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Aerosolized endotoxin is immediately bound by pulmonary surfactant protein D in vivo

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Abstract

Collectins are carbohydrate binding proteins that are implicated in innate host defense. The lung collectins, surfactant proteins A and D (SP-A and SP-D), bind a variety of pathogens in vitro and influence phagocytosis by alveolar macrophages. In this report we show that SP-D binds endotoxin (lipopolysaccharide, LPS) in vivo in a rat model of acute respiratory distress syndrome (ARDS). Intratracheal aerosolization of LPS in rats resulted in the typical features of human ARDS. Total amounts of SP-D, as well as the carbohydrate binding properties of SP-D were measured in lung lavage as a function of time. The amount of SP-D did not change during 24 h. Interestingly, SP-D in lung lavage isolated from rats during the first 2 h after LPS treatment, was not able to bind to carbohydrate. Further analysis revealed that the carbohydrate binding sites of SP-D were occupied by LPS, suggesting that SP-D is an LPS scavenging molecule in vivo. Electron microscopic analysis indicated that, 1 h after LPS aerosolization, aggregates of SP-D with LPS were found in lysosomal structures in alveolar macrophages. We conclude that the lung collectin SP-D binds inhaled endotoxin in vivo, which may help to protect the lung from endotoxin-induced disease.

Keywords: Surfactant protein D (SP-D); Lung; Lipopolysaccharide; Collectin; Alveolar macrophage; Host defense

1. Introduction

Lipopolysaccharide (LPS) is an important mediator of septic shock and acute respiratory distress syndrome (ARDS) in the setting of sepsis, a life-threatening inflammatory lung condition with mortality rates that are still unacceptably high at 50% [1]. LPS is a component of the outer membrane of Gram-negative bacteria and a powerful pro-inflammatory agent. LPS activates leukocytes to release a wide variety of inflammatory mediators, which contribute to the pathogenesis of sepsis and ARDS [2].

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The respiratory tract is continuously exposed to LPS from the cell wall of inhaled Gram-negative bacteria [3]. For this reason the epithelial surface of the lung must have an efficient defence system to protect the gas-exchange function of the alveoli, essential for breathing. The alveolar surface of the lung is lined with pulmonary surfactant, a mixture of lipids and proteins, which is thought to play a role in the first line defence against invading bacteria and other pathogens [4–6]. One of the known surfactant proteins, surfactant protein D (SP-D), is thought to be a molecule of an innate immune system to protect the lung [7–10].

Firstly, SP-D is a so-called collectin, a protein which consists of a collagen-like domain and a lectin domain that binds carbohydrates in a Ca^{2+}-dependent manner [7]. This structure allows SP-D to interact with bacteria as well as with phagocytic cells. SP-D selectively recognizes carbohydrates on the cell wall of bacterial pathogens with its carbohydrate binding site and might thus contribute to the lungs' defence against these pathogens. Furthermore, several in vitro studies suggest a role in innate defence for SP-D. It is known, for example, that SP-D is able to bind and agglutinate bacteria and viruses [11,12]. It is also known that SP-D can activate neutrophils, monocytes and alveolar macrophages [6] and that SP-D is able to bind LPS with its carbohydrate binding site in vitro [13].

Recently, it was reported that, in rats, SP-D levels are increased in lung lavage 72 h after intratracheal LPS instillation in vivo [14]. However, it is not known whether SP-D is involved in the acute response to LPS in LPS-induced disease in vivo, and whether SP-D is able to bind LPS in the lung, in vivo. In this study, we used an established model of ARDS [15], in which LPS is aerosolized intratracheally in rats, to investigate whether the lung collectin SP-D is able to bind LPS in an acute phase reaction to intratracheally aerosolized LPS.

2. Materials and methods

2.1. Rat model for human ARDS

Forty-five male albino Wistar rats (180–200 g), obtained from Charles River (Germany) were used in this study. The animals were kept at a regular 12 h light/dark cycle, with a temperature of 22 ± 2°C. Food and water were given ad libitum.

We used the animal model as described by Van Helden et al. [15]. Briefly, the rats were intubated with an aerosolizer under brief halothane (Albic BV, Maassluis, The Netherlands) anaesthesia. Endotoxin-free Dulbecco's PBS at pH 7.4 was used to dissolve LPS (rough LPS from *Salmonella Enteritidis*, Sigma, St. Louis, MO, USA) for intratracheal aerosolization. PBS (0.5 ml; control) or LPS (0.5 ml; 16 mg/kg) dissolved in PBS, was instilled with a miniature nebulizer. The solution was dispersed in the trachea, just above the bifurcation. This relatively high dose of LPS was needed to obtain a manifest clinical picture of ARDS within 24 h. ARDS was diagnosed in this model 24 h after LPS was aerosolized according to the lung injury score for patients, which includes the extent of the inflammatory density on chest X-rays, the severity of hypoxemia, the decline in lung compliance and changes in respiratory frequency [16].

At time points 0, 1, 2, 3, 5, 10, 16 and 24 h after aerosolization 3–5 rats of the PBS-treated group and the LPS-treated group were killed and broncho-alveolar lavage (BAL) was performed with 5 subsequent vol of 8 ml PBS. These vols were pooled and cells were pelleted by centrifugation (10 min, $150 \times g$). The supernatant (40 ml, stored at −20°C) was used to determine the total amounts of SP-D and the carbohydrate binding properties of SP-D.

2.2. Rat SP-D ELISA

Total amounts of SP-D in cell-free BAL were measured by enzyme-linked immunosorbent assay. The polyclonal anti-rat SP-D antibodies used in this ELISA were raised in rabbits and do not cross-react with rat SP-A. Also, the antibodies react equally well with SP-D in the presence of LPS as in the absence of LPS. The assay is linear in the range of 3–100 ng rat SP-D/ml. Rabbit anti-rat SP-D polyclonal antibodies were diluted in 0.1 M $NaHCO_3$ buffer (pH 9.6) and coated on a polystyrene 96-well microtiter plate (maxisorb, NUNC, Roskilde, Denmark; 50 μl/well, 16 h, 4°C). After coating, the microtiter plate was blocked with 3% non-fat dried milk (Protifar, Nutricia, Zoetermeer, The Netherlands) in

washing solution (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH 7.4, 200 μl/well) for 60 min at 22°C. Then the plate was washed six times in washing solution. Cell-free BAL samples were diluted in washing solution containing 0.1% BSA and 50 μl of the samples was applied to each well of the microtiter plate. After 1 h of incubation at 22°C, the plate was washed six times in washing solution and a biotin-conjugated rabbit anti-rat SP-D polyclonal antibody, diluted in washing solution containing 0.1% BSA, was applied to the plate (50 μl/well, 22°C, 1 h). After washing the plate six times in washing solution, the bound biotin-conjugated antibodies were detected with horseradish peroxidase-conjugated streptavidin (0.1 μg/ml, Sigma, St. Louis, MO, USA), diluted in washing solution containing 0.1% BSA (50 μl/well). After 1 h incubation at 22°C the plate was stained with 150 μl/well tetramethylbenzidine reagent (100 μg tetramethylbenzidine (Merck, Darmstadt, Germany)/ml, 1 mM H_2O_2 in 0.1 M citric acid buffer, pH 4.0). The reaction was stopped by adding 50 μl 2 M H_2SO_4 and absorption was measured at 450 nm.

2.3. Enzyme-linked mannan binding assay

Carbohydrate binding properties of SP-D in cell-free BAL were measured by an enzyme-linked mannan binding assay. Mannan (from *Saccharomyces cerevisiae*, Sigma, St. Louis, MO, USA) was activated by partial periodate oxidation, using conditions that result in oxidation of about 1 out of 10 mannan saccharide subunits. Briefly, 100 mg mannan was dissolved in 1.5 ml 40 mM $NaIO_4$ (pH 7.0). After incubation (1 h, 22°C), the partially oxidized mannan was dialyzed against demineralized water. The aldehyde groups of the activated mannan were coupled to primary amino groups of BSA as follows. One ml of activated mannan solution (10 mg/ml in 0.2 M H_3BO_3, 100 mM $NaCNBH_3$, pH 9.0) was mixed with 1 ml BSA solution (10 mg/ml in demineralized water). After coupling (16 h, 4°C) the non-reacted aldehyde groups were blocked by addition of 0.1 ml ethanolamine (1 M, pH 9.0) and incubated for 1 h at 22°C. The BSA-conjugated mannan was dialyzed against 50 mM Tris-HCl, 5 mM $CaCl_2$ (pH 7.5).

For the enzyme-linked mannan binding assay, the BSA-conjugated mannan was dissolved in 0.1 M $NaHCO_3$ buffer (pH 9.6) at a concentration of 20 μg/ml, and coated on a polystyrene 96-well microtiter plate for 16 h at 4°C. After coating the BSA-conjugated mannan to the plate, the procedure is the same as for the rat SP-D ELISA, except that all buffers used in this assay have a final concentration of 5 mM $CaCl_2$. To study whether the carbohydrate binding sites of SP-D were occupied by LPS after LPS aerosolization, cell-free lavage samples were pretreated with TBS-EDTA (10 mM Tris-HCl, 150 mM NaCl, 0.1% BSA, 0.05% Tween-20 and 2.5 mM EDTA) for 1 h at 37°C, prior to applying these samples to the blocked mannan-BSA coated plates in the presence of calcium ions.

2.4. Purification of rat SP-D

Rats were treated with silica to induce lipoproteinosis [17]. Lungs of these rats were washed four times with 8 ml TBS (5 mM Tris-HCl, 150 mM NaCl, pH 7.4). Cells were removed by centrifugation ($150\times g$, 10 min, 4°C), and the cell-free lavage was centrifuged for 2 h at $28\,000\times g$ at 4°C and the surfactant fraction was removed. The high speed supernatant was concentrated with an Amicon YM 30 membrane (Millipore, Etten-Leur, The Netherlands). Solid ammonium sulfate was added to the concentrated supernatant to a final concentration of 5% saturation and centrifuged (20 min, $10\,000\times g$, 4°C). The pellet was discarded and the remaining proteins of the supernatant were concentrated by addition of solid ammonium sulfate to a final concentration of 60% saturation, and precipitated by centrifugation (20 min, $10\,000\times g$, 4°C). The precipitated proteins were dissolved in TBS and extensively dialyzed against TBS (50 mM Tris-HCl, 150 mM NaCl, pH 7.4). Before the protein sample was applied to a maltosyl-agarose column (Sigma) 1 M $CaCl_2$ was added to a final concentration of 5 mM. After application of the sample to the column, the column was washed extensively with TBS-Ca (50 mM Tris-HCl, 500 mM NaCl (high salt), 5 mM $CaCl_2$, pH 7.4) and SP-D was eluted from the column with TBS-EDTA (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, pH 7.4). SP-D appeared as a single 43 kDa band on SDS-PAGE under reducing conditions.

2.5. Mannan binding of SP-D after incubation of SP-D with LPS in vitro

We investigated whether in vitro incubation of SP-D with LPS resulted in decreased binding to carbohydrate. 50 μl of purified rat SP-D (0–10 ng/well) was incubated with 50 μl LPS (0–5 μg/well), both diluted in TBS-Ca (50 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.4) containing 0.05% Tween-20, in a 96-well microtiter plate. After incubation for 1 h at 37°C, the SP-D-LPS solutions were transferred to a blocked mannan-BSA coated microtiter plate in the presence of calcium ions and the procedure for the enzyme-linked mannan binding assay was followed as described above.

2.6. FITC-labeled LPS aerosolization

Six rats were used in this experiment. We used the rat model for ARDS as described above, but in this study FITC-labeled LPS (from *S. Enteritidis*, Sigma) was intratracheally aerosolized. The FITC-labeled LPS was mixed with unlabeled LPS in a 1:3 ratio. At 1, 10 and 24 h after aerosolization rats were killed, BAL was performed, cells were pelleted and total amounts of SP-D as well as the carbohydrate binding ability of SP-D in the cell-free BAL were measured.

2.7. Electron microscopy

Cell pellets were fixed (2% (w/v) paraformaldehyde and 0.5% glutaraldehyde in PBS) overnight at 4°C. Subsequently the samples were centrifuged ($6000\times g$, 10 min) and the pellet was resuspended in PBS containing 50 mM glycine (pH 7.4) to block free aldehyde groups. After 10 min the procedure was repeated and the resuspended samples were left for 1 h in the PBS/glycine. Samples were centrifuged for 10 min ($6000\times g$) and the pellet was resuspended in 10% (w/v) gelatine in 0.1 M sodium phosphate buffer (pH 7.4) at 37°C. Subsequently, the samples were centrifuged for 2 min at $9500\times g$. Samples were cut out the tube and placed in fixative (2% (w/v) paraformaldehyde and 0.5% glutaraldehyde in PBS). Ultrathin cryosections were cut at −120°C on a Reichert UltracutS/FCS (Leika Aktiengesellschaft, Vienna, Austria) using a cryo diamond knife (Drukker, Cuyk, The Netherlands) and were transferred to formvar carbon coated nickel grids. Thawed ultrathin cryosections were incubated in the following solutions: PBS/glycine (15 min), block buffer (5% BSA, 0.1% cold water fish skin gelatin (Aurion, Wageningen, The Netherlands) in PBS, pH 7.4, 30 min), incubation buffer (0.1% BSA-C (Aurion, Wageningen, The Netherlands) in PBS, pH 7.4, 5 min), rabbit anti-rat SP-D in incubation buffer (1 h), incubation buffer (3×10 min), protein A-gold particles (10 or 15 nm, 1 h), incubation buffer (3×10 min), rabbit anti-FITC (Molecular Probes, Eugene, OR, USA) in incubation buffer (1 h), incubation buffer (3×10 min), protein A-gold particles (10 or 15 nm, 1 h), incubation buffer (3×10 min), PBS (2×5 min), 2% glutaraldehyde in PBS (2 min), PBS (2×5 min), distilled water (4×5 min), 2% uranyl acetate-oxalate (pH 7.4, 10 min). Stained cryosections were embedded in 1.8% methylcellulose containing 0.3% uranyl acetate. Immunogold-labeled cryosections were observed and photographed in a Philips CM10 electron microscope (Philips, Eindhoven, The Netherlands) at 80 kV.

3. Results

3.1. Rat model for human ARDS

Within 24 h LPS-treated rats showed typical signs of human ARDS, according to the lung injury score for patients [16]. Furthermore, the amount of protein in the cell-free BAL increased severely in the LPS-treated rats [15]. Ten h after LPS was aerosolized the amount of protein reached a plateau at which the amount of protein was 16-fold higher than before LPS aerosolization. Also, the number of neutrophils in the total lavage increased gradually in time and was significantly increased after 5 h [15]. Furthermore, TNF-α levels in the cell-free BAL increased severely in the first h after LPS was aerosolized and peaked at 5 h after treatment [15].

3.2. Total amounts of SP-D

When total amounts of SP-D were measured in cell-free BAL, no significant differences were observed between the LPS-treated group and the con-

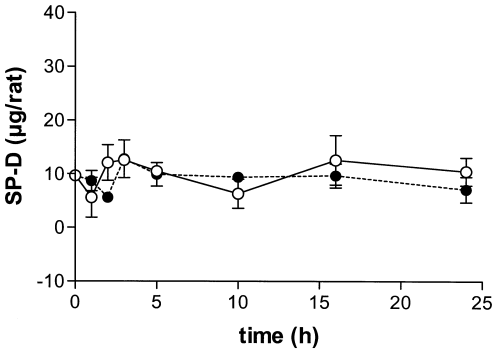

Fig. 1. Total amounts of SP-D in broncho-alveolar lavage, determined by sandwich ELISA. No difference was observed between the LPS-treated group (●) and the control group (○) ($n=3$). Data are represented as mean ± S.E.M. of triplicate determinations. The data are representatives of one of two independent experiments.

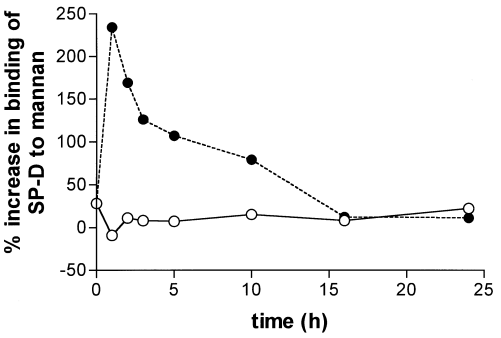

Fig. 3. Effect of EDTA pretreatment on carbohydrate binding properties of SP-D in the LPS-treated group (●) and the control group (○) ($n=3$). Results are expressed as the % increase in carbohydrate binding compared to the carbohydrate binding before EDTA pretreatment. Data are represented as mean of triplicate determinations. The data are representatives of one of two independent experiments.

trol group (Fig. 1). The amount of SP-D did not change over time (Fig. 1).

3.3. Carbohydrate binding properties of SP-D

Intratracheally aerosolized LPS decreased the ability of SP-D to bind to mannan (Fig. 2). During the first 2 h after LPS aerosolization, the percentage of SP-D bound to mannan decreased to less than 10% compared to the initial percentage of binding. At later time points, 10, 16 and 24 h after aerosolization, the percentage of SP-D bound to mannan-BSA was still significantly decreased by 30–40% in the LPS-treated rats, compared with the control group.

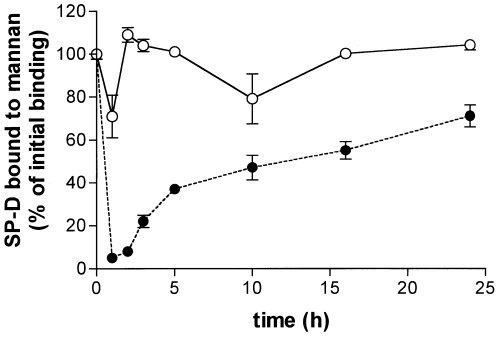

Fig. 2. Carbohydrate binding properties of SP-D in broncho-alveolar lavage, determined by the enzyme-linked mannan binding assay. SP-D from the LPS-treated rats (●) is not able to bind to mannan immediately after aerosolization compared to SP-D from control rats (○) ($n=3$). Data are represented as mean ± S.E.M. of triplicate determinations. The data are representatives of one of two independent experiments.

To study whether the decreased percentage of SP-D bound to mannan after LPS aerosolization was caused by LPS occupying the Ca^{2+}-dependent carbohydrate binding sites of SP-D, the lung lavage samples were pretreated with EDTA, prior to application to a blocked mannan-BSA coated plate in the presence of calcium ions. We found that EDTA pretreatment of the cell-free BAL samples from LPS-treated rats increased the amount of SP-D bound to mannan (Fig. 3). During the first h after LPS aerosolization, the percentage of SP-D bound to mannan was increased by 234%. This supports the previous finding that the ability of SP-D to bind mannan was especially decreased during the first h after LPS-aerosolization. At 16 and 24 h after aerosolization, EDTA pretreatment of lung lavage samples from LPS-treated rats did not increase the percentage SP-D bound to mannan. EDTA pretreatment of the lung lavage samples from the control rats did not have any effect on the percentage of SP-D bound to mannan (Fig. 3).

3.4. Mannan binding of SP-D after incubation of SP-D with LPS in vitro

Preincubation of purified rat SP-D with LPS in vitro also decreased the ability of SP-D to bind to mannan (Fig. 4). When purified rat SP-D was incubated with 5 µg LPS, prior to application to a mannan-BSA coated plate, the percentage of SP-D bound to mannan decreased to less than 5% com-

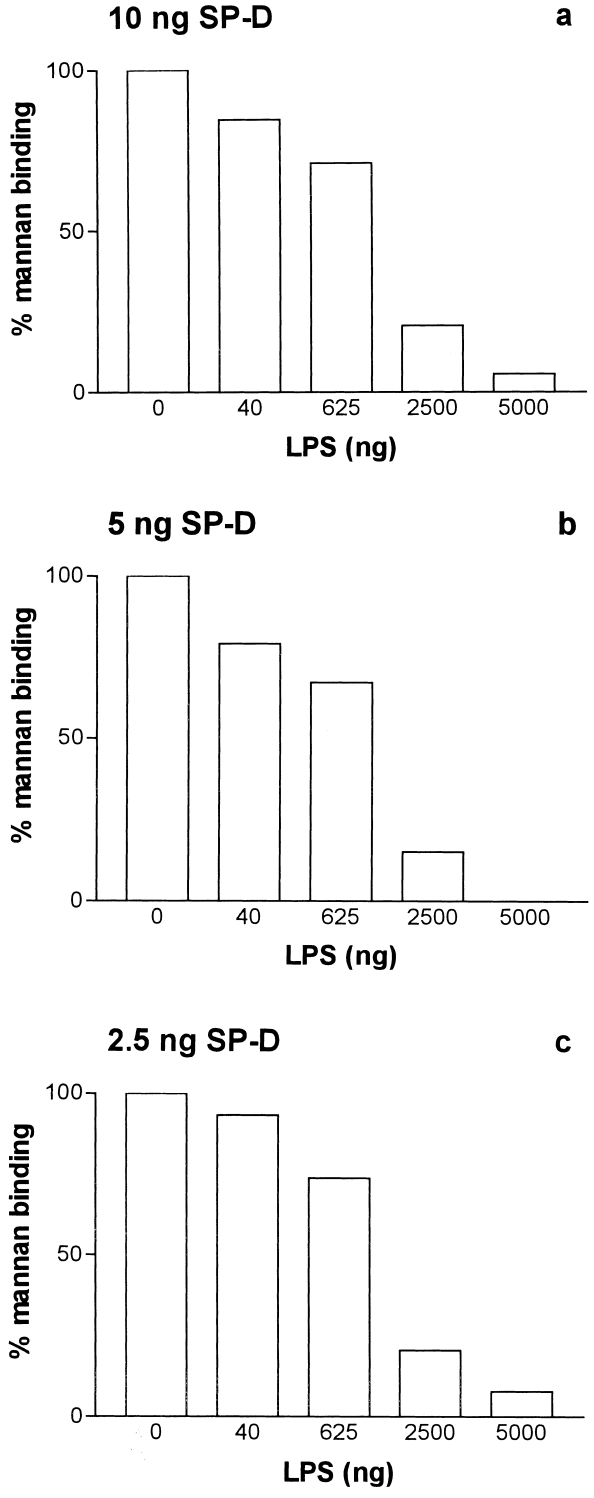

Fig. 4. Carbohydrate binding properties of rat SP-D after in vitro preincubation with LPS. Incubation of SP-D with LPS decreased the ability of SP-D to bind to mannan considerably. Data are expressed as % of binding relative to binding of 10 ng SP-D to mannan in the absence of LPS. a: 10 ng SP-D; b: 5 ng SP-D; c: 2.5 ng SP-D. The data are representatives of one of two independent experiments.
←

pared to the binding of SP-D to mannan in the absence of LPS (Fig. 4).

3.5. FITC-labeled LPS aerosolization

Rats were treated with FITC-labeled LPS to determine the fate of LPS in the lung after aerosolization. The observed effects of FITC-labeled LPS aerosolization on the lungs were similar to those observed after aerosolization with unlabeled LPS. Furthermore, the amount of SP-D in the cell-free BAL of the FITC-LPS-treated rats did not change over time, and SP-D of the FITC-LPS-treated rats was not able to bind to mannan immediately after FITC-LPS was aerosolized.

Electron microscopic analysis showed that, immediately after aerosolization of FITC-labeled LPS, SP-D colocalizes with LPS in lysosomal structures in alveolar macrophages (Fig. 5a). In contrast, 24 h after aerosolization of FITC-labeled LPS, hardly any SP-D could be observed in the alveolar macrophages, while FITC-label was still present in the macrophages (Fig. 5b). Furthermore, hardly any SP-D could be detected in the alveolar macrophages of control rats (Fig. 5c).

4. Discussion

In this study we show that SP-D is involved in the acute phase reaction to intratracheally aerosolized endotoxin in a rat model for ARDS. Evidence is provided that SP-D binds free LPS in the lung immediately after LPS was aerosolized. During the first 2 h after LPS aerosolization, 92% of the SP-D from the LPS-treated rats was not able to bind to carbohydrate, in comparison to SP-D from the control rats. Although we were not able to design a good method to show directly that LPS is bound to SP-D in vivo, the results suggest that the carbohydrate

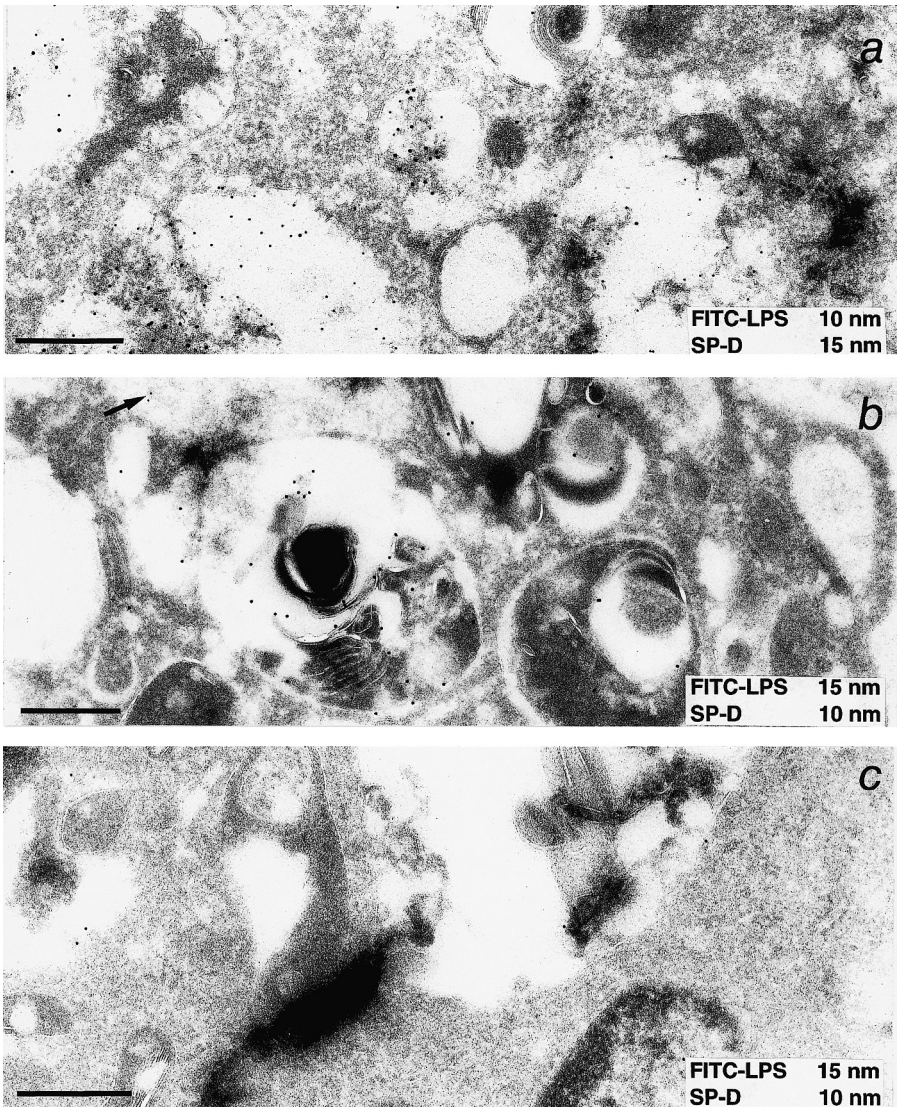

Fig. 5. Ultrathin cryosections of alveolar macrophages double labeled for SP-D and FITC. a: SP-D 15 nm gold, FITC 10 nm gold, magnification: 42 000×. One h after FITC-LPS was aerosolized, aggregates of SP-D with FITC-LPS are found in lysosomal structures in the macrophages. b: SP-D 10 nm gold, FITC 15 nm gold, magnification: 38 000×. 24 h after FITC-LPS was aerosolized, FITC-label was still present in the alveolar macrophages. In contrast, hardly any SP-D (arrow) could be detected in the alveolar macrophages. c: SP-D 10 nm gold, FITC 15 nm gold, magnification: 45 000×. In control rats hardly any SP-D was observed in the alveolar macrophages. Bars are 0.5 μm.

binding sites of the SP-D molecules in LPS-treated rats are occupied by LPS.

Furthermore, it was shown that EDTA pretreatment of lung lavage samples of LPS-treated rats increases the percentage of SP-D bound to mannan, with a maximum increase of 234%, in the first h after aerosolization. Because the binding of SP-D to carbohydrates is calcium ion dependent, EDTA pretreatment of the lung lavage samples may release the LPS from the carbohydrate binding sites of SP-D, thus enabling SP-D to bind to mannan again in the presence of calcium ions. This notion is supported by results from in vitro studies: preincubation of purified rat SP-D with LPS decreased the ability of SP-D to bind to mannan-BSA coated plates. The decreased binding of SP-D to mannan as a result of intratracheally aerosolized LPS cannot be explained by a lower amount of SP-D present in cell-free BAL

of LPS-treated rats, because the total amount of SP-D did not differ between the LPS-treated rats and the control rats during the course of the experiment. Intratracheal aerosolization of rats with FITC-labeled LPS supports the previous findings. Electron microscopic analysis of alveolar macrophages in the BAL showed that immediately after FITC-labeled LPS aerosolization SP-D colocalized with LPS in lysosomal structures in the alveolar macrophages. Based on the other experiments in this study, that show binding of LPS to SP-D immediately after LPS aerosolization, we conclude that the alveolar macrophages have phagocytosed SP-D-LPS aggregates. Although no differences in total SP-D in cell-free BAL could be detected between the groups, in preliminary experiments, we observed more SP-D in the cell fraction (mainly macrophages) of the BAL from LPS-treated rats than in the cell fraction of the BAL of control rats. This suggests that immediately after LPS aerosolization, SP-D present in the epithelial lining fluid binds the inhaled LPS, and is rapidly phagocytosed by the alveolar macrophages.

The fact that SP-D binds LPS in vivo, in an immediate reaction to intratracheally aerosolized LPS, extends previous in vitro studies [13]. The results from the present study support the growing belief that the lung collectin SP-D is a molecule of an innate immune system. Effective immunity depends on specific as well as innate immunity. The importance of specific, T-cell mediated and antibody response as defence against infection is well known. However, the innate defence mechanisms are very important to protect the lung against all kinds of potentially harmful pathogens, when the specific immune response has not yet developed. Components of innate immunity include macrophages, neutrophils, natural killer cells, cytokines and collectins [18]. In addition to the lung-specific collectins, SP-D and SP-A, serum collectins have been described: mannose binding lectin (MBL), CL-43 [19] and conglutinin (in ruminants [20]). Especially MBL is known to be an acute phase protein, important in innate immunity [21]. Because of the structural similarity between the serum and the lung collectins, the lung collectins might have a similar function in the immediate, innate defence of the lung. The results of this study show that SP-D is indeed involved in this acute phase reaction, by binding LPS immediately after aerosolization.

The biological significance of SP-D binding to free LPS remains to be established. Several possibilities should be considered. First of all, by binding free LPS, SP-D can contribute to the clearance and subsequent inactivation of free LPS that is released at sites of colonization or infection by Gram-negative bacteria. Secondly, because SP-D is able to bind LPS on the cell wall of inhaled Gram-negative bacteria and is able to affect the phagocytic functions of alveolar macrophages, either via the collectin (C1q) receptor [22] or via a specific SP-D receptor [23,24], it is possible that SP-D functions as a bridge between the Gram-negative bacteria and phagocytic cells and in this way enhances clearance and uptake of the bacteria by these cells. A third possibility is that SP-D facilitates mucociliary clearance of LPS or Gram-negative bacteria by forming complexes [13]. It was reported that SP-D can induce large aggregates of bacteria, which in some cases equalled the size of alveolar macrophages [11]. These large aggregates can easily be removed from the respiratory tract. The formation of aggregates is consistent with the structure of the SP-D molecule, which consists of several carbohydrate binding sites per SP-D molecule, separated by long collagen arms.

In the rat model for ARDS, we observed an increase of TNF-α in the LPS-treated rats that peaked 5 h after LPS was aerosolized [15]. An important effect of LPS is the induction of cytokines, like TNF-α, by macrophages. It would be interesting to study whether SP-D alone or SP-D/LPS complexes have an effect on cytokine induction. It is known, for example, that LPS binding protein (LBP) binds LPS in the blood and in this way facilitates the transfer of LPS to the CD14 receptor on monocytes and macrophages. LPS, bound to LBP is far more potent in inducing cytokine production than LPS alone [25]. In contrast, Bactericidal/permeability increasing protein (BPI) competes with LBP for LPS in the bloodstream. LPS, bound to BPI, is less potent in inducing an inflammatory response than LPS alone [26]. Current investigations in our laboratory are aimed to compare the physiological activities of SP-D with those of LBP and BPI.

We conclude that the collectin SP-D is a molecule of innate immunity in the lung. The fact that there is still no specific designated role for SP-D in the main function of pulmonary surfactant, which is lowering

the surface tension of the alveoli to prevent alveolar collapse at end-expiration, favors a role for SP-D as a molecule of an innate immune system in the lung. This innate immune system might be a very fast and important first reaction to Gram-negative bacteria and may help to protect the lung from LPS-induced disease.

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