Der f1 induces pyroptosis in human bronchial epithelia via the NLRP3 inflammasome

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Abstract. Damage to the bronchial epithelium leads to persistent inflammation and airway remodelling in various respiratory diseases, such as asthma and chronic obstructive pulmonary disease. To date, the mechanisms underlying bronchial epithelial cell damage and death by common allergens remain largely unknown. The aim of the present study was to investigate Der f1, an allergen of *Dermatophagoides farinae*, which may result in the death of human bronchial epithelial cells (HBECs). Der f1 induces BECs to undergo the inflammatory cell death referred to as pyroptosis, induced by increasing lactate dehydrogenase release and propidium iodide penetration. Stimulation by Der f1 enhances interleukin (IL)-1β cleavage and release, which is associated with caspase-1 activation. In addition, the NOD-like receptor family pyrin domain-containing 3 (NLRP3), is required for the activation of caspase-1 through increasing the formation of the inflammasome complex. Consistent with these findings, pre-treatment of HBECs with a caspase-1 inhibitor, or silencing of NLRP3 by siRNA transfection, reduced Der f1-mediated IL-1β and pyroptosis. Therefore, the common allergen Der f1 was not only found to induce allergy, but also led to pyroptosis and IL-1β secretion via the NLRP3-caspase-1 inflammasome in HBECs. This newly identified connection of the Der f1 allergen with BEC damage and inflammation may play an important role in the pathogenesis of asthma.

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Introduction

Asthma is a chronic inflammatory airway disease that is defined as reversible airway narrowing, and is characterized by episodic symptoms of dyspnea, wheezing and coughing (1). Asthma is a major health concern, affecting more than 300 million individuals worldwide (1). Bronchial epithelial cells (BECs) are the first line of defence against noxious inhalants, such as microorganisms, gases and allergens that may cause asthma (2). BECs not only constitute a physical barrier, but also play a key role in inflammatory, immune and regenerative processes in response to these noxious inhalants (3). Loss of BEC integrity is a hallmark of asthma pathogenesis (4), and sloughing of BECs has been found in bronchial biopsy samples from patients with mild to severe asthma (5,6). A number of mechanisms may explain the loss of epithelial integrity, such as cell death (7) and disrupted cell-cell and cell-extracellular matrix interactions (7). Repetitive cycles of injury and repair of BECs is a major factor in airway structural changes leading to remodelling of the airways (3,8,9). Therefore, understanding epithelial damage is a valuable approach to identify optimal treatments for chronic airway diseases.

Pyroptosis is a process of host cell death caused by microbial infections and non-infectious stimuli (stroke, chemotherapy and inflammation) (10,11). Pyroptosis differs morphologically and mechanistically from other types of cell death, such as apoptosis and necrosis. The unique aspect of pyroptosis is that it is dependent on caspase-1 activation, which mediates cell death and cleaves and secretes proinflammatory cytokines, such as interleukin (IL)-1β and IL-18 (10,12). Both cell death

and proinflammatory signals cause tissue damage, which may lead to permanent structural changes (13). Therefore, we hypothesized that pyroptosis may act as a pathogenic mechanism contributing to inflammatory injury of airway epithelia.

Allergic sensitization to inhaled allergens, such as house dust mites (HDMs), predisposes susceptible individuals to developing allergic hypersensitivity reactions and producing IgE (2), which are key to the pathogenesis of allergic airway diseases (14). Among HDMs, *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f), are the most important triggers of bronchial asthma, rhinitis and atopic dermatitis (15,16). In addition to the allergic response, HDMs may also disrupt cell adhesion, induce cell death and increase the permeability of lung epithelia (17). Furthermore, Dermatophagoides sp. peptidases have been shown to elicit apoptosis in a bronchial cell model (15). Der f1 (*D. farina* allergen 1), which displays 82% homology to Der p1 (*D. pteronyssinus* allergen 1), possesses cysteine protease activity, and it has been shown to enhance tissue damage and immune activation via downregulation of anti-protease-based lung defences (18,19). However, little is known on the effect of HDMs on BEC injury, particularly with regards to inflammation-mediated cell death. The aim of the present study was to investigate whether the common inhaled allergen, Der f1, induces pyroptosis in BECs, suggesting a connection between BEC injury and an asthmatic inflammatory microenvironment.

Materials and methods

Cell culture. Primary human BECs (pHBECs) were obtained and maintained in BECs growth medium (Lonza, Walkersville, MD, USA). HBE135-E6E7 human bronchial epithelial cells (HBE-135, CRL-2741) were obtained from American Type Culture Collection (Manassas, VA, USA) and maintained in keratinocyte-serum free medium with 5 ng/ml human recombinant epithelial growth factor and 0.05 mg/ml bovine pituitary extract (Invitrogen; Thermo Fisher Scientific, Carlsbad, CA, USA) supplemented with 0.005 mg/ml insulin and 500 ng/ml hydrocortisone (Sigma-Aldrich; Merck KGaA, St. Louis, MO, USA). Both cell lines were cultured at 37˚C, with 90% relative humidity and 5% CO_2.

Cell viability. Cells were plated in 96-well culture plates ($5x10^3$/well) and treated with common inhaled allergens, including recombinant Der f1 (rDer f1), deglycosylated rDer p1 (rDer p1 DG), natural Fel d1 (nFel d1), nCan f1 and nDer p1 (Indoor Biotechnologies Inc., Charlottesville, VA, USA) for 24 h. BEC viability was determined by premixed WST-1 cell proliferation reagent (Clontech Laboratories, Mountain View, CA, USA), according to the manufacturer's instructions.

TdT-mediated dUTP nick end labeling (TUNEL) assays and propidium iodide (PI) staining. Cells were treated with rDer f1 for 24 h and then assayed for apoptotic cell death using a commercial TUNEL assay kit (BD Biosciences, San Diego, CA, USA). Cells were harvested and fixed in 4% formaldehyde, then stained using TUNEL reaction mixture (45 μl labeling solution and 5 μl enzyme solution), followed by incubation in the dark for 1 h at 37˚C. The percentage of apoptotic cells was assessed by flow cytometry (BD Biosciences).

Cells were collected by trypsinization and then stained with PI for 30 min at room temperature. After washing with phosphate-buffered saline, the penetration of the PI dye was analyzed by flow cytometry.

Lactate dehydrogenase (LDH) release assay and cytokine measure. The release of LDH was measured in the cell supernatants of the BECs after rDer f1 treatment using a LDH assay kit (BD Biosciences). Cell supernatants (100 μl) were then transferred to a 96-well microplate and mixed with 100 μl of the reaction solution provided in the kit. Optical density was measured at 492 nm using an enzyme-linked immunosorbent assay (ELISA) reader. The level of IL-1β was determined using an ELISA-based kit (R&D Systems Europe, Ltd., Abingdon, UK). ELISA was performed according to the manufacturer's instructions.

Caspase-1 activity analysis. Caspase-1 activity in the cell lysate was determined with a caspase-1 colorimetric kit (BioVision, Inc., Milpitas, CA, USA) by cleavage of 5 μl of the caspase-1 substrate Ac-YVAD-pNA. The cleavage of pNA was monitored by changes in absorbance at 405 nm.

Immunoblot/co-immunoprecipitation (co-IP). The cells were lysed on ice with M-PER lysis reagent (Pierce Chemical Co., Rockford, IL, USA) and then centrifuged at 14,000 x g for 15 min. The supernatant fraction was collected for immunoblot analysis. Equal amounts of protein were resolved by SDS-PAGE (8-12%) and transferred to a polyvinylidene difluoride membrane. After blocking, the membrane was incubated with the desired primary antibody for 1-16 h. The membrane was then treated with the appropriate concentrations of peroxidase-conjugated secondary antibody, and the immunoreactive proteins were detected using an enhanced chemiluminescence kit (Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions. The interaction of NOD-like receptor family pyrin domain-containing 3 (NLRP3) with caspase-1 was assessed by co-IP. Antibodies against human IL-1β (pro-IL-1β) (rabbit, D3U3E, 1:1000, 12703S), NLRP3 [rabbit, D2P5E, 1:1000 (in IP, 1:100] and caspase-1 (rabbit, D7F10, 1:1000, 3866S) were obtained from Cell Signaling Technology (Beverly, MA, USA). Monoclonal antibody anti-human glyceraldehyde-3-phosphate dehydrogenase (GAPDH; rabbit, polyclonal, 1:5000, ABS16) was obtained from Millipore Corp.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RNA isolation was performed using a TRIzol reagent (Invitrogen; Thermo Fisher Scientific). cDNA was prepared using an oligo (dT) primer and RT (Takara Bio, Inc., Otsu, Japan) following standard protocols. RT-qPCR was performed using SYBR-Green on an ABI 7500 Real-Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA). Each PCR reaction mixture contained 200 nM of each primer, 10 μl of 2x SYBR-Green PCR master mix (Applied Biosystems Life Technologies), 5 μl cDNA and RNase-free water in a final volume of 20 μl. The PCR reaction was performed with a denaturation step at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 1 min. All PCRs were performed in triplicate and normalized by the

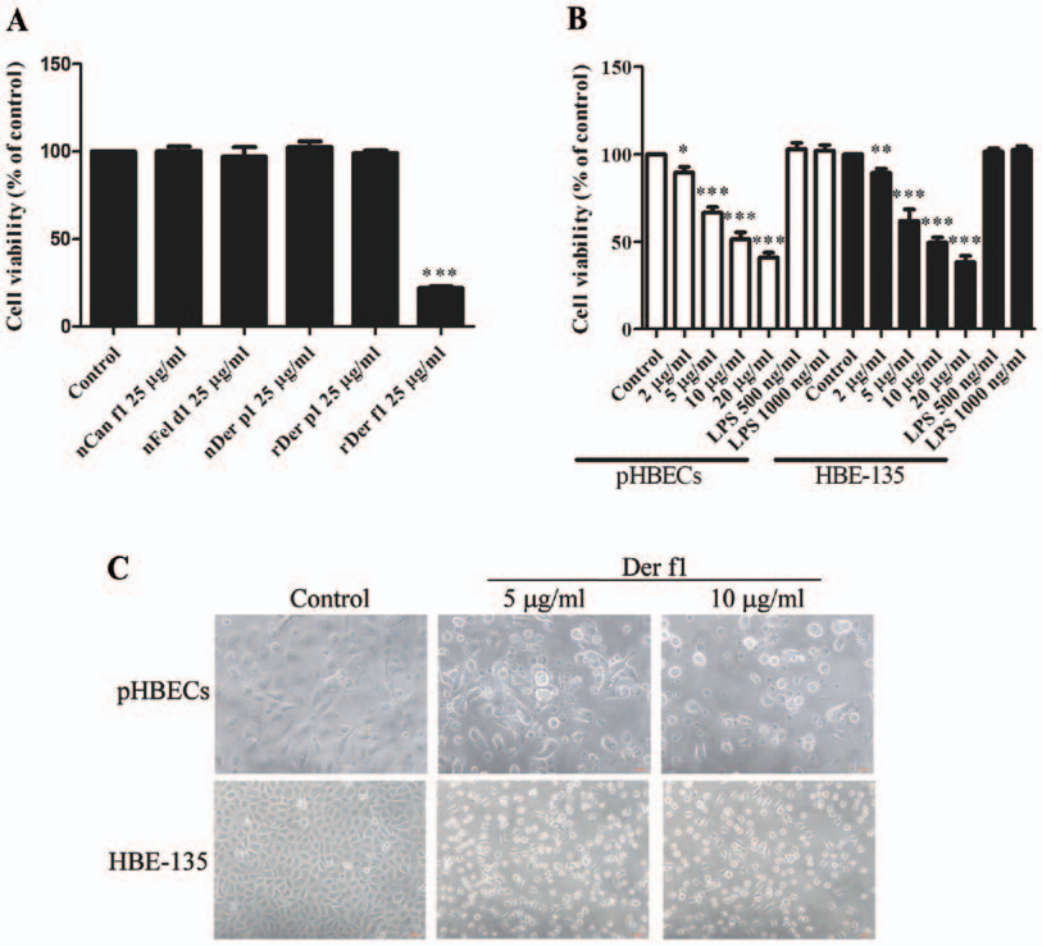

Figure 1. rDer f1 caused cell death in BECs. (A) Effect of allergens on HBE-135 cell viability. (B) rDer f1 decreased the viability of BECs. (C) rDer f1 induced cell death in BECs. pHBECs and HBE-135 cells were treated with various allergens or concentrations of rDer f1 for 24 h. The viability of the BECs was determined by the WST-1 proliferation reagent. BEC morphology was evaluated using an inverted microscope after 24 h of treatment. Data are expressed as the mean of three independent experiments ± standard deviation, $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. control. BECs, bronchial epithelial cells; pHBECs, primary human BECs; Der f1, *Dermatophagoides farin*a allergen 1; LPS, lipopolysaccharide.

internal control GAPDH. The relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method.

siRNA knockdown. BECs were transfected with 25 nM ON-TARGET plus control or NLRP3 siRNA by DharmaFECT formulation 4 reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After 24 h of transfection, the medium was changed to whole medium and the cells were treated with rDer f1 for an additional 24 h. The changes in NLRP3 were measured by RT-qPCR, as described above.

Statistical analysis. Data are presented as mean ± standard deviation. One-way analysis of variance test with Tukey's multiple comparison post hoc test was employed for comparison of all data with the control. $p<0.05$ was considered to indicate statistically significant differences.

Results

Exposure to rDer f1 decreases BEC viability by inducing cell death. Exposure to the most common allergens may result in inflammatory and allergic reactions in susceptible hosts (2). In addition, BEC damage is a critical pathogenic characteristic of airway diseases (3). Therefore, the effects of common allergens, including cat, dog and dust mites, on the viability of BECs were first assessed. HBE-135 cells were treated with a series of common inhaled allergens, including nCan f1, nFel d1, nDer p1, rDer p1 DG and rDer f1 at 25 μg/ml for 24 h. Only rDer f1 treatment exerted a prominent inhibitory effect on the viability of HBE-135 cells (Fig. 1A). This effect was dose-dependent in pHBECs and HBE-135 cells (Fig. 1B). Inverted microscopy revealed that numerous cells had assumed a round shape and had detached from the culture plates, suggesting that rDer f1 caused BEC death, but not a reduction in proliferation (Fig. 1C).

rDer f1 induces BEC pyroptosis but not apoptosis. Whether apoptosis or another means of cell death caused the decreased cell viability after rDer f1 treatment was next investigated. Apoptosis was first evaluated by the TUNEL assay. However, 5 and 10 μg/ml of rDer f1 treatment failed to induce apoptosis in either pHBECs or HBE-135 cells (Fig. 2A).

It was then investigated whether rDer f1 induced pyroptosis in BECs. Pyroptosis is characterized by the formation of pores in the cell membrane, which may be detected by LDH release and PI staining without cell membrane permeabilization (10,12). Flow cytometric analysis revealed that an increase

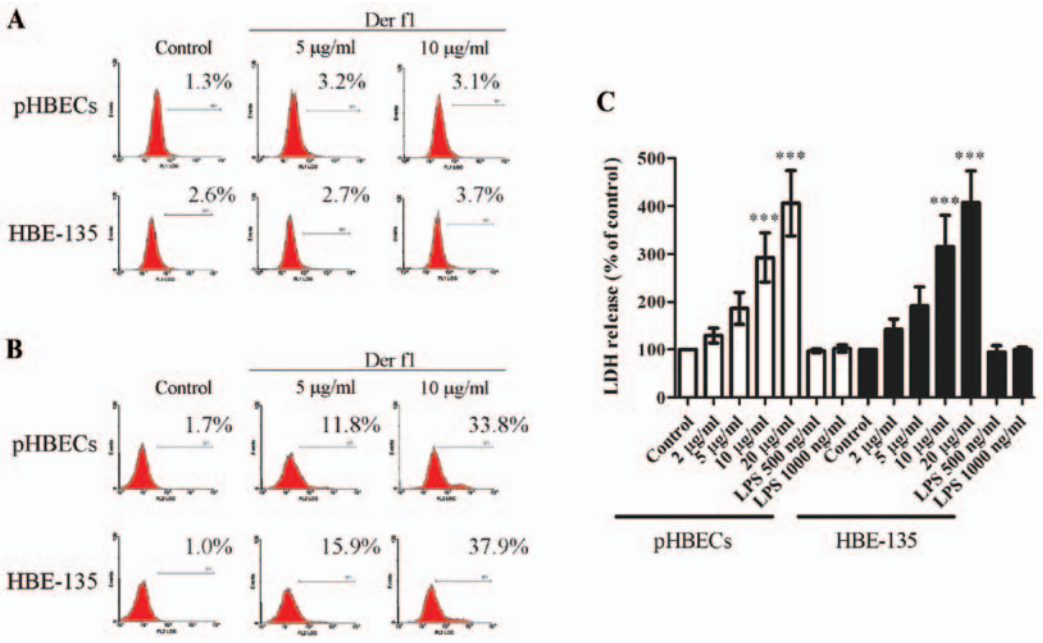

Figure 2. rDer f1 induced pyroptosis in BECs. (A) rDer f1 did not trigger cell apoptosis. rDer f1 induced pyroptosis in BECs, as determined by (B) PI staining and (C) LDH release analysis in pHBECs and HBE-135 cells treated with various concentrations of rDer f1 for 24 h. The percentage of apoptosis was assessed by terminal deoxynucleotidyl TdT-mediated dUTP nick end labeling (TUNEL) analysis to determine cellular DNA fragments. For pyroptosis analysis, the supernatants of rDer f1-treated pHBECs and HBE-135 cells were collected by centrifugation, and the levels of LDH were then determined using a cytotoxicity detection kit. rDer f1-treated BECs were harvested by trypsinization and centrifugation. The cells were then stained by PI and assessed by flow cytometry. Data are expressed as the mean of three independent experiments ± standard deviation, $^{***}p<0.001$ vs. control. LDH, lactate dehydrogenase; BECs, bronchial epithelial cells; pHBECs, primary human BECs; Der f1, *Dermatophagoides farin*a allergen 1; PI, propidium iodide; LPS, lipopolysaccharide.

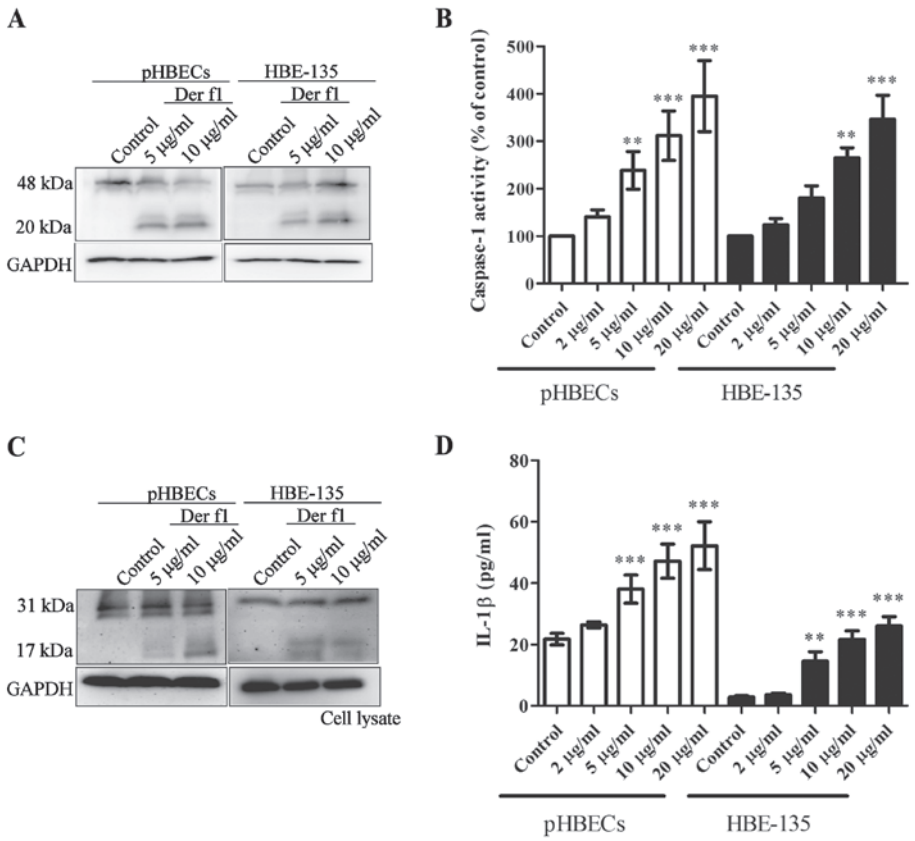

Figure 3. rDer f1 induced caspase-1 activation and IL-1β release. (A) rDer f1 increased the active cleavage of caspase-1. (B) rDer f1 enhanced the activity of caspase-1. (C) rDer f1 increased the maturation of interleukin (IL)-1β. (D) rDer f1 enhanced the release of IL-1β. pHBECs and HBE-135 cells were treated with various concentrations of rDer f1 for 12 h. Caspase-1 cleavage and IL-1β maturation in the BEC lysates were assessed by immunoblotting. Caspase-1 activity was determined using a caspase-1 activity assay kit. The supernatants of BECs treated with or without rDer f1 were collected to assay IL-1β concentrations by means of ELISA. Data are expressed as the mean of three independent experiments ± standard deviation; $^{**}p<0.01$ and $^{***}p<0.001$ vs. control. BECs, bronchial epithelial cells; pHBECs, primary human BECs; Der f1, *Dermatophagoides farin*a allergen 1.

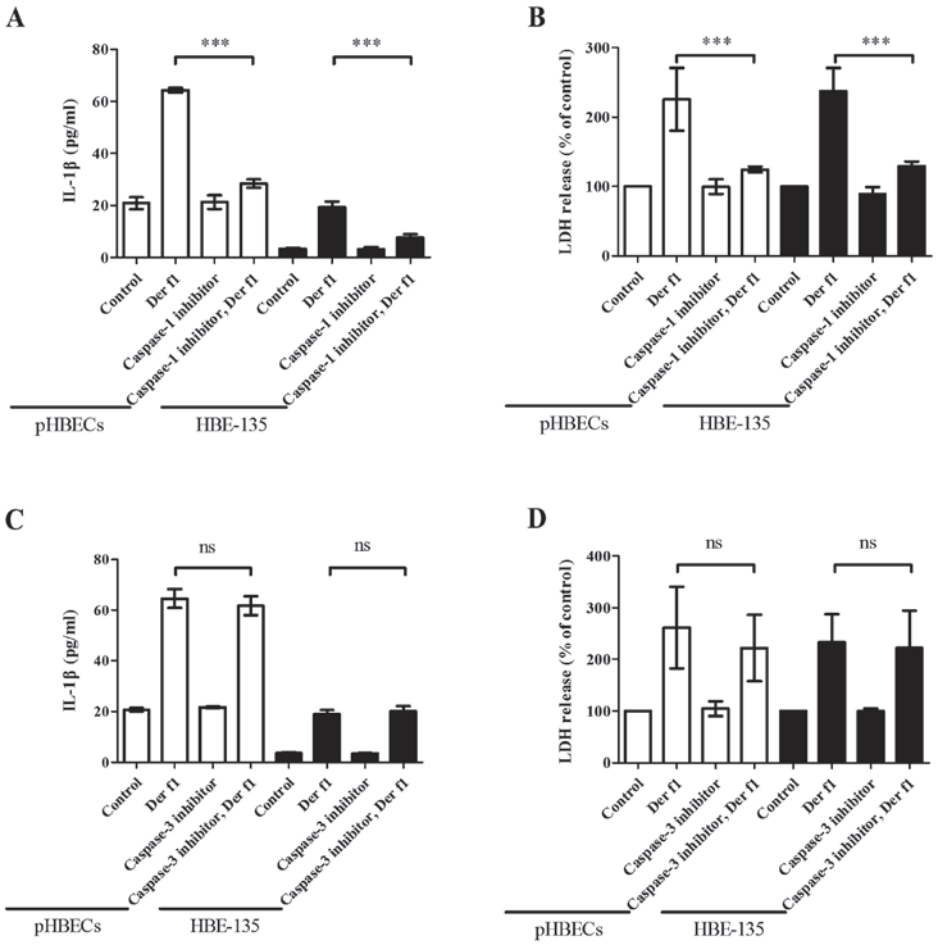

Figure 4. Involvement of caspase-1 in rDer f1-mediated pyroptosis. Specific caspase-1 inhibitor prevented rDer f1-mediated (A) interleukin (IL)-1β release (B) and LDH release. Caspase-3 inhibitor did not affect rDer f1-mediated (C) IL-1β release or (D) LDH release. pHBECs and HBE-135 cells were pre-treated with caspase-1 inhibitor (20 μM) or caspase-3 inhibitor (20 μM) for 1 h, and rDer f1 (10 μg/ml) was added for 12 h for IL-1β and 24 h for LDH analysis. The levels of IL-1β and LDH in the supernatants were assessed by ELISA and a cytotoxicity detection kit, respectively. Data are expressed as the mean of three independent experiments ± standard deviation; ***p<0.001 vs. control; ns, not significant. pHBECs, primary human bronchial epithelial cells; LDH, lactate dehydrogenase; Der f1, *Dermatophagoides. farin*a allergen 1.

in PI-positive cells was well-correlated with an increase in rDer f1 concentration (Fig. 2B). In addition, with increasing concentrations of rDer f1, more LDH was released (Fig. 2C). This finding suggested that cell death was associated with the loss of cellular membrane integrity and release of LDH, further indicating that there was pore formation during rDer f1 treatment. Lipopolysaccharide (LPS) treatment (up to 1,000 ng/ml) did not affect cell viability, suggesting that LPS contamination may not be a major concern (Fig. 2B and C). Taken together, these findings confirmed that rDer f1 induced epithelial cell death by pyroptosis, but not apoptosis.

rDer f1 triggers caspase-1 activation and IL-1β release in BECs. Pyroptosis is characterised by caspase-1 activation and IL-1β release (10,12). To confirm caspase-1 activation and IL-1β release, the pHBECs and HBE-135 cells were treated with various concentrations of rDer f1 for 12 h, and whole-cell lysates were assessed to confirm caspase-1 cleavage and activity by immunoblot analysis and caspase-1 activity kits, respectively. Fig. 3A shows that rDer f1 treatment caused caspase-1 activation, as demonstrated by the presence of cleaved caspase-1 (20 kDa). Increased caspase-1 activity was observed in the whole-cell lysates of rDer f1-treated BECs, compared with the controls (Fig. 3B).

In addition, rDer f1 treatment led to conversion of pro-IL-1β to mature IL-1β, as demonstrated by the presence of cleaved IL-1β (17 kDa) (Fig. 3C). Compared with controls, increased concentrations of IL-1β were detected in the supernatant of BECs following rDer F1 treatment (Fig. 3D). These findings suggest that rDer f1 increases caspase-1 activation and IL-1β release in BECs.

rDer f1-induces IL-1β release and pyroptosis in BECs in a caspase-1-dependent manner. Our findings indicated that exposure to rDer f1 increased IL-1β production and caused pyroptosis in BECs. To confirm that both phenomena were associated with caspase-1 activation, a specific caspase-1 inhibitor was used to assess the involvement of caspase-1. pHBECs and HBE-135 cells were pre-treated with a caspase-1 inhibitor (Z-YVAD-FMK; 20 μM) or a caspase-3 inhibitor (20 μM) for 1 h, followed by rDer f1 (10 μg/ml) for 12 h for IL-1β and 24 h for LDH analysis. In the presence of the caspase-1 inhibitor, IL-1β and LDH release caused by rDer f1 decreased to nearly the same level as that in control pHBECs

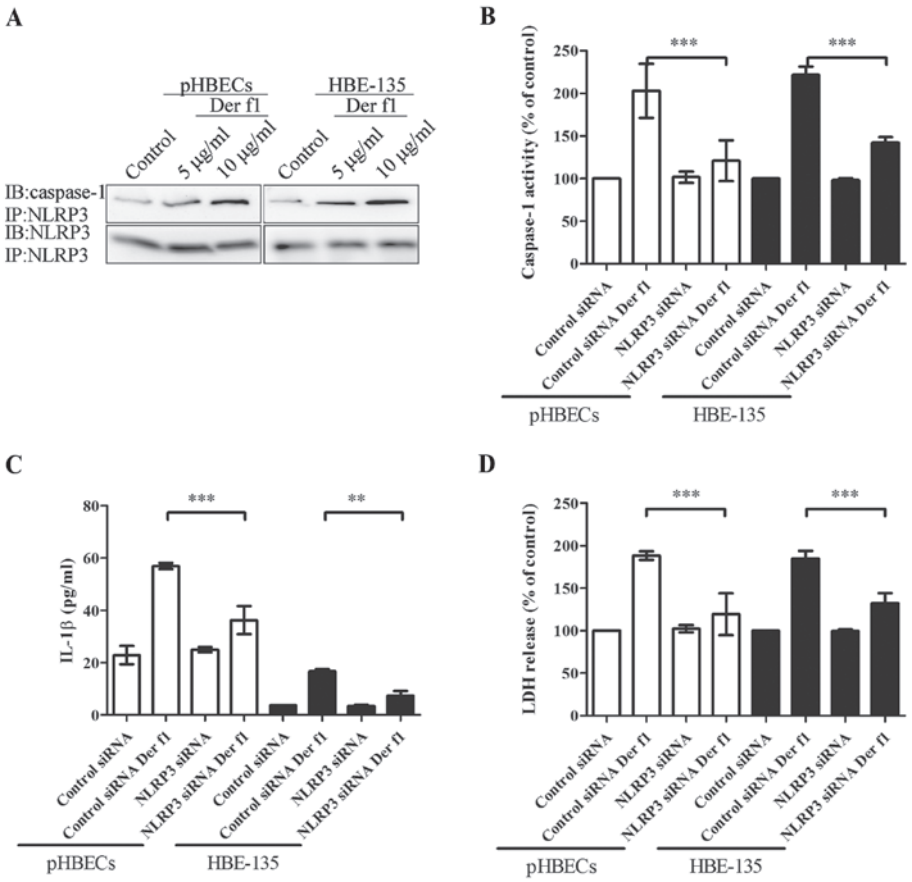

Figure 5. NLRP3 is required for rDer f1-mediated caspase-1 activation and pyroptosis. (A) Association of NLRP3 and caspase-1. (B) Inhibition of NLRP3 decreased caspase-1 activation, (C) IL-1β release and (D) LDH release. pHBECs and HBE-135 cells were treated with 5 and 10 μg/ml rDer f1 for 12 h. The interaction between NLRP3 and caspase-1 was assessed by immunoprecipitation. In the blocking study, pHBECs and HBE-135 cells were transfected with NLRP3 or control siRNA. After 24 h of transfection, the cells were treated with 10 μg/ml rDer f1 for 12 h for caspase-1 and IL-1β release, and 24 h for LDH analysis. The activity of caspase-1 was determined using a caspase-1 activity assay kit. The levels of IL-1β and LDH in the supernatants were assessed by ELISA and a cytotoxicity detection kit, respectively. Data are expressed as the mean of three independent experiments ± standard deviation; **p<0.01 and ***p<0.001 vs. control. LDH, lactate dehydrogenase; pHBECs, primary human bronchial epithelial cells; Der f1, *Dermatophagoides farina* allergen 1; NLRP3, NOD-like receptor family pyrin domain-containing 3.

and HBE-135 cells (Fig. 4A and B). However, in the presence of caspase-3 inhibitor, there was no inhibitory effect on either IL-1β secretion or LDH release (Fig. 4C and D), suggesting that the secretion of IL-1β and triggering of pyroptosis by rDer f1 are dependent on caspase-1.

rDer f1-induced pyroptosis is due to the NLRP3 inflammasome. To further elucidate the possible role of NLRP3 in rDer f1-mediated caspase-1 activation, the association of NLRP3 and caspase-1 was assessed. rDer f1 was applied to pHBECs and HBE-135 cells; subsequently, co-IP was performed to confirm the association between NLRP3 and caspase-1 (Fig. 5A). Increased concentrations of rDer f1 were well-correlated with an upregulated interaction of NLRP3 and caspase-1.

To confirm that NLRP3 mediated rDer f1-induced pyroptosis of BECs, cells were transfected with NLRP3 siRNA to inhibit its expression. Compared with control siRNA transfection, transfection of pHBECs and HBE-135 cells with NLRP3 siRNA led to ~70% inhibition of NLRP3 expression (data not shown). Transfection of BECs with NLRP3 siRNA inhibited rDer f1-mediated caspase-1 activation and IL-1β release (Fig. 5B and C). Furthermore, rDer f1-induced pyroptosis was also inhibited by NLRP3 siRNA (Fig. 5D). These findings confirmed that rDer f1 acts through NLRP3-activated caspase-1, which in turn induces IL-1β release and pyroptosis.

Discussion

Asthma is a major public health concern worldwide. The most well-known mechanism of asthma pathogenesis is allergen-induced airway inflammation (20,21). The subsequent reactions include bronchospasm, oedema and epithelial damage. Epithelial damage may enhance susceptibility to allergens and sensitivity of the airways, leading to persistent asthma and airway remodelling (2,4,6). The novel finding of the present study is that Der f1, an allergen of *D. farinae*, was found to induce cell death in BECs. The increased LDH release and intracellular PI, but not TUNEL, suggested that this cell death process was due to pyroptosis. In addition, Der f1 led to inflammasome formation characterized by caspase-1 activation and IL-1β secretion. This whole process occurred via the NLRP3 inflammasome pathway. These findings suggest that Der f1-induced epithelial pyroptosis plays a major role in asthma pathogenesis and airway remodelling.

BECs are the first line of defence against external antigens, and are considered to play an important role in the development

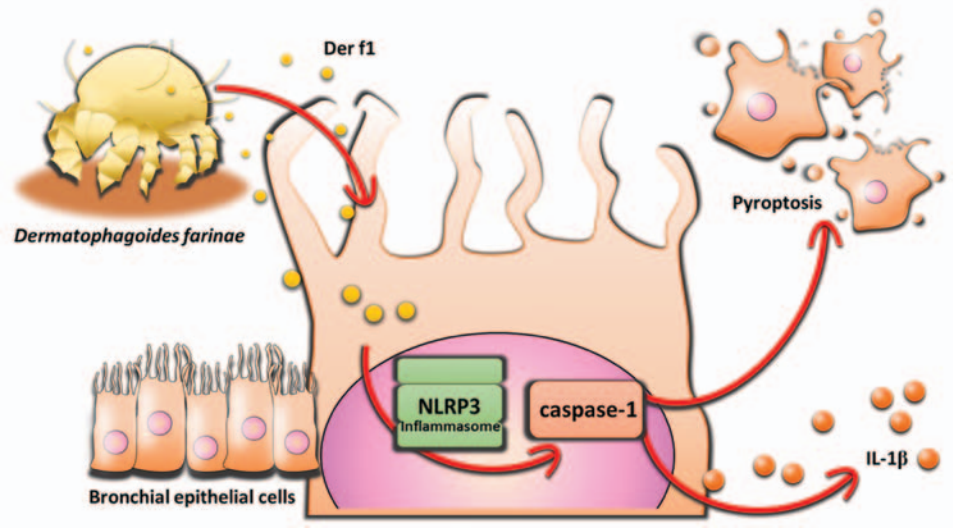

Figure 6. Proposed scheme of Der f1-induced pyroptosis in bronchial epithelial cells (BECs). The Der f1 allergen induces BEC death through the caspase-1 pathway, referred to as pyroptosis. Denuded bronchial epithelia may play a role in sensitizing susceptible individuals to develop asthmatic symptoms. Following pyroptosis, BECs secrete interleukin (IL)-1β, which may perpetuate asthma pathogenesis. Der f1, *Dermatophagoides farina* allergen 1 NLRP3, NOD-like receptor family pyrin domain-containing 3.

of airway allergic inflammation (17). Intact epithelia provide mucociliary clearance and remove noxious agents, thereby avoiding allergen sensitization and airway hyperresponsiveness. Sloughing of BECs has been found in the majority of asthmatic patients, regardless of the severity (5,6). In asthmatic patients, clumps of epithelial cells in the sputum and increased epithelial cells in bronchoalveolar lavage suggest that epithelial cell sloughing is a pathological characteristic of asthma (22). The exact mechanism underlying epithelial cell sloughing in asthma remains a matter of debate. Pyroptosis, a type of programmed cell death, is triggered by microbial infections and non-infectious stimuli (10,11). Once activated, inflammasome-mediated caspase-1 activation leads to membrane rupture followed by the secretion of pro-inflammatory cytokines, including IL-1β. Pyroptosis plays a key role in several clinically relevant conditions, including respiratory diseases. Reisetter *et al* demonstrated that carbon black nanoparticles mimicking particulate ambient pollution also led to pyroptosis of lung alveolar macrophages (23). Acute respiratory distress syndrome has been shown to be due to the induction of NLRP1-dependent pyroptosis (24). In contrast to apoptosis of bronchial epithelial cells, which lessens inflammation inside the airway, pyroptosis induces intense airway inflammation, which may lead to permanent structural changes (13). In the present study, BECs were exposed to several common allergens, but only rDer f1 treatment led to cell death. TUNEL analysis failed to provide evidence that apoptosis played a major role in Der f1-mediated cell death. By contrast, the LDH release assay as well as PI staining favoured Der f1-induced cell death by pyroptosis. To evaluate whether LPS contamination affected the results, treatment with LPS ≤1,000 ng/ml was performed. The results revealed that LPS treatment did not exert any effect on cell viability and LDH release, suggesting that Der f1-mediated pyroptosis is not due to LPS contamination.

Caspase-1 catalyses the conversion of pro-IL-1β to mature IL-1β, a key inflammatory mediator that controls both local and systemic immune responses (25). IL-1β drives diverse biological processes, including extravasation, cell proliferation and differentiation, cytokine secretion, angiogenesis, wound healing and pyrexia (26,27). Unregulated and sustained release of IL-1β has been shown to lead to a number of chronic inflammatory diseases, such as psoriasis and inflammatory bowel disease. Moreover, IL-1β has also been shown to play a role in the early phase of asthma pathogenesis and to modulate airway constriction and relaxation responses directly on the airway smooth muscles (26). Our results demonstrated that Der f1 increased the proteolytic activation and activity of caspase-1, which in turn induced the secretion of IL-1β from BECs. Moreover, only caspase-1 inhibition, and not caspase-3 inhibition, prevented Der f1-mediated IL-1β release and pyroptosis, suggesting that caspase-1 plays a critical role in Der f1-mediated cell death in BECs.

The NLRs play important roles in the recognition of exogenous microbial components or endogenous destructive cellular factors in innate immunity (28). Various inflammasomes, such as NLRP1, NLRP3/ASC and IPAF, are associated with pyroptosis and the secretion of pro-inflammatory cytokines (29). The NLRP3 inflammasome may be triggered by various pathogens, toxins, bacterial RNA and uric acid through two sensors of danger signals, toll-like receptors and P2X7 receptors (28). Following endogenous or exogenous stimulation, oligomerised NLRP3 interacts with apoptosis-associated speck-like protein containing a CARD (ASC) through homotypic protein-protein interactions of the pyrin domains. The interactions between ASC and the CARD domain of pro-caspase-1 are considered to activate caspase-1 (28,30). Our results demonstrated that Der f1 increased the association of NLRP3 and caspase-1. Following NLRP3 knockdown, the Der f1-induced increased activity of caspase-1 was shown to decrease in pHBECs and HBE-135 cells compared with that in controls. In addition, the results revealed that Der f1-induced IL-1β release and pyroptosis were reduced in pHBECs and HBE-135 cells compared with the controls. These results indicate that the NLRP-3-related inflammasome contributes to Der f1-mediated pyroptosis in BECs.

The novel finding of the present study is that Der f1 induces pyroptosis in BECs via the NLRP3 inflammasome (Fig. 6). The ensuing caspase-1 activation leads to BEC death and release of the pro-inflammatory cytokine IL-1β. Der f1 may not act solely as an allergen, and Der f1-mediated pyroptosis may represent a pathogenic mechanism contributing to inflammatory injury of airway epithelia. Further studies are required to elucidate the connection between Der f1 and NLRP3 inflammasome and Der f1-mediated pyroptosis of BECs in asthma. In conclusion, the results of the present study suggest that Der f1-induced epithelial cell pyroptosis plays a major role in the pathogenesis of asthma, providing novel evidence on the effect of HDMs on BEC injury and offering novel insight into the pathobiology of asthma and other respiratory diseases.

Acknowledgements

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References

1. Bel EH: Clinical Practice. Mild asthma. N Engl J Med 369: 549-557, 2013.
2. Lambrecht BN and Hammad H: The airway epithelium in asthma. Nat Med 18: 684-692, 2012.
3. Holgate ST, Roberts G, Arshad HS, Howarth PH and Davies DE: The role of the airway epithelium and its interaction with environmental factors in asthma pathogenesis. Proc Am Thorac Soc 6: 655-659, 2009.
4. Lloyd CM and Saglani S: Asthma and allergy: The emerging epithelium. Nat Med 16: 273-274, 2010.
5. Martínez-Girón R and van Woerden HC: Disruption of airway epithelium in asthma pathogenesis: Are protozoa responsible? Proc Am Thorac Soc 7: 161, author reply 161, 2010.
6. Chanez P: Severe asthma is an epithelial disease. Eur Respir J 25: 945-946, 2005.
7. Holgate ST: The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev 242: 205-219, 2011.
8. Holgate ST: Epithelial damage and response. Clin Exp Allergy 30 (Suppl 1): 37-41, 2000.
9. Davies DE: The role of the epithelium in airway remodeling in asthma. Proc Am Thorac Soc 6: 678-682, 2009.
10. Bergsbaken T, Fink SL and Cookson BT: Pyroptosis: Host cell death and inflammation. Nat Rev Microbiol 7: 99-109, 2009.
11. Yeretssian G, Labbé K and Saleh M: Molecular regulation of inflammation and cell death. Cytokine 43: 380-390, 2008.
12. LaRock CN and Cookson BT: Burning down the house: Cellular actions during pyroptosis. PLoS Pathog 9: e1003793, 2013.
13. Tanaka H, Miyazaki N, Oashi K, Teramoto S, Shiratori M, Hashimoto M, Ohmichi M and Abe S: IL-18 might reflect disease activity in mild and moderate asthma exacerbation. J Allergy Clin Immunol 107: 331-336, 2001.
14. Maezawa Y, Nakajima H, Kumano K, Kubo S, Karasuyama H and Iwamoto I: Role of IgE in Th2 cell-mediated allergic airway inflammation. Int Arch Allergy Immunol 131 (Suppl 1): 2-6, 2003.
15. Jacquet A: Innate immune responses in house dust mite allergy. ISRN Allergy 2013: 735031, 2013.
16. Baker SF, Yin Y, Runswick SK, Stewart GA, Thompson PJ, Garrod DR and Robinson C: Peptidase allergen Der p 1 initiates apoptosis of epithelial cells independently of tight junction proteolysis. Mol Membr Biol 20: 71-81, 2003.
17. Gandhi VD, Davidson C, Asaduzzaman M, Nahirney D and Vliagoftis H: House dust mite interactions with airway epithelium: Role in allergic airway inflammation. Curr Allergy Asthma Rep 13: 262-270, 2013.
18. Wang JY: The innate immune response in house dust mite-induced allergic inflammation. Allergy Asthma Immunol Res 5: 68-74, 2013.
19. Takai T, Kato T, Sakata Y, Yasueda H, Izuhara K, Okumura K and Ogawa H: Recombinant Der p 1 and Der f 1 exhibit cysteine protease activity but no serine protease activity. Biochem Biophys Res Commun 328: 944-952, 2005.
20. Holgate ST: Pathogenesis of asthma. Clin Exp Allergy 38: 872-897, 2008.
21. Holt PG and Sly PD: Viral infections and atopy in asthma pathogenesis: New rationales for asthma prevention and treatment. Nat Med 18: 726-735, 2012.
22. Fahy JV: Remodeling of the airway epithelium in asthma. Am J Respir Crit Care Med 164 (Suppl 2): S46-S51, 2001.
23. Reisetter AC, Stebounova LV, Baltrusaitis J, Powers L, Gupta A, Grassian VH and Monick MM: Induction of inflammasome-dependent pyroptosis by carbon black nanoparticles. J Biol Chem 286: 21844-21852, 2011.
24. Kovarova M, Hesker PR, Jania L, Nguyen M, Snouwaert JN, Xiang Z, Lommatzsch SE, Huang MT, Ting JP and Koller BH: NLRP1-dependent pyroptosis leads to acute lung injury and morbidity in mice. J Immunol 189: 2006-2016, 2012.
25. Denes A, Lopez-Castejon G and Brough D: Caspase-1: Is IL-1 just the tip of the ICEberg? Cell Death Dis 3: e338, 2012.
26. Whelan R, Kim C, Chen M, Leiter J, Grunstein MM and Hakonarson H: Role and regulation of interleukin-1 molecules in pro-asthmatic sensitised airway smooth muscle. Eur Respir J 24: 559-567, 2004.
27. Delaleu N and Bickel M: Interleukin-1 beta and interleukin-18: Regulation and activity in local inflammation. Periodontol 2000 35: 42-52, 2004.
28. Mariathasan S and Monack DM: Inflammasome adaptors and sensors: Intracellular regulators of infection and inflammation. Nat Rev Immunol 7: 31-40, 2007.
29. Aachoui Y, Sagulenko V, Miao EA and Stacey KJ: Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection. Curr Opin Microbiol 16: 319-326, 2013.
30. Miao EA, Rajan JV and Aderem A: Caspase-1-induced pyroptotic cell death. Immunol Rev 243: 206-214, 2011.