Identification of *Ulocladium chartarum* as an important indoor allergen source

To the Editor,

The constant exposure to fungal spores, which constitute the largest proportion of aerobiological particles, can cause severe health problems, including allergic diseases.1,2 Appropriate management of fungal allergies is hampered by the fact that our knowledge about fungal allergy is still limited to a small number of thoroughly investigated fungi, whereas for the majority of species, it is not yet known whether they play a role in allergic diseases.2 This lack of knowledge together with unreliable diagnostic results obtained with fungal allergen extracts of poor quality contributes to a general underdiagnosis of fungal allergy.3,4 In the present study, we investigated the allergenic potential of *Ulocladium chartarum*, an opportunistic human pathogen5 that can grow on various substrates.6,7 Exposure to *Ulocladium* species often occurs in the indoor environment as they are commonly detected inside damp buildings and are regarded as an indicator of water damages.6–9 The fact that *U. chartarum* belongs...
to the same family as the outdoor Aeroallergen source Alternaria alternata suggests that U. chartarum might also represent an allergen source.\textsuperscript{6,7}

IgE immunoblots, performed with sera from patients sensitized to different mold species (Table S1), demonstrated the high allergenic potential of U. chartarum, since patients' IgE antibodies recognized several U. chartarum proteins (Figure 1A), whereas exposure of the blotted proteins to sera from non-allergic individuals or to immunodetection reagent did not lead to any unspecific binding (data not shown). Interestingly, despite their phylogenetic relationship, the IgE-binding profile of A. alternata (Figure S1) differed significantly from the one of U. chartarum. Mold-allergic patients not only recognized more proteins in U. chartarum than in A. alternata extract, but also showed an overall stronger reactivity to them.

Peptide mass fingerprinting allowed to identify a 15 kDa U. chartarum protein (A in Figure 1A) as an Alt a 1-homologous protein, a 41 kDa protein (B) as formate dehydrogenase, a protein not yet known as an allergen, and a 48 kDa protein (C) as an Alt a 1-6-homologous enolase. The proteins were designated Ulo c 1\textsuperscript{A} (A), Ulo c 2\textsuperscript{B} (B), and Ulo c 6\textsuperscript{C} (C). The IgE-reactive protein bands D, E, and F could not yet be identified by mass spectrometry. cDNAs coding for the identified allergens was generated and cloned into a bacterial expression vector, and recombinant proteins were produced in Escherichia coli. Circular dichroism spectroscopy revealed that the recombinant allergens contain considerable secondary structures. In case of rUlo c 1, mainly beta-sheet structures (minimum of far-UV spectrum at 213 nm) were found, whereas predominantly alpha-helical structures (minima at 207 nm and 220 nm) were detected for rUlo c 2 and rUlo c 6 (Figure S2).

ELISAs performed with sera from mold-allergic patients showed the IgE-binding capacity of the three recombinant allergens, with rUlo c 1 displaying the highest levels of IgE reactivity (Figure 1B). Furthermore, IgE inhibition immunoblots, where nitrocellulose-blotted protein extracts from U. chartarum were exposed to serum pools that had been pre-incubated with the recombinant allergens, showed that the recombinant allergens were able to completely inhibit patients' IgE binding to their natural counterparts (Figure 1C). This indicates that the recombinant allergens represent well-folded proteins which contain all the IgE-binding epitopes present in their natural counterparts and suggests that rUlo c 1, rUlo c 2, and rUlo c 6 could be used as tools for in vitro diagnosis of U. chartarum sensitization.

The prevalence of the molecules' IgE reactivity was analyzed by ELISA using sera from 85 individuals sensitized to different mold species (Figure S3). rUlo c 1 was recognized by 58% of the patients, indicating that this molecule represents a major mold allergen, whereas rUlo c 2 was recognized by 43% and rUlo c 6 by 40% of the patients, suggesting minor mold allergens.

Sequence comparison of the three U. chartarum allergens with homologous A. alternata proteins revealed protein sequence identities of 89% between Ulo c 1 and Alt a 1, 99% between Ulo c 2 and its homologous A. alternata protein, and 100% between Ulo c 6 and Alt a 6. The high sequence homology between Ulo c 1 and Alt a 1 and the suggested presence of Alt a 1-homologous proteins in other species of the Pleosporaceae family\textsuperscript{10} prompted us to investigate the potential cross-reactivity between rUlo c 1 and rAlt a 1 in IgE inhibition immunoblots. As depicted in Figure 2A, pre-incubation of patients' sera with one allergen significantly reduced or even abolished IgE binding to the other molecule, whereas IgE binding was not reduced by pre-incubation of the sera with the irrelevant respiratory allergen rBet v 1. This reduction in IgE binding obtained with rAlt a 1 and rUlo c 1 was comparable to the reduction obtained by self-inhibition and indicates that the two allergens share conserved IgE epitopes. Furthermore, both recombinant allergens, rUlo c 1 and rAlt a 1, induced a dose-dependent expression of CD63 in basophils from six of the eight analyzed mold sensitized individuals (Figure 2B), which provided evidence for the molecules' biological activity.

Analysis of the release of allergens from allergen sources can provide useful information about the potential exposure to these allergens. Interestingly, our investigations on the release kinetics of Ulo c 1 and Ulo c 6 from U. chartarum and Alt a 1 and Alt a 6 from A. alternata showed that the allergens were all immediately released from the spores (Figure S4). These findings suggest that rapid allergen elution might also occur when spores reach mucosal surfaces, where they can then elicit allergic reactions.

In conclusion, in this study we provide evidence that U. chartarum represents an important, so far underestimated, allergen source that shows at least partial cross-reactivity to the evolutionarily related species A. alternata. Owing to the fact that in contrast to the outdoor mold A. alternata, U. chartarum is an important component of the indoor environment, it is especially important to raise the awareness that U. chartarum represents a potential cause of respiratory allergic diseases and to include the species into routine allergy diagnosis. We identified the species' first allergens, Ulo c 1, Ulo c 2, and Ulo c 6, and produced them as IgE-reactive recombinant molecules. These recombinant allergens will expand the repertoire of fungal allergens available for fungal allergy diagnosis, thus improving diagnosis' specificity and sensitivity.

The description of the used methods can be found in the supporting information.

**KEYWORDS**

allergy diagnosis, indoor allergens, mold allergy, recombinant allergens, ulocladium chartarum

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FIGURE 2  IgE cross-reactivity between the major fungal allergens rUlo c 1 and rAlt a 1. (A) Two micrograms of the recombinant allergens were separated by SDS-PAGE, blotted onto nitrocellulose, and exposed to a pool of sera (25–27, 42, and 78) from mold-allergic patients, which had been pre-incubated with rUlo c 1 or Alt a 1 or, for control purposes, with the non-relevant allergen rBet v 1 or with buffer only. Molecular weight markers are indicated in the left margins. (B) Basophil activation of rUlo c 1 and rAlt a 1 was determined by measuring the expression of CD63 by flow cytometry after incubation of the whole blood from eight Alternaria-sensitized patients (patients 13–16, 18, 21, 22, and 24) and from two non-atopic control individuals (control 1 and 2) with increasing allergen concentrations. The percentage of CD63-positive basophils (y-axis), based on the total amount of basophils, is displayed against the concentration of the applied allergen (x-axis).

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Reduced IL-31 receptor alpha splice variant mRNA following allergen challenge in a canine model of atopic dermatitis

To the Editor,

Interleukin 31 (IL-31) signaling is a target for therapy in atopic dermatitis (AD). Twelve isoforms of the cytokine-specific IL-31 receptor alpha (IL-31RA) produced by alternative mRNA splicing are known to have varied or opposing functions, but the physiological relevance of these has been obscured by the use of qPCR assays which are not specific to one isoform.\(^1\)\(^-\)\(^3\) If new treatments are to target IL-31RA, it is important to understand the modulation and likely functions of these different isoforms in allergic inflammation.

When Cevikbas and colleagues examined transcription of two groups of IL-31RA isoforms in skin, they found that while overall IL-31RA transcription was reduced, a subset of transcript variants was increased in the lesional skin of AD patients compared with healthy controls.\(^4\) This subset included both functional IL-31RA isoforms and a non-functional isoform lacking part of the cytokine-binding domain, so exactly which of these are upregulated in lesional atopic skin, and what effects this may have, remains unclear.\(^1\)\(^\,\)\(^4\)

Dogs (Canis lupus familiaris) spontaneously develop AD which is strikingly similar to its human counterpart and can be used to answer questions that may be challenging when studying human patients.\(^5\)\(^\,\)\(^6\)

Canine IL-31RA mRNA splice variants encode only three isoforms, two full-length and one truncated in the extracellular domain, which correspond to human homologs identified in Cevikbas’ upregulated subset. As in the human full-length IL-31RA, the extracellular region of the canine long IL-31RA isoforms includes a signal peptide and five fibronectin type III (FnIII) domains, the first two of which include the cytokine-binding domain in human IL-31RA.\(^1\)\(^\,\)\(^2\) The short canine IL-31RA is missing the signal peptide and the first FnIII domain, as in its non-functional human homolog.\(^3\) In functional studies of the human homologs of these IL-31RA splice variants by Maier and colleagues, translation of the short isoform was shown to be markedly reduced, and when expressed it was not able to activate STAT signaling in response to IL-31, suggesting a negative influence on IL-31 signaling.\(^4\) The potential role of these transcript variants during allergic inflammation can therefore be clarified by examining their transcription in atopic dogs.

The atopic beagles used in this study have been validated as suitable model for human AD which mimics natural occurring disease, with the advantage that the amount, timing, and frequency of allergen stimulation can be controlled and consecutive skin biopsies can be taken.\(^5\)\(^\,\)\(^6\) Full-thickness skin biopsies were collected from 10 atopic beagles at baseline (day 0) and at three hours (day 1, early inflammation), three days (peak inflammation), and 10 days (resolution of inflammation) following acute house dust mite (Dermatophagoides farinae) allergen stimulation. 50 mg crude D. farinae (31 mg/ml in phosphate buffered saline pH 7.2) was gently applied to the inguinal skin on days 1, 2, and 3 to provoke a moderate flare of AD which resolved by day 10. On days 1 and 3, biopsies were taken 3 h after allergen exposure. The severity of inflammation was assessed using a validated scoring system (canine atopic dermatitis extent and severity index; CADESI-03) prior to collection of each biopsy. IL-31RA splice variant transcription was measured using qPCR assays targeting total IL-31RA transcript, long IL-31RA isoforms mRNA transcript variants X1-4, short IL-31RA isoform mRNA transcript variant X5, or...