Stimulation of Leukocyte Lysophospholipase Activity by Noninfectious Agents (43279)

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Abstract. **Mouse peritoneal leukocyte lysophospholipase (LPL) activity was studied to determine whether or not noninfectious agents cause increased enzyme activity and whether neutrophils have LPL activity. In the first study, mice infected with *Ascaris suum*, a known inducer of LPL activity, were given intraperitoneal injections of proteose peptone, thioglycolate, bovine albumin, paraffin, glycogen, or *A. suum* whole worm extract (WWE). Cell populations collected from mice injected with *A. suum* WWE, proteose peptone, thioglycolate, or bovine albumin contained increased numbers of neutrophils and eosinophils. These cell populations had increased LPL activity when treated, *in vitro*, with either *A. suum* WWE, zymosan-activated complement, or with the agent they were induced with. However, the LPL activity of the different cell populations did not respond to all treatments in the same way. In a second study, *A. suum*-infected or noninfected mice were given intraperitoneal injections of paraffin, thioglycolate, glycogen, or *A. suum* WWE. Enriched cell populations containing either lymphocytes or macrophages, from infected or noninfected mice, did not have increased LPL activity following *in vitro* stimulation with *A. suum* WWE, zymosan-activated complement, or with the agent they were induced with. Enriched neutrophil populations from infected or noninfected mice had increased LPL activity following *in vitro* treatment with *A. suum* WWE or zymosan-activated complement. Results demonstrate that the LPL activity of peritoneal leukocytes can be induced by noninfectious agents and that neutrophils have increased LPL activity following *in vitro* stimulation.** [P.S.E.B.M. 1991, Vol 197]

Lysophosphatidylcholine (LPC) is a biologically active compound present as a minor phospholipid in plasma and cellular membranes (1). LPC is cytotoxic if incubated with cells in serum or artificial media (2). A sublytic amount of LPC, however, stimulates phagocytosis (3), changes the surface properties of erythrocytes (4), and is involved in hypersensitivity (5) and inflammatory reactions (6). LPC activity during inflammation could serve as a mediator for macrophage activation and for enhanced ingestion activity (7).

The biological function of lysophospholipase (LPL; E.C. 3.1.1.5), the enzyme that catalyzes the hydrolysis of LPC, has been hypothesized to be that of a protective mechanism. LPL has been shown to have a stabilizing, anti-inflammatory effect on the membranes of erythrocytes (8). It chemically degrades LPC, preventing its lytic activity on animal tissue cells (9). Phagocytic cell extracts have been shown to antagonize the edema-producing properties of chemical mediators of inflammation (10, 11). The possibility of LPL activity being increased at a time when the inflammatory process has resulted in a build-up of LPC would be indicative of a protective biological process of tissue cells or possibly of invading pathogens.

LPL activity is increased in tissues during infection with helminths, and immune-mediated LPL activity by specific worm infections is evident following reinfection with the same helminth species (12–16). Pathogens, other than helminths, can also produce increased LPL activity (17–20).

Mouse peritoneal leukocytes have been shown to have increased LPL activity when treated, *in vitro*, with helminth antigens (21). The difference between this primary LPL activity and that of immune-mediated enzyme activity following exposure to antigen is related

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to a cell cooperation between T lymphocytes, macrophages, and eosinophils (22, 23). T lymphocyte activation is a necessary part of immune-mediated LPL activity and eosinophils or basophils are thought to be the cells containing the enzyme (24–30). Our laboratory has recently shown that the tissue LPL activity associated with *Ascaris suum* infections in mice is affected by alterations in dietary iron (31), magnesium (32), or zinc (33), a finding related to T lymphocyte function.

Eosinophils or basophils are not always found in tissue with high levels of LPL activity, which suggests that other cells are sources of LPL activity (18). The heterogeneous group of pathogens that result in tissue LPL activity proposes that LPL-containing cells are stimulated by a wide variety of antigens, and possibly not by an infectious process (12–16). The present study was designed to determine whether LPL activity is also associated with neutrophils and whether antigen not found in infectious disease processes will increase LPL activity.

Materials and Methods

Experimental Animals. BALB/cByJ male mice, 6–8 weeks old (The Jackson Laboratory, Bar Harbor, ME), were fed commercial mouse food and tap water, *ad libitum*. Mice were housed in a room with a 12-hr light/12-hr dark cycle with a temperature range of 23°C to 24°C.

Infection of Mice. *A. suum* eggs were prepared as described previously (34, 35). Mice were each given 1000 infective eggs, by mouth, 6 weeks prior to harvesting peritoneal leukocytes.

Noninfectious Agents. *A. suum* whole worm extract (WWE) was prepared by homogenizing adult worms, followed by freeze-thawing, centrifugation, sterilization, heat-inactivation, and performance of a total protein assay (36). Proteose peptone (Difco Laboratories, Detroit, MI), thioglycolate (Gibco Laboratories, Madison, WI), bovine albumin and glycogen (Sigma Chemical Co., St. Louis, MO), and paraffin oil (Curtin Matheson Scientific, Houston, TX) were prepared in 3.3-mg/ml aliquots and stored at 20°C until used in experimentation.

Zymosan-activated Mouse Complement (ZAC). A suspension of 20 mg/ml of zymosan (Sigma) in distilled water was boiled for 15 min, followed by washing two times with distilled water (37). The zymosan preparation was incubated for 25 min at 37°C with mouse serum (Cooperbiomedical, Inc., Malverson, PA), v/v. The ZAC was tested for lytic activity using the method of Hudson and Hay (38). One CH_{50} unit ml^{-1} of ZAC was used for the *in vitro* treatment of mouse peritoneal leukocytes.

Peritoneal Leukocytes. Leukocytes were removed from the peritoneal cavities of sacrificed mice by lavage with cold phosphate-buffered saline (39). In the first study, peritoneal cells were collected 48 hr after intraperitoneal injection of 1.0 ml of either saline, *A. suum* WWE, proteose peptone, thioglycolate, or bovine albumin into mice infected previously with 1000 *A. suum* eggs. Results are given in Table I. In the second study, both *A. suum*-infected and noninfected mice were injected with stimulating agents to produce enriched cell populations of lymphocytes, macrophages, and neutrophils. Lymphocyte-enriched cell populations were produced by injecting ip 1.0 ml of 10% liquid paraffin into each mouse, with cell collection 48 hr later. Macrophage-enriched cell populations were produced by injection of 1.0 ml of 10% thioglycolate, followed by lavage 96 hr later. Neutrophil-enriched cell populations were produced by injection of 1.0 ml of 10% glycogen, with a cell harvest 4 hr later. Eosinophil-enriched cell populations were produced by injecting 1.0 ml of 1.0 mg/ml *A. suum* WWE into infected mice, followed by peritoneal lavage 48 hr later. Results are given in Table III.

Cell suspensions were assayed for total cells present using Turk's stain and a hemocytometer, and cell viability was determined using trypan blue dye exclusion. All cells collected were observed to be greater than 95% viable. Leukocytes were differentiated by Wright staining smears on glass slides. Total numbers of each cell type were determined by multiplying the percentage of each cell type by the total number of leukocytes. Cell suspensions containing erythrocytes were discarded. Leukocyte suspensions were washed with 12.5% glycerol medium at 4°C and used immediately in experiments.

Lysophospholipase Assay. Leukocyte suspensions, in glycerol medium, were added in 0.3-ml quantities containing 1×10^6 cells/0.3 ml to each of multiple sets of 10 test tubes (five tubes were treated with agents and five tubes were nontreated control tubes in each set). Each of the five treated test tubes in each set received 0.3 ml of the enzyme substrate LPC (6×10^{-2} *M*; Sigma) preheated to 37°C in a shaking water bath; each of the five control test tubes received 0.3 ml of distilled water. Stimulating agents, 0.3-ml amounts of *A. suum* WWE, proteose peptone, thioglycolate, bovine albumin, paraffin, glycogen, or ZAC were added to all 10 test tubes in each group according to each study. Control tubes containing either serum, zymosan, serum + zymosan, *A. suum* WWE, proteose peptone, thioglycolate, paraffin oil, glycogen, or LPC were prepared. A requirement for enzyme analysis was that all control tube mixtures have LPL activity levels of baseline or less.

Cell mixtures and control tubes were incubated in a shaking water bath at 37°C for 24 hr. After incubation, the reactions were terminated by the addition of 0.1 ml of 2.0 *N* H_2SO_4, 1.0 ml of isopropyl alcohol, 1.0 ml of distilled water, and 2.0 ml of heptane. The contents of

each test tube were mixed using a Vortex mixer and centrifuged at 2000g for 10 min. The top 1.0 ml of heptane was removed from each test tube and pipetted into a second test tube, and 10 ml of bromthymol blue indicator were added to each tube. Titration of fatty acids in the heptane solutions was accomplished by adding 0.01 N NaOH to each tube while bubbling gaseous nitrogen into each solution. The amount of NaOH used was an indication of the amount of fatty acids released by LPC into the heptane solutions. LPL activity was expressed as μM per number of cells per 24 hr.

Data Analysis. LPL activity for each group of mice was expressed as a mean ± 1SD of each of the five treated samples minus the control samples. Significant differences between LPL activity readings were determined using the paired Student's t test; a value of P less than 0.05 was considered significant (40).

Results and Discussion

The role of the immune system with regard to LPL activity has been shown to be one of a relationship between T lymphocytes and eosinophils, with helminthic parasitic infections as a cause of the increased enzyme activity (22, 23, 31–33). LPL activity has been found in large quantities in human eosinophils (24–26) and basophils (24, 28, 29) suggesting a relationship to disease states that result in increased eosinophil or basophil numbers.

Increased LPL activity due to *in vitro* incubation of mouse leukocytes with *A. suum* WWE presents a model system that can be used to test for LPL activity stimulation by other agents (21). The present study was designed to determine whether LPL activity is increased by substances other than those found in helminths and whether enzyme activity is increased in mouse peritoneal neutrophils.

In the first study, peritoneal leukocytes from *A. suum*-infected mice, injected intraperitoneally with various stimulating agents, were collected and total numbers and types were determined (Table I). *A suum* WWE was the only stimulating agent that increased total numbers of peritoneal leukocytes. Neutrophils and eosinophils were increased in all groups of injected mice when compared with the saline control group. Lymphocyte and macrophage numbers were decreased in *A. suum* WWE-injected mice, but not in any of the other mouse groups.

Results show that there was an accelerated peritoneal cell response to *A. suum* WWE due to previous infection that was not seen with any of the other stimulating agents. There was also a difference in the types of peritoneal leukocytes elicited by each of the stimulating agents, suggesting that there were no cross-reacting antigens involved in the chemotactic responses to the *A. suum* WWE and the noninfectious agents.

The populations of cells shown in Table I were treated, *in vitro*, with either saline, *A. suum* WWE, proteose peptone, thioglycolate, bovine albumin, or ZAC, and assayed for LPL activity (Table II). The LPL activity of cells collected from mice injected with saline was not increased by treatment with any of the stimulating agents and was used as a control to compare with stimulated cells. Cells from mice injected with proteose peptone showed increased LPL activity when treated *in vitro* with either proteose peptone or *A. suum* WWE, demonstrating a similarity in enzyme stimulation by the two agents. ZAC did not stimulate LPL activity in the cells, *in vitro*, suggesting a lack of a role for complement activation in proteose peptone-induced cells. LPL activity was not stimulated in cells from thioglycolate-injected mice, *in vitro*, by thioglycolate, but the same cells were stimulated by treatment with *A. suum* WWE and with ZAC. These data suggest that certain compounds that are not the specific agent of induction of cells do have a role in stimulation of *in vitro* LPL activity. *In vivo* stimulated bovine albumin cells were only stimulated by ZAC to have increased LPL activity. These results demonstrate that leukocyte LPL activity can be induced, *in vitro*, by the *in vivo* stimulating agent

Table I. Quantitation and Differentiation of Peritoneal Leukocytes from *Ascaris suum*-Infected Mice Given Intraperitoneal Injections of Various Noninfectious Agents

In vivo injection of mouse groups with various stimulating agents[a]	Number of cells/ml peritoneal fluid ($\times 10^5$)	Percentage of cell types[b]			
		Lymphocytes	Macrophages	Neutrophils	Eosinophils
Saline (control)	2.9 ± 0.8	20 ± 2	79 ± 22	1 ± 1	0 ± 0
A. suum WWE	5.6 ± 0.9[c]	3 ± 2[d]	6 ± 2[d]	7 ± 2[c]	84 ± 6[c]
Proteose peptone	3.4 ± 0.3	23 ± 13	48 ± 6	11 ± 3[c]	18 ± 8[c]
Thioglycolate	3.6 ± 0.9	12 ± 9	39 ± 19	32 ± 17[c]	17 ± 12[c]
Bovine albumin	3.2 ± 0.8	12 ± 4	56 ± 5	22 ± 6[c]	10 ± 2[c]

[a] All mouse groups were infected 6 weeks earlier with 1000 *A. suum* eggs.
[b] Average of four mice per group.
[c] Significant increase of cell numbers or types when compared with the saline-injected control group.
[d] Significant decrease of cell numbers or types when compared with the saline-injected control group.

Table II. *In Vitro* Lysophospholipase Activity of Mouse Peritoneal Leukocytes Treated with Various Noninfectious Agents

In vivo treatment of mouse groups with various stimulating agents[b,c]	*In vitro* lysophospholipase activity of leukocytes[a] ($\mu M/10^6$ cells/24 hr)			
	LPL activity of saline-treated cells (control)	LPL activity of cells treated with the *in vivo* stimulating agent	LPL activity of cells treated with *A. suum* WWE	LPL activity of cells treated with zymosan-activated complement
Saline (control)	5 ± 2	—	5 ± 2	5 ± 2
Proteose peptone	4 ± 1	12 ± 2[d,e]	18 ± 4[d,e]	6 ± 2
Thioglycolate	3 ± 1	5 ± 2	13 ± 2[d,e]	19 ± 3[d,e]
Bovine albumin	6 ± 2	7 ± 2	5 ± 2	16 ± 3[d,e]

[a] Average of four mouse groups, five repetitions per group.
[b] All mouse groups were infected 5 weeks earlier with 1000 *A. suum* eggs.
[c] Agents were injected intraperitoneally in 1.0-ml amounts containing 3.3 mg/ml.
[d] Significant increase of LPL activity when compared with the saline-injected control group.
[e] Significant increase of LPL activity when compared with the *in vitro* saline-treated leukocytes.

Table III. Comparison of *In Vitro* Lysophospholipase Activity of Different Types of Mouse Peritoneal Leukocytes Treated with Noninfectious Agents

In vivo and *in vitro* stimulating agents[b]	Mouse treatment[c]	Percentage of peritoneal cell types	*In vitro* lysophospholipase activity[a] ($\mu M/10^6$ cells/24 hr)			
			LPL activity of saline-treated cells (control)	LPL activity of cells treated with the inducing agent	LPL activity of cells treated with *A. suum* WWE	LPL activity of cells treated with zymosan-activated complement
Paraffin	Noninfected	98 Lymphocytes[d]	5 ± 1	4 ± 2	4 ± 1	4 ± 2
	Infected	97 Lymphocytes[e]	4 ± 1	5 ± 2	5 ± 2	5 ± 1
Thioglycolate	Noninfected	98 Macrophages[f]	4 ± 1	4 ± 1	5 ± 2	5 ± 2
	Infected	96 Macrophages[g]	5 ± 2	4 ± 1	6 ± 2	6 ± 2
Glycogen	Noninfected	93 Neutrophils[h]	4 ± 1	4 ± 2	17 ± 4[i]	18 ± 3[i]
	Infected	96 Neutrophils[j]	4 ± 1	5 ± 1	16 ± 3[i]	14 ± 3[i]

[a] Average of four mouse groups, five repetitions per group.
[b] Agents were injected intraperitoneally in 1.0-ml amounts containing 3.3 mg/ml or were added, *in vitro*, 3.3 mg/cell sample.
[c] All infected mouse groups were given 1000 *A. suum* eggs 6 weeks earlier.
[d] Mixed cell population containing 98% lymphocytes and 2% monocytes.
[e] Mixed cell population containing 97% lymphocytes and 3% neutrophils.
[f] Mixed cell population containing 98% macrophages, 1% lymphocytes, and 1% neutrophils.
[g] Mixed cell population containing 96% macrophages, 2% lymphocytes, and 2% neutrophils.
[h] Mixed cell population containing 93% neutrophils, 4% macrophages, and 3% lymphocytes.
[i] Significant increase in LPL activity when compared with saline-treated (control) cells.
[j] Mixed cell population containing 96% neutrophils, 3% macrophages, and 1% lymphocytes.

and also by nonspecific agents, but not in all instances. This variance in LPL activity suggests differences in the cellular or molecular mechanisms involved in the increase in enzyme activity.

To further study the effect of noninfectious agents on lymphocyte, macrophage, and neutrophil LPL activity, *A. suum*-infected or noninfected mice were given intraperitoneal injections of either paraffin, thioglycolate, or glycogen (Table III). Peritoneal cells were collected and *in vitro* LPL activity was determined following treatment with saline, the same inducing agents, *A. suum* WWE, or ZAC.

Saline-treated cells all had baseline levels of LPL activity and were used to determine increased enzyme activity in the same cell types treated with other agents (Table III). Paraffin-induced lymphocytes and thioglycolate-induced macrophages did not exhibit increased LPL activity when treated, *in vitro*, with paraffin or thioglycolate, respectively, or with *A. suum* WWE or ZAC, regardless of whether or not the mice were previously infected with *A. suum*. These findings are evidence for the discharge of lymphocytes and macrophages as sources of LPL activity in the cell population models used in the first study.

Glycogen-induced neutrophils had increased LPL activity when treated with *A. suum* WWE or ZAC, but not with glycogen (Table III). This occurred with cells from *A. suum*-infected or noninfected mice, indicating that previous exposure to *A. suum* is not necessary for cells to have LPL activity stimulated by these two agents.

The neutrophil cell populations used in the study did not contain significant numbers of other cells that could have produced LPL activity. Neutrophil cell preparations did not contain eosinophils, basophils, or mast cells as measured by cell counts of 300 cells/smear. The small numbers of lymphocytes and macrophages found in the neutrophil-enriched cell populations were previously shown not to have LPL activity stimulated by *A. suum* WWE or ZAC, thus ruling these contaminating cells out as direct sources of enzyme activity.

Together, the results from the two studies propose that compounds other than those found in infectious agents can stimulate leukocyte LPL activity. Neutrophil enzyme activity is not dependent on previous exposure of cells to the stimulating agent and occurs in the absence of sensitized T lymphocytes, a finding that differs with the model of eosinophil LPL activity proposed by Adewusi and Goven (22, 23). This nonspecific LPL activity of neutrophils suggests a difference in their role in LPL activity during an inflammatory response, in comparison to eosinophils. Activation of neutrophil LPL activity may be beneficial to invading microorganisms in that it may decrease LPC activity at a time when LPC may be lytic to the microorganisms.

Our studies have shown that noninfectious agents can stimulate LPL activity in leukocytes and that neutrophil LPL activity can be stimulated nonspecifically.

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