

This is the first study to demonstrate the therapeutic benefit of montelukast given concomitantly with loratadine in patients affected by DPU. Indeed, the favorable response to montelukast + loratadine might reflect the involvement of leukotrienes in the pathogenesis of DPU. It could also suggest that leukotriene antagonists might have a potential role in the treatment of inflammatory skin diseases. Despite the limitations of this preliminary report (eg, there was no placebo control difference in DPU with or without chronic urticaria), our results are encouraging. Therefore, we believe that more studies are needed to evaluate the efficacy of the concomitant administration of montelukast and loratadine in the treatment of DPU.

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***Penicillium nalgiovense* as an occupational and contact allergen**

To the Editor:

Molds comprise a taxonomic kingdom, with many

species ubiquitous in nature. Different studies have shown an association between respiratory allergic disease and exposure to fungi. The implication of fungi as occupational or food allergens is much more limited. Thus the exposure to *Plasmopara viticola* has been related to the respiratory symptoms of a greenhouse worker,¹ and the ingestion of champignon has been implicated in oral allergy syndrome.²

Processed cold meat manufacturers use mold cultures that are incorporated into the meat and on the outside of the casing to achieve the final aroma and flavor of the sausage and to protect it from wild mold growth. Commercially produced mold cultures of *Penicillium nalgiovense*, *Penicillium expansum*, or *Penicillium chrysogenum* are usually inoculated. This process could expose workers to high quantities of spore dust and cause hypersensitivity pneumonitis.³

We describe here a case (patient 1) of occupational asthma in a pork butcher caused by *P nalgiovense*, as well as a case (patient 2) of contact urticaria related to *P nalgiovense* sausage casing.

Patient 1 was a 45-year-old male pork butcher who had experienced cough, tightness of the chest, and sibilant dyspnea during the last 2 years. Episodes were related to the handling of sausages and inhalation of the dust coming from the sausage casings at his work in the butcher's area of a supermarket.

Patient 2 was an 18-year-old woman who reported pruritus, facial erythema, and bilateral palpebral angioedema after touching the casing of sausages, although she tolerated their ingestion.

Samples of white dust from the sausage casings were placed on Petri dishes containing bacto-potato dextrose agar supplemented with 1 g/L yeast extract. The plates were incubated at 20°C. A unique fungus grew in all the dishes. The colonies attained a diameter of 20 mm in 14 days, showing slight radial furrows that were at first white and then slightly yellow-orange in color. The colonies were identified as *P nalgiovense*.⁴ Portions of the edge of the colony were transferred to slants of the same culture medium for mycelium production.

Samples of white dust from the sausage casings were examined with an electron microscope, and the morphology of the penicillia and the conidia matched the description of *P nalgiovense*.⁴

Exposure to fungal spores at the pork butcher's area was assessed before and during his working activity by using a volumetric sampler (Biotest RCS, Biotest AG) equipped with an agar strip specific for the determination of molds (Agar strip YM, Art. No. 941200). Air volumes of 0.1 m³ were sampled at a flow rate of 50 L/min. A high concentration of fungal spores (2400 cfu/m³ of air), which were identified as *P nalgiovense*, was detected in the samples taken during the butcher's working activity. Almost no spores were observed in the samples taken before starting work.

Freeze-dried mycelium from *P nalgiovense* isolated from the sausage casings was extracted at 10% wt/vol in 1.8% NaCl for 90 minutes. After centrifuging, the super-

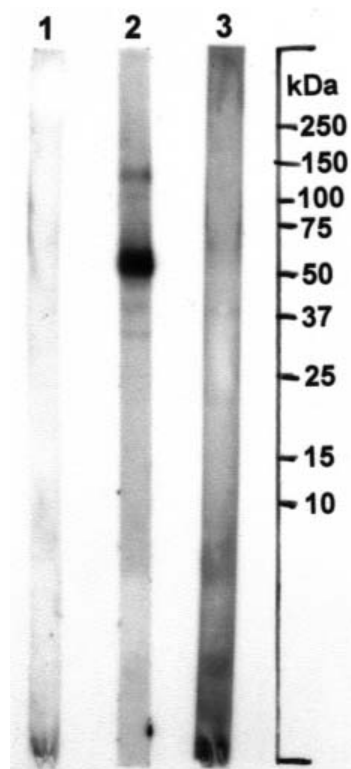


FIG 1. IgE immunoblotting of *P nalgiovense* extract: lane 1, negative control; lane 2, serum from patient 1; lane 3, serum from patient 2. The position of prestained markers run in parallel are indicated on the right.

natant was dialyzed against purified water (molecular weight cut-off point, 3500 d), sterilized by means of 0.22- μ m filtration, and freeze-dried. Protein concentration was determined by using the method of Lowry.

Skin prick tests (SPTs) were performed with a standard battery of aeroallergens (ALK-Abelló, S.A.). The results were positive to *Olea europaea* pollen in both patients and to *Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, and cat dander in patient 2. SPTs and intradermal tests were also performed with *P nalgiovense* extract at protein concentrations of 3 and 0.3 mg/mL, respectively. Both patients had positive SPT (3 $\times$ 3 mm) and intradermal test (10 $\times$ 8 mm) responses. In patient 2 a rubbing test on the forearm with the sausage casing was performed, and the result was also positive (erythema, pruritus, and wheal response after 20 minutes).

Conjunctival provocation test results with *P nalgiovense* extract⁵ were negative in both patients up to a protein concentration of 3 mg/mL. Oral provocation test results in patient 2 were also negative.

The result of a nonspecific bronchial provocation test (BPT) with methacholine⁶ was positive for patient 1 (PD₂₀ = 150 accumulated units).

A *P nalgiovense* BPT⁷ was performed in patient 1. The result was positive at a concentration of 3 mg/mL, with recordings decreasing to 22% FEV₁ and 31% peak

expiratory flow rate, respectively. No delayed reactions were detected by means of peak flow monitoring during the 24 hours after the BPT.

A peak flowmeter was given to patient 1 to carry out daily measurements for 6 weeks: 3 working and 3 out of work. The measurements showed a variability of 27%, which decreased to 9% on the days out of work. SPTs, conjunctival tests, rubbing tests, and BPTs with *P nalgiovense* extract were also performed in 3 asymptomatic butchers and 3 atopic patients with mild asthma, and no response was found.

IgE to *P nalgiovense* was determined by using the RAST technique. Only patient 1 had detectable specific IgE (RAST class 2). Specific IgE to mites and molds was determined by using the Pharmacia CAP System. Patient 1 had no IgE to mites and molds, and patient 2 had IgE to molds (class 5 for *A alternata* and *C herbarum* and class 3 for *A fumigatus*).

Tricine-SDS-PAGE was carried out in a 10% to 20% polyacrylamide gel (Novex) by using nonreducing conditions. The extract proteins separated by means of SDS-PAGE were electrotransferred onto nitrocellulose membranes, and after BSA blocking, the membranes were incubated with the patients' serum or buffer (negative control) and then with a 1:3000 dilution of mouse anti-human IgE mAb HE-28 ascitic fluid. Immunodetection of IgE-binding proteins was achieved by means of enhanced chemiluminescence (Amersham). The IgE immunoblot (Fig 1) of *P nalgiovense* extract showed mainly a protein band of approximately 50 kd that was reactive to IgE from the serum of patient 1. No IgE reactivity was observed with serum from patient 2.

It has been shown in some professional activities that the spore concentration in the environment could induce respiratory symptoms and cause occupational allergy. In our study we have demonstrated the presence of high *P nalgiovense* spore concentrations in the working area of patient 1. These spores came from the sausages handled by him. In vivo and in vitro determinations have indicated that the patient was sensitized to *P nalgiovense* isolated from the sausage casings. Furthermore, the specific BPT and peak flow measurements (at work and out of work) showed that *P nalgiovense* spores are responsible for the patient's respiratory symptoms.

Molds can also act as contact and food allergens.³ Thus in patient 2 the recurrent episodes of contact urticaria related to the handling of sausages were caused by *P nalgiovense*, as demonstrated by skin and rubbing tests. No specific IgE to *P nalgiovense* was detected in the patient's serum, but this could be related to her clinical expression.

In conclusion, in both patients *P nalgiovense* acts as an allergen present in highly consumed products, and consequently, future studies are needed to evaluate the clinical effect of *P nalgiovense* as an occupational, contact, and food allergen.

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Allergic hyperreactivity to microbial components: A trigger factor of "intrinsic" atopic dermatitis?

To the Editor:

Atopic dermatitis (AD) is a common, chronic, relapsing inflammatory skin disease characterized by typically distributed eczematous skin lesions and intense pruritus. A majority of patients with AD have high concentrations of total and allergen-specific IgE in the blood and skin. This form has been individualized as the IgE-mediated/allergic or *extrinsic* form of AD. In the so-called non-IgE-mediated/nonallergic or *intrinsic* form, which has been reported to represent 10% to 30% of all patients with AD, patients have normal total serum IgE levels and no allergen-specific IgE against aeroallergens or food allergens, although they exhibit the same clinical phenotype.² However, factors contributing to the development of this subtype of AD are completely obscure.

The skin of patients with AD exhibits a striking susceptibility to colonization and infection with microbial components, such as *Staphylococcus aureus*, *Pityrosporum ovale*, or *Candida albicans*. Recently, it has been

shown that *S aureus* strains isolated from AD skin produce enterotoxins, such as staphylococcal enterotoxins A (SEA), B (SEB), C (SEC), D (SED), and toxic shock syndrome toxin 1 (TSST-1), which display superantigenic activities. As a hallmark, superantigens facilitate the binding of MHC class II to the T-cell receptor independent of the peptide groove and stimulate T cells expressing a particular T-cell receptor β -chain. After superantigenic stimulation, skin-homing cutaneous lymphocyte antigen-positive T cells from patients with AD undergo a T-cell receptor V β expansion and perpetuate the inflammatory immune response in this way.³ Additionally, these exotoxins can act as classical allergens and induce the production of functional relevant specific IgE antibodies, which trigger mediator release from sensitized basophils after IgE antibodies have been cross-linked with the appropriate antigen.⁴

It is assumed that IgE antibodies directed against the commonly found skin yeasts *P ovale* and *C albicans* might potentially play analogous roles in triggering humoral immune responses.

Because skin colonization with microbial components is a common feature of both patients with high (ie, >150 kU/L IgE) and low serum IgE levels (ie, <150 kU/L IgE), it was the aim of our study to determine the prevalence of circulating IgE antibodies with specificity to microbial products in the intrinsic subgroup of patients with AD. For this purpose, a total of 114 patients with AD and 30 nonatopic healthy volunteers were recruited. Total serum IgE and specific IgE levels to SEA, SEB, SEC, SED, TSST-1, *P ovale*, *C albicans*, and various aeroallergens and food allergens, such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, *Alternaria tenuis*, timothy grass and birch pollen, wall pellitory, mugwort, cat dander, peanut, hazelnut, egg white, cow milk, fish, wheat, green apple, kiwi, and tomato, were assessed with the help of a CAP RAST system (Pharmacia, Germany).

As a result, 23 (20%) of 114 patients with AD had IgE serum levels of less than 150 kU/L and no allergen-specific IgE against aeroallergens or food allergens. In 53% of these patients with AD, specific IgE toward microbial components could be found (Table I). In 47% of patients displaying classical features of intrinsic AD, neither specific IgE antibodies to aeroallergens and food allergens nor to microbial components were detectable (Table I). In the extrinsic form of AD, in 85% of the patients, allergen-specific IgE against aeroallergens or food allergens in combination with allergen-specific IgE against microbial components was detectable, whereas no allergen-specific IgE against microbial components was detectable in the control group, in which the serum IgE level was 11.4 ± 5.4 kU/L. Statistical analyses with the χ^2 test demonstrated a significant difference ($P < .001$) in IgE reactivity versus no reactivity against microbial components for patients with intrinsic AD in comparison with healthy control subjects. Together, these findings underline the hypothesis that in a subgroup of patients with AD with low serum IgE levels, an allergic reactivi-