Component-resolved diagnosis of baker's allergy based on specific IgE to recombinant wheat flour proteins*

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Background: Sensitization to wheat flour plays an important role in the development and diagnosis of baker's asthma.
Objectives: We evaluated wheat allergen components as sensitizers for bakers with work-related complaints, with consideration of cross-reactivity to grass pollen.
Methods: Nineteen recombinant wheat flour proteins and 2 cross-reactive carbohydrate determinants were tested by using CAP-FEIA in sera of 101 bakers with wheat flour allergy (40 German, 37 Dutch, and 24 Spanish) and 29 pollen-sensitized control subjects with wheat-specific IgE but without occupational exposure. IgE binding to the single components was inhibited with wheat flour, rye flour, and grass pollen. The diagnostic efficiencies of IgE tests with single allergens and combinations were evaluated by assessing their ability to discriminate between patients with baker's allergy and control subjects based on receiver operating characteristic analyses.
Results: Eighty percent of bakers had specific IgE levels of 0.35 kU_A/L or greater and 91% had specific IgE levels of 0.1 kU_A/L or greater to at least one of the 21 allergens. The highest frequencies of IgE binding were found for thiol reductase (Tri a 27) and the wheat dimeric α-amylase inhibitor 0.19 (Tri a 28). Cross-reactivity to grass pollen was proved for 9 components, and cross-reactivity to rye flour was proved for 18 components. A combination of IgE tests to 5 components, Tri a 27, Tri a 28, tetrameric α-amylase inhibitor CM2 (Tri a 29.02), serine protease inhibitor–like allergen (Tri a 39), and 1-cys-peroxiredoxin (Tri a 32), produced the maximal area under the curve (AUC = 0.84) in receiver operating characteristic analyses, but this was still lower than the AUC for wheat- or rye flour–specific IgE (AUC = 0.89 or 0.88, respectively).
Conclusions: Component-resolved diagnostics help to distinguish between sensitization caused by occupational flour exposure and wheat seropositivity based on cross-reactivity to grass pollen. For routine diagnosis of baker's allergy, however, allergen-specific IgE tests with whole wheat and rye flour extracts remain mandatory because of superior diagnostic sensitivity. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Baker's asthma,* Triticum aestivum*, recombinant allergens, alpha-amylase inhibitor, cross-reactivity, grass pollen, rye flour, wheat allergy, specific IgE, component-resolved diagnosis*

Wheat *(Triticum aestivum)* is a source of numerous allergens responsible for different manifestations of IgE-mediated allergy, depending on the route of exposure.[1] Ingestion of wheat can induce food allergy or wheat-dependent exercise-induced anaphylaxis; inhalation of wheat and rye flour is the main cause of baker's asthma.

Knowledge of the relevant allergen components might help to improve diagnostics, such as skin prick tests, which have been found to lack sensitivity[2-4]; to standardize *in vitro* IgE antibody assays[5]; or to optimize immunotherapy. In addition, discrimination between different clinical manifestations of wheat allergy or different allergic phenotypes might be supported by analyzing a patient's individual IgE reaction profile to single allergens, as has been proposed previously.[6-8]

In German wheat-sensitized bakers the recombinant allergens most frequently bound by serum IgE were the wheat α-amylase inhibitors Tri a 28 and Tri a 29.01 and a thiol reductase homologue (Tri a 27).[9] In a panel of Spanish bakers with baker's asthma, microarrays with 12 wheat seed allergens purified from natural sources identified the wheat tetrameric α-amylase inhibitor subunit CM16 (WTAI-CM16), the lipid transfer protein Tri a 14, and Tri a 28 as the most frequent allergens.[10] In The Netherlands, where most of the work on wheat flour exposure assessment and influence on sensitization rates has been performed,[11-13] no data on antiwheat reaction profiles in sensitized bakers are available.

It cannot be excluded that the most important sensitizing wheat or rye flour single allergens differ per country with consequences for diagnosis. Therefore we tested the same panel of recombinant wheat flour allergens used earlier for German bakers[9] also in populations of Spanish and Dutch bakers with occupational allergy. The allergen panel was completed with the serine protease inhibitor–like allergen (SPILA),[14] which is now denominated by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies as Tri a 39, and the lipid transfer protein isoallergen wheat nonspecific lipid transfer protein (nsLTP) type I subfamily 9.1 (nsLTP 9.1; Tri a 14.0101).[15-17]

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*Allergen names, including isoallergen and variant numbers, used in this study were approved by the International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-Committee.

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Abbreviations used
AUC: Area under the curve
CCD: Cross-reactive carbohydrate determinant
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
HRP: Horseradish peroxidase
MBP: Maltose-binding protein
MUXF: Carbohydrate part of bromelain
nsLTP: Nonspecific lipid transfer protein
ROC: Receiver operating characteristic
sIgE: Allergen-specific IgE
SPILA: Serine protease inhibitor–like allergen
Tri a 12.0102: Wheat profilin
Tri a 14.0101: Wheat nonspecific lipid transfer protein type I subfamily 9.1 (nsLTP 9.1)
Tri a 14.0201: Wheat non-specific lipid transfer protein type I subfamily 9.7 (nsLTP 9.7)
Tri a 15.0101: Wheat monomeric α-amylase inhibitor 0.28 (WMAI-0.28)
Tri a 19.0101: Wheat ω5-gliadin
Tri a 21.0101: Wheat αβ-gliadin
Tri a 25.0101: Wheat thioredoxin H
Tri a 27.0101: Wheat thiol reductase homologue
Tri a 28.0101: Wheat dimeric α-amylase inhibitor 0.19 (WDAI-0.19)
Tri a 29.0101: Wheat tetrameric α-amylase inhibitor CM1 (WTAI-CM1)
Tri a 29.0201: Wheat tetrameric α-amylase inhibitor CM2 (WTAI-CM2)
Tri a 30.0101: Wheat tetrameric α-amylase inhibitor CM3 (WTAI-CM3)
Tri a 31.0101: Wheat triosephosphate isomerase (TPIS)
Tri a 32.0101: Wheat 1-cys-peroxiredoxin
Tri a 33.0101: Wheat serpin
Tri a 34.0101: Wheat GAPDH
Tri a 35.0101: Wheat dehydrin
Tri a 39.0101: Wheat SPILA

Our ultimate aim is to define which wheat flour allergens should be included in a component-resolved analysis of baker's sensitization. Not only the frequency of allergen-specific IgE (sIgE) in bakers' sera but also sIgE concentrations play a role in an allergen's effects.[18] Because IgE measurements by means of CAP-FEIA have the advantage of producing quantitative IgE values with an analytic sensitivity of 0.1 kU_A/L,[5] this was our method of choice. Furthermore, important single allergens usually constitute a relevant amount in natural extracts. It is not self-evident that allergens produced by using recombinant techniques are expressed under natural conditions in sufficient amounts to be responsible for sensitization. Therefore we investigated whether IgE binding to the detected allergens could be inhibited by flour extracts.

An additional complication in wheat allergy diagnosis is the well-known cross-reactivity of grain flours and grass pollen, although the responsible proteins sharing common epitopes have not been identified, with the exception of profilin.[19-21] Thus a positive IgE reaction to crude wheat extracts in a baker with respiratory symptoms strongly suggests but does not prove a work-related allergic sensitization. Therefore to improve the specificity of IgE antiwheat diagnostics, on the one hand, we studied cross-reactivity of wheat allergen components with grass pollen allergens in IgE inhibition experiments, and on the other hand, we included a control group of grass pollen–sensitized subjects with sIgE to wheat flour but no occupational exposure to wheat flour and evaluated their IgE reactions to the panel of wheat allergen components. This selection of negative control subjects was preferred to bakers without complaints (who might be already occupationally sensitized and become allergic later in life) or control subjects without wheat flour sIgE (which should rarely bind wheat components and would require a high number of control subjects to find any positive results). Thus the negative control group for occupational wheat flour allergy consisted of 29 selected cases from the 3 countries with cosensitization to wheat flour and grass pollen, probably because of IgE cross-reactions. The IgE reactions in the control group were compared with the "true-positive" reactions of occupational allergic sensitization to wheat flour in 101 bakers with occupational disease and specific IgE to wheat flour. IgE tests to all single allergens and several combinations of tests were evaluated by using receiver operating characteristic (ROC) analyses to assess their ability to discriminate between the 2 groups.

METHODS

Patients and sera

The 40 German bakers with work-related complaints were the same as described previously[9] and were selected based on physician-diagnosed occupational disease and wheat allergy. The 24 Spanish bakers had work-related asthma and rhinitis and a positive inhalation challenge test result to wheat or rye flour. All consecutive bakers attending La Paz Hospital in 2005 to 2008 who were given a diagnosis of baker's asthma caused by wheat flour were selected. The 37 Dutch bakers were selected during the validation study of The Netherland's health surveillance system[13] and had work-related asthma, rhinitis, or both. All bakers were sensitized to wheat flour (sIgE ≥0.35 kU_A/L), 81 (80%) had asthma, 95 (95%) had rhinitis, and 93 (92%) were male. Diagnosis of work-related asthma followed the consensus statement of the American College of Chest Physicians.[22] The mean ages of German, Spanish, and Dutch bakers were 40 ± 15, 38 ± 10, and 36 ± 9 years, respectively.

From each country, also control sera from patients with hay fever or known pollen sensitization without occupational exposure but with sIgE to wheat flour (≥0.35 kU_A/L) were obtained (10 German, 10 Dutch, and 9 Spanish control subjects). Twenty-one control subjects were male (72%), 21 (72%) had rhinitis, and 17 (59%) had asthma. The mean ages of the German, Dutch, and Spanish control subjects were 30 ± 15, 36 ± 10, and 33 ± 18 years, respectively.

Cloning of wheat allergens

By using the sequence information for the wheat SPILA published by Constantin et al[14] (GenBank accession no. EU051824), the following 5′ primer, including a restriction site for *Fsp*I (5′-tgc gca ATG AGC CCT GTG GTG AAG AAG CCG-3′), and 3′ primer, including a restriction site for *Hind*III (5′-a agc TTA GCC GAC CCT GGG GAC CTG GGC AAT-3′), were designed. The cDNA of SPILA was obtained from the wheat endosperm phage surface-displayed cultivar Wyuna cDNA library[23] and subcloned and sequenced in the pDrive-vector system (Qiagen, Hilden, Germany). After identification, SPILA was expressed as a maltose-binding protein (MBP)–SPILA hybrid in *Escherichia coli* carrying an 8-amino-acid spacer (ISEFVISA) between its carrier protein MBP and the ATG start codon of the target protein SPILA. The SPILA nucleotide sequence is published in GenBank (accession no. HE972340).

The translated protein sequence of HE972340 in comparison with EU051824 differs by 2 amino acid changes: glycine to arginine (position 11) and glycine to serine (position 16). All other wheat flour allergens, with the exception of Tri a 14.0101 and wheat ω5-gliadin (Tri a 19.0101), which were obtained by Thermo Fisher Scientific (Freiburg, Germany), were produced as described previously.[9]

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