

# Endotoxin Reduces Specific Pulmonary Uptake of Radiolabeled Monoclonal Antibody to Angiotensin-Converting Enzyme

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The biodistribution of radiolabeled monoclonal antibody (Mab) to angiotensin-converting enzyme (ACE) was examined in normal and endotoxin-treated rats. Endotoxin administration at a dose of 4 mg/kg induced mild or middle pulmonary edema. The ACE activity in lung homogenate remained virtually unchanged, while the activity of serum ACE increased 15 hr after endotoxin infusion. In normal rats, anti-ACE Mab accumulates specifically in the lung after i.v. injection. Endotoxin injection induces reduction of specific pulmonary uptake of this antibody. Even in non-edematous endotoxemia, the accumulation of anti-ACE Mab antibody (Mab 9B9) decreased from 19.02 to 11.91% of ID/g of tissue without any change in accumulation of control nonspecific IgG. The antibody distribution in other organs and its blood level were almost the same as in the control. In a case of endotoxemia accompanied by increased microvascular permeability, the lung accumulation of Mab 9B9 was reduced to 9.17% of ID/g of tissue, while the accumulation of nonspecific IgG increased to 1.44% versus 0.89% in the control.

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**W**e have previously reported that radiolabeled anti-angiotensin-converting enzyme (ACE) monoclonal antibody (Mab 9B9) is specifically accumulated in the lungs of experimental animals (1,2). An extremely high tissue selectivity (lung-blood radioactivity ratio up to 20), specificity (immunospecificity up to 40) and efficiency (up to 30% of injected dose per gram (ID/g) of tissue) of the <sup>125</sup>I-Mab 9B9 accumulation in the lung has been shown (1). Additionally, specific pulmonary uptake of <sup>111</sup>In-labeled Mab 9B9 was visualized by gammascintigraphy (2).

ACE is a carboxy dipeptidase that hydrolyzes brady-

kinin and angiotensin I to angiotensin II. This enzyme is localized on the luminal surface of the vascular endothelium and participates in the regulation of vascular tone (3). The changes in ACE activity in lung tissue, bronchoalveolar lavage, and serum in relation to various abnormalities in lung function have been reported (4). Both clinical and experimental results show that the injury to lung endothelium is accompanied by a decrease in ACE activity in lung homogenate with a transitory increase of its activity in the serum (5-8). All these data suggest that ACE might be considered as a marker of lung endothelium injury (5,9).

Bearing in mind that (a) cellular ACE in normal lung is exposed to the bloodstream only from the luminal surface of vascular endothelium; (b) ACE activity is usually altered when pulmonary disease is present; and (c) Mab 9B9 is specifically accumulated in the lungs of normal animals, we attempted to examine the antibody biodistribution and accumulation in the target organ when the lung is damaged. Previously we have analyzed the changes in ACE activity and accessibility to circulating radiolabeled Mab 9B9 in alpha-naphthylthiourea (ANTU) induced pulmonary edema. It was shown that ANTU injection induces a decrease in specific lung uptake of Mab 9B9 in contrast to an increase in the accumulation of nonspecific IgG and albumin (10). A decrease in ACE activity in lung homogenate was also found.

In the present work, we studied biodistribution of Mab 9B9 in endotoxin-challenged rats. Endotoxin (LPS) produces a complement- and leukocyte-mediated oxidative injury to the lung endothelium (11). In respect to lung injury, the rat is more resistant to LPS than dogs or sheep (12); rats were reported to die due to severe coagulatory, liver, and intestinal dysfunction (13). We have found that LPS-induced mild or middle pulmonary edema is accompanied by a decrease in specific pulmonary uptake of radiolabeled Mab 9B9. It is noteworthy that Mab 9B9 accumulation is significantly decreased in response to LPS when other markers of pulmonary damage (ACE activity, LPO products

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content) and edema (pulmonary uptake of serum proteins, lung weight coefficient) were considerably less indicative, suggesting a promise for radiolabeled Mab 9B9 as a "supersensitive" probe in the monitoring of the lung ACE status.

## MATERIALS AND METHODS

The following reagents were used: Iodo-gen (Pierce), *E. coli* lipopolysaccharide serotype 0127:B8 (LPS, endotoxin), bovine serum albumin (BSA) and thiobarbituric acid (TBA) (Sigma), trichloroacetic acid (TCA), O-phthalaldehyde, Hip-His-Leu, (all reagents from Serva), and Nonidet P-40 (Fluka).

### Preparation of Anti-ACE Mabs and Labeling of Protein with $^{125}\text{I}$

ACE from human lung was isolated as described previously (14). Mouse Mabs to ACE were obtained using human lung ACE as the antigen by a conventional hybridoma technique (15). Monoclonal antibody 9B9 (IgG<sub>1</sub>) cross-reacted with rat and monkey ACE. This antibody was specifically accumulated in the rat lung upon i.v. injection (1). Monoclonal antibody 3A5 (IgG<sub>1</sub>) cross-reacted with human and monkey ACE, but not with rat enzyme. This antibody was not accumulated in rat lung, and its biodistribution was similar to that of nonimmune mouse immunoglobulin. In the present study, Mab 3A5 was used as a nonspecific (control) IgG.

Monoclonal antibody 9B9 and BSA were labeled with  $^{125}\text{I}$  (16). Proteins (500  $\mu\text{g}$ ) were incubated with 0.5 mCi of Na  $^{125}\text{I}$  and 2  $\mu\text{g}$  of iodogen for 20 min at 0°C. Unbound radiolabel was eliminated by Sephadex G-25 gel filtration. The final specific radioactivity of Mab 9B9 and BSA was equal to  $0.3\text{--}0.6 \times 10^6$  cpm/ $\mu\text{g}$  of protein.

### Indium Labeling

Mab 3A5 (control nonspecific IgG) was labeled with  $^{111}\text{In}$  using diethylenetriaminepentaacetic acid (DTPA) as a bifunctional chelating agent (17). Briefly, a 10-fold molar excess of cyclic DTPA anhydride dissolved in dry DMSO (0.7 mg/ml) was added to 1 mg of protein (1 mg/ml in 0.1 M NaHCO<sub>3</sub>). Unconjugated DTPA was then removed by gel filtration on Sephadex G-50 and 200  $\mu\text{g}$  of DTPA-conjugated antibody were mixed with 1 mCi of  $^{111}\text{In}$  (carrier-free) added in 0.1 M citrate, pH 6.0. Unbound label was removed on a Chelex-100 microcolumn. At least 70% of added  $^{111}\text{In}$  was recovered in the protein fraction. This procedure yielded an antibody with 0.5–1.0 DTPA molecules per antibody molecule and with a specific radioactivity of 2–4 mCi/mg.

### Administration and Biodistribution of Labeled Proteins in Normal and LPS-Treated Rats.

Male (200–300 g) Wistar rats were injected intravenously with *E. coli* endotoxin (0–4 mg/kg in 0.5 ml of PBS). Radiolabeled antibodies were injected intravenously 4 or 15 hr after LPS or PBS injection. Rats received a mixture of  $^{111}\text{In}$ -labeled Mab 3A5 (2  $\mu\text{g}$ ,  $1\text{--}2 \cdot 10^6$  cpm) and  $^{125}\text{I}$ -labeled Mab 9B9 (the same dose) in 0.5 ml PBS. Radiolabeled albumin was injected in a similar manner 15 hr after LPS injection in a separate set of experiments. One hour after injection of radiolabeled proteins the animals were decapitated, blood was collected, internal organs were excised, washed with saline, and weighed. Radioactivity was measured in a Compugamma counter (LKB

Wallac-Bromma, Sweden). The Mode 1 CPM Double Label program of the 1282 LKB gamma counter was used for separation of the  $^{125}\text{I}$  and  $^{111}\text{In}$  radioactivities. Radioactivity was measured in two channels, the first had the window at 35–102 levels (20–75 keV,  $^{125}\text{I}$  channel), while the second was set at 105–235 levels (80–1300 keV,  $^{111}\text{In}$  channel). The calculation program provided subtraction of the contaminated indium radioactivity at levels 35–102 for the iodine channel and contaminated iodine radioactivity at levels 105–235 for the indium channel. Decay correction was used for both isotopes. Pure  $^{125}\text{I}$  and  $^{111}\text{In}$  were included in each assay as references.

The biodistribution of labeled proteins was estimated using the following parameters: (a) percentage of the ID/1 g of tissue; (b) localization ratio (LR) defined as the ratio between radioactivity of 1 g of tissue and 1 g of blood; (c) immunospecificity (IS) was determined as the ratio of percentage of injected Mab 9B9 dose per 1 g of tissue to that of Mab 3A5; and (d) decrement of immunospecificity was defined as a ratio of normal IS to that of LPS-treated animals. The degree of lung edema was assessed using the lung weight coefficient (lung weight:body weight)  $\times 100\%$  (18, 19).

### Determination of LPO Products

Malondialdehyde was measured using a reaction with TCA-TBA mixture (TBA-reactants) (20). Fluorescent LPO products in chloroform-methanol extracts were determined as described (21).

### ACE Activity Assay

Homogenates were prepared as follows: 0.5 g of tissue was homogenized in 4.5 ml of Tris-HCl buffer (pH 8.3) in a Polytron homogenizer for 1 min at 25°C. Nonidet P-40 was added to a final concentration of 1% (v/v). After 1 hr incubation at 25°C, the mixture was sonicated at 50 Wt for 1 min and centrifuged at 1000 g for 5 min. Specific ACE activity was determined using Hip-His-Leu as a substrate (22). Briefly, 240  $\mu\text{l}$  of 0.1 M Tris-HCl (pH 8.3) containing 0.6 M NaCl and 2.5 mM Hip-His-Leu (Serva) was incubated for 10 min at 37°C. The reaction was initiated by the addition of 10  $\mu\text{l}$  of the assayed sample. After incubation for 1 hr at 37°C, the reaction was terminated by the addition of 1.45 ml of 0.28 N NaOH. The amount of His-Leu was measured with o-phthalaldehyde (Serva) using a Hitachi spectrofluorimeter (Japan). One unit of the enzyme activity was defined as the amount of ACE catalyzing the hydrolysis of 1  $\mu\text{mole}$  of Hip-His-Leu per min under the above conditions. Protein was determined as described by Bradford (23).

### Statistical Analysis

The significance of differences between the mean values of ACE activity, antibody, and BSA distribution in control groups and LPS-treated animals was determined using the paired Student's t-test. All calculations were carried out using a Statpack 3.1 program package (North Western Analytical, Inc., Portland, OR).

## RESULTS

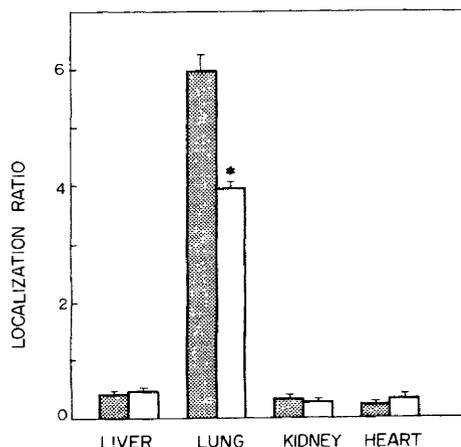
To evaluate the individual variability in biodistribution of labeled proteins, a simultaneous administration of  $^{125}\text{I}$ -Mab 9B9 and nonspecific  $^{111}\text{In}$ -IgG was per-

formed. This enabled us to relate the biodistribution of immune and control immunoglobulins in the same animal. The emission energy levels of  $^{125}\text{I}$  and  $^{111}\text{In}$  differ considerably. This allows an accurate separation of their peaks in the same sample by a computer. In a separate set of experiments, we have shown that tissue radioactivity remains virtually unchanged after simultaneous or independent injection of these isotopes (data not shown).

Endotoxin causes a decrease in the lung accumulation of Mab 9B9 but has no effect on the antibody distribution in other organs (Fig. 1). The tissue selectivity of antibody accumulation (the localization ratio) decreased approximately 1.5-fold (5.9 in the control versus 3.9 in LPS-treated animals).

The biodistributions of radiolabeled Mab 9B9, non-specific IgG and albumin in control and LPS-treated rats, and the changes in weight coefficient, a parameter reflecting the degree of the pulmonary edema (18, 19), are summarized in Table 1. After endotoxin injection, weight coefficient increased by 20%, however, this difference was statistically insignificant in each group (Table 1). The absolute lung accumulation of non-specific IgG and BSA (expressed as percent of dose per gram of tissue) increased 1.5-fold ( $1.56 \pm 0.39$  versus  $0.96 \pm 0.10$  in the control,  $n = 5$ , ns) and by 13-fold ( $1.10 \pm 0.5$  versus  $0.86 \pm 0.07$ ,  $n = 5$ ,  $p < 0.01$ ), respectively. As shown earlier, after injection of ANTU, the lung weight coefficient increased markedly (from  $0.56 \pm 0.2$  in control to  $1.0 \pm 0.1$ ) with a simultaneous 5-fold increase in IgG and BSA accumulation in the lung (10). Therefore, LPS induces only mild changes in lung microvascular permeability. However, the lung accumulation of Mab 9B9 decreased considerably, from 14.86 in control to 8.97% of ID/g of tissue (Table 1).

Thus, in LPS-treated rats, specific lung accumulation



**FIGURE 1** Changes in Mab 9B9 biodistribution in rat after LPS injection. The data were obtained 16 hr after LPS injection (4 mg/kg) and presented as  $M \pm \text{s.e.m.}$ ,  $n = 5$ , \* -  $p < 0.001$ . Open bars: LPS-treated rats, closed bars: control rats.

**TABLE 1**  
Biodistribution of Radiolabeled Proteins in the Organs of Normal and Endotoxin-Treated Rats (15 Hours After Injection)

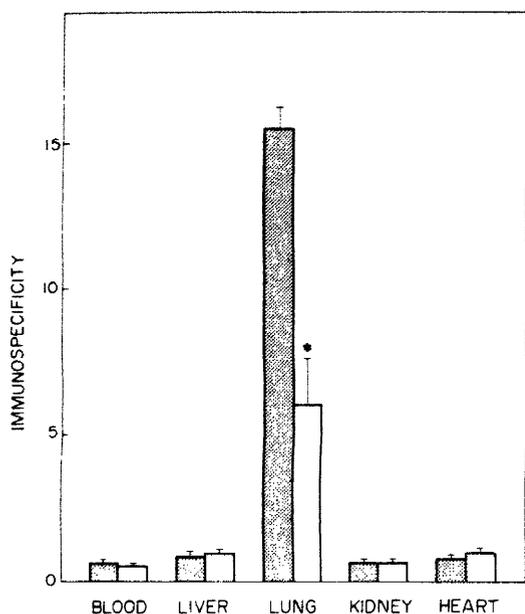
Organ	Group	Preparations		
		Mab 9B9	IgG	BSA
Blood	PBS	$2.17 \pm 0.2$	$3.68 \pm 0.3$	$2.49 \pm 0.1$
	LPS	$2.27 \pm 0.1$	$4.09 \pm 0.6$	$2.67 \pm 0.1$
Lungs	PBS	$14.86 \pm 1.5$	$0.96 \pm 0.1$	$0.86 \pm 0.1$
	LPS	$8.97 \pm 0.6^{**}$	$1.50 \pm 0.4$	$1.10 \pm 0.1^*$
Liver	PBS	$0.87 \pm 0.1$	$1.14 \pm 0.2$	$0.65 \pm 0.0$
	LPS	$0.98 \pm 0.1$	$1.17 \pm 0.1$	$0.60 \pm 0.1$
Kidneys	PBS	$0.56 \pm 0.1$	$0.88 \pm 0.2$	$1.43 \pm 0.1$
	LPS	$0.55 \pm 0.0$	$0.86 \pm 0.1$	$1.20 \pm 0.2$
Heart	PBS	$0.49 \pm 0.1$	$0.61 \pm 0.1$	$0.65 \pm 0.0$
	LPS	$0.61 \pm 0.1$	$0.78 \pm 0.0$	$0.64 \pm 0.0$
LWC	PBS	$0.65 \pm 0.0$	$0.58 \pm 0.1$	$0.59 \pm 0.1$
	LPS	$0.73 \pm 0.1$	$0.75 \pm 0.1$	$0.72 \pm 0.1$

The data are expressed as a %ID. The mean  $\pm$  s.e.m.  $N = 8$  in Mab 9B9 group and  $n = 5$  in the IgG and BSA groups. The data are statistically significant at  $p < 0.01$  (\*\*) and at  $p < 0.05$  (\*).

LWC (lung weight coefficient) differences are statistically insignificant in the three groups.

of Mab 9B9 is reduced in contrast to an increased accumulation of nonspecific IgG. One can suggest a priori that nonspecific accumulation of all immunoglobulins of this class, including both Mab 3A5 and Mab 9B9, is approximately equal. Therefore, endotoxemia causes antiparallel changes in the lung accumulation of Mab 9B9, that is (a) a decrease in specific accumulation as result of the reduction of Mab 9B9 binding to pulmonary ACE and (b) an increase in nonspecific accumulation as a result of enhanced microvascular permeability (the degree of such accumulation is similar to that of nonspecific IgG). Nonspecific accumulation may lead to underestimation of the real value of the reduction of specific accumulation. In order to quantitate this real value of the reduction of specific accumulation of Mab 9B9 in the pulmonary tissue, we have calculated the immunospecificity (IS) of the antibody biodistribution in the normal and LPS-treated rats. The IS coefficient was calculated as a ratio between the accumulations of immune and nonspecific immunoglobulins. The value thus obtained includes a contribution of nonspecific component in the value of specific accumulation of the antibody. This coefficient is a convenient parameter characterizing the specificity of the antibody accumulation in the target organ (24). The IS of the Mab 9B9 accumulation in the lung is reduced 2.5-fold after LPS injection (Fig. 2), while the IS for other organs remains virtually unchanged.

Table 2 illustrates the effect of LPS dose on the accumulation of Mab 9B9 and nonspecific IgG in the lungs and the blood. High LPS dose reduces the lung



**FIGURE 2**

Changes in immunospesificity of Mab 9B9 accumulation in the lung and other organs in rat after LPS injection. The data were obtained 16 hr after injection (4 mg/kg) and presented as  $M \pm s.e.m.$ ,  $n = 5$ , \* -  $p < 0.01$ . Open bars: LPS-treated rats, closed bars: control rats.

accumulation of Mab 9B9 and increases that of non-specific IgG. The tissue selectivity of Mab 9B9 accumulation (localization ratio) decreased drastically at high doses of LPS. It can be seen from Figure 3 that the lung weight coefficient exhibits a tendency to rise with increased LPS dose, whereas the IS of the Mab 9B9 accumulation gradually decreases.

Both ANTU and LPS are known to produce lung injury mediated by active oxygen metabolites (11, 25). ANTU causes accumulation of lipid peroxidation (LPO) products in lung homogenate (11). However, in the present work, we failed to reveal a statistically significant increase in LPO products in lung homogenates after LPS injection (MDA concentration was 0.31

$\pm 0.05$  nmole/mg versus  $0.26 \pm 0.02$  nmole/mg in the control,  $n = 5$ ). In contrast to ANTU, which reduced ACE activity in lung homogenates at constant ACE activity in the serum (10), LPS did not significantly change lung ACE activity, while the activity of serum ACE was slightly increased (Table 3). However, this increase in serum ACE activity may reflect not only pulmonary but liver disorders as well (see below). Therefore, in contrast to ANTU-induced oxidative lung injury, LPS-induced injury is not accompanied by the changes in tissue-specific markers of oxidative lung injury such as ACE activity and accumulation of LPO-products.

To determine the individual variation in rat response to LPS, we assessed the biodistribution of immune and nonspecific immunoglobulins in the same animals. Administration of a mixture of  $^{125}\text{I}$ -Mab 9B9 and  $^{111}\text{In}$ -IgG allowed us to characterize antibody accumulation in two groups of rats. In the first group of animals, LPS administration induced slight pulmonary edema 4 hr after injection (Group 1), while in the second group it provoked a moderate edema (Group 2) (Table 4). In the first group (a 1.21-fold increase in the weight coefficient) the absolute accumulation of Mab 9B9 decreased from 19.02% to 11.91% of ID/g of tissue ( $p < 0.05$ ). The IS of the accumulation decreased 2-fold. Pulmonary uptake of nonspecific IgG in the lung was similar to that in the control animals. In Group 2 rats (a 1.44-fold increase in weight coefficient), the antibody accumulation decreased to 9.17% of injected dose per g of tissue ( $p < 0.01$ ), while the accumulation of non-specific IgG increased from 0.89 to 1.44% ( $p < 0.05$ ). The IS of Mab 9B9 accumulation in the lung was reduced to 6.37, however in contrast to Group 1 animals, nonspecific accumulation exerts a considerable effect in this reduction.

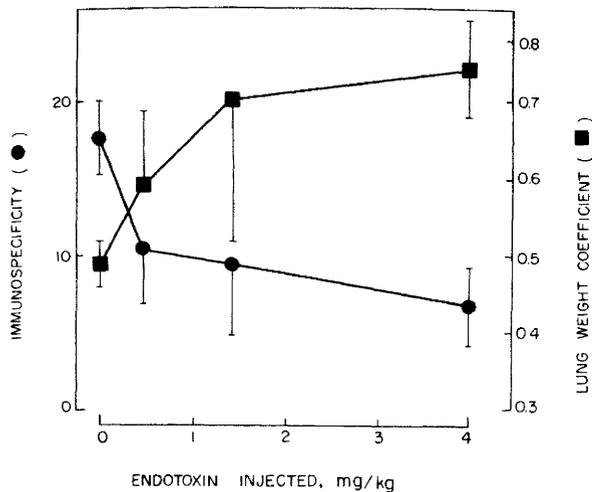
## DISCUSSION

It has been suggested that ACE may be used as a marker of lung endothelium injury (5-9). Serum, lung

**TABLE 2**  
Effect of Endotoxin Dose on Specific Lung Accumulation of Mab 9B9 and Nonimmune IgG

		Dose of endotoxin injected (mg/kg)			
		0.00	0.44	1.33	4.00
% ID/g of tissue					
Lungs	Mab 9B9	16.54 $\pm$ 1.6	11.75 $\pm$ 3.6	11.71 $\pm$ 5.6	8.07 $\pm$ 1.8
	Mab 3A5	0.93 $\pm$ 0.1	1.12 $\pm$ 0.2	1.18 $\pm$ 0.1	1.15 $\pm$ 0.3
Blood	Mab 9B9	1.26 $\pm$ 0.1	1.14 $\pm$ 0.4	1.16 $\pm$ 0.0	1.12 $\pm$ 0.1
	Mab 3A5	2.87 $\pm$ 0.1	1.89 $\pm$ 0.1	1.89 $\pm$ 0.0	1.93 $\pm$ 0.8
Localization ratio					
Lung	Mab 9B9	13.14 $\pm$ 1.4	10.30 $\pm$ 3.3	10.17 $\pm$ 5.1	7.17 $\pm$ 1.3
	Mab 3A5	0.32 $\pm$ 0.0	0.59 $\pm$ 0.1	0.62 $\pm$ 0.0	0.60 $\pm$ 0.2

The data were obtained 16 hr after endotoxin injection and are the mean of 3 determinations  $M \pm s.e.m.$



**FIGURE 3**  
Dependence of Mab 9B9 immunospecificity and lung weight coefficient on the dose of endotoxin injected. The data were obtained 16 hr after injection and presented as  $M \pm$  s.e.m.,  $n = 3$ .

homogenate, broncho-alveolar lavage and effusate were used as tissue samples for the determination of ACE activity (4). Unfortunately, though serum can be easily obtained, serum ACE level is not the best "mirror" to reflect pulmonary ACE status. In some cases, serum ACE activity is unrelated to lung injury. For example, in LPS-treated animals both enhanced [(8) and the present study] and unchanged (26) serum ACE activity have been reported. Since ACE clearance from the bloodstream is strongly dependent on liver function, some treatments may increase serum ACE activity by their hepatotoxic action, but not the injury to pulmonary endothelium (27).

The ACE level in the lung tissue (homogenate, BAL, effusate) seems to be the most informative parameter of the pulmonary endothelium status. However, certain limitations are imposed on the determination of the ACE activity in any tissue sample: (a) effect of the tissue inhibitors of ACE (28); (b) low availability of lung tissue for homogenate sample in clinics; and (c) direct action

**TABLE 3**  
ACE Activity in Lung Homogenate and Sera of Normal and Endotoxin-Treated Rats

Injection	ACE activity (units/g of tissue)	
	Homogenate	Serum
PBS	$14.28 \pm 4.7$	$0.13 \pm 0.0$
LPS	$13.28 \pm 4.7$	$0.18 \pm 0.0^*$
Percent of control	$94 \pm 10$	$134 \pm 7$

The data were obtained 16 hr after endotoxin injection and are the mean of 5 separate experiments ( $n = 3$  in each experiment)  $\pm$  s.e.m.

The results are statistically significant at  $p < 0.01$  (\*).

**TABLE 4**  
Comparison of Edematous and Nonedematous Endotoxemia in Rats 4 Hours After Injection of 4 mg/kg Endotoxin

Group	LWC	Mab 9B9	Mab 3A5	IS
PBS	$0.52 \pm 0.0$	$19.02 \pm 1.2$	$0.89 \pm 0.1$	$21.40 \pm 1.5$
LPS (1)	$0.63 \pm 0.0^*$	$11.91 \pm 0.6^*$	$0.89 \pm 0.0$	$13.41 \pm 1.9^*$
LPS (2)	$0.75 \pm 0.0^\dagger$	$9.17 \pm 1.7^\dagger$	$1.44 \pm 0.2^*$	$6.4 \pm 1.4^\dagger$

The data are presented as %ID/g of tissue; the mean  $\pm$  s.e.m. Group 1 (PBS),  $n = 4$ ; group 2 (LPS 1) and 3 (LPS 2),  $n = 3$ .

LWC = lung weight coefficient; IS = immunospecificity coefficient calculated from the percent of accumulated dose per g of the lung tissue for the antibody and IgG (Mab 3A5). LPS 1, rats with slight edema; LPS 2, rats with mild edema. The animals were divided in the two groups according to changes in lung weight coefficient.

\* Statistically significant at  $p < 0.05$ .

† Statistically significant at  $p < 0.01$ .

of aggressive agents such as oxidants and proteases leading to modification of ACE (29). Therefore, under some circumstances the assay of ACE activity in pulmonary tissue may not provide information on the status of the lung endothelium.

To overcome these obstacles a new approach to the monitoring of lung ACE activity has been developed, based on the determination of the degree of hydrolysis of ACE substrates during the first passage through the lungs (30, 31). This procedure offers information on the accessibility of active ACE to circulating compounds. However, some of the limitations related to activity of ACE and ACE inhibitors still may interfere with the analysis (28).

We have demonstrated that radiolabeled anti-ACE Mab 9B9 specifically accumulates in the lungs of normal animals. Reduced lung accumulation of Mab 9B9 was shown in ANTU-treated rats with acute pulmonary edema (10). Thus, the determination of Mab 9B9 accumulation in the lungs was employed as an alternative monitoring of lung endothelial status. This approach has some advantages over the determination of the products of ACE substrate hydrolysis. First, the Mab 9B9 epitope is not localized in the active site of ACE molecule (1) and, therefore, the antibody can react both with active and inactive ACE exposed on the endothelial surface. Second, the sizes of the antibody and ACE substrate molecules differ considerably, therefore binding of these ligands to ACE may characterize the enzyme accessibility to circulating particles of varied size. Third, the possibility of gamma scintigraphic visualization of ACE in the lungs using  $^{111}\text{In}$ -labeled anti-ACE Mab 9B9 can not be excluded (2).

In ANTU-induced pulmonary edema, the decrease in specific accumulation of Mab 9B9 coincides with a marked increase in the accumulation of nonspecific IgG and BSA (10). The accumulation of radiolabeled

albumin is a conventional test of microvascular permeability and is widely applied for quantitation of the pulmonary edema (32). Though the decrease in Mab 9B9 accumulation upon acute pulmonary edema is specific and probably reflects some changes in lung vascular endothelium, the use of antibody to assess the lung damage accompanied by severe edema has no advantages over the conventional monitoring of albumin accumulation.

Similar to ANTU, LPS damages the lungs by oxygen-radical pathway. LPS activates complement and leukocytes, and converts the endothelial layer into the target for activated leukocytes (12). Sequestration of leukocytes in pulmonary capillaries and the development of lung endothelial injury occur in endotoxemia (33). The active oxygen metabolites released from leukocytes are the major damaging agent (34). However, in contrast to ANTU-induced, LPS-induced pulmonary injury is not accompanied by severe edema in our experiments.

LPS-induced changes in the biodistribution of Mab 9B9 were characterized using several parameters. First, a percent of injected dose/g of tissue that reflects the absolute accumulation of the radiolabel. Both in edematous and non-edematous endotoxemia the absolute lung accumulation of Mab 9B9 is markedly decreased. The uptake of nonspecific IgG and albumin remains unchanged in nonedematous endotoxemia while in edematous endotoxemia it increased by 50%.

The second parameter, LR, is the ratio between tissue and blood radioactivities. This parameter reflects the tissue selectivity of accumulation. It depends directly on the blood level of antibody. Therefore, LR may be changed at a constant absolute accumulation in the target organ. This case is exemplified by an artificially accelerated clearance (35). On the other hand, if the antibody is specifically and efficiently accumulated in the target organ, its blood level is markedly reduced compared to the nonspecific immunoglobulin [antibody depletion by the target antigen (36, 37)]. In endotoxemia, Mab 9B9 LR is reduced only in the lungs, implying that LPS-induced changes of ACE accessibility are lung-specific and are recognized by the Mab 9B9 (Fig. 1).

The third parameter (i.e., immunospecificity) allows a comparison between biodistribution of the antibody and nonspecific IgG. Simultaneous injection of both proteins labeled by different isotopes enabled us to evaluate individual variations in rats within the same group. Immunospecificity index offers the estimation of nonspecific component in the antibody biodistribution, which is of particular importance when the distribution of the nonspecific IgG is also changed. In our model, for example, serum proteins, including albumin and IgG, accumulated in the lung as a result of increased vascular permeability. Under these conditions, the non-

specific accumulation of proteins in the target organ interferes with specific accumulation and thus hampers the assessment of the changes in the accumulation of specific antibody. This drawback can be overcome by comparing IS coefficients in control and endotoxin-treated rats.

Immunospecificity can be quantitatively assessed as:

1. The ratio of absolute accumulation of the antibody and nonspecific IgG in the target organ (expressed as %ID/g of tissue).
2. The ratio of LRs for these immunoglobulins in the organ.

In the present study we employed the first approach, while the second one was used by Keenan et al. (24). Immunospecificity coefficients calculated by both methods are presented in Tables 5A and 5B. As one can see from the results presented in these tables, IS calculated as the ratio of LRs is about two times higher compared to one estimated by the first method. This may be explained by the fact that LR is influenced by blood clearance; the IgG blood level is about 2-fold higher compared to the antibody level (antibody depletion by the target antigen). Changes in the IS decrement (i.e., the ratio of immunospecificities in control and LPS-treated animals calculated from LR) are more significant since LPS reduce IgG concentration in the blood (see Tables 2 and 4). Therefore, for contribution of the blood clearance two modes of the IS index

**TABLE 5**  
Changes in the Immunospecificity of Lung Accumulation of Mab 9B9 in Endotoxemia

A. Effect of the endotoxin dose (the data of Table 2 are used)				
	Endotoxin injected (mg/kg)			
	0.00	0.44	1.33	4.00
IS (1)	17.81 ± 2.6	10.50 ± 3.7	9.92 ± 4.9	7.02 ± 2.4
DIS (1)	—	1.7	1.8	2.5
IS (2)	41.05 ± 5.6	17.43 ± 5.9	16.44 ± 8.4	11.94 ± 4.4
DIS (2)	—	2.4	2.5	3.4

B. Comparison of edematous and nonedematous endotoxemia (the data of Table 4 are used)			
	Control rats	Nonedematous endotoxemia	Edematous endotoxemia
IS (1)	21.44 ± 1.5	13.45 ± 1.9	6.46 ± 1.4
DIS (1)	—	1.6	3.3
IS (2)	50.25 ± 3.6	28.13 ± 4.5	11.92 ± 2.3
DIS (2)	—	1.8	4.2

The data are the mean of 3 determinations ± s.e.m.  
IS (1) = immunospecificity coefficient calculated from %ID/g of tissue; DIS (1), decrement of IS (1).  
IS (2) = immunospecificity coefficient calculated from localization ratios of Mab 9B9 and Mab 3A5; DIS (2) = decrement of IS (2).

calculation should be employed, while determination of the real changes in the antibody accumulation in the target organ (the immunospecificity decrement) invokes the application of IS index expressed as %ID/g of tissue. In any case, in LPS-treated rats, IS of the Mab 9B9 accumulation decreases dramatically regardless of the method of its calculation (Table 5).

ANTU-treated rats displayed a greater decrease in the immunospecificity, up to 10-fold (10) versus 2.5-fold observed in case of endotoxemia. However, the decrease of IS index in ANTU-treated rats resulted primarily from a growing contribution of the nonspecific component (5-fold increase in the IgG accumulation) and reflects the increase in vascular permeability. In LPS-treated rats without edema, the immunospecificity decrement is due entirely to a reduced accumulation of Mab 9B9 (Tables 4 and 5), i.e., reflecting a reduction of interaction between the antibody and ACE exposed on the endothelial surface.

In contrast to ANTU, endotoxins cause less significant changes in lung weight coefficient and accumulation of plasma proteins, and do not affect lung ACE activity and accumulation of LPO products. Both agents reduced Mab 9B9 lung uptake and in non-dematous endotoxemia this parameter was the most sensitive as compared with the other studied markers of lung injury.

The mechanisms underlying the decrease in lung accumulation of Mab 9B9 are unclear. Some possibilities are:

1. Alterations in endothelial ACE turnover (i.e., the synthesis, recycling, and shedding) induced by oxidants (38).
2. Death and desquamation of endothelial cells (4).
3. Shielding of the endothelial surface by adherent leukocytes (11).
4. Plasma flow redistribution in pulmonary capillaries (39).
5. Oxidants and proteases may modify not only the active site, but other sites of the ACE molecule, including Mab 9B9 epitope.

All these events may lead to reduced binding of antibody to ACE in the lungs. Elucidation of these mechanisms will provide more insight into the pathogenesis of endotoxin-induced lung injury. It also may be suggested that radiolabeled Mab 9B9 can be employed for the development of monitoring ACE accessibility to the bloodstream by gamma scintigraphy.

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