

*I. Sander
M. Raulf-Heimsoth
M. Düser
A. Flagge
A. B. Czuppon
X. Baur*

Professional Associations' Research
Institute for Occupational Medicine
(BGFA), Ruhr University, Bochum,
Germany

Differentiation between Cosensitization and Cross-Reactivity in Wheat Flour and Grass Pollen- Sensitized Subjects

27415

Key Words

Allergens
Grass pollen
Wheat flour
Cross-reactivity
Baker's asthma
Pollinosis
Specific IgE
Inhibition

Abstract

Background: To diagnose baker's asthma, occupational sensitization to wheat flour should be distinguished clearly from influences of cosensitization such as it may exist in pollinosis patients. To define the route of sensitization, the cross-reactivity between wheat flour and grass pollen allergens was investigated. **Methods:** Two groups of atopic individuals with or without professional contact to wheat flour were screened by skin prick test for their sensitization to wheat flour and grass pollen and, in the case of hints for cosensitization, by Enzyme Allergo Sorbent Test (EAST). Cross-reactivity between wheat flour and grass pollen allergens was investigated by IgE binding inhibition assay using sera of 20 cosensitized individuals and by immunoblots. **Results:** The immunological cross-inhibition between wheat flour and grass pollen proves some proteins to share common allergenic determinants. Significant differences between bakers and individuals not occupationally exposed to flour could be seen in the inhibition of IgE binding to wheat flour. While the IgE binding to wheat flour allergens was only slightly inhibited by grass pollen proteins in baker's sera its inhibition was nearly complete by this extract in sera of nonexposed atopics. Immunoblots indicate that wheat proteins with a molecular weight of about 8–12 and 22 kD preferentially react with sensitized bakers' IgE and not with IgE of other individuals. IgE binding to grass pollen allergens in immunoblots and inhibition experiments showed no differences between bakers and other subjects. **Conclusion:** In the group of atopics without professional contact to flour, the positive test results from wheat flour obviously ensue from contact to cross-reacting grass pollen proteins whereas bakers are exposed and sensitized to allergens of both sources. Such cross-inhibition experiments help to identify the source of sensitization which may be difficult to obtain in case of cross-reacting allergens.

KARGER

E-Mail karger@karger.ch
Fax +41 61 306 12 34
<http://www.karger.ch>

© 1997 S. Karger AG, Basel
1018 2438/97/1124-0378 \$12.00/0

This article is also accessible online at:
<http://BioMedNet.com/karger>

Correspondence to: Dr. Ingrid Sander
BGFA, Department of Allergology/Immunology, Bürkle-de-la-Camp-Platz 1
D-44789 Bochum (Germany)
Phone: +0049 234 3074 606, Fax: +0049 234 3074 610
E-Mail Sander@bgfa.ruhr-uni-bochum.de

Introduction

Several studies described subjects allergic to grass pollen who are also sensitized to cereal grains [1–7]. Cross-reactivity between water-soluble wheat grain proteins and rye grass pollen could be clearly demonstrated in the case of 3 individuals by Donovan and Baldo [4]. In a study comprising 47 patients with grass pollen allergy, the probability of having a positive CAP (Pharmacia) to wheat grain increased with elevated concentrations of grass pollen-specific IgE [5]. On the other hand, a group of bakers with suspected allergies to inhaled flour did not show a correlation between positive radioallergosorbent test (RAST) values to wheat grain and RAST values obtained with various grass pollen [6]. In a study comprising 145 children allergic to cereal grains and grasses, Jones et al. [7] found that patients with wheat allergies alone showed little in vitro cross-reactivity to taxonomically related grasses, whereas patients with grass allergy alone revealed extensive cross-reactivity to both cereal grains and grasses. This observation was not explained. Because of these partially vague and inconsistent findings, we investigated the role of cross-reactivity between wheat flour and grass pollen regarding diagnostics of baker's asthma in more detail. We compared the cross-inhibition of IgE binding in bakers exposed to wheat flour with that of individuals with other professions exposed to grass pollen.

Materials and Methods

Patients

Fifty-five bakers from our outpatient department were selected due to workplace-related symptoms (rhinitis, conjunctivitis and/or bronchial asthma) and their status of atopy defined by skin prick test results on common allergens. The second group consisted of 34 patients from the same area (Bochum, Germany) with allergic symptoms such as rhinitis, asthma and/or conjunctivitis but not professionally exposed to flour. All of them had positive skin prick test results for more than one allergen of a panel of 20 common allergens matching our definition of atopy.

Sera of 10 individuals of each group with specific IgE antibodies to wheat flour and grass pollen (>0.7 kU/l) were used for Enzyme Allergo Sorbent Test (EAST; Phadezym RAST kit, Pharmacia, Uppsala, Sweden) inhibition studies.

Skin Prick Test

We performed skin prick tests with a panel of common allergens: house dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*; Alk, Copenhagen, Denmark), animal dander (dog, horse, cat; Alk), bird feathers (HAL, Düsseldorf, Germany), moulds (*Alternaria tenuis*, *Aspergillus fumigatus*, *Cladosporium herbarum*; Alk), three different tree pollen mixtures, bush and herb pollen mixtures (HAL), grass pollen (Alk), egg proteins and cow milk (Alk), cock-

roaches (Allergopharma, Reinbek, Germany), latex and chironomid hemoglobin (χ t 1–9). The latter allergens were prepared in our laboratory (0.01 or 0.1 mg/ml). Skin prick test results to wheat flour solutions from Alk and Bencard (München, Germany) were combined; if at least one outcome was positive, wheat flour sensitization was assumed. Skin test responses with a wheal diameter of more than a quarter of that of the histamine control (10 mg/ml) were regarded as positive.

Specific IgE Determination

Human IgE antibodies were measured by EAST. For wheat flour, commercial disks (Pharmacia) were used. Grass pollen allergen disks were produced in our laboratory with a concentrated solution (B2, Bencard) of a pollen mixture from different species of the *Gramineae* family: *Arrhenatherum elatius*, *Cynosurus cristatus*, *Dactylis glomerata*, *Lolium perenne*, *Poa pratensis*, *Anthoxanthum odoratum*, *Festuca pratensis*, *Agrostis tenuis*, *Bromus mollis*, *Alopecurus pratensis*, *Holcus lanatus*, *Phleum pratense*. For coupling, BrCN-activated paper disks were incubated with ultrafiltrated allergen extracts containing a protein concentration of 0.5 mg/ml for 5 h at room temperature, saturated by ethanolamine and washed according to the method described by Ceska et al. [8]. The allergen-coated disks were incubated with patients' sera, and bound IgE was determined as described by the manufacturer. The EAST reference curve (Pharmacia), always run in parallel, was used for calculation. EAST values of <0.35 kU/l (Phadezyme RAST units) represent absent or undetectable levels of allergen-specific IgE antibodies. Values of 0.35–0.7 kU/l correspond to RAST class 1, values of 0.7–3.5 kU/l to RAST class 2, values between 3.5 and 17.5 kU/l to RAST class 3 and values >17.5 kU/l to RAST class 4.

EAST Inhibition Experiments

Extracts of the grass pollen mixture (B2, Bencard) and of wheat flour (Alk, Bencard) were dialyzed in PBS (0.02 M sodium phosphate buffer, pH 7.4, 0.05 M NaCl) and concentrated by ultrafiltration using Centricon-3 centrifugation tubes (Amicon, Witten, Germany). The protein concentration was estimated by Bradford assay [9] with bovine serum albumin (BSA) as standard. The wheat flour inhibition solution consisted of an 1:1 mixture of Alk and Bencard wheat flour allergens (1 mg/ml protein concentrations) in PBS. The grass pollen solution used for inhibition had a protein concentration of 1 mg/ml.

Patient's sera with high levels of specific IgE according to EAST results were diluted with PBS to fit the linear range of the EAST reference curve. Sera with EAST values <0.7 kU/l were not suitable for reproducible inhibition experiments. EAST inhibition was performed by preincubating 40 μ l of the diluted serum overnight at 4°C with 10 μ l of inhibition solution or PBS for control. On the next day, the allergen disks were added, and bound IgE was measured as described before. Duplicate determinations had an average variation of 7.7%, the maximal variation was 24%.

The inhibition percentage was calculated by the following formula:

$$\% \text{ inhibition} = \frac{(\text{EAST value of PBS control}) - (\text{EAST value in presence of inhibition solution})}{(\text{EAST value of PBS control} - 0.35)} \times 100$$

When the EAST value of the inhibition experiment was as high or higher than the control value, the inhibition percentage was defined as zero, when the EAST value of inhibition was <0.35 kU/l (detection limit of defined IgE antibody levels), the inhibition was defined as being 100%.

Table 1a. Prick test results of atopic bakers (n = 55)

	Wheat flour +	Wheat flour –	Σ
Grass pollen +	24	8	32
Grass pollen –	15	8	23
Σ	39	16	55

The distribution of prick test results shows no significant correlation between wheat flour and grass pollen sensitization (Fisher's exact test, one-sided: $p > 0.17$).

For EAST dose-response inhibition, pooled sera of patients from both groups with specific IgE to wheat flour and grass pollen were used. The protein solutions described above were either concentrated or diluted to measure inhibition in the range of 0.1–60 µg inhibitor per sample.

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting

Briefly, 200 µg protein per gel of grass pollen solution (ultrafiltrated Bencard B2) or wheat flour extract (mixture of ultrafiltrated Alk/Bencard solutions) were mixed with sample buffer containing SDS, boiled for 3 min and electrophoresed in 14% polyacrylamide separation gels with 5% stacking gels according to the Laemmli procedure [10]. Molecular weight markers used were phosphorylase B (M_r 97,400), BSA (M_r 67,000), ovalbumin (M_r 45,000), carbonic anhydrase (M_r 29,000), soybean trypsin inhibitor (M_r 21,000), cytochrom c (M_r 12,500) and bovine lung trypsin inhibitor (M_r 6,500) from Serva (Heidelberg, Germany). The separated proteins were stained by Coomassie brilliant blue R-250 or transferred by semi-dry blotting [11] to PVDF membrane (Pall, Dreieich, Germany). Blotted membrane strips were stained by Coomassie brilliant blue R-250 or blocked with 1% BSA and 1% polyvinylpyrrolidone-40 in TBS buffer (15 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.8) for 7 h. Sera from bakers and other subjects diluted with PBS according to the EAST values were separately pooled for each group and preincubated for 5 h with 0.2 mg grass pollen or wheat flour protein per milliliter for inhibition or with PBS as control. The pooled sera were incubated overnight with the blot strips at room temperature and washed 3 times with TBST (TBS + 0.05% Tween-20). Bound IgE was detected after incubation for 4 h with ^{125} I-anti-human IgE antibodies (Pharmacia, Freiburg, Germany) diluted 1:20 in TBST with 2% BSA (ca. 50,000 cpm/strip) and intensive washing with TBST buffer by exposure to Hyperfilm MP (Amersham, Braunschweig, Germany) at -70°C for 1–2 weeks.

Statistical Procedures

Results of Fisher's exact test, Breslow-Day test for homogeneity of odds ratios and Wilcoxon rank sum test were calculated with the program StatXact-Turbo (Cytel Software Corp., Cambridge Mass., USA).

Table 1b. Prick test results of the atopic control group without professional contact to cereal grain flour (n = 34)

	Wheat flour +	Wheat flour –	Σ
Grass pollen +	11	16	27
Grass pollen –	0	7	7
Σ	11	23	34

Positive prick test results on wheat flour and grass pollen show a significant correlation with Fisher's exact test (one-sided: $p < 0.05$).

Results

Correlation of Skin Prick Test Results

Immunological sensitization to wheat flour and grass pollen allergens was investigated in two groups. All patients had more than one positive skin prick test result matching our definition of atopy. In both study groups, we correlated skin prick test results with wheat flour and those of grass pollen (table 1a, b). No correlation between these prick test results could be found in the group of atopic bakers (table 1a, $p > 0.17$). Atopic bakers with a positive skin prick test result with grass pollen had no higher rate of wheat flour sensitization than atopic bakers without grass pollen sensitization. On the other hand, the group of atopic subjects with other professions showed a positive correlation ($p < 0.05$) regarding the distribution of sensitization to wheat flour and grass pollen (table 1b). However, the odds ratios of both groups do not differ significantly (Breslow-Day test for homogeneity, $p = 0.1174$).

EAST and EAST Inhibition Results

Sera of 10 individuals of each group with specific IgE antibodies to wheat flour as well as to grass pollen antigens were used for detailed antibody analyses. The test results showed that for all 10 atopics without professional exposure to wheat flour the binding to grass pollen was higher than that to wheat flour proteins, whereas for bakers heterogeneous combinations were found (fig. 1). A correlation between the specific IgE values of wheat and grass pollen was not observed.

The differences between the two investigated groups can be demonstrated more clearly by inhibition experiments (fig. 2a, b). In sera of the nonbaker group IgE binding to wheat flour could be inhibited up to 100% by grass pollen proteins. In contrast, only a slight inhibition was obtained when baker's sera were tested (fig. 2a). This difference was

highly significant ($p < 0.001$). Comparing the results of single sera of the nonbaker group (N1–10) with autoinhibition results it can be stated that cross-inhibition by grass pollen proteins is as high as autoinhibition by wheat flour solution. No group-dependent differences could be found in the autoinhibition of IgE binding to wheat flour (fig. 2b). The incomplete autoinhibition of IgE binding to wheat flour seen in some sera is probably due to partial differences in the composition of solid phase-coupled wheat flour (Pharmacia) and the inhibitor (Alk/Bencard). Inhibition of binding of pooled sera from both study groups with different inhibitor amounts shows that 10 μg of protein are in the range of the maximum inhibition (fig. 3a, b). Inhibition of antibody binding to wheat flour by grass pollen is $< 50\%$ on account of the baker's sera in the pool (fig. 3a) confirming the results obtained with individual baker's sera (fig. 2a).

In experiments of IgE binding to grass pollen proteins, the inhibition by wheat flour proteins ranged from 40 to 95% without revealing significant differences between bakers and atopics with other professions (fig. 4a). Complete autoinhibition was obtained with grass pollen proteins, (fig. 4b). This was already seen with an amount of $< 1 \mu\text{g}$ grass pollen protein using a pool serum (fig. 3b).

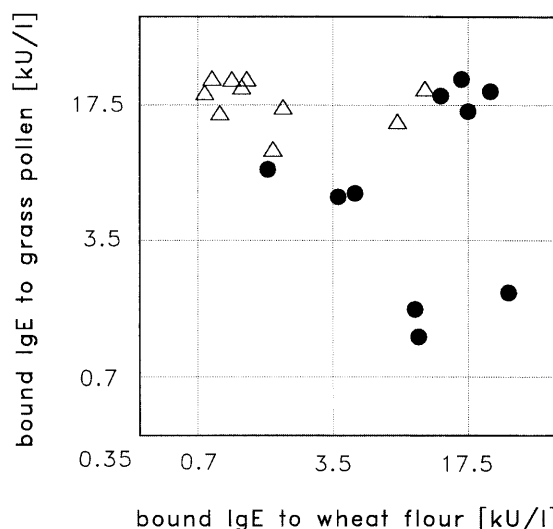


Fig. 1. Specific IgE to wheat flour and grass pollen allergens. Sera of 10 bakers (●) and of 10 employees with other professions (non-bakers; △) cosensitized to wheat flour and grass pollen were tested for specific IgE antibodies by EAST.

Fig. 2. Inhibition of IgE binding to wheat flour proteins. Sera of 10 bakers (B1–B10) and of 10 other subjects (N1–N10) were used in EAST inhibition experiments. The binding to wheat flour proteins was inhibited by 10 μg of grass pollen proteins (a) or by 10 μg of wheat flour proteins (b). The inhibition is shown as percentage of IgE binding without inhibitor. For bakers' sera, a cross-inhibition of $23 \pm 18\%$ was obtained; for atopics without professional flour exposure, $74 \pm 28\%$ inhibition was obtained (a). This group-dependent difference is highly significant according to Wilcoxon rank sum test ($p < 0.001$). Autoinhibition (b) was in the range of $84 \pm 17\%$ without significant group-dependent differences.

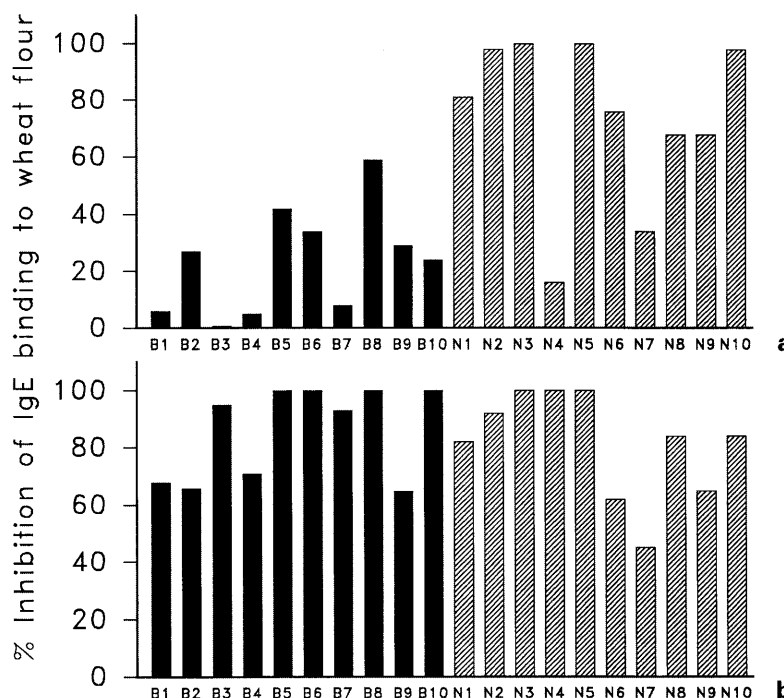


Fig. 3. Dose-dependent inhibition. IgE binding of pooled sera from both study groups to wheat flour (IgE value without inhibitor: 2.15 kU/l) (**a**) or to grass pollen (IgE value without inhibitor: 3.81 kU/l) (**b**) on solid phase was inhibited by increasing amounts of grass pollen (▼) or wheat flour □ proteins.

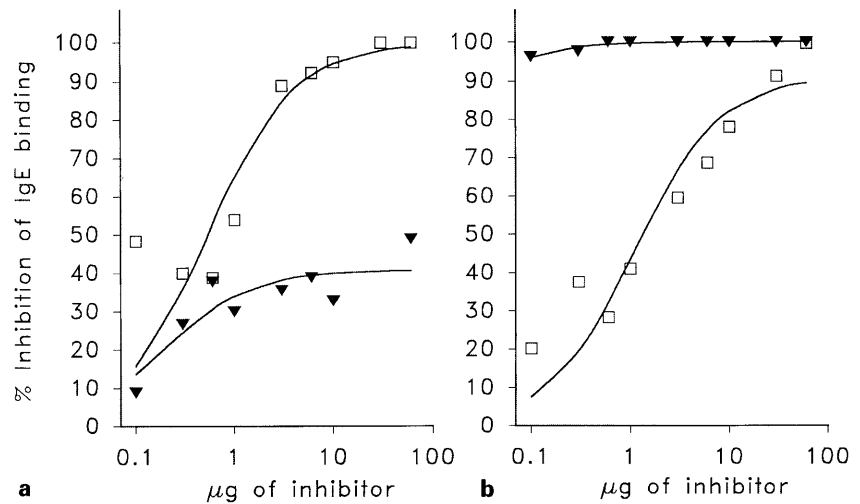
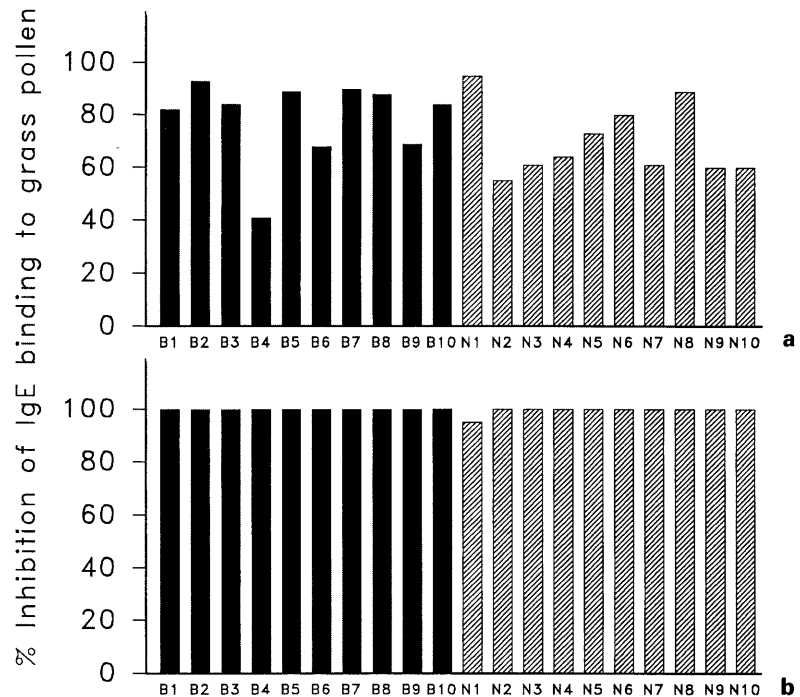


Fig. 4. Inhibition of IgE binding to grass pollen proteins. Sera of 10 bakers (B1–B10) and of 10 other subjects (N1–N10) were used in EAST inhibition experiments. Binding to grass pollen proteins was inhibited by 10 μg of wheat flour proteins (**a**) or autoinhibited by 10 μg of grass pollen proteins (**b**). Inhibition by wheat flour (**a**) was in the range of $74 \pm 15\%$ without significant group-dependent differences. Complete autoinhibition could be obtained with grass pollen proteins (**b**).



Immunoblot Experiments

To identify cross-reacting proteins, immunoblots with wheat flour and grass pollen proteins using pool sera of each group were performed. IgE antibodies of subjects not professionally exposed to wheat flour react with proteins of 30–32, 39, 60 and 69–92 kD (fig. 5). These wheat flour proteins share common epitopes with grass pollen allergens.

Inhibition with grass pollen proteins is nearly complete. The IgE of bakers reacts to wheat proteins of 8–12, 22, 27, 30, 32, 39, 60, 69 and 92 kD. While IgE binding to the protein in the range of 30 kD is nearly completely inhibited, binding to the other proteins seems to be only partially inhibited by grass pollen proteins. Wheat proteins of 8–12 and 22 kD react exclusively to baker's IgE.

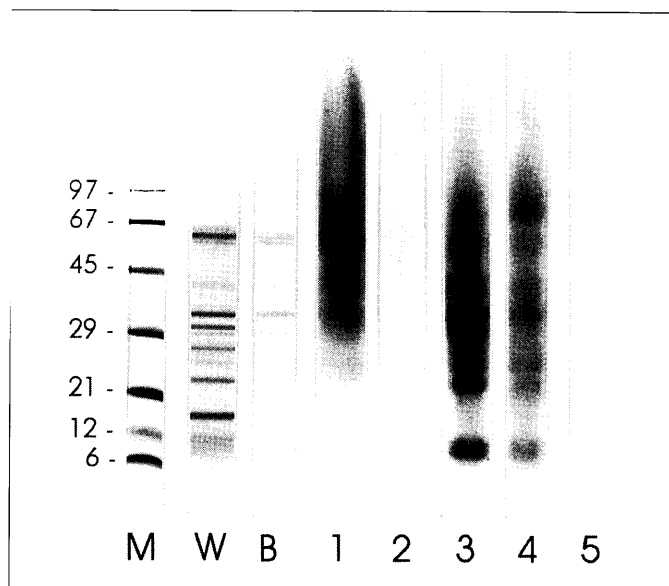


Fig. 5. Immunoblots with wheat flour proteins. Wheat flour extracts were separated electrophoretically in the presence of a molecular weight marker (M), stained in gel strips (W) or on Western blots (B) by Coomassie brilliant blue or reacted to pool sera without inhibition or in the presence of grass pollen protein. Bound IgE was detected by ^{125} I-anti-human IgE and autoradiography. 1 = Nonbakers' pool serum without inhibition; 2 = Nonbakers' pool serum inhibited by grass pollen proteins; 3 = bakers' pool serum without inhibition; 4 = bakers' pool serum inhibited by grass pollen proteins; 5 = control without serum.

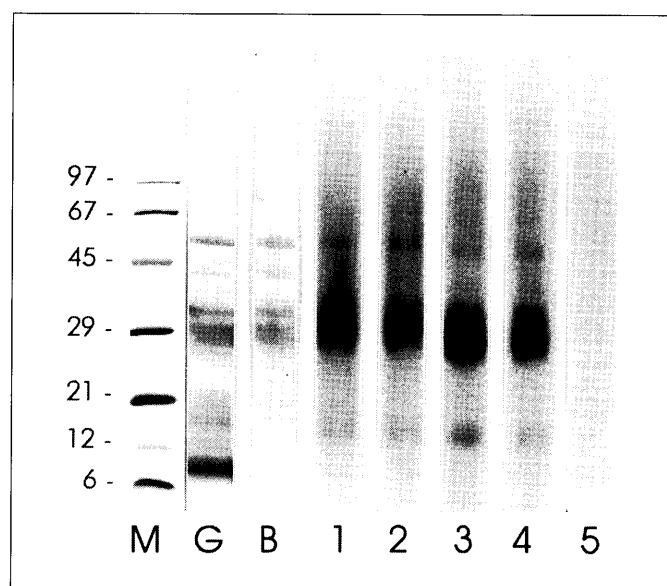


Fig. 6. Immunoblots with grass pollen proteins. Grass pollen extracts and a molecular weight marker (M) were separated electrophoretically, stained in gel strips (G) or on Western blots (B) by Coomassie brilliant blue or reacted to pool sera without inhibition or in the presence of wheat flour protein. Bound IgE was detected by ^{125} I-anti-human IgE and autoradiography. 1 = Nonbakers' pool serum without inhibition; 2 = nonbakers' pool serum inhibited by wheat flour proteins; 3 = baker's pool serum without inhibition; 4 = bakers' pool serum inhibited by wheat flour pollen proteins; 5 = control without serum.

Immunoblots with grass pollen protein do not show such group-dependent differences (fig. 6). Both pool sera react to proteins of about 10–14 kD (probably group 2 and/or group 3 allergens), 26, 28, 30 and 33 kD (group 1 and 5 allergens), 56 kD (group 4 allergens) and 80 kD. No inhibition with wheat flour proteins could be detected.

Discussion

Our results demonstrate that wheat flour and grass pollen proteins have common IgE binding epitopes. This could be expected due to the protein homologies between different species of the *gramineae* family [12]. Grass pollen and wheat flour are sources of various allergens, important to pollinosis or 'hay fever' in the case of grass pollen [13–15] and to baker's asthma in the case of wheat flour [16–21]. In agreement with other investigators showing common allergenic determinants of grass pollen and cereal grain proteins by immunoaffinity analysis and protein blotting [3, 4, 7],

allergenic cross-reactivity between wheat flour and grass pollen could be demonstrated by EAST inhibition experiments. The binding to grass pollen proteins could be inhibited by wheat flour in the range of $74 \pm 15\%$ using sera of 20 cosensitized individuals. This inhibition could not be related to single protein bands because the binding to grass pollen allergens did not differ visibly after inhibition with wheat flour in immunoblots.

An important aspect in the evaluation of baker's asthma is to prove that sensitization to wheat flour allergens has an occupational cause. Therefore, the role of cross-reactivity between wheat flour and grass pollen is crucial. Furthermore, the possibility that pollinosis may increase the risk of developing baker's asthma during working life has to be considered especially with regard to prevention. Prichard et al. [20, 21] discovered an association between atopic status (positive prick test on common allergens) and frequency of wheat flour sensitization. A significant association between skin sensitization to occupational allergens and atopy was also found in an epidemiological study on Italian bakers and

pastry makers [22]. Some authors even recommended that no person with current atopy should be employed in the bakery trade [23, 24].

To investigate whether sensitization to grass pollen increases the risk of developing grain flour allergy even beyond the atopic status, we correlated skin prick test results with these allergen sources in two selected groups. The known influence of atopy on sensitization to grass pollen [25] or wheat flour should be more or less equal in both groups since only atopic subjects were considered. As expected, positive skin prick test results on wheat flour were more frequent (70%) in the group of 55 atopic bakers than in the group of 34 atopics with other professions. In contrast, sensitization to grass pollen detected by prick test occurred in a higher frequency in the group of employees without flour contact. In the panel of atopic bakers, no correlation between grass pollen and wheat flour sensitization by prick test or by IgE binding could be detected. On the other hand, in the group of atopics without professional contact to grain flour, a significant association between positive prick test results on grass pollen and wheat flour was observed. In these cases, sensitization obviously originated from contact with grass pollen, and IgE binding to wheat flour was mostly due to common epitopes. In accordance with this interpretation, binding to wheat flour could be inhibited by grass pollen proteins to the same degree as by autoinhibition with wheat flour solution. Immunoblots show various cross-reacting wheat flour proteins in the range of 30–32, 39, 60 and 69–92 kD. Binding of nonbakers' IgE to these allergens was completely inhibitable with grass pollen extracts.

The group of bakers was exposed to both allergen sources. In these cases, the role of common epitopes was of minor influence. IgE binding to wheat flour could be inhibited by grass pollen proteins by about 23% only. In immunoblots with bakers' IgE, most wheat proteins showed little cross-reactivity due to grass pollen proteins. The same refers to wheat proteins in the range of 8–12 and 22 kD reacting exclusively with bakers' sera, while IgE binding to a protein in the range of 30 kD seemed to be inhibited completely by grass pollen allergens.

Regarding the investigated atopic bakers, the distribution of positive skin prick test results gives no evidence that sensitization to grass pollen allergens increases the risk of sensitization to wheat flour beyond the mere atopic condition. This is evident when the group of atopic bakers is regarded alone as well as when atopic bakers are compared with atopic controls.

Our investigation showed inhibition experiments to provide valuable information on the identification of the causative substances in subjects sensitized to complex organic material such as pollen and flour with partially cross-reacting components.

Acknowledgements

We thank Mrs. C. Hillenbach and Dr. P.O. Degens from our Epidemiological Department for their help with statistical data analyses and our Medical Department, especially Dr. O. Hagemeyer, Dr. H. Allmers, and E.-M. Schneider for the examination of our patients. We are indebted to Mrs. K. Weber for the helpful review of the manuscript.

References

- 1 Wraith DG, Merrett J, Roth A, Yman L, Merrett TG: Recognition of food-allergic patients and their allergens by the RAST technique and clinical investigation. *Clin Allergy* 1979;9:25–26.
- 2 de Martino M, Novembre E, Cozza G, de Marco A, Bonazza P, Vierucci A: Sensitivity to tomato and peanut allergens in children monosensitized to grass pollen. *Allergy* 1988;43:206–213.
- 3 Donovan GR, Baldo BA: Cross-reactivity of IgE antibodies from sera of subjects allergic to both ryegrass pollen and wheat endosperm proteins: Evidence for common allergenic determinants. *Clin Exp Allergy* 1990;20:501–509.
- 4 Donovan GR, Baldo BA: Immunoaffinity analysis of cross-reacting allergens by protein blotting. *Electrophoresis* 1993;14:917–922.
- 5 Vestergaard HS, Bindslev-Jensen C, Poulsen LK: Specific IgE to wheat in patients with grass pollen allergy (abstract). *Allergy Clin Immunol News* 1994;(suppl 2):445.
- 6 Sutton R, Skerritt JH, Baldo BA, Wrigley CW: The diversity of allergens involved in bakers' asthma. *Clin Allergy* 1984;14:93–107.
- 7 Jones SM, Magnolfi CE, Cooke SK, Sampson HA: Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity. *J Allergy Clin Immunol* 1995;96:341–351.
- 8 Ceska M, Eriksson R, Varga JM: Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1–9.
- 9 Bradford MM: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
- 10 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680–685.
- 11 Kyhse-Andersen J: Electrophoretic transfer of multiple gels: A simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J Biochem Biophys Methods* 1984;10:203–209.
- 12 Astwood JD, Mohapatra SS, Ni H, Hill RD: Pollen allergen homologous in barley and other crop species. *Clin Exp Allergy* 1991;25:66–72.
- 13 Matthiesen F, Löwenstein H: Group V allergens in grass pollens. II. Investigation of group V allergens in pollens from ten grasses. *Clin Exp Allergy* 1991;21:309–320.
- 14 Petersen A, Becker WM, Schlaak M: Characterization of grass group I allergens in timothy grass pollen. *J Allergy Clin Immunol* 1993;92:789–796.
- 15 Vrtala S, Grote M, Duchêne M, van Ree R, Kraft D, Scheiner O, Valenta R: Properties of tree and grass pollen allergens: Reinvestigation of the linkage between solubility and allergenicity. *Int Arch Allergy Immunol* 1993;102:160–169.

- 16 Blands J, Diamant B, Kallos P, Kallos-Deffner L, Löwenstein H: Flour allergy in bakers. I. Identification of allergenic fractions in flour and comparison of diagnostic methods. *Int Arch Allergy Appl Immunol* 1976;52:392–406.
- 17 Björkstén F, Backman A, Jarvinen KA, Lehti H, Savilahti E, Syvanen P, Karkkainen T: Immunoglobulin E specific to wheat and rye flour proteins. *Clin Allergy* 1977;7:473–483.
- 18 Baldo BA, Wrigley CW: IgE antibodies to wheat flour components. Studies with sera from subjects with baker's asthma or coeliac condition. *Clin Allergy* 1978;8:109–124.
- 19 Block G, Tse KS, Kijek K, Chan H, Chan-Yeung M: Baker's asthma. Clinical and immunological studies. *Clin Allergy* 1983;13:359–370.
- 20 Prichard MG, Ryan G, Walsh BJ, Musk AW: Skin test and RAST responses to wheat and common allergens and respiratory disease in bakers. *Clin Allergy* 1985;15:203–210.
- 21 Prichard MG, Ryan G, Musk AW: Wheat flour sensitisation and airways disease in urban bakers. *Br J Ind Med* 1984;41:450–454.
- 22 de Zotti R, Larese F, Bovenzi M, Negro C, Molinari S: Allergic airway disease in Italian bakers and pastry makers. *Occup Environ Med* 1994;51:548–552.
- 23 Jarvinen KA, Pirila V, Björkstén F, Keskinen H, Lehtinen M, Stubb S: Unsuitability of bakery work for a person with atopy: A study of 234 bakery workers. *Ann Allergy* 1979;42:192–195.
- 24 de Zotti R, Molinari S, Larese F, Bovenzi M: Pre-employment screening among trainee bakers. *Occup Environ Med* 1995;52:279–283.
- 25 Wüthrich B, Schindler C, Leuenberger P, Ackermann-Lieblich U: Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. *Int Arch Allergy Immunol* 1995;106:149–156.

Announcement

1997 Programme of European Research Conferences (EURESCO)

The Programme of European Research Conferences (EURESCO) is run by the European Science Foundation, in association with several learned societies, and with funding from the Euroconferences Activity of the European Union. Each Conference consists of a series of meetings, held typically every other year. There are neither written contributions nor proceedings. Unconventional ideas and new approaches, not yet fully explored, are encouraged.

Life Sciences

Molecular Biology of Cellular Interactions: Cell Adhesion Molecules and Receptor Cross-Talk, N. Kieffer (Luxembourg) – Granada, Spain, 31 October – 5 November

Biomedicine and Health

Hormones, Blood Cells and Immunity: Prolactin, Growth Hormone and Insulin-Like Growth Factor-I in the Immune System, R. Kooijman (Brussels) – Obernai (near Strasbourg), France, 3–8 October.

The conferences are open to all researchers, whether from industry or academia. Attendance is limited to about 100. Emphasis will be on discussion about new developments. The registration fee covers full board and lodging. Grants will be available for younger scientists, in particular those from less favoured regions in Europe. Some conferences may have limited funding for participants from Eastern and Central Europe. Deadline for applications: 3–4 months before a conference.

For information and application forms, contact the Head of the EURESCO Unit:

Dr. J. Hendekovic
European Science Foundation
1, quai Lezay-Marnésia, F-67080 Strasbourg Cedex (France)
Tel. +33 388 76 71 35, Fax +33 388 36 69 87
E-Mail euresco@esf.org
On-line information and application at: <http://www.esf.org/euresco>

Philippe Laudat Conferences

These international conferences are organised by Institut National de la Santé et de la Recherche Médicale INSERM under the responsibility of a European scientific organising committee. Proceedings are not published. The conferences are open to scientists worldwide. Attendance being restricted to approximately 100 – speakers included – applicants will be selected by the scientific organising committee. Participants will be charged a registration fee covering access to the scientific sessions and full board. All conferences will be held in Aix-les-Bains, France. Deadline for applications: **28 April**.

Activation-Induced Cell Death and Peripheral T Cell Tolerance: Prospects for Therapeutic Intervention, J.-P. Revillard (Lyons), M. Goldman (Brussels), D. Kabelitz (Langen), H. Waldmann (Oxford) – 2–6 November

Molecular Mechanisms of Infection and Pathology in Prion Diseases, J.-P. Liautard (Montpellier), J.-Y. Cesbron (Lille), D. Dormont (Fontenay-aux-Roses), J. Hope (Edinburgh), C. Weissmann (Zürich) – 16–20 November

For additional information and application forms, please contact:

Conférences Philippe Laudat
Bureau des Colloques et Conférences, INSERM
101 rue de Tolbiac, F-75654 Paris Cedex 13 (France)
Tel. +33 144 23 60 87, Fax +33 144 23 60 87
E-Mail laudat@tolbiac.inserm.fr
Internet: <http://www.inserm.fr>