

Surfactant protein D modulates pulmonary clearance of pollen starch granules

Carla Winkler,¹ Katja Hüper,¹ Ann-Carina Wedekind,¹ Sabine Rochlitzer,² Christina Hartwig,³ Meike Müller,² Armin Braun,² Norbert Krug,² Jens M. Hohlfeld,¹ and Veit J. Erpenbeck¹

¹Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany; and Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany

²Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany

³Department of Anatomy, Hannover Medical School, Hannover, Germany

ABSTRACT

Pollen starch granules (PSGs) are allergen particles that get into contact with pulmonary surfactant and phagocytes in the terminal airways. In this study, the effects of surfactant protein D (SP-D) on the interaction of PSGs with phagocytes and on the pulmonary clearance of PSGs were determined. Fluorescently labeled PSGs were incubated in vitro with murine lung macrophages or dendritic cells (DCs) ± recombinant rat SP-D (rSP-D). In addition, the effect of SP-D on uptake of PSGs by lung macrophages and DCs was studied in vivo. Furthermore, PSGs were instilled in Balb/c mice and the effects of SP-D on total lung clearance were assessed by optical imaging. SP-D treatment increased the number of PSG-positive macrophages and DCs in vitro. Furthermore, SP-D accelerated uptake/binding by alveolar macrophages and reduced the number of PSG-positive tissue macrophages and DCs at 24 hours. However, SP-D did not affect total lung clearance of PSGs and it did not enhance the T-cell proliferation induced by PSG-positive DCs. In conclusion, SP-D increased PSG-positive cells in vitro and accelerated PSG binding/uptake in vivo. The observed effects were limited to cellular clearance mechanisms and did not affect the total clearance of PSGs from the lung.

KEYWORDS allergen; dendritic cells; macrophages; T-cell proliferation; optical imaging

Surfactant protein D (SP-D) belongs to the group of collectins that modulate pulmonary host defense and cellular immune responses in the lung. Its function is highly dependent on its oligomeric structure. SP-D is composed of monomers (43 kDa) assembled by disulfide crosslinking into trimers and further multimerized to varying degrees (>1 MDa) [1, 2]. The monomers are characterized by 4 different domains: the cysteine-rich N-terminal domain, the collagen-like domain, the neck domain, and the C-terminal lectin-like domain described as carbohydrate recognition domain (CRD) [3]. With their CRD SP-D may

bind to various carbohydrate structures and glycolipids present on pathogens and allergens [4].

SP-D has been shown to bind to allergens such as *Aspergillus fumigatus* and *Dermatophagoides pteronyssinus* (Der p1) as well as to allergen particles such as pollen starch granules (PSGs). This has been demonstrated to increase uptake and binding to phagocytes [5–7]. PSGs released from grass pollen are composed of starch and complex carbohydrates and contain several major allergens. Due to their small size of 0.5 to 2.5 µm, they preferentially reach the lower airways where they can trigger allergic airway inflammation [8–10].

Pulmonary clearance of inhaled particles or particle-like material represents an essential primary defense mechanism to eliminate harmful triggers of airway inflammation. This clearance mainly consists of a rapid noncellular (mucociliary) clearance but there exists also a much slower cellular clearance that is driven by professional phagocytes [11].

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Address for correspondence to Jens M. Hohlfeld, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs Straße 1a, 30625 Hannover, Germany. E-mail: jens.hohlfeld@item.fraunhofer.de

Phagocytes represent a heterogeneous family of cells that include macrophages, dendritic cells, and neutrophils [12, 13]. Under physiologic conditions, alveolar macrophages are the largest cell population within the airways and one of their major tasks is the clearance of inhaled material from the lung. They are less efficient in presenting antigens in comparison to tissue macrophages due to a lower major histocompatibility complex (MHC) II expression [14]. Compared to macrophages, dendritic cells (DCs) are very potent antigen-presenting cells (APCs). After uptake of antigens, they process them into peptides and present them on their surface, which can lead to activation of T-cells and initiation of primary and memory immune responses. Thus, DCs play an important role for the initiation of the allergic airway inflammation [15]. Lung DCs display a heterogeneous cell population regarding localization and surface marker expression. They are characterized by their high expression of MHC II and CD11c [16].

So far, the effect of SP-D on the uptake of PSGs by pulmonary DCs has not been investigated. Furthermore, the *in vivo* effect of SP-D on cellular and noncellular clearance mechanisms of PSGs is unknown. In the present study, we assessed the effects of SP-D on the interaction of PSGs with pulmonary macrophages and DCs *in vitro* and determined the role of SP-D for the interaction of PSGs with macrophages and DCs *in vivo*. In addition, the effect of SP-D on the total pulmonary clearance of PSGs was determined.

MATERIAL AND METHODS

Recombinant rat SP-D (rrSP-D) was purified by maltose affinity chromatography from the medium supernatant of cultured Chinese hamster ovarian (CHO) cells stably transfected with a full-length rat SP-D cDNA clone as described previously [17]. *Phleum pratense* pollen was obtained from Allergon (Ängelholm, Sweden). All antibodies were purchased from BD Pharmingen (Heidelberg, Germany). All other reagents, unless otherwise specified, were purchased from Sigma-Aldrich (Deisenhofen, Germany). Female Balb/c wild-type (wt) mice (8 to 10 weeks old) were derived from Charles River, Sulzfeld, Germany. All mice were fed with laboratory food with low autofluorescence (CRD FLI; Harlan Winkelmann, Borcheln, Germany) and tap water *ad libitum* and held in regular 12-hour dark:light cycle at a temperature of 22°C. Mice were acclimatized for 7 days before starting the experiments. All animal experiments were performed in accordance with the German animal protection law under a protocol

approved by the appropriate authority (Bezirksregierung Hannover).

Isolation and staining of PSGs

PSGs were isolated from grass pollen (*Phleum pratense*) as described previously [7]. Pollen (300 mg) was vortexed in 40 mL of deionized, autoclaved water for 3 minutes. Whole pollen and pollen fragments were separated by centrifugation at $50 \times g$ for 4 minutes. The supernatant was filtered (5- μ m filter; VWR International, Hannover, Germany) and centrifuged twice at $2500 \times g$ for 10 minutes. The resulting pellet was resuspended in 1 mL of sterile 0.1 M NaHCO₃. To determine the number of PSGs, an aliquot was diluted in phosphate-buffered saline (PBS) (1:100) and counted in an improved Neubauer chamber.

Immediately after isolation, PSGs were fluorescently labeled with Alexa Fluor 488 dye for flow cytometry or Alexa Fluor 680 dye (both Molecular Probes, Eugene, OR, USA) for detection with the IVIS 200 system. About 1×10^9 to 2×10^9 PSGs in 1 mL NaHCO₃ (0.1 M) were used for the staining procedure. The suspension was transferred into the vial of reactive dye followed by rotation for 1 hour at room temperature in the dark. Excessive dye was removed by washing with 15 mL sterile PBS and centrifugation at $2500 \times g$ for 12 min. Finally, the pellet was resuspended in 1 mL of PBS. The number of PSGs was counted under fluorescence light in an improved Neubauer chamber.

Treatment and instillation of PSGs

Balb/c Mice were anesthetized with 2% propofol (Braun, Melsungen, Germany) intraperitoneally and subsequently exposed to 4% halothane for 1.5 minutes before each treatment. Intrapharyngeal instillation was performed as described previously by Rao et al. [18]. A total amount of 5 μ g rrSP-D (diluted in 50 μ L saline) or saline alone were instilled 1 hour before instillation of $2\text{--}4 \times 10^7$ fluorescently labeled PSGs resuspended in 50 μ L saline.

Isolation of lung macrophages and dendritic cells

Mice were sacrificed with an intraperitoneal injection of pentobarbital-sodium (150 mg/kg body weight; Merial, Hallbergmoos, Germany). The lungs were then perfused through the pulmonary artery with 10 mL PBS to remove intravascular cells. Detached lungs were minced with a razor blade and suspended in 5 mL of digestion buffer containing culture medium (RPMI 1640; Gibco Invitrogen, Karlsruhe,

Germany) supplemented with 175 U/mL collagenase XI and 50 U/mL DNase I (Roche, Mannheim, Germany). Digestion for 30 minutes under shaking (200 rpm) at 37°C was stopped with 0.01 M EDTA to inactivate the enzymes. A single-cell suspension was obtained by passing the digest through a 100- μ m mesh and, if needed, trace amounts of erythrocytes were lysed by incubation in Gey's lysis solution (0.83% NH₄Cl, 0.1% KHCO₃) for 1 minute. After lysis and centrifugation, the cell pellet was resuspended in PBS with 5% fetal calf serum (FCS) and 2 mM EDTA.

In vitro uptake experiments

Antigen-presenting cells were defined as CD11c⁺ cells. Isolation was performed by CD11c MACS positive selection (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were then stained with antibodies specific for mouse MHC II. Cell sorting was performed with BD FACS Aria (BD Pharmingen, Heidelberg, Germany). Macrophages were characterized and sorted as CD11c⁺ and MHC II^{low} whereas DCs were sorted as CD11c⁺ and MHC II^{high}. Macrophages and DCs (2×10^5 /mL) were cultured for 24 hours in RPMI 1640, supplemented with HEPES (25 μ M), FCS (10%), and penicillin-streptomycin (1%) and incubated with fluorescently labeled PSGs (Alexa Fluor 488) in a ratio of 1:10 for additional 15 hours (37°C, 5% CO₂) in the absence or presence of rrSP-D (1 μ g/mL). PSG-positive cells were determined by flow cytometry. For confocal analysis of PSG uptake, cells were seeded in chamber slides (Lab-Tek, Hatfield, USA) and fixed after the incubation with PSGs.

For macrophage/DC sorting and DC phenotype staining, phycoerythrin (PE)-labeled anti-MHC II (eBioscience, Hatfield, UK), APC-labeled anti-CD11c (BD Pharmingen), fluorescein isothiocyanate (FITC)-labeled anti-CD14 (eBioscience), anti-CD40, anti-CD80, and anti-CD86 antibodies (BD Pharmingen) were used.

In vivo uptake experiments

Balb/c Mice ($n = 4-8$ per group) were sacrificed 1, 4, 24, and 48 hours after intrapharyngeal PSG instillation. Bronchoalveolar lavage (BAL) was performed 3 times with 1 mL PBS and BAL cells (alveolar macrophages) were washed before staining and analysis. Lungs were harvested and digested as described above. Before flow-cytometric analysis, BAL cells and interstitial lung cells were fixed with 2% formaldehyde in PBS and stained with antibodies specific for mouse MHC II and CD11c.

Flow-cytometric analysis

Cells were analyzed by flow cytometry with a Coulter FC-500 (Beckmann Coulter, Krefeld, Germany) equipped with a 488-nm argon-ion laser and a 635-nm red diode laser. A minimum of 5000 cells (gated on positive nuclear staining) were counted from respective samples. CD11c-positive tissue cells were differentiated by their expression of MHC II and the percentage of PSG-positive cells was determined for MHC II-negative and MHC II-positive cells, respectively.

T-lymphocyte proliferation assay

Allergen-specific CD4⁺ lymphocytes were isolated from spleen of sensitized Balb/c mice. Sensitization was carried out by intraperitoneal injections of 5 μ g recombinant *Phleum pratense* major allergen Phl p5 (Biomay, Wien, Austria) adsorbed to 1 mg Alum Inject (Pierce, Rockford, USA) on days 0, 14, and 21. Mice were finally challenged on days 28 and 29 intranasally with 9 μ g *Phleum pratense* extract (Alk-Scherax, Wedel, Germany) in 50 μ L PBS as described elsewhere [19].

Lung DCs were pulsed either with PSGs with or without 1 μ g/mL rrSP-D for 5 days and subsequently cocultured with CD4⁺ T-lymphocytes for further 5 days. Lymphocyte proliferation was measured as ³H-thymidine (Amersham Pharmacia, Buckinghamshire, UK) incorporation and detected in a scintillator (Topcount NXT; Canberra Packard, Dreieich, Germany).

Optical imaging for the assessment of total lung clearance of PSGs

The Xenogen IVIS 200 system (Caliper Life Science, Hoptikon, USA) was used to determine the total pulmonary clearance of PSGs that were labeled with Alexa Fluor 680 fluorescent dye (Molecular Probes, Eugene OR, USA). Prior to the instillations, different concentrations of labeled PSGs (1×10^5 to 4×10^7) were analyzed to determine a suitable concentration. The detected fluorescence intensity was proportional to the amount of labeled PSGs and 4×10^7 labeled PSGs were instilled into mice after saline or rrSP-D instillation, as described above. However, due to the high absorbance, the fluorescence signal of instilled PSGs could only be detected ex situ. Therefore, animals were sacrificed 1, 4, 24, and 48 hours after PSG instillation. Lungs, stomachs, and intestines were dissected for optical imaging ex situ. Imaging parameters were selected and implemented through the instruments' Living Image 2.5 Software, using CY5.5

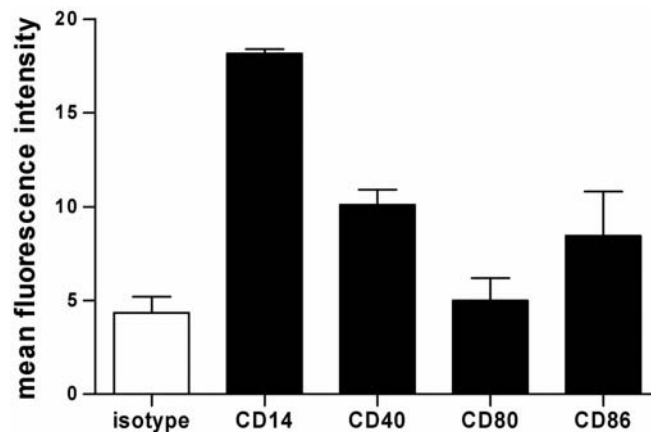


FIGURE 1 Isolated lung dendritic cells (DCs) were characterized according to their expression of CD14 and costimulatory molecules (CD40, CD80, CD86) directly after sorting by flow cytometry. The respective mean fluorescence intensities in relation to the isotype control is given. Lung DCs were CD14 positive and expressed CD40 and CD86 but did not express CD80. Representative data of 2 experiments are shown.

filters. The fluorescence images were real time, unprocessed images. After selecting appropriate regions of interest (ROIs) for the measurement, the fluorescence intensity of the organs was reported as “efficiency,” a value without a unit.

Statistical analysis

Values are given as means $\pm$ SEM. Statistical comparison between treatment groups (rrSP-D versus sham) was done with the *t* test, comparison within the group with a 1-way analysis of variance (ANOVA) and selected posttests (Dunn’s test) using GraphPad Prism, Version 4.03. In cases of multiple testing exact *P* values are given. *P* values $< .05$ were considered to be significant.

RESULTS

SP-D enhanced the binding/uptake of PSGs by pulmonary macrophages and DCs in vitro

Differentiation and sorting of the CD11c-positive macrophages and DCs from lung tissue was performed according to their low- and high-MHC II expression, respectively. The purity for both cell types was $>90\%$, confirmed by reanalysis of the sorted cell populations.

The isolated pulmonary DCs were further characterized according to their surface marker expression by flow cytometry (Figure 1). After the sorting procedure DCs expressed CD14, CD40, and CD86, but

no CD80. Therefore, these cells are considered capable to induce T-cell proliferation upon antigen presentation.

Flow-cytometric analysis of PSG binding/uptake by pulmonary macrophages and DCs is shown in Figure 2. After 15 hours of incubation with PSGs, $4.2\% \pm 1.3\%$ of the macrophages and $8.6\% \pm 2.7\%$ of the DCs were PSG positive. Addition of rrSP-D at a concentration of $1 \mu\text{g/mL}$ increased the number of PSG-positive macrophages to $26.7\% \pm 6.2\%$ ($P < .01$; Figure 2A and B) and the number of PSG-positive DCs to $14.7\% \pm 3.5\%$ ($P < .05$; Figure 2D and E). Thus, the SP-D-mediated augmentation of PSG-positive cells was greater in macrophages than in DCs.

Representative 3-dimensional images of PSG-positive cells obtained by confocal microscopy are shown in Figure 2C and F, confirming that both macrophages and DCs internalized PSGs.

T-lymphocyte proliferation was induced by PSGs but not affected by SP-D

Because PSGs contain several major allergens, it was investigated whether the uptake of PSGs by DCs has implications on their antigen presentation and subsequent activation of antigen specific T-lymphocytes. Accordingly, pulsing DCs with PSGs induced significant T-lymphocyte proliferation ($P = .03$; Figure 3). However, even though SP-D increased the number of PSG-positive DCs 2-fold, it did not modulate T-lymphocyte proliferation when compared to the respective PSG control without SP-D ($P = .61$).

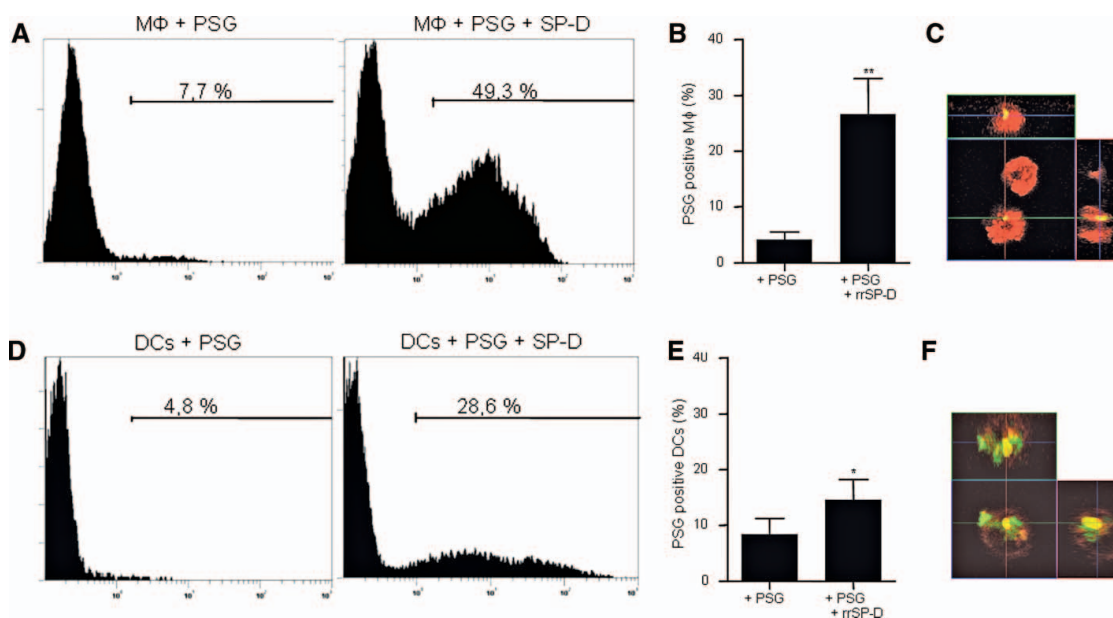


FIGURE 2 Effect of SP-D on pollen starch granule (PSG) uptake by macrophages (A–C) and dendritic cells (DCs) (D–F) isolated from lung tissue, in vitro. Cells were incubated in the presence or absence of recombinant rat surfactant protein-D (rrSP-D; 1 μ g/mL) with fluorescently labeled PSGs. Cells staining positive for PSGs were determined by flow cytometry and are shown as percentage of PSG-positive cells (C, F). Examples of histograms obtained by flow cytometry are shown in A and D. Data are shown as mean $\pm$ SEM, $n = 7$. * $P < .05$; ** $P < .01$. The uptake of PSGs into macrophages (C) and DCs (F) was exemplarily determined by confocal microscopy. Cells surfaces were either stained for CD11c (red) or CD11c (red) + MHC II (green). Fluorescently labeled PSGs are shown in yellow.

SP-D accelerates cellular uptake/binding of PSGs in vivo

Because rrSP-D increased the uptake/binding of PSGs by pulmonary macrophages and DCs in vitro,

we also investigated the cellular uptake/binding of fluorescently labeled PSGs by pulmonary macrophages and DCs in vivo. Therefore, fluorescently labeled PSGs were instilled into mouse lungs after treatment

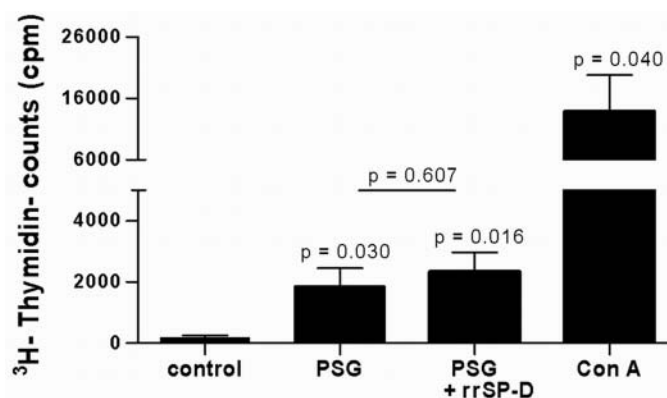


FIGURE 3 Antigen presentation by pulmonary dendritic cells (DCs) was assessed by pulsing DCs either with pollen starch granules (PSGs) (ratio of cells:PSGs = 1:10) in the presence or absence of recombinant rat surfactant protein-D (rrSP-D; 1 μ g/mL) followed by coculture with CD4⁺ T-lymphocytes from sensitized animals. Proliferation of T-lymphocytes was determined after 7 days by ^3H -thymidine incorporation. Results represent means $\pm$ SEM, $n = 4$ (in triplicates). Exact P values are given for the comparison to control. In addition, the exact P value for the SP-D effect compared to PSGs alone is provided.

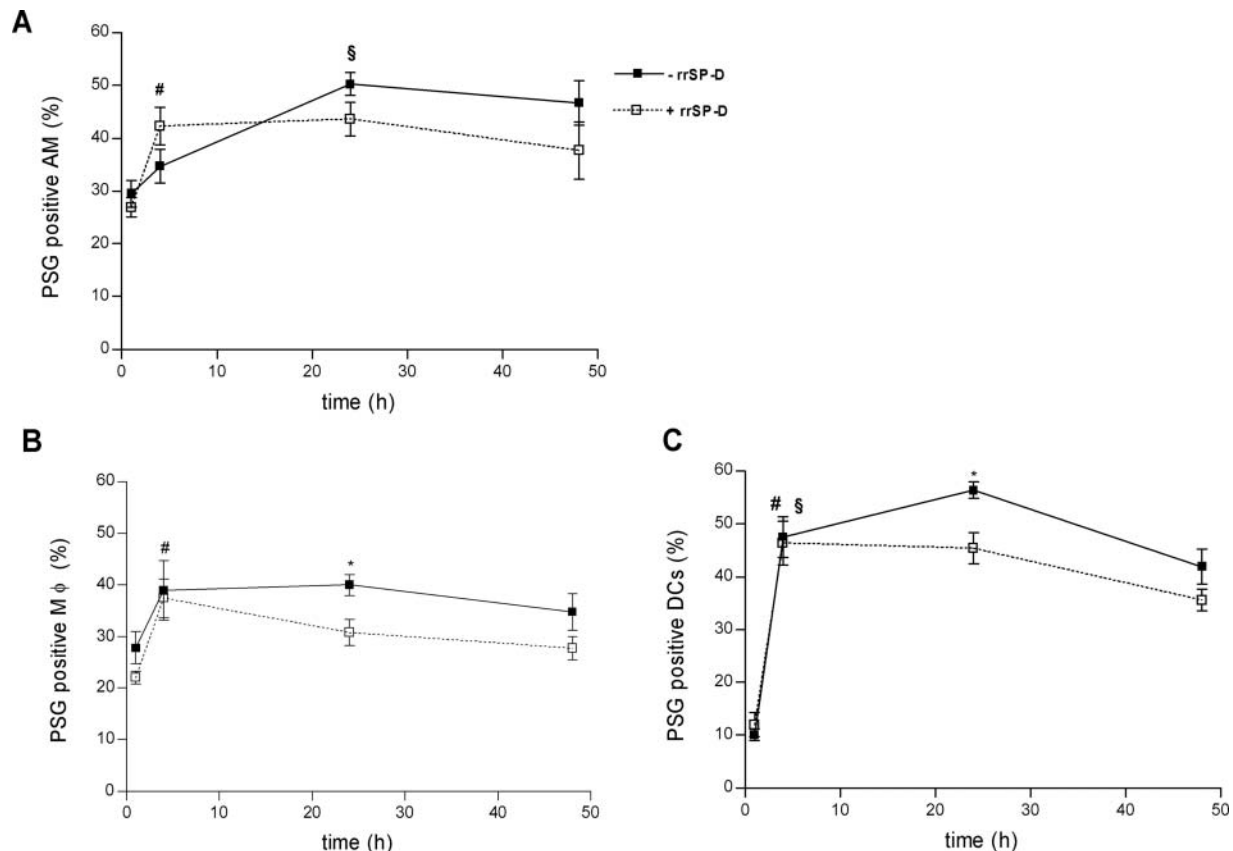


FIGURE 4 Kinetics of pollen starch granule (PSG)-positive cells in the lung after PSG instillation was determined by flow cytometry. PSG-positive alveolar macrophages (AMs) isolated from bronchoalveolar lavage taken 1, 4, 24, and 48 hours (h) following PSG instillation after pretreatment with (*open boxes*) and without (*closed boxes*) recombinant rat surfactant protein D (rrSP-D) (**A**). PSG-positive macrophages (MΦs) and dendritic cells (DCs) isolated from lung tissue are shown in **B** and **C**, respectively. Data are shown as mean $\pm$ SEM, $n = 4-8$. * $P < .05$ compared to saline treatment; # $P < .05$ compared to 1 hour within the SP-D-treated group. § $P < .05$ compared to 1 hour within the saline-treated group.

with SP-D or saline and cellular uptake/binding by macrophages and DCs was analyzed by flow cytometry at different time points up to 48 hours.

One hour after the instillation of PSGs, $29.5\% \pm 2.5\%$ of the alveolar macrophages, $27.8\% \pm 3.1\%$ of the tissue macrophages, and $10.1\% \pm 1.1\%$ of the tissue DCs were PSG positive (Figure 4). Compared to the 1-hour assessment, a significant increase of PSG-positive alveolar macrophages was observed already after 4 hours in SP-D-treated animals but not until 24 hours in untreated animals (Figure 4A). These data suggest that SP-D accelerates the uptake/binding of PSGs by alveolar macrophages *in vivo*. In line with these observations, the numbers of PSG-positive tissue macrophages and DCs already reached a peak after 4 hours in SP-D-treated mice, whereas they reached a peak only after 24 hours in untreated mice. At this time point, the number of PSG-positive tissue macrophages and DCs was higher in untreated mice when compared to the SP-D-treated mice (Figure 4B and C). We conclude that SP-D accelerated PSG up-

take by alveolar macrophages early after instillation of PSGs, which may have led to a faster cellular clearance of PSGs and subsequently resulted in decreased numbers of PSG-positive tissue cells after 24 hours.

SP-D does not influence the total pulmonary clearance of PSGs

The IVIS 200 optical imaging system was used to determine the effect of SP-D on total pulmonary clearance of PSGs. The fluorescence intensities in lungs of Balb/c mice were measured *ex situ* at different time points after instillation of labeled PSGs because the fluorescence intensities of instilled PSGs were too weak to be detected *in vivo* (Figure 5B, C). A nonlinear regression analysis was performed with a 1-phase exponential decay model. The resulting half-life was 1.9 hours without SP-D and 3.1 hours in SP-D-treated animals (Figure 6A). No differences between the groups were observed at any time point. The fluorescence intensities of the stomachs were

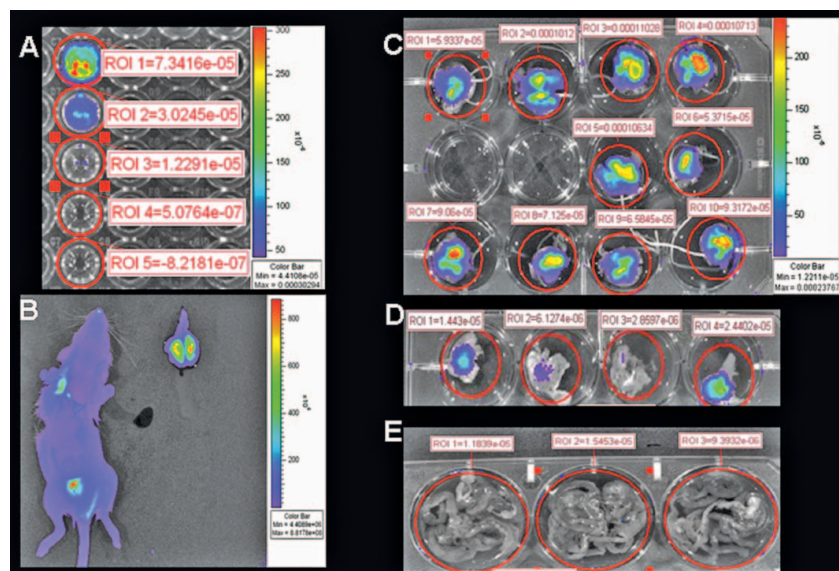


FIGURE 5 Fluorescence intensity of Alexa Fluor 680-labeled pollen starch granules (PSGs) determined with the IVIS 200 optical imaging system. Fluorescence intensities within the determined region of interest (ROI) of different numbers (ROI 1: 4×10^7 ; ROI 2: 2×10^7 ; ROI 3: 1×10^7 ; ROI 4: 1×10^6 ; ROI 5: 1×10^5) of labeled PSGs (A). Instilled labeled PSGs (4×10^7 PSG/ $50 \mu\text{L}$) were not detected in situ, but after lung harvest ex situ (B). Representative images of fluorescence intensities from different organs: lung (C), stomach (D), and intestine (E), assessed 1 hour after intrapharyngeal instillation of PSGs into the lung.

lower and the clearance data paralleled the data from the lungs (Figure 6B). The fluorescence intensities of the intestines were further reduced (data not shown).

DISCUSSION

The respiratory tract is continuously exposed to allergen particles present in the inhaled air. In sensitized individuals, allergen avoidance is pivotal to reduce the

burden of disease [20]. Besides reduced exogenous allergen exposure, effective endogenous clearance mechanisms contribute to minimize the pulmonary exposure to allergens. In this respect, mucociliary clearance is very rapid and represents the major mechanism of pulmonary clearance, whereas the cellular clearance is much slower and only accounts for a minor portion of the total clearance [11]. Because the lung collectin SP-D has been demonstrated to accelerate pathogen clearance from the lung [21], to

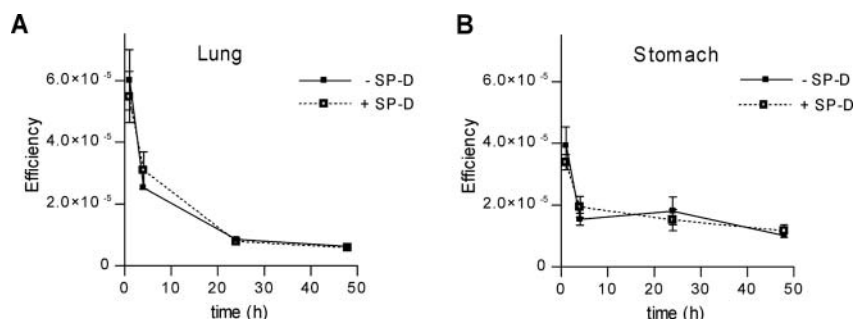


FIGURE 6 Clearance of pollen starch granules (PSGs) from lung (A) and stomach (B) determined ex situ at different time points following PSG instillation after pretreatment with (open boxes-dashed lines) or without (closed boxes-solid lines) recombinant rat surfactant protein-D (rrSP-D). The resulting half-life in the lung was 1.9 hours without SP-D and 3.1 hours with SP-D treatment (A). Data are shown as mean $\pm$ SEM of 5 experiments.

enhance uptake of particulates by macrophages in vitro [7], and to modify allergen-induced airway inflammation in vivo [5, 22], we thought to investigate the effect of SP-D on allergen particle uptake by lung cells, on subsequent allergen presentation, and on total pulmonary clearance of allergen particles. Our data demonstrate that SP-D increased the cellular uptake of PSGs by macrophages and dendritic cells in vitro and accelerated PSG uptake/binding in vivo. The resulting reduction of PSG-positive tissue DCs 24 hours after instillation may be beneficial in allergic lung disease, since in the presence of SP-D, fewer DCs may present antigen and induce inflammation at this time point.

Our experiments showed SP-D interactions with PSGs that were previously demonstrated for pathogens [21, 23, 24]. In most of the previous studies, SP-D increased the uptake of pathogens by phagocytes and subsequently improved pathogen clearance from the lung, which was thus identified as a major defense mechanism of SP-D against pathogens [25]. We hypothesized that SP-D plays a similar role for the total clearance of PSGs and, therefore, we determined the clearance of PSGs by optical imaging. Interestingly, the total clearance of PSGs was unaffected after SP-D treatment. Different reasons may have caused this finding. First, the amount of additionally administered SP-D was too small to induce a significant effect on the total clearance, since SP-D at physiologic concentrations was already present. Second, SP-D effects are limited to cellular clearance mechanisms, which contribute to total clearance only as a small fraction. The largest fraction consists of mucociliary clearance, which may not be susceptible to SP-D treatment. Furthermore, differences exist between the techniques that we used for the detection of PSG clearance and the techniques that were used for the detection of pathogen clearance in previous studies. For pathogens, no real-time clearance was determined but a surrogate was used, mostly by measuring quantitative cultures of lung homogenates at different time points after instillation [21, 23]. However, by using this technique, not only the clearance of the pathogens from the lung, but also other mechanisms of pathogen neutralization, including, e.g., the direct inhibition of the pathogen proliferation by SP-D [26] or the direct killing of pathogens by alveolar macrophages after SP-D treatment, would have been assessed, although it was only referred to as clearance [24].

In general, particulate materials are susceptible to uptake by APCs because their dimensions are comparable to those of microorganisms. Particles can—via phagocytosis—deliver antigens to APCs very efficiently [27–29]. After uptake of the particles, APCs

process the contained antigen and migrate to local lymph nodes where the antigen is presented by major histocompatibility complex (MHC) class I and class II molecules to T-cells [30]. Particulate antigens might, therefore, mediate the induction of both humoral and cellular immunity. We demonstrate that PSG uptake by DCs induced T-lymphocyte proliferation. However, the SP-D-induced increase of PSG-positive cells was not accompanied by an augmentation of lymphocyte proliferation. These findings are important because they demonstrate that SP-D does not enhance the potential inflammatory effects of the PSG allergens following uptake by antigen-presenting cells. In addition, previous studies indicated that SP-D attenuates T-cell activation by binding and sequestering antigens [31]. Therefore, we assume that the inhibitory effects of SP-D on T-cell activation could additionally compensate for possible proliferation-enhancing effects that may be triggered by an increased number of PSG-positive DCs.

The focus of our study was to better understand the fate of allergen particles in the airways and not to study the molecular mechanism through which SP-D modulated the cellular uptake of PSGs. It has been demonstrated before that SP-D binding to PSGs and uptake by alveolar macrophages is calcium and maltose dependent, suggesting interaction of the CRD of SP-D with glycosylated structures present on PSGs [7]. In addition, SP-D can also bind to carbohydrate structures on phagocytes and thereby mediate the contact of PSGs to the cells [32]. Furthermore, both macrophages and DCs can bind PSGs independently via C-type lectin and integrin receptors [33]. The molecular mechanisms of SP-D-induced uptake of PSGs by phagocytes have to be addressed in separate experiments.

In conclusion, our results demonstrate that SP-D increased the cellular uptake/binding of PSGs by macrophages and DCs in vitro and accelerated uptake/binding of PSGs by alveolar macrophages in vivo. However, the total clearance of PSGs from the lung remained unaffected after SP-D treatment. Our data suggest that targeting of PSGs into phagocytes by SP-D does not lead to an increased allergic inflammation because SP-D does not augment presentation of PSG allergens by lung phagocytes.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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