 μg of lyso-Pc/ml, a greatly enhanced Fc-mediated ingestion was observed at 3 to 8 hr after treatment (2). Such an *in vitro* treatment led to 85% of macrophages to ingest target cells via Fc receptors.

Since we found that contribution of nonadherent cells is required for activation of macrophages, we hypothesized that nonadherent cells may release a factor(s) for the ultimate activation of Fc-mediated ingestion activity of macrophages. This possibility and developmental processes for Fc-mediated ingestion activities of macrophages can be studied by reconstitution analysis with lyso-Pc-treated nonadherent cells and adherent macrophages.

In this communication, we report the contributory mechanism of lyso-PC-treated nonadherent cells to activation of macrophages for ingestion activity and analysis of signaling factor(s) by stepwise transfer of culture media among nonadherent and adherent cells.

Materials and Methods

Animals. Inbred BALB/c mice, 6–10 weeks of age, were purchased from The Jackson Laboratory, Bar Harbor, ME. The mice were housed in accredited (American Association for Accreditation of Laboratory Animal Care) animal quarters provided with tempera-

Received April 11, 1989. [P.S.E.B.M. 1990, Vol 193]
Accepted October 13, 1989.

0037-9727/90/1932-0118$2.00/0

ture and light controls. Mice were fed Purina Mouse Chow and water *ad libitum*.

Chemicals and Reagents. L-α-lysophosphatidylcholine (lyso-Pc) was purchased from Sigma Chemical Co., St. Louis, MO. The purity of lyso-Pc lot 50F-870 was greater than or equal to 99% pure, 0.5% precursor of lyso-Pc and no other compounds such as lipopolysaccharide were detectable. In addition to this information, we have tested the final preparation of lyso-Pc solution using the Limulus amebocyte lysate assay. No endotoxin was detectable in lyso-Pc samples and media (2). The lyso-Pc was dissolved in pyrogen-free saline (500 μg/ml), sterilized by Millipore filters, and stored at −20°C until use. After being thawed, appropriate dilutions of lyso-Pc were made in RPMI 1640 medium just before use. Cyclosporine, which is known to inhibit synthesis of interleukins 1 and 2, was purchased from Sandoz Ltd., East Hanover, NJ. The drug was dissolved in 95% ethanol at a concentration of 20 mg/ml. This stock preparation was used to prepare the desired concentrations of the drug in culture medium. At the highest concentration of drug tested (2 μg/ml), the amount of ethanol present in the sample was less than 0.03%. Ethanol alone at this low concentration was not inhibitory to macrophage activity.

Fluorescein-labeled monoclonal antibody specific for the Thy 1.2 antigen and unlabeled anti-Thy 1.2 antiserum (New England Nuclear, Boston, MA) were a gift from Dr. W. Weidanz, Hahnemann University. Polyvalent mouse IgG and IgM antibodies against sheep erythrocytes were purchased from Cordis Laboratories, Miami, FL. Monoclonal mouse IgG_1 and IgG_{2b} antibodies against sheep erythrocytes were purchased from Pel-Freez Biologicals, Rogers, AK.

Antihuman interleukin 2 (IL-2) was purchased from Collaborative Research Incorporated, Bedford, MA. The activity of this anti-IL-2 was verified in a standard neutralization assay. The concentration of 45 ng of antihuman IL-2 fully neutralized the stimulatory effect of one half maximal unit of human IL-2 on [^{3}H] thymidine incorporated by IL-2-dependent mouse T lymphocytes.

Macrophage Collection and Cultivation. The peritoneal cells from untreated mice were prepared according to the procedure described by Cohn and Benson (4) and Griffin and Silverstein (5). Briefly, cells were harvested by injecting 4–5 ml of cold (4°C) phosphate-buffered saline (PBS) free of Ca^{2+} and Mg^{2+} supplemented with 5–10 units of heparin/ml. The cells were then washed three times in cold PBS without heparin and resuspended at 5% in RPMI 1640 medium with 10% heat-decomplemented (56°C, 30 min) γ-globulin-free fetal calf serum (FCS; GIBCO Laboratories, Grand Island, NY). The desired cell number ($1–2 \times 10^6$/ml) was determined using a Thoma pipette and improved Neubauer hemacytometer (6). One-milliliter aliquots of the cells were layered onto 12-mm glass coverslips (Bellco, Vineland, NJ) that had been placed in the 16-mm diameter wells of tissue culture plates (Costar, Cambridge, MA). The plates were incubated at 37°C in a humidified 5% CO_2 incubator for 30 min to allow macrophage adherence. Coverslips were removed, immersed with gentle agitation in warm RPMI 1640 medium (37°C) to dislodge nonadherent cells, and placed in fresh tissue culture wells.

Enumeration of Adherent Macrophages. The percentage of macrophages in peritoneal adherent cells from BALB/c mice was determined and identified as macrophages by phagocytic and morphologic criteria (4). These criteria were assessed by latex particle (0.81 μm) ingestion and Giemsa staining, respectively. About 96% of the adhering cells were macrophages according to the above criteria.

Splenic Lymphocyte Collection. Spleen cells from BALB/c mice were collected and processed as described by Tyan and Ness (7). Briefly, using the plunger of a sterile 5-ml plastic syringe, spleens were gently pressed through a sterile Nickel-Chromium (Nichrome) stainless steel mesh screen cup (A. H. Thomas Co., Philadelphia, PA) into a sterile petri dish containing 15 ml of ice-cold PBS. A sterile plastic syringe without the needle was used to mix the medium with the cells in a tilted petri dish. The cells were washed twice, treated with tris(hydroxymethyl)aminomethane-buffered ammonium chloride to lyse erythrocytes, and then washed three times in PBS. The splenic macrophages were removed by adherence to plastic petri dishes.

Fractionation of Splenic Cells. T lymphocytes were obtained by passing the cells over nylon-wool columns as described by Julius *et al.* (8). Briefly, plastic syringes (20 ml) were packed with 1.0 g of nylon-wool. Each column was autoclaved and washed with 30 ml of PBS and followed by 30 ml of PBS with 5% FCS. The wet columns were then incubated for 1 hr at 37°C. Prior to loading the cells, each column was flashed with 30 ml of warm PBS-FCS. Two ml of PBS-FCS containing 1.5×10^8 cells were loaded in the column and incubated for 45 min in a humidified 5% CO_2 environment. About 85% of the effluent cells (nylon-wool-nonadherent cells) were T lymphocytes as determined by immunofluorescence using a fluorescein-labeled monoclonal antibody specific for the Thy 1.2 alloantigen. The binding capacity of B cells to nylon-wool was used as a method to obtain enriched B cell population as described by Trizio and Cudkowicz (9). The B cell population was further depleted of contaminating T cells by treatment with anti-Thy 1.2 antiserum and guinea pig complement (GIBCO). The enriched B cell population lacked T cells as determined by immunofluorescence.

Treatment of Peritoneal and Splenic Lymphoid Cells. *Treatment of adherent cells or mixture of adherent and nonadherent cells.* Peritoneal cells ($1–2 \times 10^6$ cells/ml) were layered onto coverslips and incubated for 30 min to affect adherence. After incubation, 20 μg of lyso-Pc/ml were added to the peritoneal cell culture

or to the adherent cells in RPMI 1640 medium supplemented with 10% FCS (FCS medium) and incubated for 30 min. Nonadherent cells and residual lyso-Pc were removed. The adherent cells were incubated in a fresh FCS medium for 3 hr and then assayed for ingestion activity.

Treatment of splenic lymphoid cells. Nonadherent cells were treated with 20 μg of lyso-Pc/ml of FCS medium for 30 min. After being washed, the treated nonadherent cells were admixed with untreated macrophages for time course analysis of development of macrophage ingestion activity. For the preparation of conditioned medium, the treated B or T cells were resuspended in FCS medium and incubated for 3 hr. The resultant supernatant (conditioned medium) was used as a conditioned medium (0.5 ml/well) and then added to untreated macrophages and incubated for 2 hr prior to ingestion assay.

Ingestion Assay. The ingestion of sheep erythrocytes (E) coated with immunoglobulin G, EIgG, and with immunoglobulin M, EIgM, in the presence or absence of complement was determined according to Bianco *et al.* (10). Briefly, washed erythrocytes (Dutchland, Denver, PA) were coated with subagglutinating dilutions of purified rabbit anti-E, IgG, or IgM fractions (Cordis Laboratories, Miami, FL). After being washed, a portion of the EIgM conjugate was mixed with an equal volume of C5-deficient complement containing DBA/2 mouse serum (1/20 dilution) and incubated for 15 min at 37°C to obtain EIgMC complexes. For Fc or C3b receptor-mediated ingestion assay, a 0.5 ml of 0.5% suspension of EIgG or EIgMC in RPMI 1640 medium without FCS was overlaid on each macrophage-coated (monolayer) coverslip and cultured at 37°C in a humidified 5% CO_2 incubator for 1 hr. Noninternalized erythrocytes were lysed by immersing the coverslips in a hypotonic solution (1/5 PBS) for 5 to 10 sec. The macrophages were fixed with methanol, air dried, and stained with Giemsa stain, and ingestion was quantified microscopically. The data are expressed as the ingestion index according to Bianco *et al.* (10).

Results

Combined Action of Lysophosphatidylcholine-Treated Adherent and Nonadherent Cells for Enhanced Ingestion Activity of Macrophages. Treatment of adherent cells (macrophages) alone with 20 μg of lyso-Pc/ml in FCS medium for 3 hr (even up to 24 hr) did not significantly enhance ingestion activity of macrophages (Table I). Since treatment of mice with 20 μg of lyso-Pc/mouse induced greatly enhanced ingestion activity of peritoneal macrophages (1–3), contribution of nonadherent cells to the activation of macrophages was considered. Lyso-Pc treatment of peritoneal cells (mixture of adherent and nonadherent cells) showed a greatly enhanced ingestion of IgG-coated target cells but only a low level ingestion of IgM-coated target cells with complement (Table I). Therefore, contribution of nonadherent cells is essential for the activation process of macrophages for Fc-mediated ingestion.

Detailed Analysis of Receptor Specificity with IgG Subspecies for Ingestion Activity of Lysophosphatidylcholine-Activated Macrophages. Since lyso-Pc-activated macrophages preferentially ingest IgG-coated target cells via Fc receptors, target cells coated with specific subclasses of IgG were analyzed for ingestion. As illustrated in Figure 1, macrophages of lyso-Pc-treated peritoneal cells efficiently ingested IgG_1-coated target cells whereas no significant ingestion of IgG_{2b}-coated target cells was observed. Target cells coated with polyvalent IgG are more efficiently ingested than IgG_1-coated target cells. However, ingestion activities with polyvalent IgG-coated or monovalent IgG_1-coated target cells may not be comparable due to unknown relative concentration of IgG_1 in the polyvalent IgG and monovalent IgG_1 samples. The possibility that contribution of other subclasses of IgG being utilized by lyso-Pc-activated macrophages cannot be ruled out.

Temporal Development of Ingestion Activity of Macrophages after Mixing Lyso-Pc-Treated or Untreated Adherent and Nonadherent Cells. Since cultivation of lyso-Pc-treated mixed culture of nonadherent and adherent cells is required for the development of the ultimate ingestion activity of macrophages, a signal transmission from the lyso-Pc-treated nonadherent cells to adherent cells (macrophages) must have occurred. Accordingly, time course analysis was performed with lyso-Pc-treated nonadherent cells mixed with untreated adherent cells. These mixed cells were then cultured for indicated periods and assayed for macrophage ingestion of IgG-coated target cells. As shown in Figure 2, ingestion activity of macrophages increased as early as 3 hr after treatment with the maximal ingestion activity at 5 hr. This observation supports the hypothesis that lyso-Pc-treated nonadherent cells release a signal(s) for activation of macrophages. Surprisingly, however, untreated nonadherent cells added to lyso-Pc-treated adherent cells induced a greatly enhanced ingestion activity at 5 hr after treatment (Fig. 2). It should be noted here that an adherent peritoneal cell population is likely to contain a small number of B cells. Therefore, the B cells present in a treated adherent cell population can provide a signal which interacts with untreated nonadherent cells (probably T cells) and in turn nonadherent cells provide the second signal to activate macrophages. To circumvent the contribution of the contaminating nonadherent cell population to macrophage activation mechanism, we further purified the T or B cell population using anti-Thy 1.2 antibody with complement and prepared conditioned medium of lyso-Pc-treated or untreated B and T cells.

Effect of Conditioned Medium of Lyso-Pc-Treated Nonadherent Cells on Adherent Cells for Development of Macrophage Ingestion Activity.

Table I. Lyso-Pc Treatment of Adherent Cells[a] with or without Nonadherent Cells for Stimulation for Ingestion Capacity of Macrophages[b]

Dose of lyso-Pc (μg/ml)	Ingestion index[c] of immunoglobulin-coated target cells					
	Adherent cells only			Mixed adherent and nonadherent cells		
	Uncoated E	EA(IgG)	EA(IgM)C	Uncoated E	EA(IgG)	EA(IgM)C
0	19 ± 6	36 ± 15	50 ± 10	21 ± 9	43 ± 17	40 ± 15
20	28 ± 9	60 ± 21	45 ± 10	50 ± 18	323 ± 80	152 ± 30

[a] Mixed adherent and nonadherent cells were treated with lyso-Pc for 3 hr whereas adherent cells only were treated up to 24 hr.
[b] Sheep erythrocytes (E) coated with subagglutinating levels of IgM with complement (C) were used as targets for ingestion. E or antibody (A)-coated E, EA(IgG), or EA(IgM)C were incubated with macrophages for 1 hr to allow ingestion activity in medium without serum.
[c] The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip. The data shown are representative of two separate experiments.

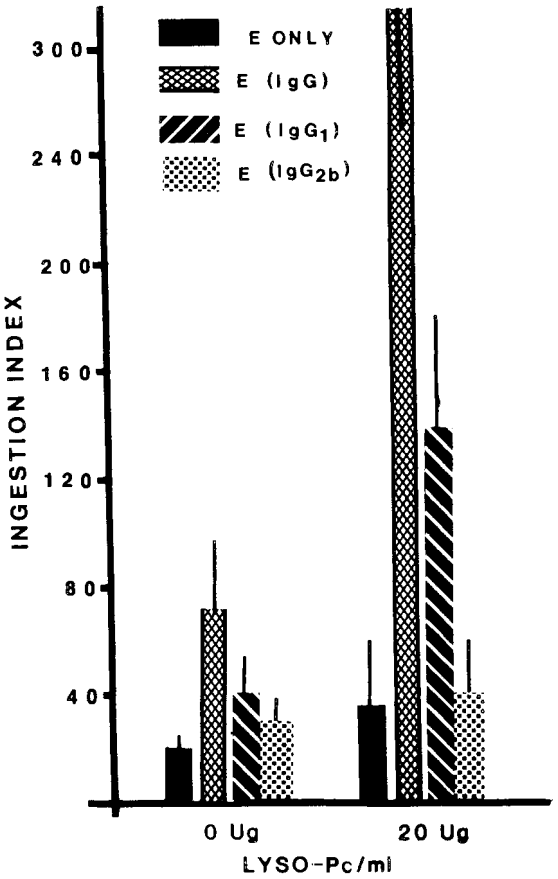

Figure 1. Analysis of receptor specificity with IgG subspecies for ingestion activity of lyso-Pc-stimulated macrophages. Resident peritoneal cells were treated with 20 μg of lyso-Pc/ml for 30 min. After being washed, the adherent cells were incubated for 2 hr before the ingestion assay. Sheep erythrocytes (E) coated with subagglutinating IgG subspecies were used as targets. The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip.

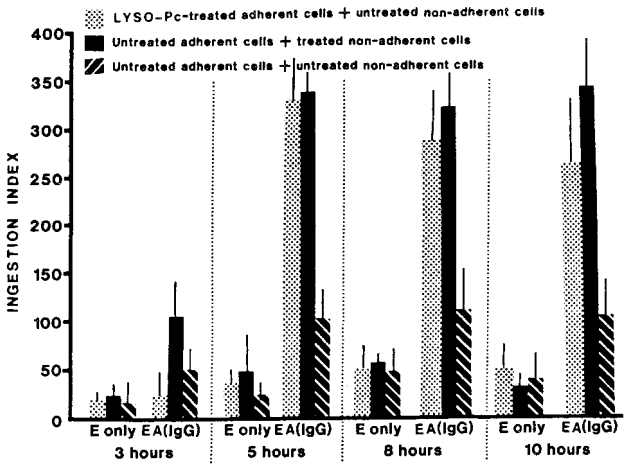

Figure 2. Kinetics of *in vitro* stimulation of mouse peritoneal macrophages for ingestion activity after treatment with lyso-Pc. Resident peritoneal adherent or nonadherent cells were treated separately with 20 μg of lyso-Pc/ml for 30 min in RPMI 1640 medium with 10% FCS. After being washed, the lyso-Pc-treated macrophages were admixed with untreated nonadherent cells or vice-versa. These mixtures were then incubated in RPMI 1640 medium with FCS for up to 10 hr. Sheep erythrocytes coated with subagglutinating IgG were used as targets. The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip. The data shown are representative of three separate experiments.

Since development of enhanced macrophage ingestion required co-cultivation of nonadherent and adherent cells, conditioned medium (culture medium) of the lyso-Pc-treated nonadherent cells was tested for macrophage activation by admixing it with adherent cells. Nonadherent cells were treated with 20 μg of lyso-Pc/ml in FCS medium for 30 min, washed in PBS, and cultured in FCS medium for 3 hr. When the conditioned medium of lyso-Pc-treated nonadherent cells was admixed with adherent cells and cultured for 2 hr, a greatly enhanced ingestion activity of macrophages was observed (Fig. 3). However, conditioned medium from untreated nonadherent cells admixed with adherent cells showed an enhanced ingestion activity of macrophages (Fig. 3). Therefore, we unequivocally confirmed that lyso-Pc-treated nonadherent cells release a signal(s) for activation of macrophage ingestion activity.

Stepwise Transfer of Signaling Factor(s) for Activation of Macrophages. By the use of conditioned media of lyso-Pc-treated nonadherent cells, we confirmed our previous hypothesis that signaling factor(s) for activation of macrophages may be derived from lyso-Pc-treated nonadherent cells (B or T cells) (1, 2). When individually lyso-Pc-treated B or T cells were

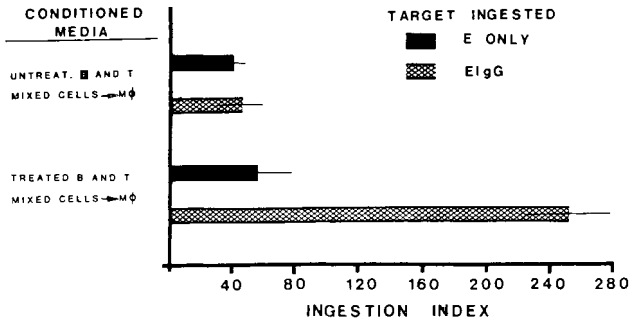

Figure 3. The effect of conditioned medium of lysophosphatidylcholine-treated nonadherent (mixture of B and T cells) cells on macrophage stimulation for ingestion activity. B and T cell mixtures were treated with 20 μg of lyso-Pc/ml for 30 min, washed in PBS, and then incubated for 3 hr. The condition medium (→) was added to untreated macrophages and incubated for 2 hr before the ingestion assay. Sheep erythrocytes coated with a subagglutinating level of IgG were used as targets. The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip. The data shown are representative of three separate experiments.

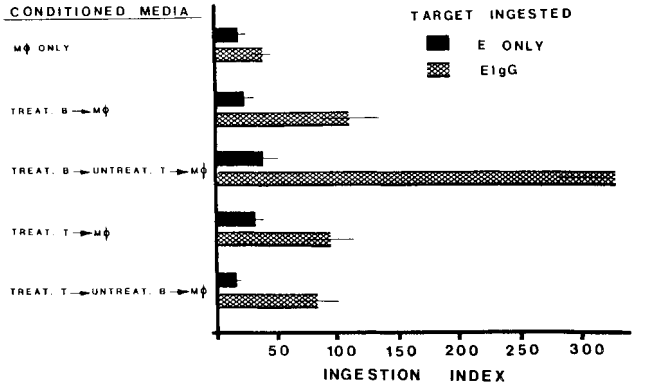

Figure 4. Analysis of signaling factor exchange by transferring conditioned media among nonadherent cells for activation of macrophages for ingestion activity. The B or T cell population was treated with 20 μg of lysophosphatidylcholine/ml for 30 min, washed, and then incubated in fresh medium for 3 hr. The resultant conditioned medium (Treat. B or Treat. T medium) was admixed with an untreated T or B cell population and incubated for an additional 2 hr. The resultant conditioned medium (Treat., B → Untreat. T or Treat. T → untreat. B) was admixed with untreated macrophages and incubated for 2 hr before the ingestion assay. Sheep erythrocytes coated with a subagglutinating level of IgG were used as targets. The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip. The data shown are representative of three separate experiments.

cultured in FCS medium for 2 hr, the resultant conditioned medium of either cell type was unable to activate macrophages by 3-hr cultivation with adherent cells (data not shown). Thus, induction of ultimately active signaling factor(s) seems to require a combined action of B and T lymphocytes. Therefore, the sequential stimulation steps of signaling factor(s) among nonadherent cells should be tested to determine the following: (i) a factor is released from T cells and modified on B cells, (ii) a factor is released from B cells and modified on T cells, or (iii) a factor is released from T and B cells separately. Accordingly, lyso-Pc-treated T cells were incubated in FCS medium for 3 hr. The resultant culture medium (conditioned medium) was then added to untreated B cells and cultured for an additional 2 hr. When the treated T/untreated B cells conditioned medium was added to untreated adherent cells and incubated for 2 hr, no significant enhancement of macrophage ingestion activity was observed (Fig. 4). Similarly, lyso-Pc-treated B cells were cultured in FCS medium for 3 hr. The B cell-conditioned medium was added to untreated T cells and incubated for an additional 2 hr. When the treated B/untreated T cell-conditioned medium was added to adherent cells for 2-hr cultivation, a greatly enhanced ingestion activity of macrophages was observed (Fig. 4). These observations suggested that the signaling factor(s) is originating from lyso-Pc-treated B cells and modified on T cells or a new signaling factor(s) from T cells was induced for macrophage activation.

Effects of Cyclosporine and Anti-Interleukin 2 on Macrophage Activation by the Factor(s) Derived from Lyso-Pc-Primed Nonadherent Cells. The initial signaling factor(s) for the activation of macrophages is derived from lyso-Pc-treated B cells within 3 hr after treatment and requires contribution of T cells in order to activate macrophages for ingestion activity. These intercellular communications among T, B, and macrophages may involve the actions of macromolecules such as interleukins secreted from these cells. This possibility can be tested by an inhibitor for production of interleukins. When peritoneal cells were treated with 20 μg of lyso-Pc/ml of FCS medium in the presence of cyclosporine (0.5 and 2 μg/ml) for 30 min and washed and then cultured for 3 hr in the presence of cyclosporine, a slightly reduced ingestion activity was observed (Fig. 5A). Since no complete inhibition was demonstrated with cyclosporine at a concentration as high as 2 μg/ml, a slight reduction in the macrophage ingestion activity may be attributed to the toxicity of this drug. Thus, cyclosporine, an established inhibitor of T cell function (11–13), had no significant inhibition of the lyso-Pc-induced macrophage-activating signal factor(s). This observation indicated to us that interleukins 1 and 2 are not involved in the lyso-Pc-induced activation of macrophages.

To further substantiate this proposition, we tested the effect of anti-interleukin 2 on the macrophage-activating factor(s). When conditioned medium from lyso-Pc-primed peritoneal nonadherent cells was pretreated with anti-IL-2 for 15 min and admixed with untreated adherent cells and then incubated for an additional 2 hr, no significant reduction in ingestion activity was noted (Fig. 5B). This result also suggests that the macrophage-activating signal factor produced by lyso-Pc-treated nonadherent cells is different from the established interleukin 2.

Discussion

Our previous observations show that adherent cell population alone cannot be stimulated by *in vitro* lyso-

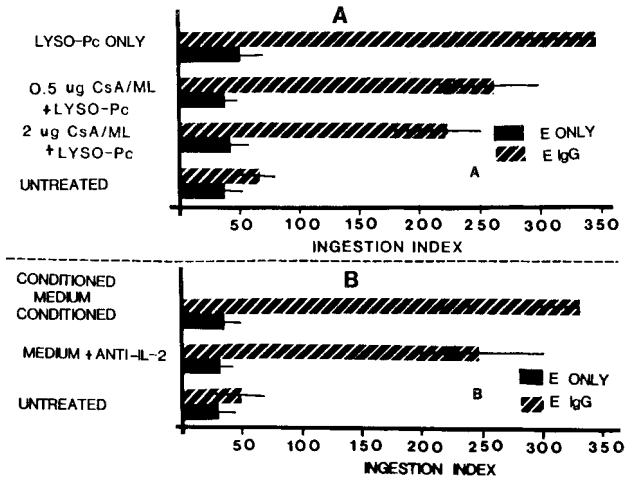

Figure 5. Effects of cyclosporine A (CsA) and antihuman IL-2 on macrophage activation by the factor derived from lyso-Pc-primed peritoneal cells. Cells were treated with 20 μg of lyso-Pc for 30 min in the presence of cyclosporine, washed, and then the adherent cells were cultured for 3 hr with cyclosporine A before the ingestion assay (A). A 3-hr conditioned medium from washed lyso-Pc-treated peritoneal cells was pretreated with anti-IL-2 for 15 min and then used to culture macrophages for 2 hr before the ingestion assay (B). Sheep erythrocytes (E) coated with subagglutinating IgG were used as targets. The ingestion index is expressed as the mean value of triplicate samples ± SD for 500 macrophages counted at random on each coverslip.

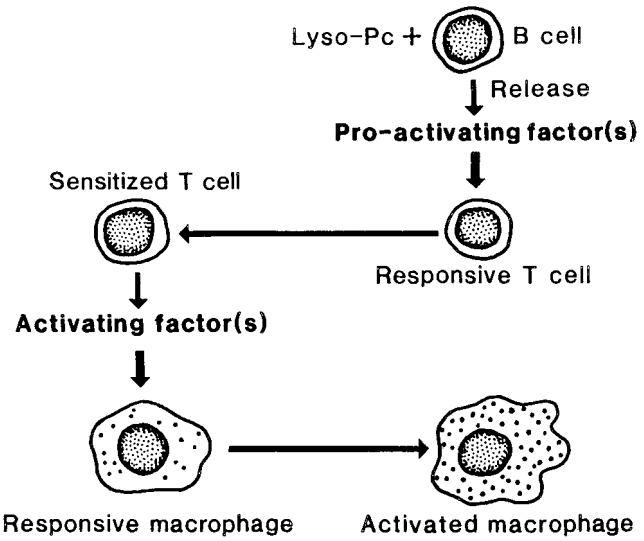

Figure 6. Schematic representation of biochemical pathway for the activation mechanism of macrophages by lysophosphatidylcholine.

Pc treatment to a functionally active macrophages for ingestion (1, 2). Treatment of a mixed culture of adherent and nonadherent cells with lyso-Pc produced a greatly enhanced ingestion activity of macrophages (1, 2). This observation suggested that collaborative action between adherent and nonadherent cells develops the mechanism of ingestion machinery of macrophages.

The present data with mixed cultivation of nonadherent (B and T) cell and macrophages indicate that treatment of nonadherent cells with lyso-Pc develops the signal(s) for activation of macrophages for ingestion activity (Figs. 2 and 3). Two pathways for signal transmission for ultimate activation of macrophages may be considered: (i) cell-to-cell contact or (ii) a signaling factor(s) is released from nonadherent cells into culture medium and transmitted to adherent cells. The former possibility is unlikely because efficient stimulation of macrophages for ingestion was observed, although the rate of adherent and nonadherent cell collision under stand culture (nonshaking) was expected to be very low. In fact, even a very small number of nonadherent cells co-cultured with adherent cells during a brief treatment (30 min) with lyso-Pc was able to stimulate macrophages for ingestion activity (data not shown). The presence of both B and T cells in the culture is required for development of ingestion activity. A trace amount of B cells present in a treated T-enriched cell population may be sufficient to release the initial signaling factor(s) for activation of macrophages. Accordingly, we further purified the B cells using anti-Thy 1.2 antiserum and complement. The purified B cells were treated with lyso-Pc, and their conditioned medium was used for culturing untreated T cells. When the resultant culture medium was admixed with untreated macrophages, a greatly enhanced Fc-mediated ingestion was observed (Fig. 4). Therefore, it may be concluded that lyso-Pc treatment of B cells triggers development of the signaling factor for macrophage ingestion capacity. The treated B cells initiate the process by releasing a signal factor(s) (macrophage proactivating factor) which stimulates T cells to modify the factor or release a new signaling factor. The resultant factor(s) (macrophage-activating factor) is required for the ultimate activation of macrophages for Fc-mediated ingestion capacity (Fig. 6). The activity of the macrophage-proactivating factor(s) released by lyso-Pc-treated B cells and its subsequent processing or modification by T cells, with the resultant release of an ultimate macrophage-activating factor(s) (Fig. 6), were not abrogated by either cyclosporine A (an established inhibitor of T cell function) or anti-interleukin 2. Therefore, it is concluded that lyso-Pc-induced macrophage-activating factor(s) is distinct from the established interleukins 1 and 2. This conclusion is further substantiated by the evidence that only a few hours of co-cultivation of lyso-Pc-treated nonadherent cells with adherent cells is required for the development of macrophage ingestion capacity. This rapid developmental process does not support a possible contribution of other established interleukins because development and secretion of interleukins by mitogen-activated murine lymphocyte cells require more than 24 hr (14, 15). The chemical nature of the signaling factor(s) remains to be characterized as to the precise processing mechanism of signaling factor(s) among B and T cells and macrophages.

1. Ngwenya BZ, Yamamoto N. Activation of peritoneal macrophages by lysophosphatidylcholine. Biochim Biophys Acta **839**:9–15, 1985.

2. Ngwenya BZ, Yamamoto N. Effects of inflammation products on immune systems: Lysophosphatidylcholine stimulates macrophages. Cancer Immunol Immunother **21**:174–182, 1986.
3. Yamamoto N, Ngwenya BZ. Activation of mouse peritoneal macrophages by lysophospholipids and ether derivatives of neutral lipids and phospholipids. Cancer Res **47**:2008–2013, 1987.
4. Cohn ZA, Benson B. The differentiation of mononuclear phagocytes, morphology, cytochemistry, and biochemistry. J Exp Med **121**:153–169, 1975.
5. Griffin FM, Silverstein SC. Segmental response of the macrophages plasma membrane to a phagocytic stimulus. J Exp Med **139**:323–336, 1974.
6. Davidson I, Nelson DA. The blood. In: Davidson I, Henry JB, Eds. Todd-Sanford Clinical Diagnosis by Laboratory Methods. 14th Ed. Philadelphia: WB Saunders, pp120–330, 1969.
7. Tyan ML, Ness DB. Mouse blood leukocytes: *In vitro* primary and secondary responses to two synthetic polypeptides. J Immunol **106**:289–291, 1971.
8. Julius MH, Simpson E, Herzenberg LA. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur J Immunol **3**:645–650, 1973.
9. Trizio D, Cudkowicz G. Separation of T and B lymphocytes by nylon wool columns: Evaluation of efficacy by functional assay in vivo. J Immunol **113**:1093–1097, 1974.
10. Bianco C, Griffin FM, Silverstein SC. Studies of macrophage component receptors. Alteration of receptor function upon macrophage activation. J Exp Med **141**:1278–1290, 1975.
11. Lafferty KJ, Borel JK, Hodgkin P. Cyclosporine-A(CsA): Models for the mechanism of action. Transplant Proc **15**:2242–2247, 1983.
12. Perfect JR, Durack DT. Effects of cyclosporine in experimental cryptococcal meningitis. Infect Immun **50**:22–26, 1985.
13. Palay DA, Cluff CW, Wentworth PA, Ziegler HK. Cyclosporine inhibits macrophage-mediated antigen presentation. J Immunol **136**:4348–4353, 1986.
14. Gearing AJH, Johnstone AP, Thorpe R. Production and assay of the interleukins. J Immunol Methods **83**:1–27, 1985.
15. Threshsel U, Evequoz V, Fleish H. Stimulation of interleukin 1 and 3 production by retinoic acid *in vitro*. Biochem J **230**:339–344, 1985.