

Flour protein antigens in occupational flour hypersensitivity

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Thirty serum samples from clinical cases of flour hypersensitivity were analyzed for wheat or rye flour protein antibodies. The patients included 20 bakers and 10 others who also had occupational flour exposure. Twenty-three cases had antiflour antibodies which recognized antigens other than control sera in the flour protein patterns. The immunologic response of individual cases seemed very variable in view of the numerous differences between the cases in the antigen-antibody reactions. For the practical purposes, the flour protein antigens were divided in three groups, *i.e.*, those larger than 80 kDa, those between 80 and 50 kDa and those smaller than 50 kDa. Cases with flour-induced dermatitis ($n = 8$) showed sensitization towards antigens in all size classes while those with rhinitis or asthma showed more antigens with a molecular weight less than 50 kDa. The test offers a possibility to independently verify an exposure to flour while it does not substitute for the conventional immunologic diagnostic tests.

Key words: Asthma; dermatitis; flour; IgG; immunoblotting; rye; wheat.

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INTRODUCTION

Exposure to flour is one of leading causes of occupational asthma in many national surveys for occupational diseases.^{1–3} The disease may be acquired at a young age after a relatively brief exposure. In fact, a death certificate study in Chicago shows that young bakers of 20–35 years of age had markedly higher risk for a fatal asthma than expected.⁴

Many bakers work in small firms with resources which may not allow extensive protective equipment in the form of hoods and local exhausts. Even the use of personal respiratory protectors may be rare in family businesses and other small bakeries.

Immunologic techniques allow the determination of the concentrations of relevant aeroallergens in bakeries.^{5,6} Flour proteins are the most important antigens while allergy towards technical additives, like α -amylase,⁷ and to various parasites⁸ is also possible.

An abundance of antigenic determinants have been described in the flour protein fractions^{9–11} while for a

rapid screening for the presence of serum flour protein antibodies a simple immunoblotting technique may be most suitable.¹² The latter is complementary to the so-called RAST test as it gives an idea of the molecular weight of the antigens because the flour protein species are electrophoretically separated before the immunologic detection.

That property has been exploited in this study in order to look for the variability in the immunologic response in occupational hypersensitivity to flour. It also has provided an idea of the incidence of the disease over a period of 3 years as confirmed independently by the rather specific antibody detection.

SUBJECTS AND METHODS

All cases referred to us during a period of 3 years, *i.e.*, 1 January 1994 through 31 December 1996, for a serum test to detect IgG antibodies towards flour protein were included in this study. There were five women and 25 men. Each year 10 individual samples were analyzed. The catchment area corresponded to a population base of 0.7 millions inhabitants, and our laboratory was the only one offering this specific

analytical service. Private practitioners including generalists, pneumologists and dermatologists sent serum samples after having established the clinical diagnoses according to the consensus guidelines published by the Swiss Society for Allergology and Immunology.¹³ It can be estimated that a majority of new cases were referred to the test.

Serum was separated from the 5 ml blood samples by centrifugation and it was analyzed for antiflour IgG as described before.¹² Each time a control serum from a healthy unexposed office clerk was also analyzed. The test flours included the four most utilized kitchen varieties in the region, *i.e.*, complete wheat flour, semi-white and white wheat and rye flour. The flour samples were bought in a local supermarket. All immunoblots were photographed and compared with the simultaneously analyzed control patterns.

RESULTS

Thirty serum samples were analyzed during the 3-year period. There were 10 new cases each year. Bakers were the greatest group, 20 cases, followed by three pastry makers, two baker's apprentices, two sales persons, two general employees from a bakery and one cook. Asthma and upper respiratory tract symptoms were present in more than half of the cases while eight persons had dermatitis only (Table 1). Those suffering from simple rhinitis tended to be younger than those presenting with asthma (Table 1). The ages of dermatitis cases varied from 17–54 years.

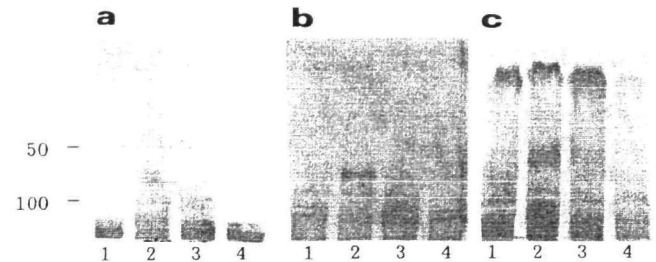
All control immunoblots contained staining of flour protein with a molecular weight of higher than 100 kDa (Figure 1). Twenty-three patient serum samples reacted with additional protein fractions in the four tested flours (Figure 1). For analytical purposes, the immunologic reactions were divided according to the antigen molecular weight in three major groups, *i.e.*, antigens greater than 80 kDa, between 80 and 50 kDa and smaller than 50 kDa. Immunologic staining almost always produced several reactive protein bands in case of patient sera (Figure 1). In seven patients, the staining pattern was similar to controls.

Table 1. Subjects with flour hypersensitivity

Diagnosis	n	Mean age	Age range
Rhinitis	3	19	16–23
Rhinitis and asthma	8	39	24–55
Asthma	4	35	30–64
Dermatitis	8	30	17–54
Dermatitis and rhinitis	2	25	21–29
Dermatitis, rhinitis and asthma	2	32	30–34
Others	3	40	24–50
All	30	35 ± 13 ^a	

^a Mean ± SD. Others include a case of allergic alveolitis, a case of conjunctivitis and a case of sinusitis.

Figure 1. Immuno-electrophoretic analysis of complete wheat (1), rye (2), semiwhite (3) and white wheat (4) flour protein antigens by control serum (a), by a serum from a case of flour dermatitis (b) and by serum from a case of baker's asthma (c). The flour protein was solubilized and electrophoresed on polyacrylamide gel slabs and then blotted on polyvinylidene fluoride (PVDF) membrane for a reaction with the respective serum. The antigen-antibody complexes were then visualized with antihuman immunoglobulins raised in mouse. The origin of the electrophoresis is on the bottom, and the scale on the left indicates the migration of 100 and 50 kDa molecular weight protein standards. Note the prominent reaction with rye protein fractions at 70 kDa (lane 2).



When the staining patterns by patient sera were compared with the clinical diagnoses, it appeared that rhinitis and asthma cases were associated more with staining of flour antigens with molecular weight of less than 50 kDa (Figure 1; Table 2). It is also noteworthy that there was important crossreactivity between the flour varieties (Figure 1c). The staining of flour antigens with sera of dermatitis cases was more evenly distributed according to the molecular weight of protein antigens. Specifically, the staining of a rye flour protein at a molecular weight of 70 kDa was always very prominent (Figure 1b).

DISCUSSION

Table 2. Occurrence of antigens and symptoms

Symptom	Antigen molecular weight ^a		
	≥ 80	80–50	< 50
Dermatitis	3	4	5
Rhinitis	1	5	10
Asthma	2	3	10

^a kDa. Note that the frequency of symptoms is greater than the number of cases because many had combined disease. Sera from seven subjects (23%) gave results comparable to those of controls.

The incidence of flour hypersensitivity in this study is comparable to investigations elsewhere.¹⁴ If the studied region is thought to be sufficiently representative of the whole of Switzerland (population 7 million) then 100 to 200 new cases of flour hypersensitivity should be found each year in the country. This study also confirms the idea that flour and its proteins are among the major antigens in baker's asthma^{15,16} although in 23% of patients the test revealed no additional antigens as compared to controls.

In all cases, a rye flour antigen was very prominently stained. This compares well with the properties of a 13.5 kDa antigen sequence isolated by others¹⁷ and which elicits a greater immunologic response than its barley analogue.¹⁷ Whereas the rye flour may be more immunogenic than other cereals, important crossreactivity between flours exists also. This agrees with the clinical notion that changing the flour type does not resolve the respiratory problems in baker's asthma.

The idea has been put forward that rhinitis may precede asthma in occupational hypersensitivity to flour. This may well be the case as the patients with rhinitis tend to be younger than those with asthma or asthma and rhinitis combined. Otherwise, the age range of cases corresponds to that found in other studies.^{18,19}

Flour dermatitis may also appear as an independent problem, and even its immunologic mechanisms may differ from those of the respiratory tract allergy in view of the greater variability of the antigen staining in this study. In a Finnish study during one year, there were 19 cases of flour dermatitis vs. 81 cases of asthma.²⁰ In this study, there were eight cases of isolated dermatitis vs. 15 cases of upper respiratory allergy in the 3-year period. It is possible that the present study attracted relatively more dermatitis patients as its diagnostics may pose particular problems and therefore the practitioners wished to compare their data more with the proposed serum test.

The use of specific immunologic tests makes the verification of exposure to offending agents possible.²¹ This is of particular importance in the compensation of the disease costs, as in many countries occupational diseases are accorded a special status. However, the determination of the circulating IgG antibodies does not substitute for the analysis of the specific IgE antibodies towards flour, parasites or α -amylase protein. Comparison with the latter would be especially interesting as the enzyme has become a common additive since the 1970s.¹⁶ A prospective clinical investigation is being planned on the basis of this descriptive feasibility study.

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