Lung to blood passage of different-sized molecules during lung inflammation in the rat

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FOLKESSON, HANS G., BJÖRN R. WESTRÖM, STEFAN G. PIERZYNOWSKI, AND BÖRJE W. KARLSSON. *Lung to blood passage of different-sized molecules during lung inflammation in the rat.* J. Appl. Physiol. 71(3): 1106–1111, 1991.—The passage of different-sized marker molecules over the lower respiratory tract into the blood circulation during pulmonary inflammation induced by dextran, endotoxin [i.e., lipopolysaccharide from *Escherichia coli* (LPS)], or ferritin was assessed in the rat. Bovine immunoglobulin G (BIgG, mol wt = 150,000 Da), bovine serum albumin (BSA, mol wt = 67,000 Da), and the nonapeptide 1-deaminocysteine-8-D-arginine vasopressin (dDAVP, mol wt = 1,067 Da) were used as permeability markers after intratracheal instillation. The pathophysiological indexes of a proceeding lung inflammation were increased total cell number, changed leukocyte proportions and increased total protein content obtained in bronchoalveolar lavage, and lung edema formation shown as an increased lung wet-dry weight difference. Intratracheal instillation of dextran induced a moderate neutrophil invasion into the lungs but had no effect on the passage of the different markers over the lungs (BIgG 1.8 ± 0.6%, BSA 3.5 ± 1.2%, dDAVP 26.1 ± 20.7%) compared with control rats instilled with the markers alone (1.8 ± 0.4%, 4.1 ± 1.3%, 20.0 ± 3.8%, respectively). Endotoxin administration resulted in markedly higher lavage cell counts and lung edema concomitantly with an increased lung passage of the markers (3.2 ± 0.9%, 22.0 ± 6.1%, 33.3 ± 12.0%, respectively; $P < 0.01$–$P < 0.001$). The highest marker passage was obtained when the inflammation was most severe, i.e., after ferritin administration (17.6 ± 2.3%, 60.0 ± 6.7%, 41.6 ± 6.9%, respectively; $P < 0.001$), which resulted in markedly elevated lavage cell numbers and protein content as well as edema formation. Results from experiments where the iron chelator desferrioxamine (Desferal) was administered together with ferritin suggest that iron released from ferritin plays a crucial role in the observed changes in the lung passage, as a significant decrease in the lung transfer was observed in these rats (11.9 ± 7.1%, 26.4 ± 5.0%, 15.2 ± 5.5%, respectively; $P < 0.01$–$P < 0.001$) compared with ferritin-exposed rats. These results show that the inward lung passage of different-sized marker molecules was increased during inflammatory conditions apparently correlated to the severity of the lung injury.

instillation; lung injury; lung passage; dextran; endotoxin; ferritin; macromolecules; peptides

THE RESPIRATORY TRACT MUCOSA, a large surface exposed to the environment, restricts the entrance of particles and molecules into the body's internal milieu. However, injuries and inflammation in this mucosa may affect its barrier functions. The defense mechanisms of the respiratory tract are the mucociliary clearance system and the migratory inflammatory cell system cooperating with several antioxidant defense mechanisms (3, 12). The extent to which these defense mechanisms are breached by inhaled foreign materials may induce or affect the severity of several respiratory diseases (12).

Earlier studies have shown that during an inflammation, increased amounts of macromolecules, (e.g., serum albumin) pass from the blood circulation over the mucosal barrier into the respiratory tract lumen (26, 30, 31). Fewer studies, however, have concerned the inward passage of macromolecules from the air space into blood and the factors influencing this passage during inflammation. The low-molecular-weight marker diethylenetriamine pentaacetate passes over the lungs to a higher degree in patients with the adult respiratory distress syndrome and in smokers than in normal subjects (5) while clinically stable asthmatic patients do not demonstrate an increased permeability to the same compound (7). It has also been shown that after an aerosol challenge with a specific antigen the plasma levels of intratracheally administered macromolecules as well as of mannitol were increased (28). Ozone increased the tracheal permeability for proteins in the rat by increasing the vesicular transport, i.e., adsorptive endocytosis (2). The same transport route was also suggested in the canine bronchial epithelium by Johnson et al. (16) and in the bullfrog alveolar epithelium by Kim et al. (17). Known inflammatory mediators, such as platelet-activating factor, tumor necrosis factor, and lysophosphatidyl choline also alter the permeability to different-sized molecules in the respiratory tract (4, 19, 31).

In this investigation, we have studied the passage of different-sized marker molecules into the blood circulation after an intratracheal instillation during experimentally induced inflammatory conditions in rat lungs. The inflammatory inducing agents used were dextran, to which Sprague-Dawley rats are naturally hypersensitive (32), the endotoxin *Escherichia coli* lipopolysaccharide (LPS) (8), and ferritin, presumed to be inflammatory due to its iron content (1).

METHODS

Experimental animals. Sprague-Dawley rats (ALAB, Solna, Sweden), 100–120 days old and weighing 250–400 g, were used as recorded in the figures and tables. The animals were kept in separate polycarbonate cages during the experimental period under a 12:12-h day-night rhythm at 20 ± 2°C and a relative humidity of 50 ± 10%

and were given a laboratory rat chow (R3, EWOS, Södertälje, Sweden) and water ad libitum.

Some rats were chronically catheterized in the right jugular vein as described elsewhere to allow repeated blood sampling (9). The blood taken was replaced with the same volume of sterile 0.9% NaCl at each sampling.

Tracheal instillation of markers. Marker solutions were administered in a volume of 1.0 ml/kg body wt to the rats under light ether anesthesia by using a tracheal instillation technique as described previously by Folkesson et al. (9). The marker solution consisted of bovine immunoglobulin G (BIgG; G-5009, Sigma, St. Louis, MO, 15 mg/ml), bovine serum albumin (BSA; A-4503, Sigma, 5 mg/ml), and 1-deaminocysteine-8-D-arginine vasopressin, (dDAVP; Ferring, Malmö, Sweden, 100 μg/ml) dissolved in 0.9% NaCl.

Experiments. The rats were intratracheally instilled with the marker solution alone (controls) or together with one of the three inflammatory-inducing substances, dextran 70,000 (Dextran T70, Pharmacia Fine Chemicals, Uppsala, Sweden, 5 mg/kg body wt), LPS (L-2143, Sigma, 50 μg/kg body wt) or horse spleen ferritin (F-4503, Sigma, 5 and 50 mg/kg body wt). In some experiments the iron chelator desferrioxamine (Desferal, Ciba Geigy, Basel, Switzerland, 250 mg/kg body wt) or mannitol (BDH, Poole, UK, 18 mg/kg body wt), functioning as a radical scavenger (24), was instilled together with the marker solution and ferritin. The osmolalities of the solutions instilled were similar (256–294 mosmol/kg), except when mannitol (391 mosmol/kg) or desferrioxamine (1,039–1,057 mosmol/kg) was added.

Single blood samples were taken 16 h after instillation by heart puncture. After clotting, the samples were put on ice and centrifuged at 3,000 *g* for 15 min (+4°C). The serum was harvested and stored at −20°C until analysis. The lungs were dissected out, rinsed, weighed, dried over night at +80°C and weighed again to determine their percent water content (wet-dry weight difference). In other animals a bronchoalveolar lavage was performed 16 h after the instillation, mainly according to Holt (14). Briefly, directly after the death the rats were tracheotomized and the lavage fluid, 0.9% NaCl containing 3.2 mg/ml lidocaine (Xylocaine, Astra, Södertälje, Sweden), was allowed to enter the lungs during 3 min at 10 cm pressure, whereafter the lavage was extruded into a sampling tube by self-pressure. This procedure was repeated three times, and the recovered fluid was pooled and the volume measured. Typically, a lavage volume of 30 ml was obtained under control conditions but during severe inflammations the volume could decrease to about one-half of the control. After centrifugation of the lavage, the pelleted cells were differentially stained in May-Grünewald Giemsa stain and counted, while the lavage fluid was stored frozen (−20°C) until analysis.

To obtain serum marker levels vs. time curves, catheterized rats were intratracheally instilled with the marker solutions with or without the inflammatory-inducing substances added. Similarly, to obtain serum elimination time curves of the markers, the inflammatory substances were given intratracheally. Simultaneously the permeability markers (BIgG, BSA, and dDAVP) were given intravenously via the jugular catheter in the same doses as when given intratracheally. Repeated blood samples of 0.5 ml were taken 0–168 h after administration.

Analysis. BSA was quantified by electroimmunoassay (18) and qualitatively analyzed to check for degradation by crossed immunoelectrophoresis (10), using rabbit antibodies to BSA (Dakopatts, Hägersten, Sweden) and purified BSA (A-4378, Sigma) as a standard.

BIgG was quantified by radial immunodiffusion (15), using rabbit antibodies to BIgG (ICN BioMedicals, Lisle, IL) and purified BIgG (I-5506, Sigma) as a standard and qualitatively analyzed by double-gel diffusion (23).

dDAVP was quantified using a specific radioimmunoassay as described in detail previously (21).

The protein concentration in the bronchoalveolar lavage fluid was determined by use of the Lowry method (20), modified to be performed on 96-well microtiter plates and with BSA (A-7638, Sigma) as the standard. The total protein content in the obtained bronchoalveolar lavage fluid was calculated, and the remaining marker amounts were subtracted.

Calculations and statistics. The percentages of markers transferred over the lungs (F) were calculated by dividing the area under the serum level time curve obtained after intratracheal instillation by the respective area under the elimination time curve obtained after intravenous administration by using the formula

$$F = AUC(lung) \times dose(iv)/AUC(iv) \times dose(lung)$$

where AUC(lung) is area under the respiratory tract passage time curve, AUC(iv) is area under the elimination time curve, dose(iv) is dose administered intravenously, and dose(lung) is dose given intratracheally (6). The areas under the time curves were estimated using the linear trapezoidal rule.

Student's *t* test was used for the statistical evaluations. The significance levels used are described in Tables 1–4 and Fig. 1.

RESULTS

The lung inflammatory indexes presented in Tables 1 and 2 show that there are no differences in either the bronchoalveolar lavage parameters or the wet-dry weight difference of the lungs between uninstilled rats and control rats intratracheally instilled with the marker solution only. Only minor indications of protein marker breakdown were found in blood after lung passage as controlled by crossed immunoelectrophoresis for BSA and by double-gel diffusion for BIgG (see Ref. 9).

As presented in Table 3, BIgG and BSA marker serum levels 16 h after intratracheal instillation did not differ between the control rats instilled with the markers alone and rats instilled with the markers together with dextran. However, for the rats instilled with LPS and for those given ferritin the serum levels of BIgG and BSA were significantly higher. The increase was more pronounced for the high dose of ferritin (50 mg/kg body wt); however, a mortality of 100% was obtained during the first 24 h postinstillation. None of the rats instilled with the lower dose of ferritin (5 mg/kg body wt) died. In animals instilled with the chelator desferrioxamine, to-

TABLE 1. *Total protein content and total cell counts obtained in bronchoalveolar lavage and water content in lungs from uninstilled rats and rats 16 h after intratracheal instillation with marker molecules BSA, BIgG, and dDAVP administered without or with inflammatory-inducing substances dextran, endotoxin (LPS), or ferritin*

Experiment	Bronchoalveolar Lavage			Lungs	
	n	Protein, mg	Cell number, $\times 10^6$	*n*	Water content, %
Uninstilled	9	7.5±0.9	4.0±3.2	6	78.8±0.2
Control	9	7.1±2.3	3.7±4.2	9	79.4±0.5
Dextran	6	6.2±0.9	5.1±3.9	6	79.2±0.3
LPS	6	8.2±3.2	19.0±9.4*	6	80.2±0.5†
Ferritin (50 mg/kg)	11	45.4±25.0*‡	39.0±33.1†	6	81.9±0.5*
Ferritin (5 mg/kg)	11	17.5±5.3*	36.1±9.8*	6	80.9±0.9†
Ferritin (5 mg/kg) + Desferal	10	19.0±5.8*	51.7±24.9*	6	83.9±0.8*§
Desferal		ND	ND	6	84.5±1.3*§
Ferritin (5 mg/kg) + mannitol	6	18.6±4.8*	65.3±17.2*§	6	80.9±0.7*

Values are means ± SD; *n*, no. of rats. Water content refers to wet-dry weight difference. BSA, bovine serum albumin; BIgG, bovine immunoglobulin G; dDAVP, 1-deaminocysteine-8-D-arginine vasopressin; LPS, lipopolysaccharide; ND, not determined. * Significantly different from control rats, $P < 0.001$. † Significantly different from control rats, $P < 0.01$. ‡ Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.01$. § Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.001$.

gether with ferritin, the 16-h-marker serum levels were significantly lower compared with those instilled with ferritin alone, but the serum levels did not return to that of the control rats. Intratracheally instilled desferrioxamine did not by itself change the serum levels of BSA, while it slightly increased BIgG serum levels compared with the control levels 16 h after instillation. Treatment of ferritin-instilled animals with low doses of the radical scavenger, mannitol, resulted in lower serum levels of BSA, while no effect was seen for BIgG compared with rats given ferritin alone.

The total protein content in the lavages from dextran- and LPS-exposed animals was similar to that for the control rats, but markedly increased in rats instilled with ferritin (Table 1). Although the total lavage cell numbers were unchanged in dextran-exposed rats (Table 1), there were higher numbers of neutrophils in the lavage from these animals (Table 2). Significantly higher total cell numbers were obtained in LPS- and ferritin-exposed rats (Table 1). These increases were due to a recruitment of both macrophages and neutrophils (Table 2). Mucus particles were consistently observed in the lavage fluid from the endotoxin-exposed animals. Rats instilled with ferritin together with desferrioxamine or mannitol had higher lavage cell numbers compared with animals instilled with ferritin alone (Table 1) due to a recruitment of macrophages (Table 2), while the total protein contents in the lavage did not differ.

The water content of lungs from dextran-instilled rats did not differ from the control rats, while the endotoxin- and ferritin-instilled rats showed significant increases, indicating edema formation in the lungs in both these groups (Table 1). Even higher lung wet-dry weight differences were obtained in ferritin-exposed rats given desferrioxamine and in rats given desferrioxamine together with the markers alone, indicating that this increased edema formation was due to the desferrioxamine treatment.

The marker blood serum concentration vs. time curves (Fig. 1, *A–C*) showed that BIgG and BSA levels increased up to a maximum at 16–24 h (48–96 h for desferrioxamine-treated rats), while for dDAVP maximal serum levels were obtained 1–4 h after instillation. The calculated AUC generally confirmed the differences obtained

TABLE 2. *Cell types obtained in bronchoalveolar lavage from uninstilled rats and rats instilled with marker solution without or with inflammatory-inducing substances dextran, endotoxin (LPS), and ferritin*

Experiment	*n*	Macrophages	Neutrophils	Eosinophils
Uninstilled	7	4.0 (1.2–9.4)	0.02 (0.0–0.1)	0.01 (0.0–0.04)
Control	7	3.6 (0.5–10.8)	0.1 (0.0–0.5)	0.01 (0.0–0.1)
Dextran	6	4.0 (1.4–9.8)	0.9 (0.2–2.6)	0.1 (0.0–0.4)
LPS	5	14.2 (6.9–23.6)	4.7 (2.9–10.1)	0.1 (0.0–0.4)
Ferritin (50 mg/kg)	8	17.4 (7.6–36.0)	21.2 (1.4–71.0)	0.3 (0.0–1.3)
Ferritin (5 mg/kg)	9	22.0 (3.6–46.6)	13.5 (0.5–28.9)	0.7 (0.0–3.5)
Ferritin (5 mg/kg) + Desferal	7	48.2 (26.1–94.1)	3.5 (0.0–12.6)	0.0 (0.0–0.0)
Ferritin (5 mg/kg) + mannitol	6	55.2 (39.4–85.1)	6.7 (0.0–30.0)	3.4 (0.0–14.8)

Values are means $\times 10^6$ with range within parentheses; *n*, no. of rats.

TABLE 3. *Serum levels of BIgG and BSA in uninstilled rats and rats 16 h after intratracheal instillation with marker molecules alone or together with inflammatory-inducing substances dextran, endotoxin (LPS), or ferritin*

Experiment	*n*	Marker Serum Levels, μg/ml	
		BIgG	BSA
Uninstilled	15	0	0
Control	21	2.7±1.1	1.5±0.9
Dextran	12	2.2±0.4	2.3±1.0
LPS	12	8.8±0.7*	22.7±3.5*
Ferritin (50 mg/kg)	17	69.7±20.7*†	49.8±14.6*‡
Ferritin (5 mg/kg)	17	39.5±7.9*	39.6±4.8*
Ferritin (5 mg/kg) + Desferal	17	7.2±1.0*†	4.2±2.1*†
Desferal	6	4.1±0.9§	1.4±0.8
Ferritin (5 mg/kg) + mannitol	12	35.9±9.1*	27.9±3.2*†

Values are means ± SD; *n*, no. of rats. * Significantly different from control rats, $P < 0.001$. † Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.001$. ‡ Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.01$. § Significantly different from control rats, $P < 0.01$.

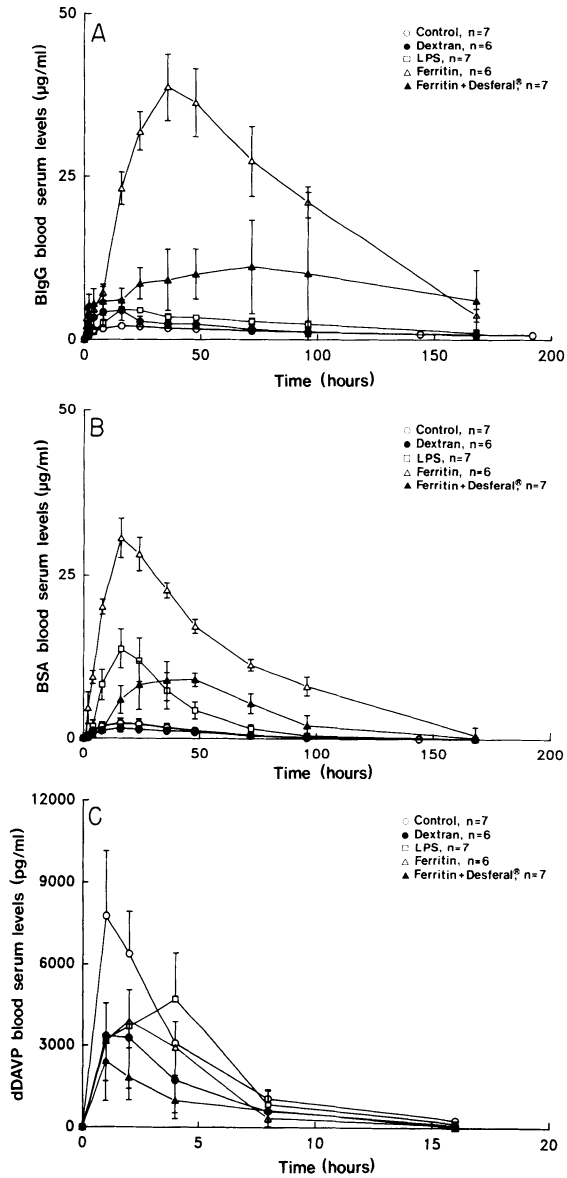

FIG. 1. Blood serum concentrations of bovine immunoglobulin G (BIgG, *A*), bovine serum albumin (BSA, *B*), and 1-deaminocysteine-8-D-arginine vasopressin (dDAVP, *C*) in adult rats 0–168 h after intratracheal instillation of 15 mg BIgG, 5 mg BSA, and 100 μg dDAVP/kg body wt without or with the inflammatory substances, dextran (5 mg/kg body wt), LPS (50 μg/kg body wt), or ferritin (5 mg/kg body wt). Values are means ± SD. In some rats the iron chelator desferrioxamine (Desferal, 250 mg/kg body wt) was intratracheally instilled together with ferritin.

from the single blood samples between the control rats and the rats given the inflammatory-inducing substances (Table 4). For dDAVP the AUC values obtained after intravenous administration were significantly lower in the rats that received the inflammatory-inducing substances intratracheally than in the control rats, while no such differences were found for BIgG and BSA.

Calculations of the total amount of markers transferred over the lungs from the AUC values, as presented in Table 4, showed that instillation of the markers together with dextran had no effect on the marker transfer. Instillation of LPS resulted in a significantly higher transfer of all the markers compared with the control rats. The highest lung transfer was obtained when using ferritin as the inflammatory-inducing substance. Instillation of ferritin together with desferrioxamine resulted in significantly lower transfers for all three markers as compared to the ferritin-exposed rats, with dDAVP returning to control levels (Table 4).

DISCUSSION

The present investigation shows that the proteins BIgG and BSA and the nonapeptide dDAVP pass via the lower respiratory tract into the blood circulation of unexposed rats after intratracheal instillation to an extent depending on their molecular weights. These findings in the normal rat are in accordance with previous data from our laboratory (9). Moreover, this study shows that the inward passage increased after experimentally induced inflammation, apparently correlated to the severity of the injury.

Intratracheal instillation was chosen as the method for marker delivery because it is a convenient method for achieving an accurate and controlled deposition (9, 27). The instillation procedure per se did not cause any changes in the inflammatory parameters investigated compared with uninstilled rats, and thus there were no indexes of a lung injury that could be accounted to the instillation procedure or the permeability markers used.

An influx of leukocytes, mainly neutrophils and macrophages/monocytes, into the luminal side of the respiratory tract has been reported after lung exposure to injurious substances (8, 13). We have therefore used increased cell numbers and changes in the cell populations in the bronchoalveolar lavage as indexes of a proceeding inflammation in the lungs. Furthermore, total protein content in the lavage fluid was used as an indicator of exudation of blood plasma into the lungs and the lung wet-dry weight difference was used as an edema indicator to characterize the severity of the experimentally induced inflammation (13).

In our study, intratracheal instillation of soluble dextran resulted in no marked effects on the marker passage in the lower respiratory tract. However, evidences of inflammation, such as neutrophilia and a tendency to increased total cell numbers in the lavage, were found in these rats, indicating some deleterious effects. In a study on liquid and protein clearance from the sheep lung, Matthay et al. (22) showed that intratracheal instillation of only saline resulted in a neutrophilia in the lavage, indicating that the presence of fluid itself can cause inflammatory changes. However, this was not true in the present study as the marker solution alone did not evoke any neutrophilia. Instead, the effects can be explained by the fact that Sprague-Dawley rats are hypersensitive to dextran (32) and some inflammatory reactions may take place in the lungs, which, however, are not severe enough to affect the molecular passage.

A more pronounced lung damage was obtained after local endotoxin exposure with LPS as significantly increased lavage cell numbers were found, mainly due to an influx of neutrophils and macrophages/monocytes. Moreover, a moderate increase was found in the lung wet-dry weight difference, indicating some edema formation. Similar cellular changes after exposure to an endo-

TABLE 4. *AUC calculated from marker serum level vs. time curves obtained after either intravenous or intratracheal marker administration and total lung transfer of BIgG, BSA, and dDAVP in control rats only given marker solution and in rats during inflammatory conditions induced after intratracheal instillation of dextran, endotoxin (LPS), or ferritin with and without Desferal*

Experiment	n	AUC			Lung Transfer		
		BIgG	BSA	dDAVP	BIgG	BSA	dDAVP
Control							
it	7	358±76	111±38	36,269±7,445	1.8±0.4	4.1±1.3	20.0±3.8
iv	4	19,400±3,601	2,668±155	181,670±16,188			
Dextran							
it	6	351±125	81±29	16,148±14,013*	1.8±0.6	3.5±1.2	26.1±20.7
iv	3	19,450±1,192	2,308±229	61,909±2,501†			
LPS							
it	7	512±153	474±141†	25,683±10,010	3.2±0.9*	22.0±6.1†	33.3±12.0*
iv	3	16,248±1,071	2,152±86*	77,032±25,859†			
Ferritin							
it	6	3,524±512†	1,751±215†	18,253±3,322†	17.6±2.3†	60.0±6.7†	41.6±6.9†
iv	3	19,978±1,189	2,975±524	43,851±17,181†			
Ferritin + Desferal							
it	7	1,618±1,050*‡	584±119†§	10,418±4,126†‡	11.9±7.1*	26.4±5.0†§	15.2±5.5§
iv	3	13,609±2,499	2,185±313	68,360±19,210*			

Values are means ± SD; *n*, no. of rats. Lung transfer is expressed in % of given dose. AUC, area under the curve [calculated using trapezoidal rule (9)]; it, intratracheal; iv, intravenous. * Significantly different from control rats, $P < 0.01$. † Significantly different from control rats, $P < 0.001$. ‡ Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.01$. § Significantly different from ferritin-exposed rats (5 mg/kg), $P < 0.001$.

toxin aerosol have been found in guinea pigs, and endotoxin has been shown to activate the inflammatory cells in the airways within a few hours after exposure (8). A hypersecretion of mucus from the nasal epithelium after endotoxin exposure was reported by Harkema et al. (11), and in our study mucus particles were consistently found in the lavage fluid from the endotoxin-exposed rats. The inflammatory changes obtained in endotoxin-exposed rats resulted in an increased lung passage of all the markers. Although the inward passage into the blood was increased there were no indications of damage to the endothelial barrier resulting in an exudation of plasma proteins because the protein contents in the lavage fluid remained unchanged.

The most marked increase in the lung passage of all the markers was obtained in the rats exposed to ferritin. A severe lung inflammation and edema formation were obtained since the total lavage cell numbers were markedly increased due to an influx of both macrophages/monocytes and neutrophils together with an increased exudation of plasma proteins. When the high dose of ferritin was used, an even more pronounced inflammation occurred causing 100% mortality within 24 h after instillation.

The crucial role of iron in the observed changes in the marker passage over the lungs after ferritin exposure was shown by addition of the iron chelator desferrioxamine. This treatment resulted in a decreased lung transfer that approached the passage in the control rats. No effects on the lavage cell counts and protein exudation were obtained, while an increase in the lung wet-dry weight difference was seen in the desferrioxamine-treated ferritin-exposed rats compared with the ferritin-exposed rats. The latter effect was shown to be due to desferrioxamine itself, as similar lung edema was obtained after the intratracheal instillation of only desferrioxamine. This and the increase in the BIgG passage obtained might be due to the hyperosmolaric desferrioxamine solution used.

Iron bound in the ferritin molecule is believed to be relatively inert and harmless to the tissue (1), but if it is released it is likely to participate in the iron-catalyzed Haber-Weiss reaction, leading to deleterious radical formation (24). Iron may be released from ferritin after leukocyte stimulation, by degradation of the ferritin molecules, and after exposure to $\cdot O_2$ and other reducing radicals (1, 27). An extended radical formation in the lungs results in an increased lipid peroxidation of the tissue and may finally lead to the observed increased marker passage over the lungs. In fact, radical formation by ferritin was indicated in this study as the hydroxyl radical scavenger, mannitol, decreased the passage of the markers via the lungs in ferritin-exposed rats.

During inflammatory conditions lung leukocytes generate and release free radicals, proteolytic enzymes, basic proteins, and other active metabolites that all can cause damage to the epithelial-endothelial barriers in the lungs (12). Such damage has been shown to lead to a loss of the structural integrity of the endothelial barrier, which in turn results in extravascular solute and protein flux, ending in a protein-rich edema (30). Similar damage might also occur in the epithelial barrier, resulting in the observed changes in the marker passage from the air spaces into the vascular compartment. We conclude that inflammatory agents deposited in the respiratory tract lead to an increase in the passage of different-sized molecules in the molecular weight range 1,000–150,000 Da, apparently dependent on the severity of the induced inflammation. The passage of BIgG and BSA is affected to a higher degree by the inflammatory changes in the lungs than the passage of dDAVP (cf. Table 4). These differences indicate that the macromolecular markers and the peptide marker traverse the lung epithelium by different

routes. Small molecules, such as dDAVP, are believed to pass predominantly paracellulary through the tight junctions (25). Our results may suggest that the paracellular passage is affected by the inflammatory changes, opening this route also for macromolecules. However, an increased active transcellular transport of the marker macromolecules can not be excluded as it has been shown that BSA passes over the canine bronchial epithelium via an endocytotic pathway (16). Similar changes in the passage of macromolecules and mannitol were obtained after an aerosol challenge with a specific antigen, indicating that both increases in paracellular as well as in transcellular passage of molecules across this barrier occurs during inflammation (28).

Our thanks to Inger Mattsson and Brita Nilsson for excellent and skillful work with the analyses, Dr. Stefan Lundin for the dDAVP analysis, Prof. Håkan Bergstrand (Draco) for valuable ideas and advice, and Dr. Cynthia de Wit for reading the text.

This investigation was supported by the Swedish Council for Forestry and Agricultural Research, Draco Foundation for Scientific Research, the Swedish Council for Natural Sciences, and the Hierta Retzius Foundation.

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Received 26 September 1990; accepted in final form 30 April 1991.

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