

Original article

Pen ch 13 allergen induces secretion of mediators and degradation of occludin protein of human lung epithelial cells

Background: Alkaline serine proteases from six prevalent airborne *Penicillium* and *Aspergillus* species have been identified as a group of major allergens (group 13). After entering human airways, the allergens are in initial contacts with respiratory epithelial cells. The purpose of this study is to investigate interactions between the Pen ch 13 allergen from *P. chrysogenum* and human lung epithelial cells.

Methods: A549 cells, 16HBE14o- cells and primary cultures of human bronchial epithelial cells (HBEPc) were exposed to purified Pen ch 13 and mediators released into culture supernatants were assayed with enzyme-linked immunosorbent assay (ELISA) kits. Cleavage of occludin in 16HBE14o- cells was analysed by immunofluorescent staining of whole cells and immunoblot analysis of whole cell extracts. Fragments generated by incubating Pen ch 13 and a synthetic peptide carrying the occludin sequence were analysed by mass spectrometry.

Results: Pen ch 13 induced productions of prostaglandin-E₂ (PGE₂), interleukin (IL)-8 and transforming growth factor (TGF)-β1 by A549 cells, 16HBE14o- cells and primary cultures of HBEPc. The protease activity of Pen ch 13 is needed for the induction of PGE₂, IL-8, TGF-β1 and cyclo-oxygenase (COX)-2 expression. A tight junction protein occludin of 16HBE14o- cells can be cleaved by Pen ch 13 at Gln202 and Gln211 which are within the second extracellular domain of the protein.

Conclusion: Pen ch 13 may contribute to asthma by damaging the barrier formed by the airway epithelium and stimulating the release of mediators that orchestrate local immune responses and inflammatory process from HBEPc.

H.-Y. Tai^{1,*}, M. F. Tam^{1,*}, H. Chou², H.-J. Peng², S.-N. Su², D.-W. Perng³, H.-D. Shen²

¹Institute of Molecular Biology, Academia Sinica, Taipei; ²Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei;

³Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

*These authors contributed equally to this study.

Key words: human lung epithelial cells; interleukin-8; occludin; Pen ch 13 allergen; prostaglandin-E₂; transforming growth factor-β1.

Horng-Der Shen PhD

Department of Medical Research and Education
Taipei Veterans General Hospital

Shih-Pai

Taipei 11217
Taiwan

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The prevalence of atopic asthma is increasing worldwide. The disease is featured by Th2-type inflammation of the airways and airway tissue remodelling (1–3). In recent decades, numerous environmental allergens have been characterized through immunological, proteomic, molecular and structural approaches (4–6). However, the precise mechanism(s) utilized by these allergens to induce atopic asthma remains unclear. After entering human bodies, the environmental allergens are in initial contacts with the respiratory epithelial cells. The bronchial epithelium acts as a physical barrier to the allergens but might also contribute to the pathogenesis of bronchial asthma (7, 8). Upon contact with allergens, the epithelial cells secrete mediators that might play a significant role in orchestrating local immune responses and the inflammatory process (2, 8, 9). Nonetheless, information regarding the interactions between purified allergens and the human airways is still limited.

In allergic sensitization, causative allergens must cross the airway epithelium to interact with antigen-presenting cells. However, the mechanism(s) by which the allergens

cross the airway lining has not been completely elucidated. Among various allergens, a cysteine protease (Der p 1) and a serine peptidase (Der p 9) from the house dust mite *Dermatophagoides pteronyssinus* have been shown to facilitate the transepithelial delivery of allergens by disrupting the tight junctions (10–12). In addition, Der p 1 and Der p 9 enhance the release of cytokines from bronchial epithelial BEAS-2B cells as well as primary cultures of human bronchial epithelial cells (HBEPc) (13). Proteases present in fungal extracts were also found to interact with epithelial cells and induce proinflammatory cytokines (14).

We have previously identified an alkaline serine protease that is a major allergen of six prevalent airborne *Penicillium* and *Aspergillus* species (15, 16). Immunoblotting data showed that immunoglobulin E (IgE) antibodies against the group 13 serine protease allergens could be detected with a frequency of > 60% in the sera of patients allergic to these fungal species (15). In addition to its reactivity with IgE antibodies, the alkaline serine protease allergen of *P. chrysogenum* (Pen ch 13) induced the release

of histamine from peripheral blood leucocytes of asthmatic patients (16). In animal studies, the group 13 alkaline serine protease allergen from *As. fumigatus* (Asp f 13) induced IgE antibody formation, pulmonary inflammation and enhanced the number of eosinophils in the peripheral blood of Asp f 13-sensitized BALB/c mice (17).

To understand the role of Pen ch 13 in human allergic asthma, the effects of Pen ch 13 on human lung epithelial cells were determined in the present study. Our results show that the Pen ch 13 allergen induces the release of Prostaglandin-E₂ (PGE₂), interleukin (IL)-8 and transforming growth factor (TGF)-β1 from A549 cells, 16HBE14o- cells and primary cultures of HBEPc. In addition, Pen ch 13 can cleave occludin tight junction protein of the 16HBE14o- cells. These effects may contribute to the allergenic potential of Pen ch 13.

Material and methods

Purification of Pen ch 13 allergen

Pen ch 13 was isolated as previously described (16). In brief, proteins in *P. chrysogenum* culture medium were precipitated with ammonium sulphate then separated by diethylaminoethyl (DEAE; DE SeptraSorb, Sepragen, Hayward, CA, USA) and CM Sephadex C50 (Pharmacia Biotech AB, Uppsala, Sweden) ion exchangers. The proteolytic activity of the purified Pen ch 13 was analysed with casein (L-5890; Sigma, St Louis, MO, USA) as the substrate as described (16).

Human bronchial epithelial cell culture

A549 cells (a human alveolar type II epithelium-like cell line) were obtained from the Food Industry Research & Development Institute, Hsinchu, Taiwan. The SV40-transformed immortalized bronchial epithelial cell line 16HBE14o- was kindly provided by Dr D. Gruenert (Cardiovascular Research Institute, University of California, San Francisco, CA, USA). Cells were cultured at 37°C in an atmosphere of 5% CO₂ in RPMI-1640 (Life Technologies Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS; Hyclone, Logan, UT, USA) and antibiotics (penicillin 100 U/ml, streptomycin 100 µg/ml; Gibco). Cryopreserved HBEPc were obtained from the Cell Applications, Inc. (San Diego, CA, USA). HBEPc are primary cells derived from normal human bronchi. The bronchial epithelial cells were cultured according to instructions provided by the manufacturer.

Mediators release by bronchial epithelial cells

A549 and 16HBE14o- cells in 24-well culture plates (Costar, Cambridge, MA, USA) at a density of 5×10^4 /well were allowed to grow individually to 80–90% confluence. They were washed with Hank's balanced salt solution (HBSS; Sigma) and incubated in serum-free RPMI-1640 for 24 h. The cells were then incubated with Pen ch 13, Pen ch 13 plus a serine protease inhibitor phenylmethylsulphonyl fluoride (PMSF; Sigma), or with medium alone for 16 h. Mediators including PGE₂, IL-8 and TGF-β1 released into culture supernatants were measured with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (R & D systems, Minneapolis, MN, USA). When the protease inhibitor PMSF was used, Pen ch 13 was preincubated with the inhibitor at

37°C for 20 min before adding to the culture medium of the epithelial cells. The possible cytotoxic effect of Pen ch 13 on cells was assessed by the exclusion of trypan blue dye (Biochrom AG, Berlin, Germany). HBEPc were cultured in bronchial epithelial cell growth medium (Cell Applications) and were treated with Pen ch 13 as described above.

Immunoblot analysis

Expression of cyclo-oxygenase (COX)-2 and cleavage of the tight junction protein occludin in Pen ch 13-treated epithelial cells were determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)-immunoblotting essentially as described by Chou et al. (16). Proteins were extracted from whole cells by adding lysis buffer [1% NP40, 150 mM NaCl, 1 mM Na₃VO₄, 1 mM PMSF, 50 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM β-glycerophosphate, 10 mM NaF] to cell pellets and incubated at 4°C for 30 min. The extracts were clarified by centrifugation and the protein content was determined with a BCA protein assay kit (Bio-Rad, Richmond, CA, USA). Proteins in the whole cell extracts were separated by SDS-PAGE and transferred electrophoretically onto polyvinylidene difluoride (PVDF) membranes (0.45 µm; Millipore, Bedford, MA, USA). After blocking with 1% skimmed milk, the blots were incubated with monoclonal mouse anti-COX-2 (Cayman chemical, Ann Arbor, MI, USA), antioccludin (Zymed laboratories Inc., South San Francisco, CA, USA) or antiactin (Chemicon, Temecula, CA, USA) antibodies for 1 h at room temperature. The blots were washed and incubated with peroxidase-conjugated affinitypure goat antimouse IgG (H + L) antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for 1 h at room temperature. For detection of COX-2 and actin, the blots were visualized by developing in a substrate solution containing 2 mM of 3-amino-9-ethyl-carbazole (Sigma). For occludin, the blots were developed by using an enhanced chemiluminescence (ECL) kit (Amersham Bioscience Ltd, Piscataway, NJ, USA).

Fluorescent antibody staining

The 16HBE14o- cells were seeded on a chambered coverglass (Nalge Nunc International Corp, Naperville, Rochester, NY, USA) at a density of 5×10^3 /well (0.8 cm²/well). They were allowed to grow to 80–90% confluence and incubated in serum-free RPMI-1640 for 24 h. After washing, cells were stimulated with 0.1 µg/ml of Pen ch 13 in a serum-free RPMI-1640 medium at 37°C for 3 h. The cells were fixed for 20 min in phosphate-buffered saline (PBS) containing 10% formaldehyde (J.T. Baker, Phillipsburg, NJ, USA) and were permeated with 2% Triton X-100 at 4°C for 2 min. Cells were incubated with a mouse antioccludin antibody (Zymed) solution at 4°C overnight and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat antimouse immunoglobulin antibodies (Jackson ImmunoResearch) for 1 h at room temperature. After washing, the fluorescent images of Pen ch 13-treated and nontreated control cells were obtained using a confocal laser-scanning microscope (FluoVIEW, Olympus, Tokyo, Japan) with excitation/emission conditions set for FITC detection.

Peptide HPLC/electrospray ionization mass spectrometry

A synthetic peptide, Occl-1, with sequence covering the second extracellular loop of the human occluding (18) (residues 198–215, NPTAQSSGSLSYGSQIYAL) was purchased from Genemed Synthesis Inc. (San Francisco, CA, USA). The peptide was digested with Pen ch 13 at 37°C for 30 min with a substrate to enzyme ratio

of 4000 : 1. The resulting fragments were separated on a C₁₈ reverse-phase column that was online with a quadrupole mass spectrometer (Fisons Instruments, VG Biotech, Altrincham, UK) equipped with an electrospray ion source. The column was eluted with an acetonitrile gradient using trifluoroacetic acid as ion pairing reagent. The mass spectrometer was set in the positive ion mode. Scanning was in the multichannel analyzer mode from *m/z* 500 to 2000. Data were summed according to the total ion current profile and processed by the MAXENT program (19).

Data analysis

The Mann-Whitney U test was used for comparing the mediator release between the Pen ch 13-treated and nontreated control cells. Experiments were performed in triplicates. Data from three different experiments (for A549 and 16HBE14o- cells) were collected for each mediator analysed. The Statistical Package for Social Science (SPSS) version 12.01 software was used for statistical analysis. Significance was inferred from a *P*-value of <0.05.

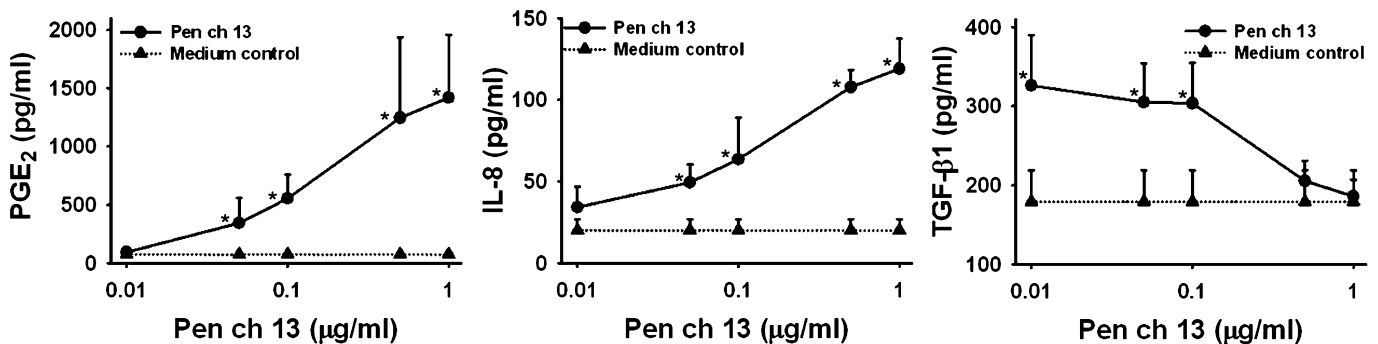
Results

Release of PGE₂, IL-8 and TGF-β1 from Pen ch 13-treated cells

To determine the effects of the Pen ch 13 allergen on human respiratory epithelial cells, cultured cells were incubated in media with various amounts of Pen ch 13 for

16 h. Significant increases in PGE₂, IL-8 and TGF-β1 production were detected in the culture media of Pen ch 13-treated A549 cells (Fig. 1, upper panel), 16HBE14o- cells (data not shown) and primary cultures of HBEpC. A similar result was obtained from two separate determinations on mediator releases by Pen ch 13-treated primary bronchial epithelial cultures. One representative result was shown in the lower panel of Fig. 1. The increases in the production of PGE₂ were statistically significant at Pen ch 13 concentrations ≥0.05 μg/ml and ≥0.5 μg/ml for A549 cells and primary cultures of HBEpC, respectively (Fig. 1). The increases in the production of IL-8 were statistically significant at Pen ch 13 concentrations ≥0.05 μg/ml. Significant differences in the production of TGF-β1 were also detectable at Pen ch 13 concentrations ≥0.01 μg/ml. However, a negative slope for TGF-β1 production at increasing amounts of Pen ch 13 was also observed (Fig. 1). Our results also showed that 0.001 but not 0.0001 μg/ml of Pen ch 13 induced significantly TGF-β1 production by A549 cells (*P* ≤ 0.05, data not shown). For negative control, bovine serum albumin (BSA, 1.0 μg/ml; Sigma) did not induce significantly mediator releases from cultured human respiratory epithelial cells (*P* ≥ 0.05, data not shown).

A549 cells



Primary culture of human bronchial epithelial cells

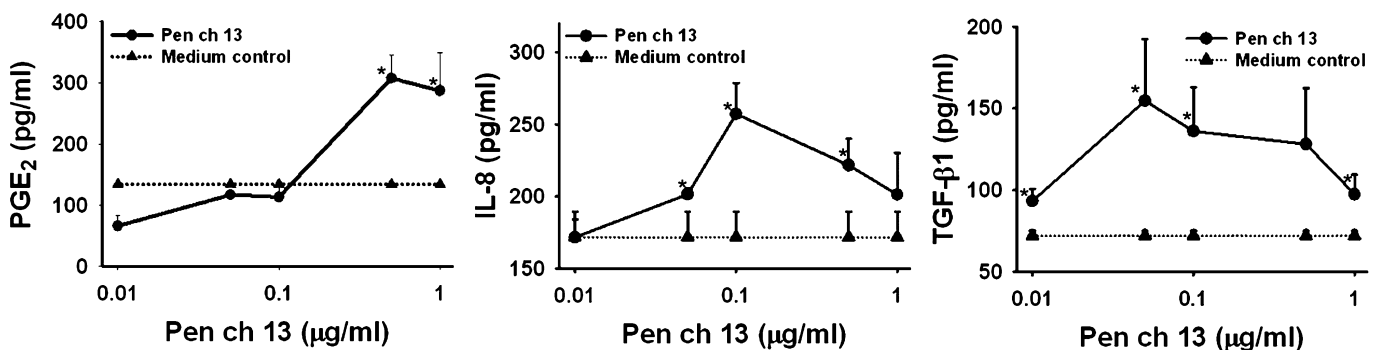


Figure 1. Dose-response curve of Pen ch 13-induced production of prostaglandin-E₂ (PGE₂), interleukin (IL)-8 and transforming growth factor (TGF)-β1 in cultured human A549 cells (upper panel) and primary culture of human bronchial epithelial cells (lower panel). *Indicates a significant difference was observed between the Pen ch 13-treated and control cells.

Viability of Pen ch 13-treated A549 cells

A549 cells that have been treated with Pen ch 13 for 16 h at 37°C were scrutinized with an inverted microscope. A portion of the cells was detached from culture plates. Cell detachment became evident at 0.1 µg/ml of Pen ch 13 (data not shown). The percentage of cells detached were 11%, 44% and 82% at Pen ch 13 concentrations of 0.1, 0.5 and 1.0 µg/ml, respectively. Upon staining with trypan blue, most of the detached cells showed dye exclusion as those of the attached A549 cells. Viability of the detached cells obtained by incubating with 1.0 µg/ml of Pen ch 13 in the culture medium was 95%.

Mediators secretion from A549 cells depends on the enzymatic activity of Pen ch 13 allergen

Pen ch 13-induced PGE₂ secretion from A549 cells was inhibited dose dependently by preincubating Pen ch 13 with increasing amounts of PMSF, a serine protease inhibitor. Pen ch 13 (0.1 µg/ml) was preincubated at 37°C for 20 min with 1.0, 10 or 100 µM PMSF. They were then added to A549 cells and the secretion of PGE₂ from cells was inhibited by 0, 80 and 98%, respectively. Our results also showed that preincubation of Pen ch 13 with 1.0, 10 and 100 µM of PMSF inhibited the secretion of IL-8 and TGF-β1 from A549 cells by 32, 80 and 98% (for IL-8) and by 15, 94 and 100% (for TGF-β1), respectively. In addition, Pen ch 13-induced cell detachment was also inhibited by preincubating Pen ch 13 with PMSF (data not shown). Control experiments showed that PMSF and the solvent dimethyl sulphoxide (DMSO) had no significant effects on mediator release or viability of the A549 cells (data not shown).

Expression of COX-2 in Pen ch 13-treated A549 cells

COX-2 expression in Pen ch 13-treated A549 cells was detected by immunoblotting with anti-COX-2 antibody. COX-2 expression was increased in A549 cells that have been treated with 1.0 µg/ml of Pen ch 13 for 16 h (Fig. 2). PMSF (0.1–100 µM) inhibited dose dependently the Pen ch 13-induced COX-2 expression. As a control, changes in actin expression were not detected in cells treated with Pen ch 13 or Pen ch 13 that has been preincubated with PMSF (Fig. 2). The Pen ch 13-induced PGE₂ release from A549 cells was inhibited by 89% in the presence of the COX-2 inhibitor NS398 (1.0 µM; Calbiochem, San Diego, CA, USA).

Pen ch 13 degraded the tight junction protein occludin of 16HBE14o- cells

The effects of Pen ch 13 on the tight junction protein occludin of 16HBE14o- cells were determined by immunofluorescent staining of cultured 16HBE14o- cells and immunoblot analysis of the whole cell extracts.

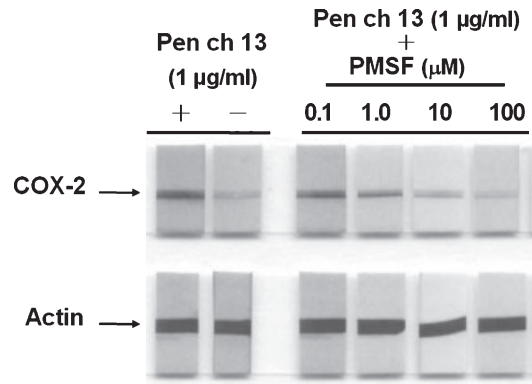


Figure 2. Immunoblot analysis of the expression of the cyclooxygenase (COX)-2 protein in A549 cells exposed to Pen ch 13 and Pen ch 13 pretreated with phenylmethylsulphonyl fluoride (PMSF), a serine protease inhibitor. The expression of actin was included in the analysis as controls.

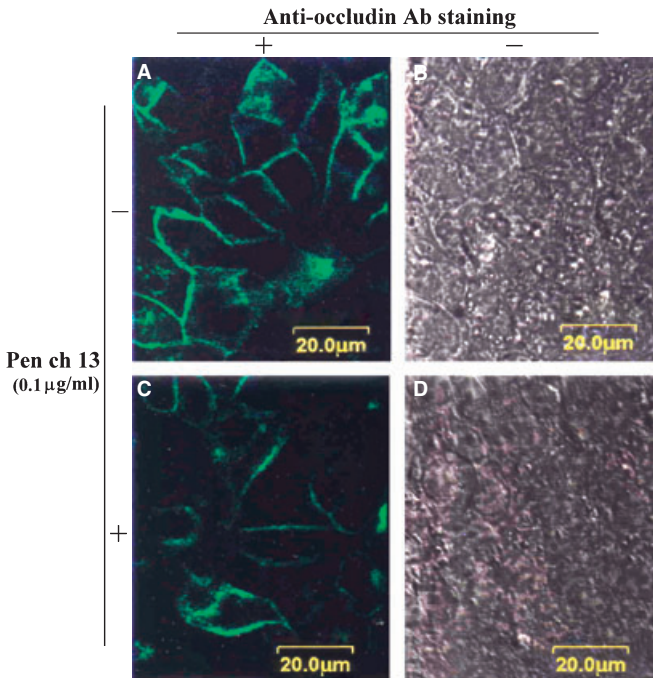


Figure 3. Immunofluorescent antibody staining of the occludin protein in the Pen ch 13-treated (panel C) and nontreated (panel A) 16HBE14o- cells. Panels B and D show the Pen ch 13-treated (panel D) and nontreated (panel B) unstained 16HBE14o- cells, respectively.

16HBE14o- cells were treated with 0.1 µg/ml of Pen ch 13 for 3 h and they were still attached onto the culture plates (data not shown). However, the intensity of the immunofluorescent stain of the occludin in Pen ch 13-treated cells (Fig. 3, panel C) was less than that of cells incubated with culture medium alone (Fig. 3, panel A).

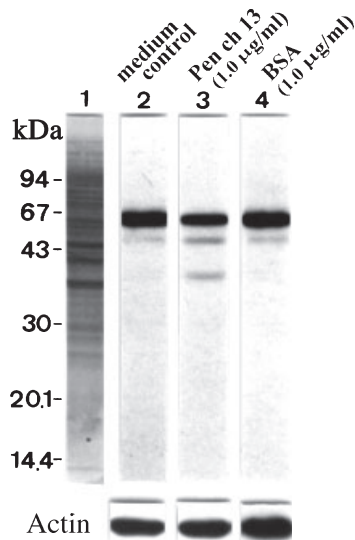


Figure 4. Immunoblot analysis of occludin protein from 16HBE14o- cells treated with Pen ch 13. Strip 1 depicts the dye-stained protein profile of whole cell extracts from 16HBE14o- cells incubated with medium. Immunoblot analysis of whole cell extracts of 16HBE14o- cells incubated for 16 h with medium, Pen ch 13 and bovine serum albumin (BSA) are shown in strips 2, 3 and 4, respectively.

The 16HBE14o- cells were treated with 1.0 µg/ml of Pen ch 13 for 16 h and the occludin protein in the cell extracts was analysed for degradation. The immunoblot intensity of the 64 kDa occludin protein in the treated cells was noticeably decreased (Fig. 4, strip 3) compared to that of control cells incubated with medium alone (Fig. 4, strip 2) or with 1.0 µg/ml of BSA for 16 h (Fig. 4, strip 4). In addition, fragments of 50 and 38 kDa that were reactive to occludin antibodies were detected in extracts of Pen ch 13-treated 16HBE14o- cells (Fig. 4, strip 3). The 50 kDa component can also be detected in control cells but at a lower intensity. The 38 kDa fragment can only be observed in the Pen ch 13-treated 16HBE14o- cells. The immunoblot intensity of actin was about the same in cells treated with Pen ch 13, BSA or incubated with medium alone (Fig. 4).

Human occludin is a 522 amino acid protein with a molecular mass of 59118 Da. It has four transmembrane and two extracellular domains (18). To address whether Pen ch 13 cleaves occludin at its extracellular domains, a peptide with sequence encompassing the second extracellular domain of human occludin ($^{198}\text{NPTAQSSGSLYGSQIYAL}^{215}$) was digested with Pen ch 13. The resulting peptides were separated on a reverse-phase column and the masses were obtained with an online quadrupole mass spectrometer. The identity of the peptide fragments was inferred from the masses obtained. The mass of the major fragments obtained that can be matched with that generated from the Occl-1 sequence is 844 and 755 Da. The 844 Da

fragment could represent $^{202}\text{QSSGSLYGS}^{210}$ and/or $^{203}\text{SSGSLYGSQ}^{211}$. The fragment $^{203}\text{SSGSLYGS}^{210}$ has a theoretical mass of 756 Da. Apparently, Pen ch 13 prefers to cut before and after Q of the Occl-1 sequence. However, minor ions that possibly represent peptides carrying a Y at the C-terminus were also detected ($^{203}\text{SSGSLY}^{208}$, $^{201}\text{AQSSGSLY}^{208}$ and $^{203}\text{SSGSLYGSQIY}^{213}$). Therefore, Pen ch 13 is not a specific protease, even though it has shown some preference for Q and Y.

We used antibodies that are specific for the C-terminal fragment (150 amino acids) of occludin in immunoblot analysis. Data from *in vitro* digestion of Occl-1 peptide suggest that Pen ch 13 possibly cleaves occludin at residues 202 and/or 211. The resulting C-terminal fragments are approximately 37–36 kDa and close to that observed on the immunoblot of the Pen ch 13-treated 16HBE14o- cell extracts (Fig. 4, lane 3).

Discussion

The bronchial epithelium may play a dynamic role in regulating the immunological and inflammatory processes in asthma. In the present study, we analysed the potential effects of the purified Pen ch 13 allergen on human respiratory epithelial cells. Our results showed that Pen ch 13 induced the production of PGE₂, IL-8 and TGF-β1 in cultured A549, 16HBE14o- and primary HBEPc. The mediators release and the expression of COX-2 were inhibited dose dependently by treating the Pen ch 13 with PMSF, a serine protease inhibitor. The results imply that the protease activity of Pen ch 13 mediates these effects. Although Pen ch 13 also induced cell detachment, the viability of the detached A549 cells as determined by trypan blue staining was >95%. Proteases present in fungal extracts of *As. fumigatus*, *Alternaria alternata* or *Cladosporium herbarum* have been found to interact with epithelial cells, leading to morphological changes, cell desquamation and induction of proinflammatory cytokines such as IL-6, IL-8 and monocyte chemotactic protein-1 (14, 20). Results obtained from this study suggest that the alkaline serine protease major allergen of *As. fumigatus* (Asp f 13) (15, 17) may be one of the proteases responsible for the effects detected.

The PGE₂ has been implicated in the development of Th2 T cells (21). PGE₂ is a potent inhibitor of human IL-12 production (22). It inhibits Th1 (23) and enhances Th2 T-cell responses (24, 25). In addition, the Th2 dominant immune responses in BALB/c mice have been attributed to an increased sensitivity of the animals to PGE₂ (26). Thus, the increased production of PGE₂ from Pen ch 13-treated lung epithelial cells may play a significant role in inducing the local immune responses towards Th2 and contributes to allergic asthma.

The production of IL-8 by epithelial cells may also be important for the inflammatory processes. The IL-8 C-X-C

chemokine is a potent chemoattractant for neutrophils (27) and its levels are elevated in the sputum and lavage fluid of patients with asthma (28, 29). The massive infiltration of neutrophils might result in the production of proteolytic enzymes that subsequently cause tissue inflammation and airway injury in asthmatic patients (29).

Chronic asthma is associated with an airway wall remodelling including subepithelial fibrosis and extracellular matrix proteins deposition (1–3). Airway epithelial cells express a variety of growth factors that might be associated with these pathogenic changes. In the present study, TGF- β 1 production was detected in culture supernatants of Pen ch 13-treated human respiratory epithelial cells. The decrease in TGF- β 1 production in cultured cells treated with higher doses of Pen ch 13 (0.5 and 1.0 μ g/ml) may be due to the degradation of TGF- β 1 by Pen ch 13. Pen ch 13 at a concentration of 1.0 μ g/ml (37°C for 16 h) reduced 85% of the TGF- β 1 as determined by ELISA (data not shown). The TGF- β 1 accumulation was related to remodelling of the airway wall in chronic asthma (30). In addition, TGF- β 1 are increased in the bronchoalveolar lavage fluid of atopic asthmatics and the level can be further increased upon allergen exposure (31). Our results suggest that the airway epithelial cell-derived TGF- β 1 may contribute to remodelling responses in asthmatic airways and play a crucial role in the pathogenesis of asthma.

We observed that desquamation induced by Pen ch 13 did not affect significantly the viability of A549 cells. Control experiments showed that BSA up to 1.0 μ g/ml did not increase mediator release or cause cell detachment (data not shown). It was also found that, with the exception of E-ColCF extracts (culture filtrate of *As. fumigatus* grown on collagen-containing medium), desquamation induced by most enzyme-containing fungal extracts did not affect the viability (trypan blue exclusion assay) of A549 cells (14, 20). Thus, results obtained from this study and before indicate that fungal enzymes (extracts) can induce mediators release and detach epithelial cells from the culture surface. Nonetheless, dose–response curves generally show mediator production at enzyme (fungal extracts) concentrations before the onset of morphological changes (8, 14, 20, 32). Proteolytic degradation of cellular adhesion structures may explain the morpho-

logical changes (shrinking of cells and cell desquamation) observed (14). However, at higher concentrations of fungal extracts, the observed morphological changes of A549 cells and corresponding cytoskeleton rearrangement may also contribute to the production of cytokines (14, 33).

The maintenance of tight junctions is crucial to the normal functioning of respiratory epithelium. Occludin is a transmembrane protein that participates in tight junction adhesion and sealing (34, 35). In the present study, Pen ch 13 degraded the occludin of cultured 16HBE14o-HBEpC. In studies of mite allergens, a cysteine protease, Der p 1, has been implicated in modulating the respiratory mucosal permeability (36) and facilitates the transepithelial allergen delivery by disrupting the tight junctions (10, 12).

In conclusion, results obtained from the present study indicate that Pen ch 13 might disrupt the epithelial barrier and facilitate the passage of itself and possibly other allergens to antigen-presenting cells residing in the submucosa. Pen ch 13 induces the production of PGE₂ in epithelial cells. The PGE₂ may subsequently activate and polarize the mucosal immune system towards a Th2 phenotype. The chemokine IL-8 released may recruit inflammatory cells to the airways and lead to exaggerated inflammatory responses that result in disturbing the structure and function of the epithelium. The antiproliferative and profibrogenic factor TGF- β 1 released may contribute to the induction of damaged epithelium in sustained repair cascades. It stimulates the secretion and deposition of interstitial collagens beneath basement membranes and contributes to the development of airway wall remodelling in asthma. Our results suggest that the Pen ch 13 allergen may lead to atopic asthma through all these events that are essential prerequisites for the development of asthma.

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