

Original article

Allergic inflammation induced by a *Penicillium chrysogenum* conidia-associated allergen extract in a murine model

Background: Recent evidence has shown that viable conidia from the fungus *Penicillium chrysogenum* induce allergic effects in mice. The present study was conducted to determine the specific allergic dose response of C57BL/6 mice to the protease extract, *Pen ch*, isolated from viable *P. chrysogenum* conidia.

Methods: Mice were treated with primary intraperitoneal (IP) injections of 10 or 100 µg of *Pen ch* adsorbed to alum, followed by weekly IP injections of 0.1, 1.0, or 10.0 µg *Pen ch* with alum for 4 weeks, and with 10.0 µg of *Pen ch* by intranasal (IN) inoculations the final 2 weeks before killing.

Results: Intraperitoneal injections of 10 and 100 µg of *Pen ch* for 5 weeks followed by 2 weeks of IN instillation of 10 µg induced significant increases of total serum immunoglobulin (Ig)E and IgG₁. Bronchoalveolar lavage cell counts revealed increased numbers of eosinophils and neutrophils. Histopathological examination of lungs detected perivascular inflammation by eosinophils and neutrophils and increased mucous production.

Conclusions: The data presented in this study indicate that sensitization to protease allergens released by viable *P. chrysogenum* conidia *in vivo* induce a strong allergic inflammatory response in a murine model, which could have implications for people exposed to high levels of conidia of this organism.

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Sick building syndrome (SBS), a term describing various symptoms including allergic rhinitis, difficulty breathing, and tightness in the chest associated with time spent in certain buildings, has been shown to be associated with the presence of certain fungi and their spores or conidia (1–3). Few studies have been conducted to elucidate the roles of fungal conidia associated with SBS in inducing or propagating allergic inflammation. Numerous studies have shown correlations between the presence of various fungi, fungal spores, mildew, and dampness in buildings with various allergic symptoms (4–6). Many of these studies have implicated various *Penicillium* sp., and recent evidence by our laboratory and others has shown a correlation of allergic symptoms in people working or living in buildings contaminated with *Penicillium chrysogenum* (2, 3, 7). An animal model was established to examine the *in vivo* roles of these conidia in inducing allergic effects (8, 9). This animal model has also been used to characterize the role of allergens released by viable *P. chrysogenum* conidia. As we have recently described, viable *P. chrysogenum* conidia release proteolytic enzymes which we have extracted and termed *Pen ch*. This conidia-associated protease extract has been shown to both induce and propagate allergic inflammation in a

mouse model (10), and has not been previously characterized by other investigators.

Numerous other protease and enzyme allergens have been characterized from various organisms including the common house dust mite (11, 12), *Penicillium* sp. (13–15), and *Aspergillus* sp. (16, 17). Dust mite allergens have been thoroughly studied in animal models as they were believed to be the major allergens in homes. Some recent studies have looked at the allergic responses to various *Aspergillus fumigatus* allergens and conidia in mice (18–20), but no other studies have been published examining the effects of exposure to other species of fungi and conidia using animal models.

The current study was conducted in order to determine the specific concentrations of the protease extract *Pen ch* required to induce allergic effects in mice and further characterize the allergic inflammation induced by this organism in comparison with the well-characterized house dust mite protease allergen *Der p 1* (11). We determined that a combined protocol of intraperitoneal (IP) sensitization to 10 µg of *Pen ch* followed by intranasal (IN) challenge with 10 µg of the same material results in strong induction immunoglobulin (Ig)E and protease- and conidia-specific IgG₁. This protocol also

produced marked airway eosinophilia as well as increased mucus production in some animals. The *Pen ch*-sensitized mice also developed perivascular and peribronchial inflammation in the lungs by eosinophils and neutrophils. These results provide evidence of allergic inflammation induced by novel protease-allergens released by viable *P. chrysogenum* conidia that should be studied further to determine exact mechanisms inducing allergic effects.

Materials and methods

Production of viable *P. chrysogenum* conidia

Viable *P. chrysogenum* conidia were propagated on cellulose-based ceiling tile to retain wild-type characteristics as described in (10). Freshly isolated conidia routinely consisted of 25% viable conidia.

Conidia-associated protease extract preparation

The protease extract, *Pen ch*, was isolated from viable *P. chrysogenum* conidia and characterized as described in (10). Average specific activity of *Pen ch* extracts was 2584 units/mg.

Inoculations

Female C57BL/6 mice 3 weeks old were purchased from Charles River (Portage, MI) and divided into groups of six mice each for the *Pen ch* inoculations. Mice were housed in suspended steel cages under constant high efficiency particle arrestor (HEPA)-filtering. Animal protocols were approved by the local Institutional Animal Use and Care Committee (IAUCC), and the animal facility was supervised by a full time veterinarian. House dust mite (*Dermatophagoides pteronyssinus*) allergen *Der p* 1 (11) was purchased commercially (Indoor Biotechnologies, Ltd., Charlottesville, VA) to be used as a positive control treatment. A summary of the treatment protocol is shown in Table 1. Mice were treated by IP injections with phosphate-buffered saline (PBS) mixed with aluminum hydroxide (alum; Pierce, Rockford, IL, USA), *Pen ch* adsorbed to alum, or *Der p* 1 adsorbed to alum 1 day/week. *Pen ch* was given initially at doses of either 10 or 100 µg. *Der p* 1 was given at an initial dose of 10 µg followed by weekly injections of 10 µg for 4 weeks. Each protease-extract treated group was given weekly injections IP with 0.1, 1.0, or 10.0 µg *Pen ch*. After week 5 of IP injections, the animals were subjected to 2 weeks of IN instillation with 25 µl per nare of PBS, 10 µg *Pen ch*, or 10 µg *Der p* 1 in 50 µl total volume. The animals were killed 18–20 h after the final IN inoculations.

Collection of blood and lung lavage

Each animal was killed by overdose of anesthetic followed by cardiac puncture with a syringe. Sera were obtained from clotted blood and stored at –20°C. Bronchoalveolar lavage (BAL) was conducted on each animal as described. Briefly, the lungs were lavaged with 4 ml of sterile Hank's balanced salt solution with 0.5 ml intervals. The lungs were removed and placed in 10% neutral buffered formalin without inflation for pathological examination. The BAL fluid was centrifuged at 1000 × *g* for 10 min to pellet the cells. The remaining BAL fluid was filtered and stored at –20°C. Cytospin slides were prepared of BAL cells, and the slides were fixed in absolute methanol and stained with Wright–Giemsa for cell differential counts. The numbers of eosinophils, neutrophils, and macrophages were counted from each BAL sample and compared with controls to determine levels of airway inflammation.

Analysis of lung sections

Formalin-preserved lungs were embedded in paraffin and sectioned for microscopic examination, as described in (10). The tissue was stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) to characterize the specific cells and inflammation present and production of mucin, respectively. Each specimen was labeled in a manner that blinded the pathologist to prevent observational bias. The following numerical scores were assigned by the pathologist to describe the peribronchial and perivascular inflammation for each specimen: 0 = normal, 1 = few cells noticeable at low power, 2 = diffuse rings of cells noticeable only at higher power, 3 = diffuse to numerous rings of cells visible at low power, 4 = numerous cells visible at low power throughout tissue.

Assays for specific cytokines, leukotrienes, chemokines, and serum antibodies

Levels of cytokines and chemokines were determined using monoclonal antibody-based sandwich enzyme-linked immunosorbent assays (ELISAs) as described in (10). Matched-pair antibodies or ELISA sets were purchased for the following serum antibodies, cytokines, and chemokines: IgE, IgG₁, IgG_{2a}, tumor necrosis factor-α, IL-4, IL-5, IL-6, IL-10, interferon-γ (Pharmingen, San Diego, CA), and IL-13, MIP-2, KC and eotaxin (R&D Systems, Minneapolis, MN).

Immunoblot

A sample of the protease-extract *Pen ch* was subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (21). The proteins

Table 1. Treatment protocol for sensitization to *Pen ch* protease extract

Group	Description of treatment	Dose of treatment
1	PBS/alum IP weekly for 5 weeks, PBS IN weekly for 2 weeks	200 µl PBS/alum IP, 50 µl PBS IN
2	10 µg <i>Pen ch</i> IP primary, 0.1 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
3	10 µg <i>Pen ch</i> IP primary, 1.0 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
4	10 µg <i>Pen ch</i> IP primary, 10 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
5	100 µg <i>Pen ch</i> IP primary, 0.1 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
6	100 µg <i>Pen ch</i> IP primary, 1.0 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
7	100 µg <i>Pen ch</i> IP primary, 10 µg <i>Pen ch</i> IP for 4 weeks, 10 µg <i>Pen ch</i> IN for 2 weeks	200 µl <i>Pen ch</i> /alum IP, 50 µl <i>Pen ch</i> IN
8	10 µg <i>Der p</i> 1 IP weekly for 5 weeks, 10 µg <i>Der p</i> 1 IN for 2 weeks	200 µl <i>Der p</i> 1/alum IP, 50 µl <i>Der p</i> 1 IN

PBS, phosphate-buffered saline; IP, intraperitoneal; IN, intranasal.

were transferred from the gel to a polyvinylidene difluoride (PVDF) membrane and blocked in Tris-buffered saline with 0.05% Tween-20 (TTBS)/0.2% casein for 1 h at room temperature. After blocking, the membrane was immersed with serum from sensitized mice diluted 1 : 100 and incubated for 2 h at room temperature. The membrane was washed with TTBS and then incubated with a secondary anti-mouse IgG antibody conjugated with horseradish peroxidase (HRP; Pierce) diluted 1 : 10 000 for 1 h at room temperature. Finally, the membrane was exposed to chemiluminescent reagents (SuperSignal West Pico; Pierce) for 30 min, followed by detection with autoradiographic film.

Protease extract-specific ELISA

An antigen specific ELISA was conducted as described in (10). Briefly, 10 µg of the protease extract *Pen ch*, 1×10^6 viable *P. chrysogenum* conidia (25% average viability), and 10 µg bovine serum albumin (BSA) diluted in PBS pH 7.2 were added to an immunoplate (Maxisorp, Nunc, Denmark) and incubated overnight at 4°C for the assay. The plate was blocked with blocking buffer (PBS pH 7.2 + 1% BSA) for 1 h at room temperature. Sera from protease-sensitized animals or mice treated with PBS only were pooled and diluted 1 : 2 for IgE and 1 : 5 for IgG₁ in blocking buffer, and monoclonal antiserum PCM39 specific for the 34 kDa *P. chrysogenum* major allergen (a generous gift from Dr Horng-Der Shen) (15) was not diluted. Primary antisera were incubated for 2 h at room temperature and plates were washed with PBS + 0.05% Tween-20. Biotinylated monoclonal antibodies specific for either mouse IgE or IgG₁ were diluted to 2 µg/ml in blocking buffer and incubated for 1 h at room temperature. HRP-streptavidin (Zymed, San Francisco, CA, USA) was diluted 1 : 2500 in blocking buffer and incubated for 30 min at room temperature, followed by addition of tetramethylbenzidine (TMB) (Dako, Carpinteria, CA, USA) for 20 min, and the reaction was stopped with 2 N H₂SO₄. Absorbance readings of each well were determined by a microplate reader (Dynatech, McLean, VA, USA) using a 450 nm filter. Absorbance readings of BSA wells were subtracted from protease and conidia absorbance readings and compared with wells incubated with control sera from mice inoculated with PBS only.

Statistics

Statistical analyses were performed using Sigma Stat 2.0, a statistical program designed by Jandel. The data were subjected to analysis of variance (ANOVA) to determine the significance of differences of test groups compared with controls. Significant results were subjected to a *post hoc* Tukey multiple comparisons test to determine which groups of mice had significant results compared with controls. Significance levels were determined with $\alpha = 0.05$.

Results

IgE and protease-specific IgG₁ responses of *Pen ch*-sensitized mice

Mice primed with 10 or 100 µg of the protease extract *Pen ch* and sensitized to 10 µg *Pen ch* produced significant levels of total serum IgE ($P < 0.001$) (Fig. 1). Significant levels of IgE ($P < 0.02$) were also detected in sera from mice primed with 100 µg protease extract and sensitized to 0.1 µg *Pen ch*. Significant levels of IgG₁ ($P < 0.001$)

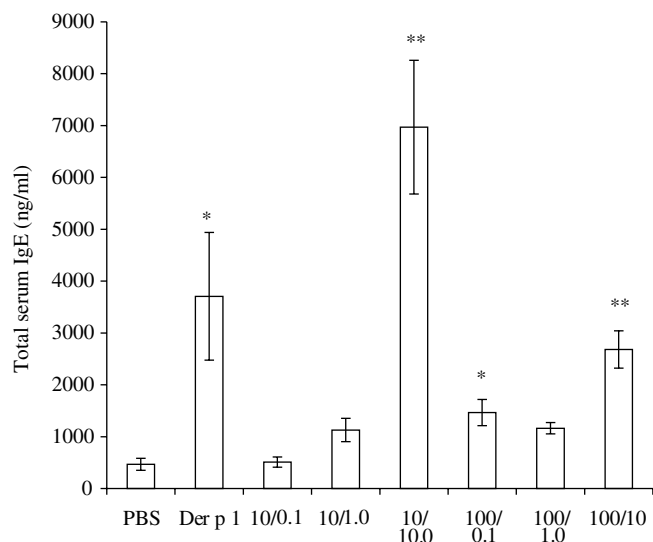


Figure 1. Serum immunoglobulin (Ig)E levels after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of *Pen ch*. Bars represent mean serum total IgE concentrations from each treatment group ($n = 6$). * $P < 0.02$ and ** $P < 0.001$ compared with the negative control animals (phosphate-buffered saline). Error bars represent SEM. Data are representative of two independent experiments (0.1, 1.0, 10, 100 = *Pen ch* in µg).

were detected in sera from mice primed with 10 and 100 µg *Pen ch* and sensitized to 10 µg *Pen ch* (Fig. 2A). Positive control mice treated with *Der p 1* produced significant levels of IgE ($P < 0.02$) and IgG₁ ($P < 0.001$) as well. Significant levels of serum IgG_{2a} ($P < 0.001$) were detected in *Der p 1*-sensitized positive control animals only but not in any other groups of mice (data not shown). Figure 2B shows significant binding of both viable *P. chrysogenum* conidia (25% viability) and protease extract *Pen ch* ($P < 0.001$) by protease-sensitized sera IgG₁ as compared with control sera. Positive control sera IgG₁ from *Der p 1*-treated mice bound significantly to immunoplate-immobilized *Der p 1* ($P < 0.001$), and did not cross-react with *P. chrysogenum* conidia or protease extract *Pen ch* (data not shown). A prominent band was detected by immunoblot analysis that corresponded to a stained protein band in the PAGE gel with an apparent molecular weight of 52 kDa (data not shown). No significant binding of *Pen ch* was detected by immunoblot analysis with the monoclonal antisera PCM39 (data not shown).

Airway cytokine and chemokine production by *Pen ch*-sensitized mice

Significant levels of airway IL-13 ($P < 0.02$) were detected in *Der p 1*-sensitized positive control animals only (data not shown). Significant levels of airway eotaxin

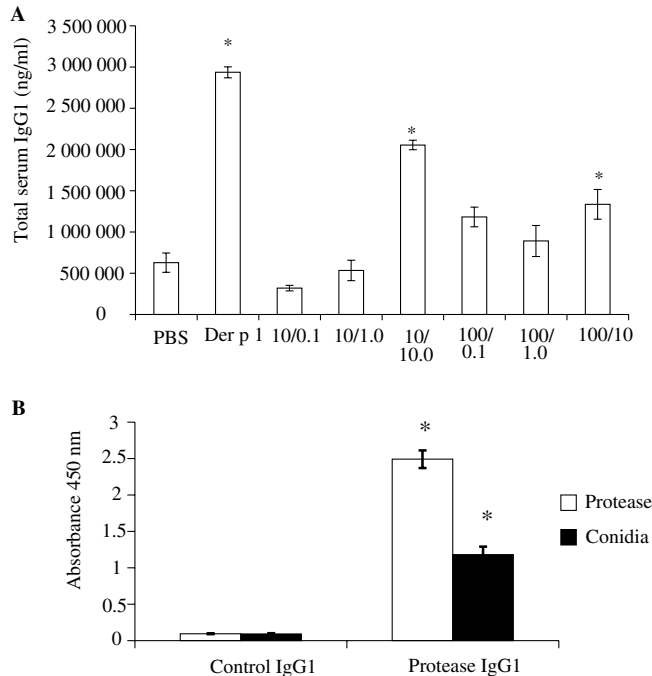


Figure 2. Total serum and *Pen ch*-specific immunoglobulin (Ig)G₁ after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of *Pen ch*. Data bars in (A) represent mean total serum IgG₁ concentrations from each treatment group ($n = 6$). Data bars in (B) represent absorbance at 450 nm as described in Materials and methods using pooled sera from mice sensitized to 10 μ g of *Pen ch* and incubated with either 10 μ g of *Pen ch* or 1×10^6 conidia bound to immunoplates. * $P < 0.001$ for sera from mice sensitized to various concentrations of *Pen ch* compared with sera from mice inoculated with phosphate-buffered saline (PBS) only (PBS in A) and pooled sera from mice sensitized to 10 μ g of *Pen ch* compared with sera from mice treated with PBS only (Control IgG₁ in B). Error bars represent SEM. Data are representative of two independent experiments (0.1, 1.0, 10, 100 = *Pen ch* concentrations in μ g).

($P < 0.002$) were detected in mice primed with 100 μ g protease extract and sensitized to 10 μ g protease extract (data not shown). No significant levels of airway IL-5 or other airway cytokines, chemokines, or leukotrienes assayed were detected by ELISA (data not shown).

BAL cell counts

Mice sensitized IP to *Der p 1* and challenged with 10 μ g *Pen ch* after 10 μ g of priming with *Pen ch* developed significant BAL eosinophilia ($P < 0.002$) (Fig. 3). Each group of mice primed with 100 μ g *Pen ch* IP and challenged with different concentrations of antigen also developed significant eosinophilia ($P < 0.002$). In addition, significant numbers of neutrophils ($P < 0.002$) were induced by priming and challenge with 10 μ g *Pen ch* as

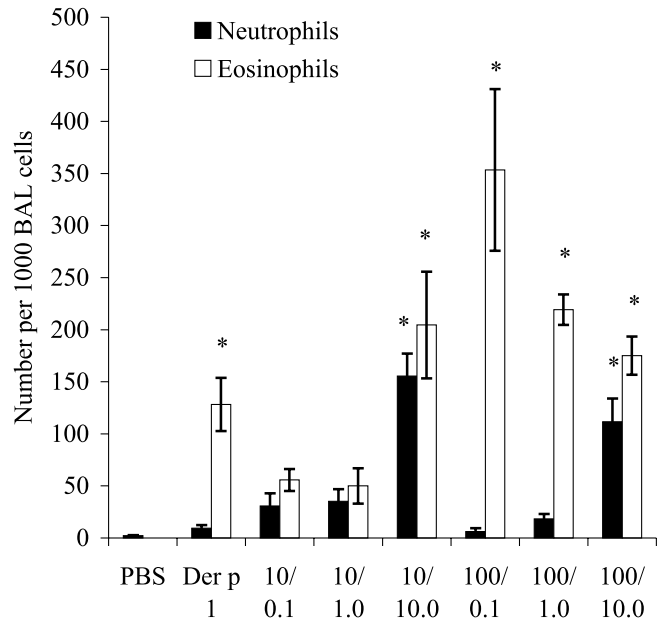


Figure 3. Airway [bronchoalveolar lavage (BAL)] eosinophils and neutrophils after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of *Pen ch*. Bars represent the mean number of each cell type per 1000 BAL cells counted from each treatment group ($n = 6$). * $P < 0.002$ compared with negative control animals (phosphate-buffered saline). Error bars represent SEM (0.1, 1.0, 10, 100 = *Pen ch* concentrations in μ g).

well as by challenge with 10 μ g *Pen ch* after initial IP injections of 100 μ g *Pen ch* (Fig. 3).

Lung histopathology

Figure 4 consists of several photographs of lung tissues from normal mice treated with PBS and mice primed with and sensitized to 10 μ g of the protease extract *Pen ch*. Figure 4A shows normal lung tissue stained with H&E, indicating no inflammation or structural changes. Figure 4B shows normal lung tissue stained with PAS. Low amounts of magenta staining in the epithelium indicate basal production of mucin as expected in normal airways. Figure 4C is an H&E slide at higher magnification of tissue from mice sensitized to and challenged with 10 μ g *Pen ch* for 7 weeks, which shows a significant influx of eosinophils and neutrophils in response to the protease extract. Figure 4D shows a similar area of lung tissue from *Pen ch*-sensitized mice stained with PAS, and arrows point to magenta staining of most of the cells of the epithelium that indicates a significant increase in mucin production caused by mucus cell hyperplasia.

Lungs from mice sensitized to and challenged with 10 μ g *Pen ch* received significant inflammatory scores of 2–3 ($P < 0.001$) for peribronchial and perivascular eosinophilia and neutrophilia (Fig. 5). Interstitial airways near the alveoli from these mice also had significant

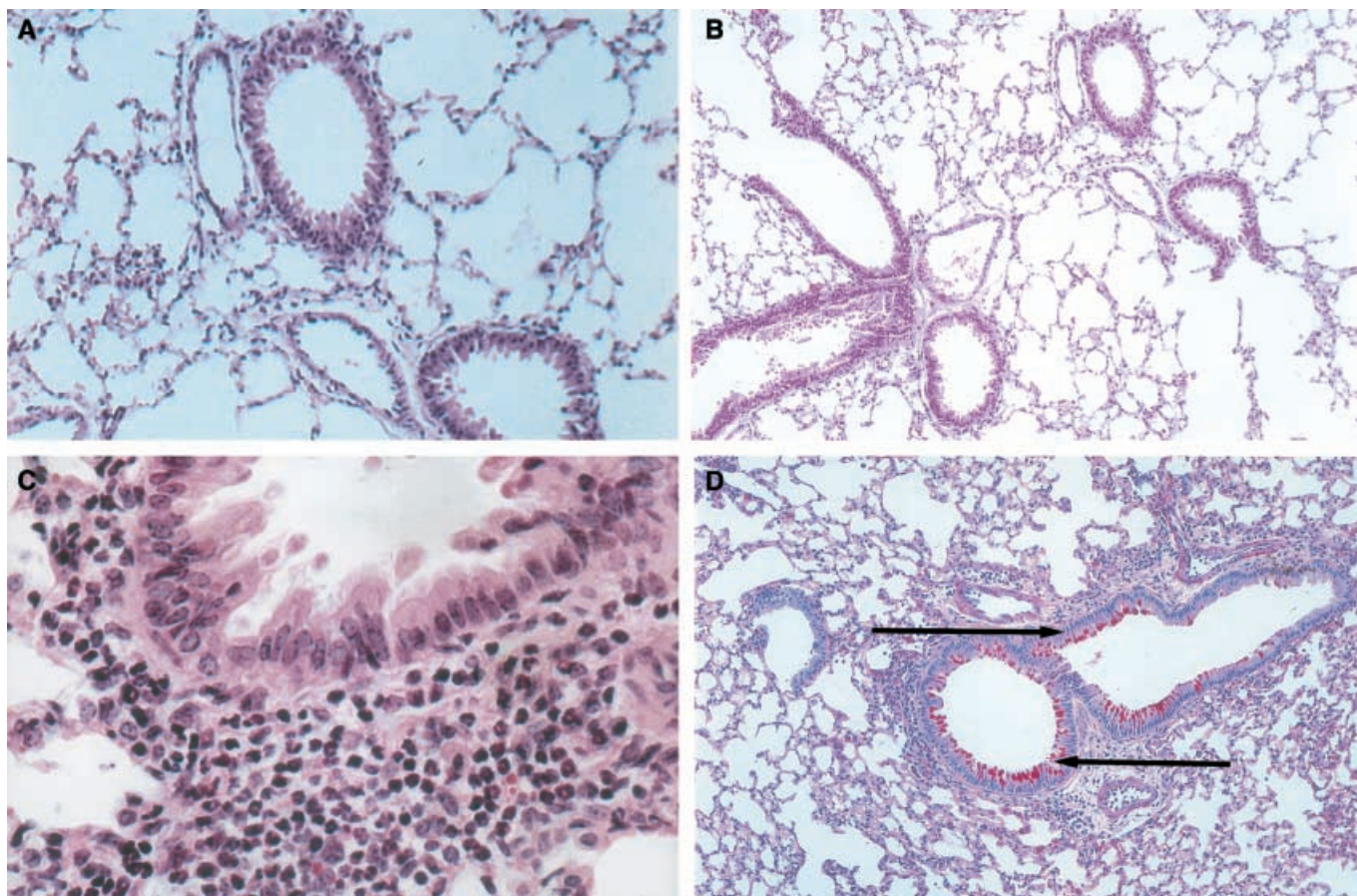


Figure 4. Histopathological examination of lungs from mice after seven weeks of intraperitoneal and intranasal inoculations with 10 μ g of *Pen ch*. Significant perivascular and peribronchial eosinophilia and neutrophilia are evident in mice sensitized to *Pen ch* (C) (H&E $\times$ 500) compared with mice treated with phosphate-buffered saline (PBS) only (A) (H&E $\times$ 125). Arrows indicate increased mucus cell hyperplasia in mice sensitized to *Pen ch* (D) [periodic acid Schiff (PAS) $\times$ 125] compared with mice treated with PBS only (B) (PAS $\times$ 125).

increases in macrophages, lymphocytes, and plasma cells. Mice primed with 10 μ g and sensitized to either 0.1 or 1.0 μ g *Pen ch* exhibited no significant differences in lung pathology compared with controls. Mice primed with 100 μ g and challenged with 0.1 μ g *Pen ch* showed evidence of significant medium and terminal airway neutrophilia and eosinophilia and perivascular neutrophilia ($P < 0.01$) with inflammatory scores from 1 to 3 (Fig. 5). Mice primed with 100 μ g and challenged with 1.0 μ g *Pen ch* exhibited significant perivascular and both medium and terminal bronchial eosinophilia and neutrophilia ($P < 0.001$) with inflammatory scores of 2–3 (Fig. 5). These mice also exhibited increased mucin production (data not shown). Mice primed with 100 μ g and challenged with 10 μ g *Pen ch* exhibited significant perivascular and peribronchial neutrophilia and eosinophilia ($P < 0.01$) with inflammatory scores of 1–3 (Fig. 5) and minor mucus cell hyperplasia (data not shown).

Discussion

Recent evidence by numerous investigators has shown the association of fungi and their conidia with indoor air quality problems and SBS (1, 3, 22). Recent evidence by our laboratory and others has correlated the presence of or sensitization to *Penicillium* sp., including *P. chrysogenum*, with symptoms consistent with SBS (2, 3) and various allergic conditions (6, 7, 23). In another study, *Penicillium* sp. were the most commonly isolated molds in indoor dust samples (24). Previous evidence by our laboratory showed an increase in serum IgG₁ specific for conidia-allergens as well as increased airway eosinophilia and neutrophilia in response to high levels of viable *P. chrysogenum* conidia (9). These studies also showed that nonviable *P. chrysogenum* conidia produced by exposure to methanol did not induce similar allergic reactions in mice (9).

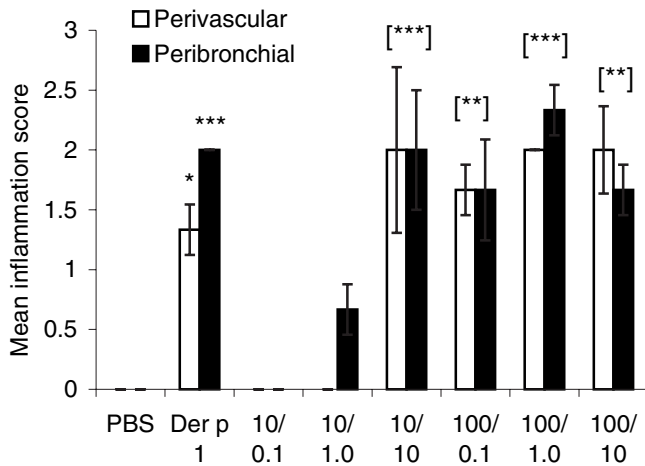


Figure 5. Mean airway inflammation of mice after 7 weeks of intraperitoneal and intranasal inoculations with various concentrations of *Pen ch*. Bars represent the mean peribronchial and perivascular inflammatory score for each treatment group ($n = 6$) as determined by a blinded pathologist and described in Materials and methods. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with negative control animals (PBS). Error bars represent SEM (0.1, 1.0, 10, 100 = *Pen ch* concentrations in μg).

Several allergens have been characterized from various *Penicillium* sp., many of which are proteases (13, 25, 26). Although many of these allergens cross-react with patient IgE indicating sensitization, they were isolated from mycelial cultures and not conidia alone. Therefore, we decided to focus on the roles of allergens released by viable *P. chrysogenum* conidia only.

Few animal studies have been conducted to elucidate the mechanism of sensitization to fungal allergens released by molds implicated in SBS. Recent studies characterized the induction of allergic asthma in BALB/c mice by inoculations with recombinant *A. fumigatus* allergens, some of which have been characterized as proteases (18, 19). These allergens were shown to induce airway hyperreactivity, airway inflammation by eosinophils, increased production of IgE, and expression of Th2 cytokines. Although *A. fumigatus* is a well-characterized opportunistic mold that infects primarily immunocompromised patients, the organism is not a common contaminant of buildings that have had water events. Therefore, the current studies were necessary to address the symptomology seen in sick buildings contaminated with various *Penicillium* sp.

We conducted this study to compare the *in vivo* effects of a protease-allergen extract *Pen ch* isolated from viable *P. chrysogenum* conidia with *Der p 1* using C57BL/6 mice. Mice sensitized by IP inoculations for 5 weeks followed by IN challenge with *Pen ch* developed significant levels of serum IgE and protease-extract specific IgG₁. Protease-extract sensitized mice also developed significant airway eosinophilia and neutrophilia as well as mucus cell hyperplasia. The BAL cell counts determined that airway

inflammation was predominated by eosinophils, however, histopathological examination of lung tissue determined that both eosinophils and neutrophils were present in significant numbers. The presence of neutrophils in the current study was only significant in the groups challenged with the highest dose of *Pen ch*. Other studies showed that eosinophils predominate in BAL fluid while neutrophils are more prominent in lung tissues after repeated challenges with protease allergens from *A. fumigatus* (20). The inability to detect BAL cytokines and chemokines was possibly because of denaturation of the cytokines during lyophilization of the BAL fluid or the BAL cytokines being too dilute for the detection limits of the ELISA. The presence of eosinophils and increased IgE and IgG₁ indicate the induction of a Th2 immune response regardless of the inability to detect any Th2-associated factors in the BAL fluid.

Protease-sensitized sera recognized and bound to immunoplate-immobilized viable *P. chrysogenum* conidia and *Pen ch*. This was an important finding as it indicated that viable *P. chrysogenum* conidia might contain protease allergens within the conidia surface. Although protease-extract specific IgE was not detected, antigen-specific IgE is difficult to detect compared with IgG₁ as it is produced in much lower amounts. It is interesting to note that monoclonal antisera specific for a previously characterized protease allergen *Pen ch* 13 from *P. chrysogenum* (26) did not bind to immobilized *Pen ch* or viable conidia. *Pen ch* 13 has a molecular weight of 34 kDa, which is significantly different from the apparent molecular weight of 52 kDa of the primary *Pen ch* conidia protein component we determined by immunoblot analysis (data not shown).

Viable *P. chrysogenum* conidia travel into lower airways because of their small size, and they also act as both an adjuvant and carrier for the protease allergens. According to our hypothesis, the protease extract *Pen ch* allergens are released by viable *P. chrysogenum* conidia upon attempted germination while in the respiratory tract after they are inhaled. As previous studies showed that conidia remained intact for up to 36 h in the lungs before being cleared by macrophages (8), the conidia would have time to release the protease allergens which could then be processed by dendritic cells (DCs) in the lower airways (27). A recent study confirmed that fungal conidia and cellular material are ingested by DCs and carried to the regional lymph nodes for antigen presentation to lymphocytes (28). Although 5 weeks of IP injections of the protease extracts with alum is intense priming, this protocol was utilized in the current study to match a previous study (10) in which mice developed significant allergic inflammation in response to the conidia specific protease extract *Pen ch*. One possible reason such intense priming is required could be the extreme instability of the protease *in vitro* while in solution, which would be inhibited somewhat by intact conidia.

Sensitization to the *P. chrysogenum* conidia-specific allergens in the protease extract *Pen ch* induces strong allergic inflammation in a murine model, suggesting that avoidance of *P. chrysogenum* conidia should help reduce exacerbation of symptoms. Contamination of buildings with *Penicillium* sp., especially *P. chrysogenum*, is a growing problem and will certainly require more extensive approaches to prevent and treat sensitization to allergens from fungal spores that can lead to allergic inflammation and asthma.

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