Elevated Plasma Activity of Lactate Dehydrogenase Isoenzyme-3 (LDH_3) in Experimentally Induced Immunologic Lung Injury.

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Normal rats injected intravenously with rabbit antiserum to rat lung develop acute pulmonary lesions characterized by an altered vascular permeability. In the present study, an increase in plasma LDH_3 activity is shown to be positively correlated with the different levels of circulating antilung antibodies and with the morphologic severity of lung injury elicited by these pathogenic immunoglobulins. Within 24 hours, the acute lung changes are resolved, accompanied by a return of the activities of plasma LDH isoenzymes to normal. It is proposed that the plasma LDH_3 isoenzymes are released into the circulation from injured alveolar capillary endothelial cells. (Amer J Path 64:575–584, 1971)

INCREASED PLASMA ACTIVITIES of certain of the five lactate dehydrogenase (LDH) isoenzymes are indicative of biologic stress on the tissues in which the major portion of these isoenzymes are compartmentalized. Notable examples are the elevation of LDH_1 activity after myocardial stress or injury [1,2] and the elevation of LDH_5 activity in hepatic disease.[1] Recent studies [3–6] demonstrated the presence of LDH_3 in pulmonary tissue and indicated that changes in its plasma activity might reflect acute pulmonary damage. The present study was undertaken to observe changes in the plasma activity of LDH isoenzymes in an experimental model of antibody-mediated acute pulmonary injury.

The experimental model comprises the injection of pneumotoxic rabbit antiserum (PN serum) to rat lung into normal rats, with an elicited response consisting of alveolar hemorrhage and perivascular hemorrhage and edema, which are changes that resemble those seen in Goodpasture's syndrome and certain other idiopathic lung diseases in humans.[7,8] In the present study, an increase in plasma LDH_3 activity is shown to be positively correlated with the different levels of circulating antilung antibodies and with the morphologic severity of lung injury elicited by these pathogenic immunoglobulins. Adsorp-

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tion of PN serum with lung tissue removes its ability to elicit a pneumotoxic response and to cause a rise in plasma LDH_3 activity. Release of LDH_3 from injured pulmonary parenchyma into the circulation presumably produces the observed rise in plasma LDH_3 activity.

Materials and Methods

Stock pools of PN serum and control serum were prepared by methods reported elsewhere.[7,8] Unanesthetized, normal, male, CD strain rats weighing 200–400 g each were used in this study. Groups of 5–10 rats were injected intravenously with doses of serum in the range of 5 to 100 $\times$ 10^{-4} ml/g body weight. Sera were heated at 56 C for 30 minutes and injected without further treatment or after adsorption with lung, kidney, liver or spleen tissue antigens (20 mg/ml) at 37 C for 30 minutes. These tissue antigens were prepared by previously reported methods.[7,8] Thirty minutes after injection, the animals were anesthetized with ether. Plasma samples were obtained from the inferior vena cava and handled as previously described [9] for analysis of LDH activity. The lungs were excised for morphologic studies.

Total plasma activity of LDH was determined by the quantitative method of Wroblewski.[10] An electrophoretic procedure [11] was used to determine the relative percentages of LDH isoenzyme activities in plasma. The absolute activity of each LDH isoenzyme was calculated by multiplying its percentage times the total plasma LDH activity and was expressed in milli-International Units of LDH activity/ml of plasma (mIU/ml).

Lungs were removed, weighed and fixed for light and electron microscopy. Changes in the ratio of lung weight to body weight were used as indices of the presence of pulmonary edema. Tissues for light microscopy were fixed in 10% neutral formalin and processed routinely for staining with hematoxylin and eosin. Tissues for electron microscopy were fixed with 0.1 M cacodylate-buffered 4% glutaraldehyde, pH 7.0, with intermittent negative pressure. Secondary fixation and *en bloc* staining was with 1% osmium tetroxide and 0.5% uranyl acetate. After dehydration, tissues were embedded in Epon, sectioned and poststained with uranyl magnesium acetate and lead citrate.

Because the acute lung changes elicited by PN serum are resolved within 24 hours,[7,8] LDH activities may change during this time. In order to check this possibility, serial plasma samples were analyzed in 2 additional groups. One group (3 rats) received 75 $\times$ 10^{-4} ml/g body weight of PN serum. A second group (3 rats) received an equivalent dose of control serum. Plasma samples for assays of LDH activity were obtained from the femoral vein on each of 3 successive days after injection.

Results

Table 1 presents the morphologic findings and plasma activities of LDH observed in animals injected with PN and control sera. Doses of PN serum are relative only to the potency of our stock serum pools. A dose of 100 $\times$ 10^{-4} ml/g body weight of PN serum is lethal for 30% of injected animals. Two minutes after injection of a lethal dose, dyspnea and cyanosis were manifest. Massive pulmonary hemorrhage and edema followed, accompanied by foaming at the nose and

Table 1—Dose-Related Plasma LDH and Lung Tissue Changes in Rats Injected with Rabbit Antiserum to Rat Lung

PN serum dose (10^{-4} ml/g body wt)	No. of animals	$\frac{\text{Lung}}{\text{Body}}$ wt $\times 10^{-4}$*	Presence of pulmonary hemorrhage or edema			Plasma LDH activities (mIU/ml)*†					
			Gross	Light microscopic	Electron microscopic	Total LDH	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
control‖	5	49 ± 2	—	—	—	53 ± 2	11 ± 3	.8 ± .4	2 ± 1	3 ± 1	36 ± 3
5	5	59 ± 6	—	—	—	131 ± 10‡	25 ± 4‡	6 ± 2	3 ± 2	9 ± 4	87 ± 12‡
25	5	56 ± 2§	—	±	+	240 ± 34‡	24 ± 5	13 ± 1‡	17 ± 6§	29 ± 8§	157 ± 23‡
50	5	56 ± 2§	—	+	+	330 ± 45‡	29 ± 4‡	8 ± 3§	18 ± 5‡	34 ± 9§	243 ± 32‡
75	9	110 ± 5‡	±	+	+	390 ± 116§	19 ± 7	7 ± 3§	13 ± 2‡	35 ± 8‡	317 ± 104‡
100	10	128 ± 9‡	+	+	+	372 ± 61‡	49 ± 7‡	11 ± 3‡	15 ± 3‡	34 ± 10§	264 ± 51‡

* Mean ± SE.

† Significance of the differences between experimental mean values and control mean values were determined using Students' **t** test.

‡ $P > 0.01$.

§ $P > 0.05$.

‖ Animals received 100×10^{-4} ml/g body wt control serum.

mouth, and death ensued within 30 minutes. At necropsy, ratios of lung to body weight were significantly increased ($P < 0.01$). On gross examination, the lungs showed marked pulmonary hemorrhage and edema. Microscopic changes consisted of diffuse alveolar and perivascular edema and hemorrhage.[7] Capillary endothelial cells were damaged and torn from their supporting membranes and the interstitial space was filled with plasma proteins, including fibrin and occasionally erythrocytes.[8] Less severe but similar patterns of injury were elicited within 30 minutes by doses of PN serum as low as 25×10^{-4} ml/g body weight. The spaces between basement membranes and endothelial cells were filled with proteinaceous fluid. The low-dose response was characterized by numerous pouches formed by endothelial cells (Fig 1). These contained fluid, but rarely erythrocytes. Electron microscopic examination of lung tissues from rats given less than 25×10^{-4} ml/g body weight of PN serum or as much as 100×10^{-4} ml/g body weight of control serum did not reveal any evidence of acute damage to lung tissue.

Plasma activities of all LDH isoenzymes were elevated after injection of pneumotoxic doses of PN serum. Significant increases in LDH_{1-5} activities were seen in all animals given at least 25×10^{-4} ml/g body weight of PN serum (Table 1). In contrast, when injections of control serum were given to normal rats, there was no significant change in any of the LDH isoenzyme activities.

Serial assay of plasma LDH activities in the group receiving 75×10^{-4} ml/g body weight of PN serum revealed that total LDH activity and all isoenzyme activities returned to control levels within 24 hours after injection. The time sequence of this return of plasma LDH activity corresponded with that of the morphologic resolution of the acute lung lesions.[7] Equivalent doses of control serum produced no significant changes in LDH activities and isoenzyme patterns up to 3 days after injection.

Table 2 shows plasma activities of LDH isoenzymes elicited 30 minutes after the injection of 75×10^{-4} ml/g body weight doses of PN serum adsorbed with different tissue antigens prior to injection. After adsorption with lung antigen, PN serum did not produce a rise in plasma LDH_3 activity. Morphologic examinations of lungs from animals receiving lung-adsorbed PN serum did not reveal changes characteristic of the pneumotoxic response. PN serum adsorbed with kidney antigen did not cause a rise in the plasma activities of LDH_1 and LDH_2, but did result in increased plasma activities of LDH_3, LDH_4 and LDH_5. Pulmonary lesions were also present but were less

Table 2—Plasma LDH_{1-5} Activities in Rats Observed after Injection of Adsorbed Rabbit Antiserum to Rat Lung (75×10^{-4} ml/g Body Weight)

Adsorbing antigen	No. of animals	Plasma LDH isoenzyme activities (mIU/ml)*				
		LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
None	9	19 ± 7	7 ± 3	13 ± 2	35 ± 8	317 ± 104
Lung	5	27 ± 3	13 ± 1	1 ± 1	37 ± 3	211 ± 22
Kidney	5	10 ± 1	1 ± 1	16 ± 2	29 ± 4	270 ± 42
Liver	3	25 ± 3	13 ± 2	17 ± 2	26 ± 2	251 ± 62
Spleen	3	49 ± 5	11 ± 2	17 ± 2	34 ± 2	264 ± 23

* Mean ± SE.

severe than those seen after an equivalent dose of nonadsorbed PN serum. These changes were revealed only by electron microscopy and resembled those elicited by doses of PN serum in the range of 25×10^{-4} ml/g body weight. After adsorption with liver and spleen antigens, PN serum still elicited the pneumotoxic response and increases in LDH isoenzyme activities.

Discussion

The role of antibodies, specifically IgG, in the genesis of lesions elicited by PN serum has been established.[7,8] Although these antibodies localize in the lung, kidney, liver and spleen, they damage only the lung and kidney. Acute pulmonary damage is transient and disappears within 24 hours after a sublethal dose of PN serum is injected. Early renal changes occur 4–6 hours after injection and are characterized by the presence of polymorphonuclear leukocytes in glomeruli. The cellular reaction subsides within 24 hours and in followed, 7–10 days after injection, by changes that reflect a membranous glomerulonephritis.

It has been postulated from the results of radioiodination experiments that antilung serum possesses at least three organ specificities: for antigens of the lung alone, for antigens of the kidney alone and for antigens shared by both lung and kidney.[12,13] This postulation is supported by our present findings that kidney-adsorbed PN serum elicits a rise in plasma activities of LDH_{3-5} and pulmonary damage, albeit not as severe as with unadsorbed serum. Although kidney damage is not apparent in specimens examined 30 minutes after injection of PN serum,[7,8] the rise in $LDH_{1 \text{ and } 2}$ activities indicates that renal cells are altered by PN antibodies.

The significance of increased plasma $LDH_{4 \text{ and } 5}$ activities after injection of either adsorbed or unadsorbed PN serum is not understood.

Studies to determine the sources for release of these isoenzymes into plasma after the injection of PN serum are inconclusive to date.

Several lines of evidence support the assertion that plasma LDH_3 activity is a reliable and sensitive measure of certain kinds of acute pulmonary injury: (1) The lung is rich in LDH_3 isoenzyme in comparison with other organs, although $LDH_{4 \text{ and } 5}$ are also present.[3,4] These observations have been confirmed in our laboratory using lung tissue extracts from CD strain rats. (2) In a reported case of human pulmonary embolism, the only appreciable alteration in the distribution of plasma LDH isoenzymes was in LDH_3 activity.[3] (3) A rise in plasma LDH_3 is correlated specifically with lung injury in experimentally induced autologous pulmonary embolism.[5] (4) LDH_3 is a specific index in the early detection of pulmonary irradiation injury during the latent period in experimental dogs.[6] (5) LDH_3 is not contained in erythrocytes and platelets which, as a result of cytolysis, are inevitable sources of error in assays of other LDH fractions.[9]

The findings that the plasma activity of LDH_3 is not elevated after injection of PN serum adsorbed with lung tissue antigen and that this adsorbed serum does not elicit a pneumotoxic response suggest that LDH_3 isoenzymes are released into the circulation from cells in the lung that are damaged by antilung antibodies. Electron microscopic observations reported here and elsewhere[8] indicate that these injured cells may be endothelial cells of the pulmonary vasculature.

This report presents the third distinct line of evidence supporting the hypothesis that increased plasma LDH_3 activity reflects acute lung injury.[5,6] A rise in plasma LDH_3 activity is seen in all rats receiving either adsorbed or unadsorbed doses of PN serum when pneumotoxic responses are elicited. As a result of the present study, the view is advanced that LDH_3 isoenzyme activity may be a useful biochemical index of acute immunologic lung injury, with potential diagnostic and prognostic use in humans or in other experimental models of pulmonary disease.

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[Illustrations follow]

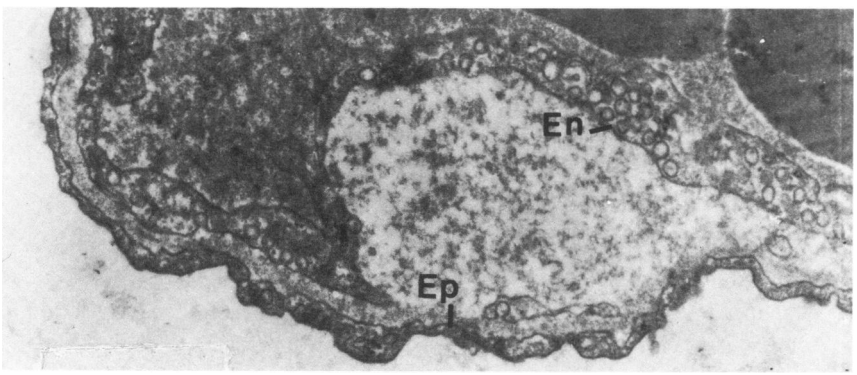

Fig 1—Electron micrograph of an alveolar septum from a rat 30 minutes after injection of 25 × 10^{-4} ml/g body weight of PN serum. The response to this low dose is characterized by the appearance of many similar pouches or blisters formed by endothelial cells. These pouches represent dissections of the endothelial cells from their supporting basement membrane and presumably contain plasma. *En* indicates endothelial cell; *Ep*, epithelial cell (× 19,000).

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