

Occupational protein contact dermatitis from wheat flour with IgE reactivity against α -amylase inhibitor

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Protein contact dermatitis (PCD) is an uncommon and often underdiagnosed condition (1) that can be provoked by wheat protein and its derivatives. Only a few studies have been performed to identify the allergens responsible for the development of wheat PCD. α -Amylase has been found to be a possible causative allergen for PCD (2). In a recent study performed in 3 patients with wheat PCD (3), immunoblotting showed IgE reactivity against three glycoproteins (an allergen of MW 27 000, peroxidase, and

purple acid phosphatase). Here, we report a case of wheat PCD with identification of IgE reactivity to α -amylase inhibitor.

Case Report

A 38-year-old male baker presented with a 1-year history of recurrent dermatitis of the hands and forearms refractory to topical corticosteroids. He reported that such lesions notably improved during prolonged work-free periods. The patient's history showed the presence of a long-lasting atopic dermatitis. The patient was patch tested with the Italian Society of Allergological, Occupational and Environmental Dermatology (SIDAPA) standard series, with negative results. Prick tests with common aeroallergens and a large panel of commercial

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food extracts (Lofarma, Milan, Italy) were carried, and gave positive results for *Parietaria*, grass, cypress, olive tree pollens, wheat, peanut, soybean, and egg white and yolk. Prick test results were confirmed by measurement of serum-specific IgE detected by ImmunoCap assay (IgE antibodies against wheat were present at 9.76 kUA/l). Prick and patch tests with materials used by the patient at work were also performed. Positive results were obtained with a prick test for wheat flour in an aqueous suspension. Patch testing showed a strong reaction to wheat flour only when performed on previously damaged skin. Wheat flour was extracted at 20% (wt/vol) in 0.5 M NaCl for 2 hr at room temperature. The extract was centrifuged at 10 000 g for 30 min at 4°C to remove precipitate, and the supernatant was dialysed through membranes (cut-off MW 3500; Spectrum, VWR, Milan, Italy) against 0.1 M phosphate-buffered saline, pH 7.4, for 48 hr. The extract protein content was measured according to Bradford (4) (Bio Rad, Milan, Italy), and proved to be 1.6 mg/ml. Purified α -amylase, α -amylase inhibitor and bromelain were purchased from Sigma (Milan, Italy). An enzyme-linked immunosorbent assay (ELISA) for specific IgE detection performed with the patient's serum showed significant IgE reactivity against both the wheat flour extract and α -amylase inhibitor. In contrast, IgE reactivity against α -amylase or bromelain was absent. The serum from the patient and sera from 3 healthy subjects were tested against wheat flour extract with immunoblotting, according to Towbin et al. (5). The immunoblotting profile showed the presence of IgE reactivity to different components of wheat flour, with the greatest intensity in the range between MW 14 000 and MW 35 000 (Fig. 1). Immunoblotting IgE reactivity was evaluated after preabsorption of the serum with 15 μ g of α -amylase inhibitor and unrelated protein (mite extract) (200 μ l of the serum pool and 800 μ l of saturation buffer containing different extracts). This profile changed when α -amylase inhibitor was used as the inhibitor in immunoblotting. In particular, the IgE-reactive band at MW $\sim$ 14 000 completely disappeared, whereas the component at MW 28 000 was partially inhibited. As expected, the unrelated extract (mite extract) did not induce any change in the immunoblotting profile of wheat extract.

Discussion

We have described an atopic baker with wheat PCD. The clinical diagnosis was confirmed by the results of patch testing on previously affected skin, prick testing, ELISA, and immunoblotting. Moreover, ELISA and immunoblotting identified α -amylase inhibitor as one of the candidate allergens of wheat PCD in our patient.

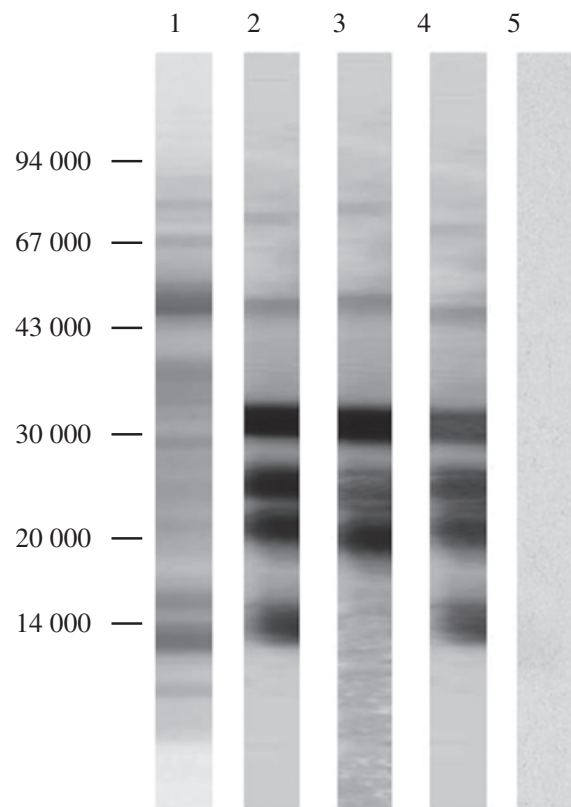


Fig. 1. Sodium dodecylsulfate–polyacrylamide gel electrophoresis of wheat flour extract stained with Coomassie colloidal blue (Invitrogen, Milan, Italy). Lane 2: IgE reactivity to wheat flour extract. Lanes 3 and 4: IgE reactivity to wheat flour extract after preabsorption of serum with α -amylase inhibitor and mite extract, respectively. Lane 5: IgE reactivity to wheat flour extract of non-atopic serum.

α -Amylase inhibitor is recognized as one of the main allergens in baker's asthma (6). However, our patient showed only cutaneous signs without any symptoms at the respiratory level, as well as no symptoms after wheat ingestion.

The results obtained in the immunoblotting study showed, after preabsorption of the patient's serum with α -amylase inhibitor, the complete disappearance of the MW 14 000 component, corresponding to the α -amylase inhibitor monomer, and partial inhibition of the MW 28 000 component. The latter component might correspond to the dimeric form of the enzyme.

Bromelain was included in the ELISA study to assess whether carbohydrate determinants could be involved in IgE reactivity, as observed in a previous report (3). The negative result obtained in the ELISA against bromelain excluded the involvement of glycan moieties in IgE binding in the PCD of our patient.

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