

Intranasal exposure to a damp building mould, *Stachybotrys chartarum*, induces lung inflammation in mice by satratoxin-independent mechanisms

M. Leino*, M. Mäkelä†, K. Reijula‡, T. Haahtela†, H. Mussalo-Rauhamaa†, T. Tuomi‡, E.-L. Hintikka‡ and H. Alenius*

*Finnish Institute of Occupational Health, Helsinki, Finland, †Division of Allergy, Helsinki University Central Hospital, Finland and ‡Uusimaa Regional Institute of Occupational Health, Helsinki, Finland

Summary

Background *Stachybotrys chartarum* is a damp building mould and a potent toxin producer that has been related to serious cases of respiratory health problems. However, the direct link between exposure and health symptoms has not been established.

Objective To examine the mechanism by which exposure to spores of satratoxin producing and non-producing *S. chartarum* strains induce inflammatory responses in murine lungs.

Methods BALB/c mice were intranasally exposed for 3 weeks to spores of a satratoxin-producing and a non-producing *S. chartarum* strain. Inflammatory cell infiltration was characterized from bronchoalveolar lavage (BAL) fluid. Cytokine and chemokine mRNA expression in lung tissue was measured with real-time PCR. Bronchial responsiveness to methacholine (MCh) was determined by whole-body plethysmography and serum antibody levels by ELISA.

Results A dose-dependent increase in monocytes, neutrophils and lymphocytes was observed in BAL fluid after intranasal (i.n.) instillation of *S. chartarum* spores. There was no difference in the BAL between exposure to the satratoxin-producing and the non-producing strains. Infiltration of inflammatory cells was associated with an induction of pro-inflammatory cytokine (IL-1 β , IL-6 and TNF- α) and chemokine (CCL3/MIP-1 α , CCL4/MIP-1 β and CCL2/MCP-1) mRNA levels in the lungs. Interestingly, CXCL5/LIX was the only chemokine that showed significantly higher mRNA levels after exposure to the satratoxin-producing strain compared with the non-producing strain. MCh-induced bronchial responsiveness was not altered significantly after mould instillation. Moreover, no significant increase in total or specific IgE, IgG2a and IgG1 antibody levels were found after *S. chartarum* exposure.

Conclusion These results indicate that lung inflammation induced by i.n. instillations of *S. chartarum* spores is regulated by the induction of pro-inflammatory cytokines and leucocyte-attracting chemokines. The data also imply that *S. chartarum*-derived components, other than satratoxins, are mediating the development of this inflammatory response.

Keywords: chemokines, cytokines, damp building mould, inflammation, lung, satratoxin, *Stachybotrys chartarum*

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Introduction

People exposed to moulds in their indoor environment complain about a variety of symptoms, including cold- and flu-like symptoms, irritation and inflammation in the upper and lower airways, headache and tiredness [1, 2]. These symptoms are often misinterpreted as being caused by allergy to moulds. *Stachybotrys chartarum*, a fungi well known for its ability to produce toxin, has been isolated occasionally in large quantities from moisture-damaged and mouldy buildings. In 1986, Croft et al. [3] described the first case where

Stachybotrys was suspected to have caused health problems in exposed persons in water-damaged buildings. Serious pulmonary haemorrhage (PH) was associated with fungal exposure in a cluster of 10 infants in Cleveland, OH in the middle of the 1990s with a mortality rate of 30% [4]. *S. chartarum* was suggested to play a role in this disease [5]. Several other epidemiological studies suggest a role for *S. chartarum* in causing health problems [6, 7]. However, the direct link between *S. chartarum* exposure in the indoor air and the symptoms has not been established.

S. chartarum is a saprophytic fungus that utilizes cellulose as a carbon source and therefore grows well on wet building materials [8, 9]. Several *S. chartarum* strains are capable under certain conditions of producing macrocyclic trichothecenes such as satratoxin H and G, immunosuppressants such

Correspondence: Harri Alenius, PhD, Finnish Institute of Occupational Health, Topeliuksenkatu 41 b, 002500 Helsinki, Finland.
E-mail: Harri.Alenius@occuphealth.fi

as stachybotrylactones and stachybotrylactams, atranones and also haemolytic compounds such as haemolysin [10–12]. Macrocytic trichothecenes are potent protein synthesis inhibitors and are cytotoxic to eukaryotic cells, while the lactones and lactams have immunosuppressant effects [13, 14]. Because of its ability to produce toxins, especially satratoxins, *S. chartarum* has often been considered to be the cause of health problems in indoor environments.

We have shown previously that intranasal (i.n.) instillation of *S. chartarum* spores elicited lung inflammation in a murine model characterized by histological methods [15]. Similarly, Rao et al. [16] showed recently that a single dose of a high concentration of *S. chartarum* spores caused acute inflammatory pulmonary effects in rats. In the present study, we examined the mechanisms by which exposure to spores of satratoxin-producing and non-producing *S. chartarum* strains induce inflammatory responses in murine lungs.

Materials and methods

Animals

BALB/c female mice, age 6–8 weeks, free of specific pathogens, were obtained from M&B, Ry, Denmark. The mice were housed under specific pathogen-free conditions and maintained on an ovalbumin-free diet. All the experiments conducted were approved by the Animal Experiment Committee of the Finnish Institute of Occupational Health.

Microbe preparations

The *S. chartarum* strains (Ehrenberg ex Link) Hughes (synonym to *S. atra* and *S. alternans*) s. 29 and s. 72 were grown on rice flour agar for 2 weeks in room temperature (RT) and after that for another 2 weeks in +4 °C to induce toxin production. The spores were suspended in phosphate-buffered saline (PBS, pH 7.2) from the agar plates and counted (spores/mL PBS).

The two strains of *S. chartarum* have previously been characterized [15, 17]. Strain s. 29 under the conditions mentioned above produced no satratoxins and only minor amounts of stachybotrylactam and stachybotrylactone, <1 ng/10⁵ spores (referred as the non-satratoxin-producing strain). Under the same conditions, s. 72 (NRRL 6084) produced satratoxin G and H, stachybotrylactone and stachybotrylactam in amounts of 4 ng, 10 ng, 8 µg and 2 µg, respectively, per 10⁵ spores (referred as satratoxin producing strain). The spore suspensions were γ-irradiated with 10 kGy before use.

For antibody analysis, an antigen suspension was prepared from the spore suspensions. The spores were sonicated for 2 × 3 min (50 W) on ice using an ultrasonic processor (Branson Sonic Power Comp, Danbury, Connecticut, USA). The unbroken spores were centrifuged down (2100 g, +4 °C, Minifuge RF Heraeus Sepatech GmbH, Osterode, West Germany) and the supernatant was filtered (0.45 µm pore size). The protein concentration of the supernatant was determined and used as the antigen suspension.

A benign microbe, *Saccharomyces cerevisiae*, was used as a control to evaluate the specificity of microbe-induced responses. The bakers yeast suspension was kindly provided

by Professor Mirja Salkinoja-Salonen. The yeast (Rajamäki, Alko) was grown on malt extract agar for 48 h in 37 °C; the cells were suspended into PBS and counted. The cell suspension was γ-irradiated with 10 kGy before use.

Preparation and analysis of mycotoxin samples

Mycotoxins were extracted from the spore suspension used for i.n. exposure with aqueous 95% methanol, purified by a hexane wash and solid-phase extraction, separated by reverse-phase HPLC, identified by tandem mass spectrometry (MS), and quantified using electrospray ionization (ESI) on a quadrupole ion trap mass analyser, as described previously [18]. Briefly, the analytes were introduced to the MS detector through an HPLC system consisting of an Alliance 2690 separations module (Waters Assoc., Milford, MA, USA) connected to a Lichrocart 250-3 Purospher RP18 column (Merck, Darmstadt, Germany) on-line with a 4 × 4 Purospher precolumn (Merck). A methanol–aqueous buffer (10 mM ammonium acetate) solvent system was used. Twenty micromolars of sodium acetate was added to solvents for enhancement of cationization in ESI-MS. Mass spectral analysis was performed on a Finnigan LCQ (Finnigan Corp., San Jose, CA, USA) that was fitted with an ESI probe. The operating conditions were optimized using T2-toxin, RDRA and T2-tetraol. To each sample, 2 µg of the alkaloid reserpine was added as an internal standard prior to the extraction procedure. Each sample series of six samples contained one or more blank samples to exclude the possibility of false positives. Blank samples were analysed prior to injecting the actual samples and once more after the last sample had been analysed. Irrespective of the compound, the intensity of at least two major fragments was used for quantitation purposes.

Mass spectrometric analysis verified that the non-satratoxin-producing strain (s. 29) used in the present experiments did not produce detectable amounts of either satratoxin G or H. On the contrary, the satratoxin-producing strain (s. 72) produced 28 ng of satratoxin G and 6 ng of satratoxin H per 1 × 10⁵ spores.

Intranasal administration of the microbe preparations

The fungal spore and yeast cell suspensions were administered intranasally (i.n.) under light anaesthesia using methoxyflurane (Metofane™, Schering-Plough Animal Health Corp., Union, NJ, USA) two times a week for 3 weeks. The fungal spores (strain s. 29 and s. 72) and yeast cells were administered into the mouse nostrils in two different concentrations (1 × 10³ and 1 × 10⁵ in 25 µL PBS), the control group received PBS only. Eight mice per group were treated with each administration. The airway responsiveness or bronchoconstriction was measured 48 h after the fifth i.n. instillation. Twenty-four hours after the last spore injection, the mice were killed and the blood and the lung samples were collected.

Determination of airway responsiveness

Airway responsiveness was assessed using a single chamber whole-body plethysmograph system obtained from Buxco (Troy, NY, USA) as described [19]. The airway responsiveness was measured 2 days after the fifth i.n. microbe instillation to obtain as reliable results as possible. Briefly,

the animal was placed, unrestrained, into a chamber and the mice were exposed for 5 min to nebulized PBS and subsequently to increasing concentrations of methacholine (MCh) (Sigma) in PBS using an AeroSonic 5000 D ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA). After each nebulization, recordings were taken for 5 min. The enhanced pause (Penh) values measured during each 5 min sequence were averaged and were expressed for each MCh concentration as the percentage of baseline Penh values following PBS exposure (% baseline Penh). Baseline Penh values did not differ significantly between any of the seven groups examined.

Sample collections and lung preparations

The mice were killed by CO₂ asphyxiation. The blood was drained from the hepatic vein. The chest cavity was opened and the lungs were lavaged with PBS via the tracheal tube (1 × 800 µL for 10 s). The bronchoalveolar lavage (BAL) sample was cytocentrifuged (Cytospin, Shandon Ltd, Pittsburgh, PA, USA) on a slide and the cells were stained with the MayGrünwald–Giemsa (MGG) stain. The cell population on the cytospin slide was counted under light microscopy. The left lung was removed for RNA isolation, quick-frozen and kept at -70 °C.

For histologic examination, the right lung was perfused with 10% formalin and then excised *en bloc*. The lungs were embedded in paraffin, cut into 5-µm-thick sections, affixed to microscope slides and deparaffinized. The slides were stained with haematoxylin and eosin (HE) and examined under light microscopy.

Real-time quantitative RT-PCR assay

Total RNA from lungs was extracted using Trizol Reagent (GIBCO BRL, NY, USA) according to the manufacturer's protocol. The total amount of RNA was quantitated by spectrophotometry (GeneQuant II/Pharmacia Biotech, Cambridge, UK). RT-PCR was performed with a two-step PCR method. First, the RNA was reverse transcribed into cDNA with MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA, USA), and random hexamers were used as primers according to the manufacturer's instructions. cDNA synthesis was performed for 30 min at 48 °C, after which the enzyme was inactivated in 95 °C for 5 min in a thermal cycler, Mastercycler gradient (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany). The real-time quantitative PCR was performed in special optical tubes in a 96-well microtitre plate (Applied Biosystems) with an AbiPrism 7700 Sequence Detector System (SDS) (Applied Biosystems), according to the manufacturer's instructions. PCR primers and probes were obtained as predeveloped assay reagents (IL-1β, IL-6, TNF-α, IL-4, IL-5, IL-12p40, IFN-γ, IL-10, CCL5/RANTES, CCL3/MIP-1α, CCL2/MCP-1 and CXCL5/LIX) or were generated (CCL4/MIP-1β) by PrimerExpress version 1.5 software and were ordered from Applied Biosystems. The primer–probe pairs for CCL4/MIP-1β were designed to span intron–exon borders. For amplification, a 10-min incubation at 95 °C was performed to activate the enzyme (AmpliTaQ-Gold); this was followed by 40 cycles with 15 s at 95 °C and 1 min at 60 °C for each cycle. PCR amplification of the housekeeping gene 18S rRNA was performed for each sample

to control for sample loading and to allow normalization between samples.

The relative quantity (relative units (RU)) was calculated by the comparative C_T method according to the manufacturer's instructions. First, the C_T for the target amplicon and the C_T for the internal control (18S rRNA) was determined for each sample. Differences in the C_T for the target and the C_T for the internal control, termed ΔC_T, were calculated to normalize the differences in the amounts of total nucleic acid added to each reaction mixture. The ΔC_T for each experimental sample was subtracted from the ΔC_T of the calibrator. This difference is termed ΔΔC_T. The amount of target, normalized to an internal control (18S rRNA) and relative to the calibrator, was then calculated by the equation 2^{-ΔΔC_T}. A FAM C_T value 40, reflecting the situation in which the gene is not expressed at the mRNA level, was used as the calibrator.

Measurement of serum antibodies

Serum levels of total IgE and *S. chartarum*-specific IgE, IgG1 and IgG2a were measured by ELISA. Briefly, a 96-well plate (Immunoplate, NUNC, Roskilde, Denmark) was coated with either *S. chartarum* antigens (20 µg/mL) or purified anti-IgE (02111D, Pharmingen, San Diego, CA, USA). The serum samples (diluted in 1:50, 1:100 and 1:200 for total IgE, 1:20 for specific IgE and 1:60, 1:180, 1:540 and 1:1620 for specific IgG1 and IgG2a) were added to the plate and incubated overnight. Biotinylated anti-mouse IgE (02122D, Pharmingen), IgG1 (02232D, Pharmingen) or IgG2a (02012D, Pharmingen) and streptavidin horseradish peroxidase (554066, Pharmingen), together with the substrate (ABTS Microwell Peroxidase Substrate System, KPL, Gaithersburg, MD, USA), was used to detect the bound antibody levels measured with an ELISA plate absorbance reader (Multiskan MS, Labsystems, Titertek Multiscan, Eflab, Turku, Finland). Total IgE levels were calculated by comparing with known mouse IgE standards (557079, Pharmingen).

Statistical analysis

The data were analysed with the GraphPadPrism Software. Differences between groups were analysed by non-parametric ANOVA, the Kruskal–Wallis test. Single-group comparisons were performed by the Mann–Whitney test. The results are expressed as individual values (Figs 1–3) or as mean ± standard error of the mean, SEM (Fig. 4). *P*-values <0.05 were considered to be statistically significant.

Results

S. chartarum instillation induces inflammatory cell infiltration to the lung

An influx of inflammatory cells into the BAL was observed after instillations of both the non-satratoxin-producing and the satratoxin-producing strains of *S. chartarum* into the lungs. The higher spore concentration, 1 × 10⁵ spores/instillation, caused a significant increase in monocytes, neutrophils and lymphocytes into the lungs for both the non-satratoxin-producing and the satratoxin-producing strains compared

with the lower spore concentration and the control mice. There was no difference in the population of cells in the BAL fluid between the satratoxin-producing and the non-

satratoxin-producing strains. The lower spore concentration (1×10^3 spores/institution) did not induce infiltration of inflammatory cells into the lungs. Figure 1 shows the cell population in the BAL fluid of the control mice (received PBS

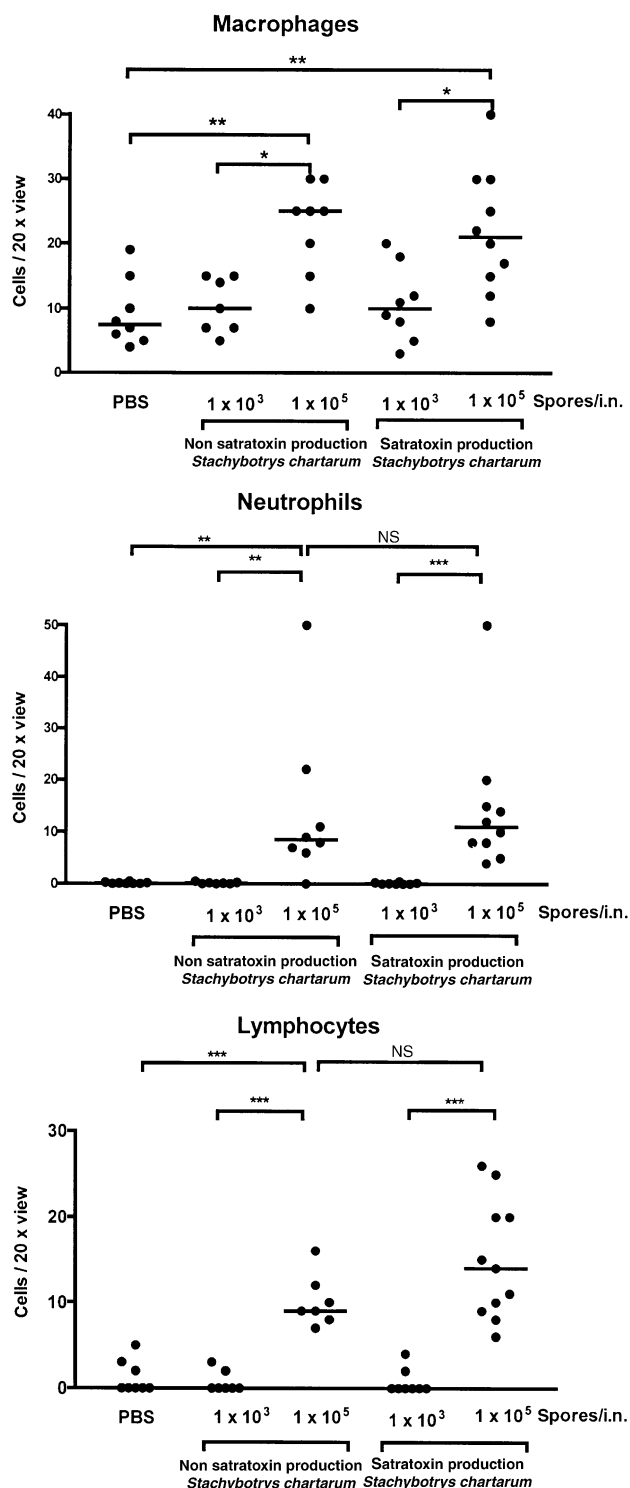


Fig. 1. Presence of macrophages, neutrophils and lymphocytes in bronchoalveolar lavage from mice 24 h after the last intranasal instillations of *Stachybotrys chartarum* spores. Two different strains and two different concentrations were used (satratoxin-non-producing strain and satratoxin-producing strain in the spore concentrations of 10^3 and 10^5 per intranasal instillation). Control mice received phosphate-buffered saline only. Data are expressed as individual values with median. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

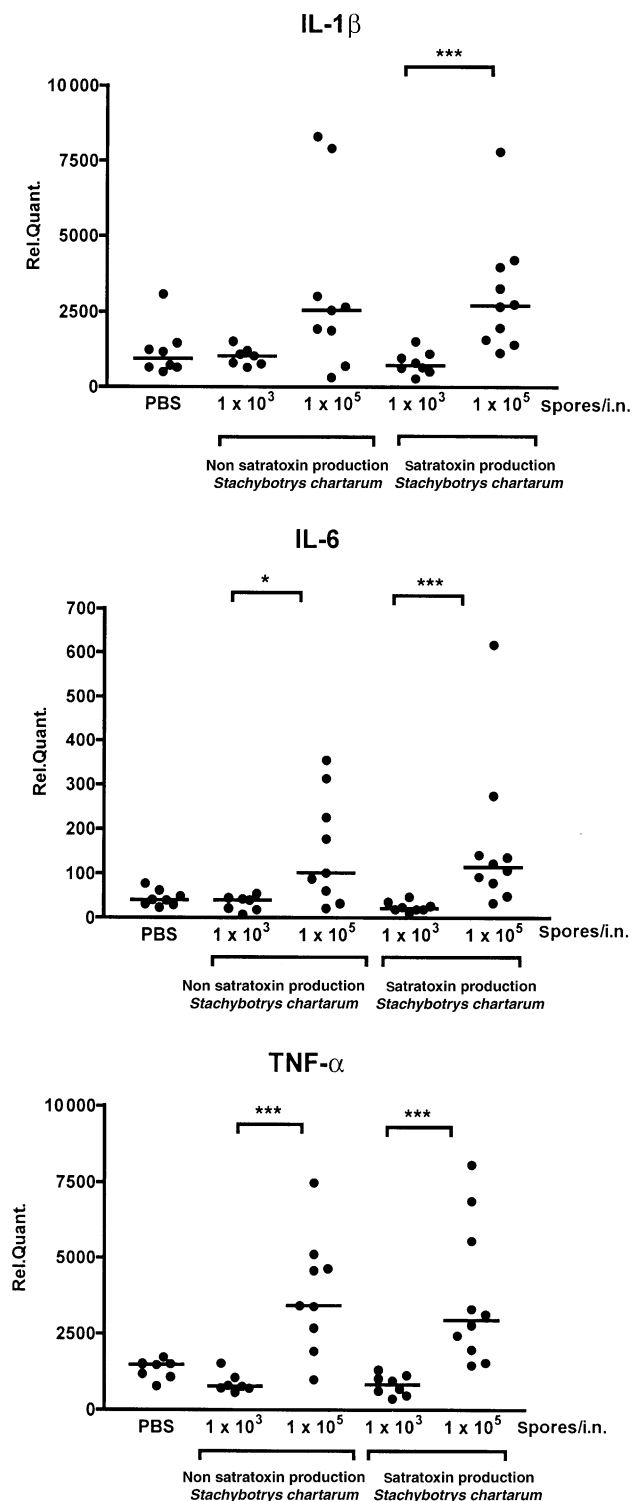


Fig. 2. Pro-inflammatory cytokine (IL-1 β , IL-6 and TNF- α) mRNA expression levels in the lung tissue of mice after repeated *Stachybotrys chartarum* spore or phosphate buffered saline instillations. Data are expressed as individual values with median. * $P < 0.05$, *** $P < 0.001$.

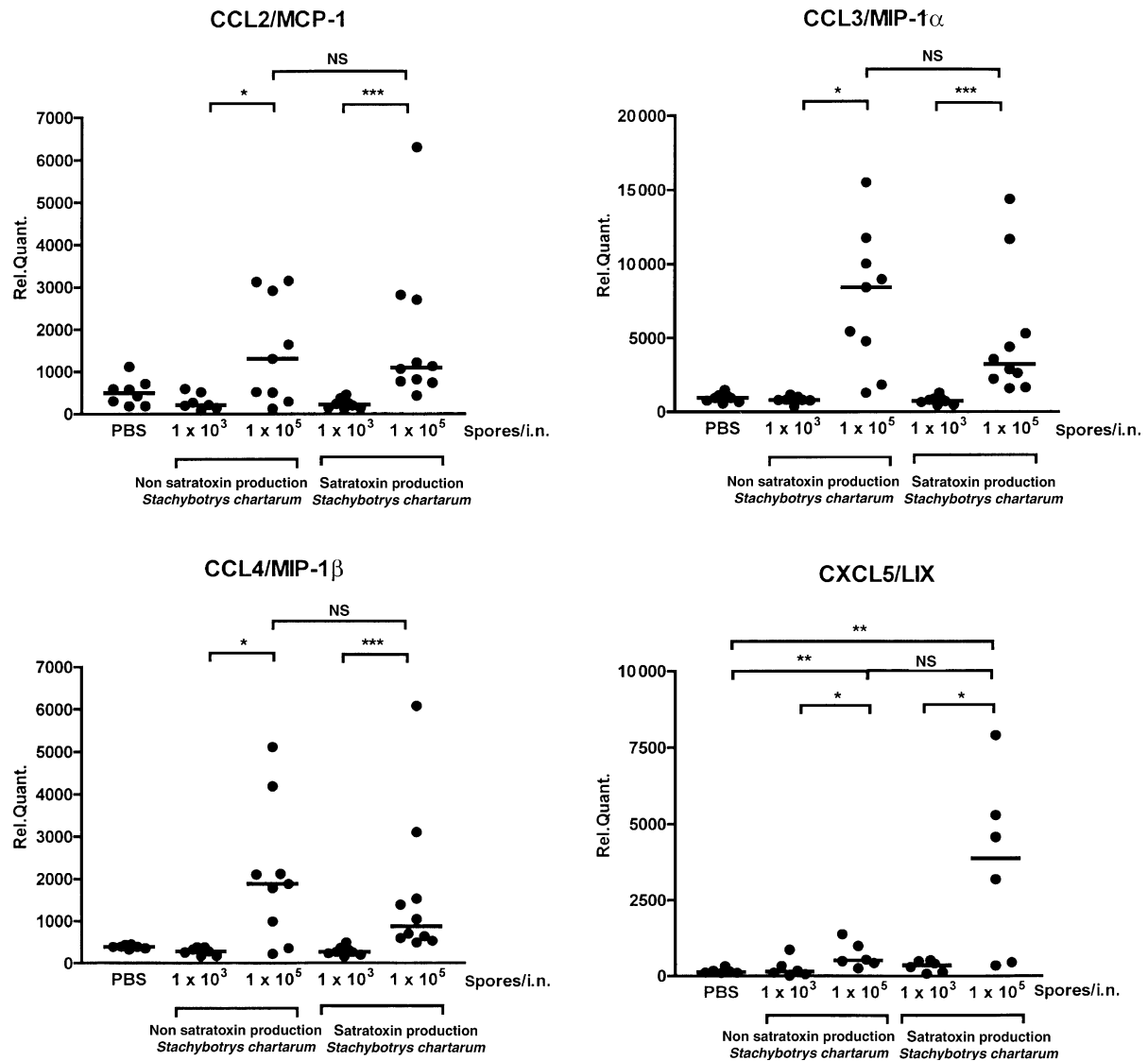


Fig. 3. Chemokine induction (CCL2/MCP-1, CCL3/MIP-1 α , CCL4/MIP-1 β and CXCL5/LIX) in mice lung after *Stachybotrys chartarum* spore or phosphate-buffered saline exposure. Data are expressed as individual values with median. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

only), the mice that had received the non-satratoxin-producing strain (s. 29) and the satratoxin-producing strain (s. 72) of *S. chartarum* spores. No influx of inflammatory cells in the BAL was found after instillation of yeast (1×10^3 and 1×10^5 cells/instillation) (data not shown).

Pro-inflammatory cytokines are significantly induced in mould-exposed lungs

Cytokines such as IL-1 β , IL-6 and TNF- α are pro-inflammatory cytokines that are induced by activated macrophages to start a cascade of inflammatory reactions. As shown in Fig. 2, there was a significant induction in the pro-inflammatory cytokine mRNA expression in the lungs after repeated instillations of *S. chartarum* spores. The higher spore concentration of both the non-satratoxin-producing and the satratoxin-producing mould strain induced a significant increase in IL-1 β , IL-6 and TNF- α mRNA

expressions in the lungs. There was a fourfold increase in TNF- α mRNA in the mice that had received 1×10^5 spores of *S. chartarum* per instillation compared with control mice. IL-6 (sixfold) and IL-1 β (threefold) gene expression levels were also elevated. There was no significant increase in the expression of major Th1 (IFN- γ and IL-12 p40) or Th2 cytokines (IL-4, IL-5 and IL-10) (data not shown) after mould exposure.

Chemokine expression is increased in mould-exposed lungs

Chemokines are small cytokines that regulate the recruitment of inflammatory cells to the site of inflammation. Expressions of several chemokines were significantly induced after *S. chartarum* instillation as shown in Fig. 3. Macrophage-inflammatory protein (MIP)-1 α /CCL3 showed almost 10 times higher mRNA expressions in the lungs of mice that had

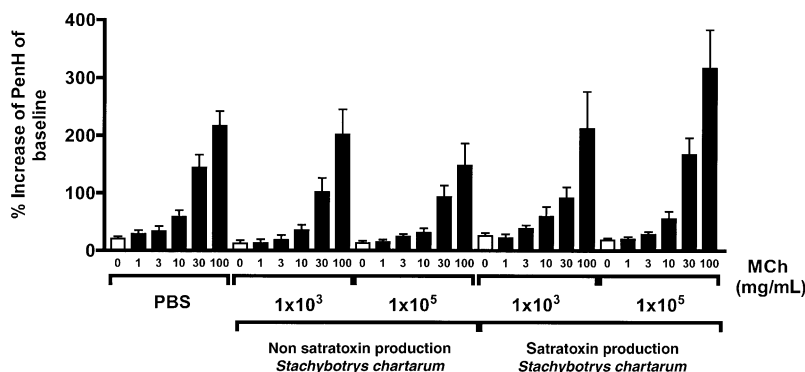


Fig. 4. Bronchial responsiveness in mice 48 h after the fifth intranasal instillation of mould spores or phosphate-buffered saline. The bronchial responsiveness was assessed as a change in the Penh value (% increase) after challenge with increasing amounts (mg/mL) of aerosolized methacholine. The columns and error bars represent means $\pm$ SEMs.

received the higher concentration of both the satratoxin-producing and the non-satratoxin-producing *S. chartarum* strains, while MIP-1 β /CCL4 and monocyte-chemoattractant protein (MCP)-1/CCL2 both showed about six times higher expression levels. The lower spore concentration did not induce significant changes in the mRNA expression levels of these chemokines compared with the control mice. Lipopolysaccharide-induced chemokine (LIX)/CXCL5 showed a dose-dependent increase in mRNA expression for both strains, but with a considerable higher expression for the satratoxin-producing strain in relation to the non-satratoxin-producing one. There was a 16-fold increase in the CXCL5/LIX mRNA expression levels after exposure to the satratoxin-producing strain compared with control mice, while the non-satratoxin-producing strain induced only a threefold increase. In addition, a small, but insignificant, increase in CCL11/eotaxin-1 mRNA was found after instillation of the satratoxin-producing *S. chartarum* strain (data not shown). The baseline expression of CCL5/RANTES mRNA was high in all samples, but was not affected by mould exposure (data not shown).

Bronchial reactivity to inhaled MCh and humoral response was not affected after mould exposure

MCh-induced bronchial reactivity was not altered significantly after mould instillation as measured by the whole-body pletysmograph (Fig. 4). Histological examination revealed only minor changes in the lungs after the spore instillation (data not shown). No differences were found between histological lung samples from mice that had received spores from the satratoxin-producing and the non-satratoxin-producing strains. No significant increase in the levels of total IgE was found after *S. chartarum* exposure (data not shown). Moreover, no detectable levels of specific IgE, IgG2a and IgG1 levels were found after the spore instillation (data not shown). No detectable changes in the MCh-induced bronchial reactivity or histological signs of inflammation were found after yeast exposure (data not shown).

Discussion

Exposure to fungi and other microbes in water-damaged buildings causes irritation of the airways such as sore throat, eye irritation and cough in some subject [20]. Long-time

exposure has been linked with increased susceptibility to respiratory infections, worsening of asthma and possibly even development of asthma [21, 22]. Moreover, it is well documented in experimental settings that certain airborne fungi may cause allergic and inflammatory pulmonary reactions when entering the respiratory tract [15, 16, 23–27]. However, the mechanisms underlying the inflammatory responses of the airways after mould exposure have not been fully elucidated.

In the present study, a marked increase in the number of macrophages, neutrophils as well as lymphocytes in the BAL fluid was observed after repeated *S. chartarum* spore instillations indicative of inflammatory infiltration. *S. chartarum*-induced lung response was specific since exposure to a benign yeast, *S. cerevisiae*, caused neither influx of inflammatory cells into BAL nor histological signs of lung inflammation. The present findings confirm those of our previous study, in which we showed that i.n. administration of *S. chartarum* spores induced inflammation in the mouse lungs examined by histological methods. Similarly, Rao et al. [16] showed recently that a single, but high, dose of satratoxin-producing *S. chartarum* spores induced inflammation with haemorrhagic exudates and elevated macrophage concentrations in the BAL sample in rats. The present findings should, however, be interpreted with caution, since spore concentrations used in the present study are rather high compared with real-life situation for humans. Nevertheless, it should be noted that only a small fraction of spores installed intranasally reach the mice alveoles. In addition, the duration of exposure to moulds in a moisture-damaged building can be substantially longer compared with 3 weeks exposure period used in the present study.

In order to examine in detail the mechanisms of how inflammatory cells accumulate into the lungs during fungal exposure, we studied the expression of major pro-inflammatory cytokines that are secreted from alveolar macrophages and other resident lung cells in the first line of defence. The expression of IL-1 β , IL-6 and TNF- α mRNA was elevated in a dose-dependent manner in the lungs of mice that had received repeated instillations of 10⁵ *S. chartarum* spores, with no significant distinction between the satratoxin-producing and the non-producing strain. The expression levels of major Th2 and Th1 cytokines were low and there were no differences between the groups. Recently, Purokivi et al. [28] reported elevated levels of IL-1, TNF- α and IL-6 in the nasal lavage fluid of subjects from a moisture-damaged

school building, which is in line with the present findings. To our knowledge, no studies on cytokine expression in response to *S. chartarum* exposure in experimental animal models have been published.

To further investigate the mechanisms of leucocyte infiltration in our model of spore-induced lung inflammation, we characterized chemokine mRNA expression in mice lung after *S. chartarum* exposure. A significant and dose-dependent induction of CCL3/MIP-1 α , CCL4/MIP-1 β and CCL2/MCP-1 mRNA was found after repeated i.n. exposure to *S. chartarum* spores. CCL3/MIP-1 α acts as a chemoattractant through CCR1 and CCR5 receptors, CCL4/MIP-1 β through the CCR5 receptor and CCL2/MCP-1 through the CCR2 receptor [29]. CCR1, CCR2 and CCR5 are expressed on the surface of a variety of cells involved in the host defence, such as T cells, monocytes and dendritic cells. No differences in mRNA expression levels were observed between exposure to satratoxin-producing and non-producing *S. chartarum* strains.

Interestingly, the induction of CXCL5/LIX chemokine was significantly elevated after exposure to the satratoxin-producing *S. chartarum* strain compared with the non-producing strain. CXCL5/LIX is reported to act through the CXCR2 receptor in a specific manner in relation to neutrophils. It is of interest that the neutrophil infiltration was comparable after exposure to the satratoxin-producing and non-producing strains. Neutrophil accumulation into the lung must, therefore, also be mediated by chemokine receptors other than CXCR2. In agreement with this hypothesis, it has been recently reported that in response to certain inflammatory stimuli, neutrophils can also be recruited by CCL3/MIP-1 α , CCL4/MIP-1 β and CCL2/MCP-1 through their corresponding receptors, CCR1, CCR2 and CCR5, on neutrophils [30, 31].

In the classical experimental allergic asthma model, considerable changes in the increased bronchial responsiveness to inhaled MCh can be measured in mice sensitized to ovalbumin compared with non-sensitized control mice [19]. In the present study, bronchial responsiveness to inhaled MCh measured by whole-body plethysmography was not altered significantly between different mice groups. In standard asthma models, the leucocyte infiltrate in lungs is mainly composed of eosinophils and Th2 cells and sensitized mice also produce allergen-specific IgE antibodies [32–34]. In contrast, the influx of inflammatory cells in our study consisted predominantly of macrophages and neutrophils, and no *S. chartarum*-specific IgE antibodies were found. Differences in leucocyte infiltrate in lungs and in allergic vs. inflammatory status may therefore explain why no considerable changes in airway responsiveness were observed.

Exposure to *S. chartarum* has been linked to non-specific symptoms such as eye irritation, headache, cough, PH and dermatitis [3, 35–37]. The ability of the fungus to produce trichothecenes, such as satratoxin G and H, has been suggested to be responsible for part of these symptoms. Interestingly, in the present study both the satratoxin-producing and the non-producing strain elicited comparable lung inflammation in exposed mice, suggesting that satratoxin G and H are not major mediators of lung inflammation in our model. It should be noted, however, that *S. chartarum* also produces toxins other than satratoxins [10, 38, 39], which may

play critical roles in the development of inflammation. Finally, *S. chartarum*-derived agents other than toxin, e.g. fungal cell wall components or other immunostimulatory substances might induce the inflammatory response.

Taken together, our results indicate that lung inflammation induced by repeated i.n. instillations of *S. chartarum* spores is regulated by the induction of pro-inflammatory cytokines and leucocyte-attracting chemokines. The present findings also suggest that *S. chartarum*-derived components, other than satratoxins, mediate the development of this inflammatory response.

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