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Effects of Viral Pneumonia on Lung Macrophage Lysosomal Enzymes

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During viral pneumonitis in mice, lung fluid protein and free lysosomal enzyme activity are increased while macrophage lysosomal enzymes are decreased.

In a murine model of viral pneumonia, pulmonary antibacterial defenses become suppressed, and secondary bacterial infections are a common occurrence. This enhanced susceptibility to bacterial pneumonia is maximal approximately 6 to 8 days after initiation of the virus infection (7, 9). During this time period, the alveolar macrophages, which are the major phagocytic defense against bacterial infection of the lung (6), have an impairment in intracellular bactericidal activity (8, 9). The mechanism for this defect is unknown; however, since lysosomal enzymes play a prominent role in cellular bactericidal mechanisms (4, 5), a reduction in the levels of these enzymes may account, in part, for the observed defect in intracellular killing activity.

In two separate experiments, a total of 54 outbred female Swiss mice were infected by aerosol inhalation with a sublethal dose of parainfluenza 1 (Sendai) virus by previously described methods (8). The control groups consisted of a total of 126 noninfected mice which were housed separately from the experimental animals. Noninfected animals and animals infected for 6, 7, and 8 days were sacrificed, and their lungs were excised in toto and lavaged with a total volume of 4.5 ml of sodium bicarbonate buffered (pH 7.2) isotonic saline containing 0.1% ethylenediaminetetraacetic acid. Lavage fluids were collected and kept at 4°C for further processing.

The recovered fluid volumes from the lungs of the virus infected animals did not vary significantly from those recovered from the controls (3.5 ± 0.1 ml, mean ± standard error of the mean). Lavage fluids from control animals contained a total of $6.5 \pm 0.3 \times 10^5$ macrophages per animal, whereas those from virus infected animals contained $1.8 \pm 0.3 \times 10^6$ macrophages per animal. To increase the number of cells in each sample to be assayed (for lysosomal enzyme activity), cell suspensions from 10 to 14 control mice and 3 to 6 infected mice were pooled separately. The lavage samples were then centrifuged at 180 × *g*, and the cells were collected and prepared on Ficoll-Hypaque gradients as described by Boyum (2), to remove erythrocytes and polymorphonuclear leukocytes. The mononuclear cells were recovered, washed, and resuspended in 1 ml of 0.1% Triton X-100 to lyse the cells for release of lysosomal enzymes. The cell lysates and cell-free lavage supernatant fluids were then frozen at −70°C until assayed.

Protein content of the supernatant fluids and cell lysates were quantitated by the method of Lowry and associates (10) with a bovine serum albumin standard. Quantitation of lysozyme was carried out by the method of Osserman and Lawlor (12), with chicken egg white lysozyme of known enzymatic activity as a standard. Acid phosphatase and β-glucuronidase quantitations were performed by the method of Mitchell and associates (11), with *p*-nitrophenyl phosphate and *p*-nitrophenyl β-glucuronidase as the respective substrates. The above assays were performed two or more times on each sample, and the average values were calculated.

Comparisons of individual enzyme values between the day-6, -7, and -8 virus-infected groups were found to be insignificant. Therefore, the results of each assay were pooled, and mean values were calculated. The normal lung fluids contained 488 ± 32 μg of protein per ml, a portion of which was comprised of lysozyme, acid phosphatase, and β-glucuronidase (Table 1). During viral pneumonia a significant increase was observed in the quantity of lung fluid, total protein, and enzyme values. However, on the basis of the specific activity (i.e., activity per microgram of protein), the lysozyme activity was reduced to the low normal range, and acid phosphatase activity remained within the high normal range. In contrast, the β-glucuronidase activity was significantly ($P < 0.001$, Student's *t* test) elevated above the normal values. These data suggest a selective increase or decrease in specific enzymes in relation to other proteins in the lung

TABLE 1. *Lung lavage fluid protein and lysosomal enzyme values*[a]

Mouse group	Protein[b] (μg/ml)	Lysozyme[c]		Acid phosphatase[d]		β-Glucuronidase[e]	
		U/ml	U/μg of protein	nmol/h per ml	nmol/h per mg of protein	nmol/3 h per ml	nmol/3 h per mg of protein
Noninfected (n = 12)[f]	488 ± 32	1,153 ± 291	2.6 ± 0.8	12.2 ± 1.3	24.7 ± 2.0	4.0 ± 0.5	8.1 ± 0.9
Virus infected (n = 17)	1,040 ± 88	1,574 ± 178	1.6 ± 0.3	32.6 ± 7.5	33.5 ± 8.0	35.1 ± 8.1	36.0 ± 8.4
	(213.1)[g]	(136.5)	(61.5)	(267.2)	(135.6)	(873.1)	(444.4)
P Value[h]	<0.001	0.20	0.20	<0.01	0.20	<0.001	<0.001

[a] Values expressed as mean ± standard error.
[b] Protein values based on bovine serum albumin equivalents.
[c] Lysozyme values determined by comparison with known concentrations of egg white lysozyme of known activity. One unit = −0.001 optical density unit per min at 450 nm, pH 6.2, 25°C.
[d] Acid phosphatase activity expressed as nanomoles of p-nitrophenol liberated per hour.
[e] β-Glucuronidase expressed as nanomoles of p-nitrophenol liberated in 3 h.
[f] Number of samples assayed. Each sample represents a pool of 3 to 6 virus-infected mice or 10 to 14 noninfected mice; see text.
[g] Values in parentheses are percent control.
[h] P values determined by Student's t test.

TABLE 2. *Lung macrophage protein and lysosomal enzyme values*[a]

Mouse group	Cell protein[b] (μg/10^6 MØ)	Lysozyme (U/μg of protein)[c]	Acid phosphatase[d] (nmol/h per mg of protein)	β-Glucuronidase[e] (nmol/3 h per mg of protein)
Noninfected (n = 9)[f]	90.1 ± 7.6	25.8 ± 3.5	96.0 ± 16.0	118.0 ± 11.0
Virus Infected (n = 9)	89.0 ± 3.9	2.9 ± 0.5	56.0 ± 5.6	38.0 ± 2.2
	(98.9)[g]	(11.3)	(58.3)	(32.2)
P Value[h]		<0.001	<0.025	<0.001

[a] Values expressed as mean ± standard error.
[b] Protein values based on bovine serum albumin equivalents. MØ, Lung macrophage.
[c] Lysozyme values determined by comparison with known concentrations of egg white lysozyme of known activity. One unit = −0.001 optical density unit per min at 450 nm, pH 6.2, 25°C.
[d] Acid phosphatase activity expressed as nanomoles of p-nitrophenol liberated per hour.
[e] β-Glucuronidase activity expressed as nanomoles of p-nitrophenol liberated in 3 h.
[f] Number of samples assayed. Each sample represents a pool of 3 to 6 virus-infected mice or 10 to 14 noninfected mice; see text.
[g] Values in parentheses are percent control.
[h] P values determined by Student's t-test.

fluids. The increase in total lung enzyme content may be a result of either cytolysis or exocytosis from the lung phagocytes (14). In support of this argument, the number of macrophages in the infected lungs increased twofold or greater; thus, more cells were available to release their enzymes into the lung environment. Upon release into the extracellular environment, the lysosomal enzymes may participate in bactericidal mechanisms (1), an enhanced inflammatory response (3), and/or, in some instances, suppression of immune mechanisms (13).

The values of the total cellular protein were unaltered by the virus infection (Table 2). However, the activities of all three enzymes quantitated were significantly reduced after the infection. The greatest decrease occurred in lysozyme activity (11.3% control values), followed by β-glucuronidase (32.2% control values), whereas acid phosphatase demonstrated the least reduction in activity (58.3% control values). The mechanism(s) responsible for the decreased lysosomal enzyme activity is unknown, but because of the differential reduction in these three enzymes, selective secretion and/or anabolic inhibition may have occurred. In summary, these reductions in lysosomal enzymes of the macrophage may be one mechanism through which viral pneumonia results in suppression of the lung's antibacterial defenses, as demonstrated by increased susceptibility of the lung to infection by opportunistic bacteria (7, 9).

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