Epithelial Permeability to Ole E 1 Is More Dependent on Functional Bronchial Epithelium-State than on Der P 1-Protease Activity That Acts As Adjuvant to the Bystander Allergen

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A dysfunctional epithelial barrier has been widely associated with allergy [1], although it is not clearly established whether this dysfunction is the cause of or the consequence of the disease. Moreover, the airway epithelial barrier integrity can be impaired by environmental proteases –most of them are allergens- derived from a wide variety of biological sources such as mites, cockroaches, food plants, fungi and pollens [2,3]. Among them, cysteine-proteases represent potent adjuvants for promoting Th2-type immune responses in the airways. Although it is well known that the cysteine-protease Der p 1, a major allergen from house dust mite, exerts different effects on the airway epithelium that could contribute to allergic airway diseases [4-6], the cleavage of tight junction (TJ) proteins was the first activity described for it [5]. Der p 1 also cleaves several molecules related to immune response, among others CD23, CD25 and IL33-alarmin [4,6]. The identification of new targets for Der p 1 could be important to define the molecular mechanisms for the initiation and exacerbation of respiratory allergies after allergen exposure.

In this work, we have analysed the effect of the Der p 1 cysteine-protease activity on the bronchial epithelial permeability to Ole e 1, the main allergen of olive pollen [7]. Exposure of air-liquid interface (ALI)-cultured Calu-3 cells to Der p 1 for 24 h decreased transepithelial electrical resistance (TEER) values, and promoted a discontinuous staining pattern for ZO-1 (TJ) in an epithelium state-dependent manner: this occurred on day 2 of culture when the establishment of a functional barrier is in progress, but not on day 7 when the barrier is well-formed (Fig. 1A, Fig.S1 and Fig. S2A). Interestingly,

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TEER values were restored to control ones in 10 min after protease removing. It is well documented that Der p 1 can disrupt the airway epithelial barrier by cleaving apical junctional complex proteins, (including ZO-1) [8], but the novelty of our findings highlights the functional state-dependence of the airway barrier disruption caused by Der p 1. In this sense, López-Rodríguez *et al.* [9] found that Ole e 1 induced the cytokine release from ALI-cultured NHBE cells in a differentiation state-dependent manner. As previously reported, Ole e 1 did neither alter TEER values nor ZO-1 staining pattern over the time. Then, ALI-cultured cells were apically exposed to Ole e 1 on days 2 and 7, and the presence of the allergen was analysed on the basolateral medium at different time points by immunoblotting -using an anti-Ole e 1 antiserum- to determine whether the protease could facilitate the allergen passage across the cell layer: a requirement for allergen sensitization [10] (Fig. 1B).

Despite the potential role of protease activity on allergen sensitization indicated by numerous studies, we demonstrated that epithelial permeability to Ole e 1 was more dependent on functional epithelium-state than on protease activity. The passage of the allergen across the epithelial barrier was higher on day 2 than on day 7 at ALI -with or without the protease-, as indicated by its detection on the basolateral side after 0.5 h of culture, and its levels increased over the time. The 18 (non-glycosylated form) and 20 (glycosylated form) kDa bands characterize its SDS-PAGE pattern [7]. Again, our data provide evidence that the functional epithelium-state at the time of contact highly determines its allergen response.

Both the remarkable decrease on Ole e 1 levels at the apical side and the alteration of its electrophoretic pattern after 24 h with Der p 1 suggested its proteolytic cleavage by this protease. Therefore, Ole e 1 was incubated with the protease for 24 h, and aliquots were taken at different time points and analysed by mass spectrometry (MS) and immunoblotting (Fig. S2B). In the absence of protease, the allergen-MS spectrum had two majority peaks; while with Der p 1, the intensity of the 17518.4 m/z-peak

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(monoprotonated specie of glycosylated form) decreased over incubation time as both the 16197.4 m/z-peak (monoprotonated specie of non-glycosylated form) and the <12500 m/z-area increased. Immunoblot analyses supported MS data, indicating that Ole e 1 is a novel target of Der p 1. Edman degradation assays indicated that Der p 1 removed the first 10 amino acid residues of Ole e 1 after 0.5 h-treatment: EDVP and FHIQ were the obtained N-terminal sequences (Fig. S2B). Although the N-terminal of Ole e 1 does not display Der p 1-consensus cleavage sequence [11], the allergen was efficiently cleaved by this protease, and following 24 h-exposure, it was degraded into short peptides. These findings suggested that other factors might be involved in the cleavage by Der p 1 [11].

Finally, inhibition ELISA assays showed that both ≤3 and >3kDa fractions obtained by ultrafiltration of Ole e 1-cleavage products were able to bind specific IgG (rabbit antiserum), but only the >3 kDa fraction exhibited IgE-reactivity with sera from 3 olive pollen allergic patients (Fig. 1C, Table S1). Moreover, both fractions activated basophils from 3-4 out of 5 of patients tested in a dose-dependent manner (Fig. 1D). The absence of IgE-reactivity of the ≤3 kDa fraction could be explained in part by the sensitivity of the assay since this fraction contained peptides with right molecular masses for activated basophils from allergic patients, although in lower levels. The identification of IgG- and IgE epitopes in Ole e 1 (using short-overlapping synthetic peptides covering the full polypeptide chain [12]) supported our data. The cleavage of Ole e 1 by the Der p 1 may also generated T cell-peptides that mediate immunomodulation of the host`s response. In this sense, Wildner *et al*. [13] have reported that peptides derived of Ole e 1-cleavage by cathepsin S -an endolysosomal cysteine-protease- substantially overlapped with T cell-epitopes of this allergen [14].

In conclusion, we have shown that the epithelial permeability to Ole e 1 shows a stronger dependence on the functional state of the bronchial epithelium than on Der p 1 protease activity. The finding that Ole e 1-derived peptides by Der p 1 cleavage

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effectively bind to IgG and IgE, and activate basophils from olive pollen allergic patients, suggests an additional mechanism by which environmental proteases may facilitate sensitization to other allergens, acting synergistically with their ability to both disrupt epithelial barrier and activate epithelial cells. In this sense, Der p 1 has been recognized as an “initiator allergen” of IgE sensitization, since it exhibits the required properties to promote an allergic response to both itself and other unrelated allergens [15]. This study highlights a new understanding of the early events that could contribute to allergy in humans.

Previous presentations

The data from this study have not been presented previously.

Supplementary data

Supplementary data are available at *Journal of Investigational Allergology and Clinical Immunology* online.

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Conflicts of interest

The authors have no financial conflicts of interest.

REFERENCES

1. Hellings PW, Steelant B. Epithelial barriers in allergy and asthma. J Allergy Clin Immunol. 2020;145:1499-509.
2. Takai T, Ikeda S. Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases. Allergol Int. 2011;60:25-35.
3. Jacquet A. Interactions of airway epithelium with protease allergens in the allergic response. Clin Exp Allergy. 2011;41:305-11.
4. Chevigné A, Jacquet A. Emerging roles of the protease allergen Der p 1 in house dust mite-induced airway inflammation. J Allergy Clin Immunol. 2018;142:398-400.
5. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest. 1999;104:123-33.
6. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, *et al*. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. Nat Immunol. 2018;19:375-85.
7. Villalba M, Rodriguez R, Batanero E. The spectrum of olive pollen allergens. From structures to diagnosis and treatment. Methods. 2014;66:44-54.
8. Steelant B, Farré R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, *et al*. Impaired barrier function in patients with house dust mite-induced allergic

doi: 10.18176/jiaci.0603

rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. J Allergy Clin Immunol. 2016;137:1043-53.

9. Lopez-Rodriguez JC, Solis-Fernandez G, Barderas R, Villalba M, Batanero E. Effects of Ole e 1 on human bronchial epithelial cells cultured at the air-liquid interface. J Investig Allergol Clin Immunol. 2018;28:186-9.
10. Anderson JM, Van Itallie CM. Tight junctions and the molecular basis for regulation of paracellular permeability. Am J Physiol. 1995;269:G467-75.
11. Jacquet A, Campisi V, Szpakowska M, Dumez ME, Galleni M, Chevigne A. Profiling the extended cleavage specificity of the house dust mite protease allergens Der p 1, Der p 3 and Der p 6 for the prediction of new cell surface protein substrates. Int J Mol Sci. 2017;18:e1373.
12. Gonzalez EM, Villalba M, Quiralte J, Batanero E, Roncal F, Albar JP, *et al*. Analysis of IgE and IgG B-cell immunodominant regions of Ole e 1, the main allergen from olive pollen. Mol Immunol. 2006;43:570-78.
13. Wildner S, Elsässer B, Stemeseder T, Briza P, Soh WT, Villalba M, *et al*. Endolysosomal degradation of allergenic Ole e 1-like proteins: analysis of proteolytic cleavage sites revealing T cell epitope-containing peptides. Int J Mol Sci. 2017;18:e178.
14. Cardaba B, Del Pozo V, Jurado A, Gallardo S, Cortegano I, Arrieta I, *et al*. Olive pollen allergy: searching for immunodominant T-cell epitopes on the Ole e 1 molecule. Clin Exp Allergy. 1998;28:413-22.
15. Zhang J, Chen J, Robinson C. Cellular and molecular events in the airway epithelium defining the interaction between house dust mite group 1 allergens and innate defences. Int J Mol Sci. 2018;19:e3549.

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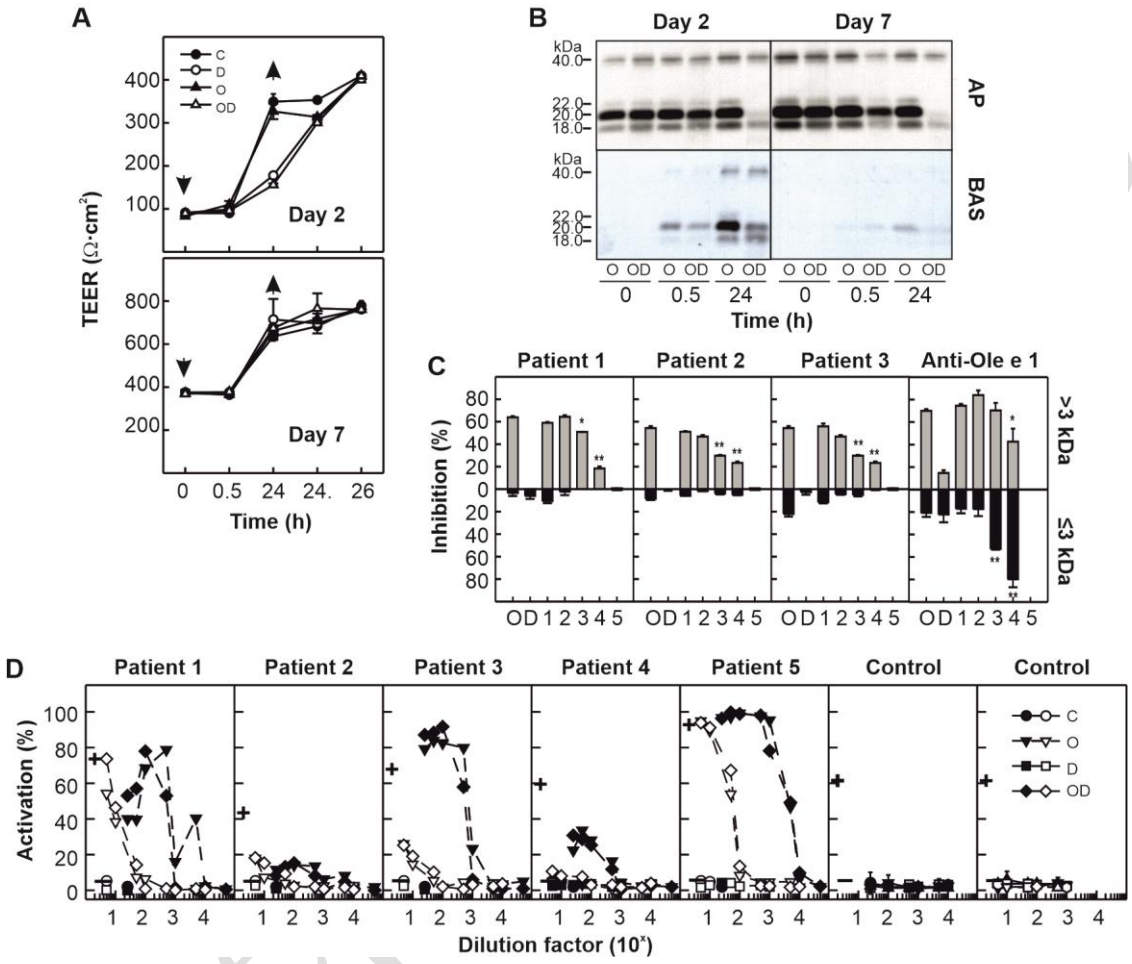
Figures

Figure 1. Effect of Der p 1 co-exposure on the response to Ole e 1 of ALI-cultured Calu-3 cells on days 2 and 7.



A) Time course of TEER values of ALI-cultured Calu-3 cells exposed to Ole e 1 and/or Der p 1, compared to unexposed cells. TEER was measured at the indicated time points, and shown as the mean value ± SD of triplicate determinations. Down and up arrows indicate the time of addition and removal of the allergen, respectively. B) Effect of Der p 1 co-exposure on bronchial epithelial permeability to Ole e 1. ALI-cultured Calu-3 cells were co-exposed to Ole e 1 and Der p 1 on days 2 and 7, and the presence of the Ole e 1 was determined by immunoblotting in the medium at different time points (0 h, 0.5 h, and 24 h).

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Molecular masses of Ole e 1 forms in kDa are indicated: 40, dimer; 22, hyperglycosylated; 20, glycosylated and 18, non-glycosylated. AP, apical medium; BAS, basolateral medium; O, exposure to Ole e 1; and OD, exposure to Ole e 1 in combination with Der p 1. For immunological characterization, Ole e 1-derived products by Der p 1 cleavage were separated into two fractions by Nanosep®-3K: >3 kDa and ≤3 kDa. C) Inhibition ELISA of Ig-binding to Ole e 1-coated-wells using cleavage products as inhibitors (1 μg/mL) and serum IgE from 3 olive pollen allergic patients (Table S1) or IgG from a specific polyclonal anti-Ole e 1 antiserum. Data are expressed as inhibition percentage (%) respect to control, and shown as the mean ± SD of duplicate determinations. Protease-treatments: 1, 0 h; 2, 0.5 h; 3, 8 h; and 4, 24 h; and 5, HCl-hydrolysed Ole e 1. O, Ole e 1; D, Der p 1. Significant differences: *$p < 0.05$, **$p < 0.01$. D) Analysis of basophil activation by flow cytometry in Ole e 1-allergic patients (but not to Der p 1, n = 5,) compared to control group (n = 5, the average response is shown) (Table S1). Cells were stimulated with different doses of >3 kDa (black symbols) and ≤3kDa (white symbols) fractions obtained from PBS (C), Ole e 1 (O), Der p 1 (D), and Ole e 1-derived products by Der p 1 cleavage (OD). Data are given as the percentage (%) of activation (CD63 expression). +, anti-IgE control; -, basal control.

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