Lung Lysophospholipase Activity in Specific-Pathogen-Free Rats Infected with *Pasteurella pneumotropica* or *Mycoplasma pulmonis*

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The effects of *Pasteurella pneumotropica* and *Mycoplasma pulmonis* infections in specific-pathogen-free rats were studied to determine whether or not bacterial infections could cause an increase in rat lung lysophospholipase activity and/or changes in bone marrow eosinophil levels. Lung lysophospholipase activity levels of *M. pulmonis*-infected rats were elevated with increasing infection dosages, but enzyme levels were not accompanied by a lung tissue eosinophilia or an increase in bone marrow eosinophils. Rats infected with *P. pneumotropica* showed neither an increased lung lysophospholipase activity level nor an increased tissue or bone marrow eosinophilia. Ottolenghi et al. (12) infected rats with the pneumonic helminth *Angiostrongylus cantonensis* and demonstrated elevated lysophospholipase activity levels in the lung tissues. Increased lysophospholipase activity correlated in time with inflammation of the lungs, worms in the tissue, and increased numbers of eosinophils in the bone marrow and lung tissues. Whether this increase in lung lysophospholipase activity could have been effected by conditions produced by concurrent pneumonic bacterial infections was not investigated.

The purpose of this research was to determine if infection with bacterial pathogens results in an elevation of lung lysophospholipase activity levels and, if so, if increased bone marrow eosinophilia or lung tissue eosinophilia accompanies the infections. *Pasteurella pneumotropica* and *Mycoplasma pulmonis* were the lung pathogens used in this study.

Specific-pathogen-free male rats, 21 days of age, were obtained from A.R.S. Sprague-Dawley, Madison, Wis. Rats were divided into groups of five animals each, infected with pneumonic agents, and maintained in pathogen-free isolator units.

Lung lysophospholipase activity was assayed by homogenizing lung tissue and incubating it with lysophosphatidylcholine at 37°C for 30 min. The free fatty acids that were released were quantitated by titration with sodium hydroxide and bromothymol blue (10). Lung tissue was analyzed for the presence of eosinophils by fixation with Formalin, staining with hematoxylin-eosin, and observation of tissue sections with bright-field microscopy.

Bone marrow eosinophil levels were determined by removing the bone marrow from the right tibia of each rat (12), suspending the cells, and counting the cells with a hemacytometer (2, 9).

Changes in lysophospholipase activity and bone marrow eosinophilia of infected rats were statistically compared to control (uninfected) rats, using the grouped Student *t* test (13). *t*-table values greater than 0.05 were regarded as not significant.

Elliott Goldstein, Davis, Calif., provided a *P. pneumotropica* that was virulent in mice and was found to be virulent in rats in our laboratory. Rats were given 0, 102, 104, 106, and 108 *P. pneumotropica* colony-forming units, intranasally, and killed 50 days after infection. Lung lysophospholipase activity levels and bone marrow eosinophil levels of infected groups of rats were compared to those of uninfected rats (Fig. 1) and were not found to differ significantly (*P* > 0.05). Group comparisons were of rats that were killed on day 50, having fully recovered from infection but still harboring *P. pneumotropica*. Rats that died before day 50 had pathological changes similar to previous findings (3, 4) and were omitted from the experiment.

The low levels of lysophospholipase activity could have been due to the lowered pathogenicity of *P. pneumotropica* at 50 days post-infection. This observation is not helpful in studying inflammatory conditions, but it is sig-
nificant in that a large proportion of conventional rats harbor *P. pneumotropica*, and the pathogen should be eliminated when studying the enzyme activities of inflammatory responses.

A second experiment was performed to determine if infection with *M. pulmonis* could result in changes in lung lysophospholipase activity, lung eosinophil levels, and bone marrow eosinophil levels. A virulent culture of *M. pulmonis* was provided by Dennis Kohn, Morgantown, W. Va. Rats were intranasally inoculated with 0, 10^2, 10^4, 10^6, and 10^8 *M. pulmonis* colony-forming units and killed 50 days after infection.

Lung lysophospholipase activity levels (Fig. 2) of the control group were significantly different from those of the 10^6 *M. pulmonis*-infected rats (*P < 0.05*), i.e., rats that had pathological changes. The lung lysophospholipase activity of the control group of rats was not significantly different from that of the 10^2 or 10^4 *M. pulmonis*-infected rats (*P > 0.05*), i.e., rats that did not have pathological changes. Bone marrow eosinophil levels of infected groups of rats were not significantly different from those of the uninfected rats (*P > 0.05*).

Pathological changes observed in *M. pulmonis*-infected rats at 50 days after infection were similar to findings of previous researchers (6, 8). The pneumatic lesions were characterized grossly by red and gray areas of consolidation and by mucopurulent exudate in bronchiectatic bronchi. The most consistent microscopic lesion found was a marked perivascular lymphoid cuffing in those portions of lung with gross lesions. These findings were characteristic of the rats infected with 10^6 colony-forming units and were not found in the 10^2, 10^4, or uninfected rat groups. *M. pulmonis* was the only pathogenic bacterium isolated from the lungs of infected rats.

The differences between helminth- and bacteria-induced inflammatory lysophospholipase activity could possibly be due to different enzymatic mechanisms and to different types of lysophospholipases.

Larsh et al. (7) postulate that inflammation due to helminth larvae stimulate the bone marrow for increased eosinophil production and then blood eosinophils migrate to the area of inflammation, aided by the release of eosinophil chemotactic substances from memory T-cells present in the inflamed tissue. Once the eosinophils accumulate in the inflamed tissue, the lysophospholipase of the tissue is increased due to the presence of lysophospholipase in the granules of the eosinophils (11).

The absence of eosinophils carrying lysophospholipase in *M. pulmonis*-infected lung tissue demonstrates a second method of increased lysophospholipase activity due to the inflammatory response. This eosinophil-independent lysophospholipase activity may be due to cell damage to erythrocytes that come into the area of inflammation and are lysed. Klibansky and De
Vries (5) have demonstrated that erythrocytes release lysophospholipase when damaged.

Lysophospholipases associated with the inflammatory response may be mixtures of enzymes with different substrate preferences. Differences in substrate specificity have been demonstrated for pancreatic lysophospholipase (1), and the same phenomenon could be occurring with lung lysophospholipases during the inflammatory process. The lysophospholipases of infected lung tissues need to be further characterized with regard to their biological and chemical roles.

In summary, increases in lung lysophospholipase activity during A. cantonensis and M. pulmonis infections demonstrate that lung lysophospholipase activity can be increased by both helminthic and bacterial infections, with or without an increase in bone marrow or lung tissue eosinophils. Lysophospholipase activity of tissue does not serve as a measurement of one type of inflammatory response to infection. Therefore, enzyme activity needs to be measured with monoinfected, pathogen-free animals.

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LITERATURE CITED

FIG. 2. Effect of varying dosages of M. pulmonis on the lung lysophospholipase activity levels (○) and the bone marrow eosinophil levels (●) of M. pulmonis-infected rats, 50 days after infection; bone marrow eosinophil level of uninfected rats (●); lysophospholipase activity level of uninfected rats (●). The points represent the mean (± 1 standard deviation).