

Identification of wheat gliadins as an allergen family related to baker's asthma

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Background: Flour is still one of the most common causes of occupational asthma worldwide. Thus far, little is known about the relevant allergens causing baker's asthma. Therefore the reliability of current diagnostic procedures is insufficient. Only few of the suspected causative wheat allergens have been hitherto characterized on the molecular level.

Objective: The aim was to identify and characterize unknown wheat allergens related to baker's asthma to improve the reliability of diagnostic procedures.

Methods: A wheat pJuFo cDNA phage display library was created and screened for IgE binding to wheat proteins with pooled sera from patients with baker's asthma. After identifying an $\alpha\beta$ -gliadin, the frequency of sensitization was investigated by means of ELISA screening of 153 bakers' sera with the recombinant $\alpha\beta$ -gliadin. Furthermore, the allergenicity of native total gliadin ($\alpha\beta$, γ , ω) was analyzed by means of ImmunoCAP.

Results: One cDNA clone was identified as an $\alpha\beta$ -gliadin. Serum IgE antibodies to the recombinant allergen were found in 12% of bakers with occupational asthma. Of the asthmatic bakers, 33% showed sensitization to native total gliadin; 4% of them had negative results on routine IgE testing with wheat extract.

Conclusions: Gliadins represent a newly discovered family of inhalable allergens in baker's asthma. This finding demonstrates that water-insoluble proteins might also represent causative allergens.

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Key words: Baker's asthma, wheat, gliadins, recombinant $\alpha\beta$ -gliadin, recombinant allergens, cDNA phage display

Baker's asthma is still a serious kind of occupationally related obstructive airway disease worldwide, affecting 4% to 25% of bakery workers.¹⁻⁴ In the West wheat is the primary cereal used in bread baking. Sixty percent to 70% of bakers with rhinitis or

asthma have increased specific IgE levels to wheat, rye, or both flour extracts.^{5,6} Further causative allergens are derived from taxonomically unrelated soybean flour, buckwheat, and other noncereal components, such as fungal α -amylase, spices, baker's yeast, egg powder, and other additives.^{7,8}

Reliable diagnostic tools for baker's asthma and especially wheat sensitization are still not available. Commercially available native wheat extracts commonly used for skin prick tests and serologic analyses differ in antigen concentration and composition because of the nonstandardized allergen sources and preparation procedures. This might lead to false-negative results. Therefore there is a need for the improvement and standardization of diagnostic tools for baker's asthma (ie, by means of highly specific and sensitive recombinant diagnostic tests).

Wheat proteins are subdivided into the water/salt-soluble fraction (including albumins and globulins) and the water/salt-insoluble gluten (containing gliadins and glutenins). The latter represent about 80% of all wheat proteins. The mainly monomeric gliadins exhibit intramolecular disulfide linkages, thus being compact and of globular shape. The subunits of the gliadin family are the $\alpha\beta$ -, γ -, and ω -gliadins based on their electrophoretic mobility. The different gliadin subunits share amino acid sequence and 3-dimensional structure homologies.⁹

By means of Western blotting and 2-dimensional immunoblot analyses, preliminary studies showed bakers' sera reacting with a wide range of wheat proteins from less than 6.5 kd up to 100 kd, with a great interindividual heterogeneity in sensitization patterns.^{10,11} Thus far, only few flour allergens have been described at the molecular level: α -amylase inhibitors,¹² acyl-CoA oxidase and fructose-bisphosphate aldolase,¹³ a wheat glycoprotein with peroxidase activity,¹⁴ triose-phosphate isomerase (TPIS),¹⁵ and recently thioredoxin,¹⁶ all of which belong to the water/salt-soluble protein fraction.

Studies by Walsh et al,¹⁷ Weiss et al,^{13,18} and Sandiford et al¹⁹ demonstrated IgE binding in the water-insoluble glutenin fraction (including the gliadin families) of wheat flour. Nevertheless, the water-soluble albumin fractions were believed to represent the most relevant allergens.^{11,20,21}

The aim of our study was to identify and characterize unknown wheat allergens related to baker's asthma to improve diagnostic tests. We used the phage display technique, which has been previously demonstrated to be a promising method in the molecular characterization of allergens from birch and grass pollens and peanut proteins.^{22,23}

METHODS

Patients' sera

Sera for IgE immunodetection were obtained from 153 bakers with rhinitis and bronchial asthma caused by type I allergy to flour (kindly

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supplied by Professor K.-Ch. Bergmann, formerly of the Allergie- und Asthma Klinik, Bad Lippspringe, Germany). The diagnosis was made by means of clinical history and immunologic testing. In 36 cases in which no agreement between clinical history and immunologic testing was found and airway resistance was normal or only slightly increased (specific airway resistance [sRaW] ≤ 1.2 kPa $\times$ s), the diagnosis was confirmed by means of inhalation challenge tests with wheat flour. None of the patients reported symptoms after eating wheat-derived products. All of the 153 bakers had signed an informed consent form. The 153 subjects (20 female and 133 male subjects) had an average age of 40 years. For the biopanning procedure, pooled sera from 21 of the 153 bakers with occupational asthma (2 female and 19 male subjects) were used; all of them were classified as ImmunoCAP class 3 (relevant sensitization) for wheat flour. The control sera were from 100 subjects (40 female and 60 male subjects with an average age of 42 years) not occupationally exposed to wheat flour (80 subjects worked in a psyllium-processing pharmaceutical industry and 20 worked in a coffee roaster). In none of the control sera had an obstructive airways disease ever been diagnosed; furthermore, none were sensitized to occupational allergens. All sera had been stored at -20°C until analysis was performed.

Construction of the wheat phage display library

A phage display library of developed wheat caryopses (*Triticum aestivum* L.) was produced with the cDNA synthesis kit from Stratagene (Amsterdam, The Netherlands). A modification of the producer's protocol was achieved by using 5 μg of total RNA to perform a first-strand synthesis. After ligation of the *EcoRI* adaptors to the double-stranded cDNA, a PCR reaction to amplify the obtained cDNA was carried out by using the following primers (obtained from Invitrogen, Karlsruhe, Germany): ZAP5' long, GGTAAGTTATGA-GAATTCGGCAGGAGG; ZAP3', GAGAGAGAACTAGTCTCGAG.

PCR probes contained 100 mmol/L Tris HCl, 100 mmol/L KCl, 2.5 mmol/L MgCl_2 , 300 mmol/L betaine, 10 pmol of each primer, and 10 pmol of each deoxyribonucleoside triphosphate. The PCR program was a touchdown PCR (3 minutes at 94°C for 19 cycles [94°C for 45 seconds, 66°C for 45 seconds, and 72°C for 120 seconds] with a temperature decrease of 0.3°C , a subsequent 9 cycles [94°C for 45 seconds, 57°C for 45 seconds, and 72°C for 120 seconds], and a final step of 72°C for 5 minutes).

The resulting PCR fragments were ligated into the pCR2.1 Topo vector (Invitrogen) and transformed into 100 μL of *Escherichia coli* OneShot (Invitrogen) by means of electroporation. The transformed cells were incubated in 250 μL of SOC medium and subsequently used to inoculate 350 mL of LB medium containing 50 $\mu\text{g}/\text{mL}$ ampicillin. Plasmid preparations were performed to gain the plasmids. Five hundred micrograms of plasmid was cut with *EcoRI*/*XhoI*, and the resulting inserts were ligated into pJuFo-2 (kindly supplied by Professor R. Cramer, Schweizerisches Institut für Allergie- und Asthmaforschung, Davos, Switzerland) according to the protocol of Cramer et al.²⁴

Biopanning

This strategy covalently links gene products displayed on the phage surface as fusion proteins to the respective cDNAs integrated into the phage genome. For the selection of IgE-binding proteins from the phage display cDNA library, 3 to 5 rounds of affinity enrichment (biopanning) were carried out. Biopanning was performed with a modification of the protocol of Cramer et al.²⁴ Briefly, overnight coating at 4°C with the antibody solution (mouse mAb, anti-human clone MH25-1; Progen, Heidelberg, Germany) was used to capture IgE from the pooled sera. The cDNA library was added after the blocking, incubation with diluted sera, and washing steps. After phage removal and extensive washing, binding phages were eluted with elution buffer (0.1 mol/L glycine, pH 2.2). The eluate was immediately neutralized with 14 μL of Tris base (0.5 mol/L), and the eluted phagemide was used to infect 5 mL of freshly grown *E coli* XL1-Blue (Stratagene) for 30 minutes at 37°C . One microliter of ampicillin (100 mg/mL) was added to the first infection culture. After the next incubation time (32°C at 250 rpm for 30 minutes), another 4 μL of ampicillin was added (incubation overnight at 32°C and 300 rpm). The amplification of the infected *E coli* cells was completed by infection with the VCSM13 helper phage

(Stratagene). After incubation (8 hours at 30°C and 300 rpm), the culture was spun down (5000 rpm at 4°C for 20 minutes), and the phage was precipitated by adding one-quarter volume of 20% (wt/vol) polyethylene glycol-8000 and 15% (wt/vol) NaCl overnight at 4°C to the supernatant. In a further centrifugation step (5000 rpm at 4°C for 20 minutes), the precipitated phages were spun down and dissolved in 2 mL of cold Tris-buffered saline followed by a cleaning step (14,000 rpm at 4°C for 10 minutes). The phages were precipitated for a second time with one-quarter volume of the cold PEG-NaCl solution (14,000 rpm at 4°C for 10 minutes), and resuspended in an adequate volume of Tris-buffered saline. The phages were stored at 4°C . For long-term storage, PEG-NaCl solution was added.

Cloning of an $\alpha\beta$ -gliadin

After affinity selection, a total of 89 clones from the enriched repertoires were randomly hand-picked, and plasmid DNA was prepared. The cDNA inserts were analyzed by means of restriction analysis with *EcoRI* and *XhoI*. Repeatedly appearing clones with cDNA inserts of the same size were sequenced. Sequencing reactions were performed on an ABI Prism sequencer by using the ABI Prism Big Dye reaction kit (Applied Biosystems, Darmstadt, Germany). The sequences were analyzed with the software Chromas Lite and BLAST searches run on the National Center for Biotechnology Information Web page (<http://www.ncbi.nlm.nih.gov/BLAST>). The inserts from the pJuFo clones were subcloned into the high-level expression vector pQE-30 UA (Qiagen, Hilden, Germany) after amplifying with the following oligonucleotides: forward, 5'-GAA TTC GGC ACG AGA GCA-3'; backward, 5'-CAT TAA GCG CGG CGG GTG-3'.

All primers used in the PCR amplification were synthesized by Invitrogen. The ligation was carried out with the QIAexpress UA cloning kit (Qiagen) and transformed into M15 electrocompetent *E coli* cells by means of electroporation. Recombinant clones in the correct direction containing an insert of the correct size were selected, and the sequence was rechecked.

Expression and purification of recombinant proteins and SDS-PAGE

For the expression of recombinant proteins, the high-level expression vector pQE-30 UA and the *E coli* strain M15 (both from Qiagen) were used. The vector encodes a fusion protein with an aminoterminal hexahistidine-tag ($6\times$ His-tag), the prerequisite of which is purification with Ni-NTA. For the production of the recombinant proteins, 100 mL of media containing antibiotics was inoculated with an overnight culture and grown at 37°C until an OD_{600} of 0.6 was achieved. Gene expression was induced by adding isopropyl- β -D-thiogalactopyranoside in a final concentration of 1 mmol/L for 4 hours. The cell culture was harvested, and the pellet was stored at -20°C . The recombinant protein was purified over Ni-NTA under denaturing conditions in the presence of 8 mol/L urea buffer and eluted finally through pH-shift in a volume of 4 fractions of 500 μL , according to the instructions of the manufacturer. The protein content was measured by using the Bradford assay.²⁵ The purity of the eluates was determined by means of SDS-PAGE (NuPAGE, 10% bis-tris, Invitrogen). Before electrophoresis, the samples were prepared with LDS sample buffer (Invitrogen) and heated for 10 minutes to 70°C . Electrophoresis was run at a 200-V constant current until the tracking dye bromophenol blue reached the bottom of the gel. Afterward, the gel was stained with Coomassie. The molecular weights of standard proteins (Invitrogen) were myosin at 188 kd, phosphorylase at 98 kd, BSA at 62 kd, glutamic dehydrogenase at 49 kd, alcohol dehydrogenase at 38 kd, carbonic anhydrase at 28 kd, myoglobin red at 17 kd, lysozyme at 14 kd, aprotinin at 6 kd, and insulin b chain at 3 kd.

His-tag detection

The detection of the His-tagged fusion protein was done with the InVision His-tag In-gel Stain (Invitrogen). This method is sensitive and highly specific and enables the visualization of His-tagged fusion protein bands directly in a polyacrylamide gel after electrophoresis without Western blotting detection. It uses a fluorescent dye conjugated to Ni^{2+} , which binds with a high affinity to

the oligohistidine sequence, and thereby a clear and specific visualization of the His-tagged protein is obtained. After electrophoresis, the proteins were fixed in the gel, followed by 2 washing steps. Staining was performed at room temperature for 1 hour with the ready-to-use InVision His-tag In-gel Stain. The stain was washed, and His-tagged fusion protein bands were visualized with a UV transilluminator. Afterward, the gel was stained with Coomassie.

ELISA

Because interfering buffers, disturbing conditions concerning the pH, and the insolubility of gliadin in aqueous solutions made the biotinylation procedure and therefore binding of the allergen to the Streptavidin ImmunoCAP impossible, IgE reactivity of the recombinant $\alpha\beta$ -gliadin could not be detected by means of ImmunoCAP (the gold standard method of serologic IgE measurement in allergology). For that reason, the determination of the IgE-binding capacity of expressed recombinant proteins was performed by using an allergen-specific ELISA. Multiwell Nunc-Immuno plates (Maxisorp Surface) were coated overnight at 4°C with 2 μ g of the recombinant protein. After every step, 3 washes were performed with PBS containing 0.05% Tween-20. The wells were saturated with 2% BSA in the washing buffer for 1 hour at 37°C and incubated with the diluted patient sera. All the following incubation steps were performed with 100- μ L solutions per well for 1 hour at room temperature. Allergen-specific serum IgE was detected by means of incubation with mouse anti-human IgE (Progen, Heidelberg, Germany) diluted 1:500 and incubated further with alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulins (DAKO, Hamburg, Germany) in a 1:1500 dilution. IgE binding was visualized by incubating with 4-nitrophenyl phosphate (Fluka, Seelze, Germany) and dissolving in substrate buffer (1 mol/L diethanolamine and 0.5 mmol/L $MgCl_2$, pH 9.8). The enzymatic hydrolysis was monitored at a wavelength of 405 nm with an automated ELISA reader after 1.5 hours. The development was carried out by incubating in darkness. As described above, sera from 100 subjects occupationally not exposed to flour served as controls. The mean OD value (A_{405}) plus 3 SDs of the healthy control sera for the respective antigen was chosen as the cutoff point.

Phadia CAP system

The detection of total IgE and allergen-specific IgE antibodies in 153 sera from patients with baker's asthma to the well-known baker's allergens wheat flour, rye flour, and α -amylase (all Phadia, Freiburg, Germany) was performed with the ImmunoCAP system (Phadia), according to the manufacturer's instructions. For the detection of gliadin IgE antibodies, CAP analysis was performed with commercial gliadin IgA/IgG ImmunoCAP (native total gliadin, f98, Phadia) with detection for IgE. The results were expressed as CAP scores of class 0 to 6 and the concentration of IgE. The cutoff value for significant positivity was 0.35 kUA/L (class 1).

Statistical analysis

Sensitivity and specificity based on 2×2 tables were calculated for ELISA with recombinant $\alpha\beta$ -gliadin, ImmunoCAP with native total gliadin, and ImmunoCAP with wheat flour.

RESULTS

Construction of the wheat phage display cDNA library

Using total RNA from developing wheat kernels, we created a cDNA library in the phage display vector pJuFo. Equal amounts of caryopses from 5, 10, 15, and 20 days after anthesis were chosen to overcome the high amount of interfering storage protein cDNAs. After ligating the cDNA inserts into the pJuFo vector, the primary titer of the library amounted to 2.5×10^5 independent clones. A final titer of a total of 8×10^8 clones was obtained after

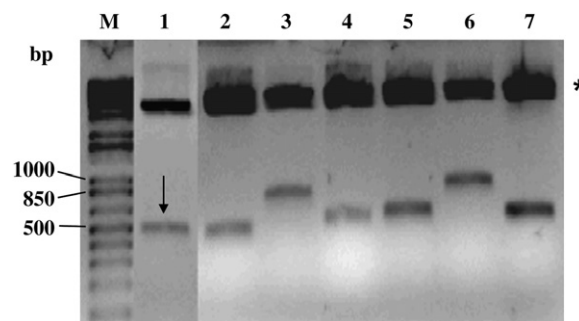


FIG 1. Selection of the enriched clones after 3 rounds of affinity selection. After preparation of plasmid DNA, a restriction analysis was performed with *EcoRI* and *XhoI*. A selection of the inserts, ranging between 500 and 800 bp, is shown. The gliadin insert is indicated with an arrow (lane 1). The residue of pJuFo is indicated with an asterisk. Lane M, Size standard; lanes 1 through 7, pJuFo clones after *EcoRI/XhoI* digestion.

amplification of the library. After restriction analysis, 90% of the clones showed an insert of at least 200 bp, with an average size of 400 bp resulting in a number of about 1×10^5 independent clones with a usable open reading frame. This library was used for selective enrichment of phage-displaying IgE-binding proteins.

Cloning, identification, expression, and purification of $\alpha\beta$ -gliadin

After 3 rounds of affinity selection with a serum pool of 21 patients with baker's asthma, a total of 89 clones from the enriched repertoires were randomly hand-picked, and plasmid DNA was prepared. The cDNA inserts were analyzed by means of restriction analysis with *EcoRI* and *XhoI*. The size of the inserts ranged mostly between 500 and 800 bp. An example of the enriched repertoire of the different large inserts is shown in Fig 1. Nucleotide sequence analyses of 435 bp as a part of clone PI-63 (Fig 2, A) showed a significant homology of 90% to an $\alpha\beta$ -gliadin (accession no. X02540) between sequences 1064 and 1534 (data not shown). The significant identity with an $\alpha\beta$ -gliadin in the amino acid sequence alignment is shown in Fig 2, B. The characterization of the other clones is in preparation. After amplifying the cDNA encoding for the $\alpha\beta$ -gliadin by means of PCR, the insert was subcloned into the high-level expression vector pQE-30 UA, and the protein was expressed as His-tagged protein in the *E coli* strain M15. The expressed proteins carrying a 6 $\times$ His-tag at their N-terminus were purified by means of Ni-NTA affinity chromatography under denaturing conditions, and an SDS-PAGE after the purification showed the apparent molecular masses of the recombinant protein.

As shown in Fig 3, A, for the $\alpha\beta$ -gliadin, a molecular mass of 20 kd was obtained in accordance with the calculated molecular masses from the predicted amino acid sequences. In the induced *E coli* culture in lane 1, after purifying, the gliadin band was clearly shown in lane 2 beside bands of the bacterial lysate. Lane 3 showed the bacterial lysate without induced wheat protein as a control. After staining the SDS-PAGE with InVision Stain in the induced lysate, a clear and specific signal of the His-tagged protein with a size according to gliadins was produced with an even stronger luminosity after purification with Ni-NTA affinity chromatography. Fig 3, B, demonstrates the His-tag detection; no signal was detectable in the noninduced control (lysate).

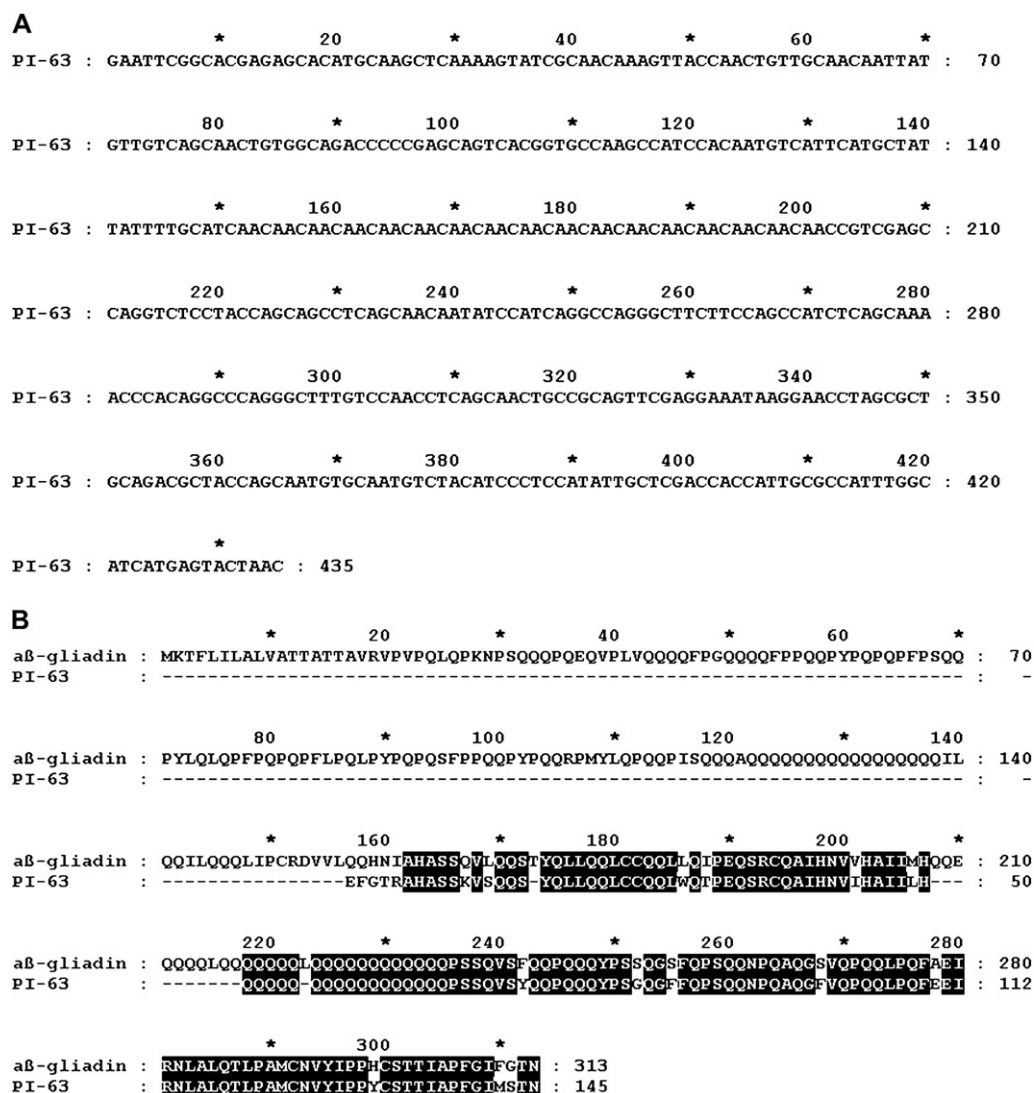


FIG 2. Nucleotide and deduced amino acid sequence of the gliadin clone. **A**, The nucleotide sequence analyses of 435 bp as a part of the clone PI-63 is shown. **B**, sequence alignment of $\alpha\beta$ -gliadin (accession no. X02540) and our clone (PI-63) is demonstrated. Residues shared by both of them are shaded in *black*.

Immunoreactivity of well-known baker's allergens, the recombinant $\alpha\beta$ -gliadin, and native total gliadin

Table I presents the total IgE level increase in 92 (61%) of 153 sera. The sensitization to wheat flour demonstrated by means of commercial CAP analyses was seen in 110 (72%) of 153 cases (and in 5 [5%] of 100 control sera), to rye flour in 110 (78%) of 142 tested sera, and to α -amylase in 20 (17%) of 120 tested sera. ELISA identified 18 (12%) of 153 bakers with IgE reactivity to the recombinant $\alpha\beta$ -gliadin clone. In only 2 (2%) of the 100 control sera was IgE reactivity to the $\alpha\beta$ -gliadin measured. IgE binding to the native commercially available gliadin-CAP ($\alpha\beta$, γ , ω) was seen in 50 (33%) of 153 cases, whereas in the occupationally unexposed control sera, only 3 (3%) of 100 exhibited such IgE antibodies. The validity of the tests was calculated as follows: in the test with wheat flour, the sensitivity was 72.6%, and the specificity was 95%; in the test with $\alpha\beta$ -gliadin, the sensitivity was 11.8%, and the specificity was 98%; and in the test with total gliadin, the sensitivity was 32.6%, and the specificity was 97%.

Forty-eight (95%) of the 50 bakers with positive results had positive results on the wheat flour ImmunoCAP, and 2 (4%) had negative results (Fig 4).

DISCUSSION

Baker's asthma occurs mainly as a result of work-related inhalation of wheat flour. In this study we were able to present cloning and characterization of a recombinant wheat $\alpha\beta$ -gliadin as a new allergen related to baker's asthma. $\alpha\beta$ -Gliadins are a subunit of wheat storage proteins belonging to the gliadin family.

Gliadins are known to have an IgA- and IgG-binding capacity with significance in celiac disease. Of the gliadin family, ω -5-gliadin (Tri a 19) has been identified as a major IgE-binding allergen in food allergies and exercise-induced asthma.^{26,27} The relevance of water-insoluble allergens in baker's asthma warrants caution: in baker's asthma, however, the water/salt-soluble proteins, representing only about 20% of whole wheat proteins,

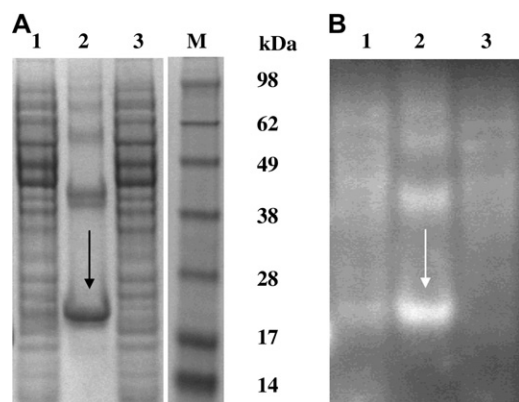


FIG 3. SDS-PAGE analysis of the recombinant $\alpha\beta$ -gliadin. The heat-denatured proteins were separated in an SDS gel by means of electrophoresis and stained either with Coomassie Brilliant Blue (**A**) or InVision His-tag In-gel Stain (**B**). The enriched recombinant $\alpha\beta$ -gliadin is indicated with an arrow. Lane M, Molecular mass marker; lane 1, induced *E coli* culture; lane 2, purified eluate; lane 3, noninduced *E coli* culture as control.

have been considered as the important allergen sources. Up to now, only water-soluble allergens have been described at the molecular level.¹²⁻¹⁶ Mittag et al¹¹ assumed a relation between protein solubility and the various clinical manifestations of wheat allergy: salt-insoluble proteins might be resistant to digestion during the passage through the stomach and intestines and might cause specific symptoms of food allergy. Mainly salt-soluble proteins can be reabsorbed in the respiratory tract, eliciting IgE-mediated allergic reactions. It has to be assumed that among bakers, whole flour particles are deposited on the mucosa of bronchi. There the allergens most readily dissolve in bronchial lining fluids. Water-soluble proteins can reach mast cells and initiate IgE-mediated reactions. In addition, however, some insoluble allergens on the surface of the foreign particles are likely to trigger IgE reactions, either because of direct contact with local mast cells or after enzymes (particularly proteases) or detergents contained in alveolar surfactant or present on the mucosa permit access of allergens that are less readily extractable. In the end, the possibility of bronchial responses to water-insoluble wheat allergens has to be evaluated in a cohort of patients with the clinical diagnosis of baker's asthma.

Eighteen (12%) of 153 sera of our asthmatic bakers showed IgE reactivity against the recombinant $\alpha\beta$ -gliadin in ELISA. (The routine methodology used for CAP coupling that relies on the covalent binding of dissolved proteins to a solid support is unsuitable for coupling of water-insoluble gliadin proteins.) This indicates that α -type gliadins are possible allergens affecting bakers. Because IgE binding to the $\alpha\beta$ -gliadin occurred, allergenicity of the whole gliadin family with similar molecular structures was feasible. Testing the allergenicity to the complete gliadin family (native gliadins) by using the commercial ImmunoCAP led to IgE binding in 50 (33%) of 153 individuals. Only 3 (3%) of 100 sera reacted in the control group, indicating that the relevance to bakers is significantly higher than in the occupationally nonexposed population.

The validity of the test assays shows that for baker's asthma, wheat flour with a sensitivity of 72.6% and a specificity of 95% is the most relevant assay of the 3 described. The high specificity of the test assays with native total gliadin (97%) and recombinant

TABLE I. Percentage of positive test results in symptomatic bakers

Test (IgE)	Method	Positive test results (no. of positive results/ no. of performed tests)
Total IgE >100 kU/L	CAP	61% (92/153)
Wheat flour	CAP	72% (110/153)
Rye flour	CAP	78% (110/142)
α -Amylase	CAP	17% (20/120)
Recombinant $\alpha\beta$ -gliadin	ELISA	12% (18/153)
Total gliadin (native)	CAP	33% (50/153)

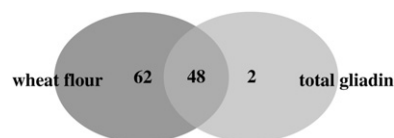


FIG 4. IgE reactivity to wheat flour/total gliadin of the CAP-positive sera. The IgE reactivity to wheat flour and gliadin was determined by means of ImmunoCAP. One hundred ten of 153 sera from patients with baker's asthma revealed antibodies to wheat flour, and 50 showed antibodies to total gliadin. Two (4%) of the 50 sera positive to gliadin were negative to wheat flour (false-negative result).

$\alpha\beta$ -gliadin (98%) shows that bakers are affected, although with a low sensitivity of 32.6% (native total gliadin) and 11.8% (recombinant $\alpha\beta$ -gliadin), which is of little relevance. In any case, 4% (2/50) of gliadin-positive bakers had negative test results on the wheat ImmunoCAP. Obviously, the commercially available wheat ImmunoCAP does not contain the water-insoluble gliadins recognized by antibodies present in these sera.

It is known that gliadin epitopes relevant to allergic reactions to food are stable to digestive procedures.²⁸ The fact that none of the 153 subjects reported gastrointestinal symptoms after ingestion of gliadin-containing food implies different mechanisms for food and airway allergens. The gastrointestinal tolerance to food proteins might still work in gliadin-sensitized subjects, even if specific IgE, functional IgE, and the relevant allergen are present. On the other hand, procedures during the baking process, such as enzymatic fragmentation caused by cellulase and actinase, intense heating, or both, as described by Watanabe et al,²⁹ might destroy antibody-binding sites before nutrition.

Recombinant allergens are of great purity and show higher specificity than native extracts. The latter are, because of their natural origin, usually contaminated by other components, and their composition varies from batch to batch. Recombinant proteins can be used to produce "designed" allergen extracts with high sensitivity and specificity. Therefore and in the view of therapeutic options by means of hyposensitization procedures, the highest benefit results from the use of recombinant gliadins in ImmunoCAP. Hence our aim is to develop a recombinant ImmunoCAP test including the complete gliadin family. It is recommended to apply gliadins also to skin testing, which is restricted to aqueous solutions. Chemical or enzymatic modification of insoluble allergens might be required to make them available for skin testing.

The results of this study demonstrate that allergens related to baker's asthma also occur in the water-insoluble gliadin fraction. Palouso²⁷ already mentioned that the gliadin fraction should be

added to the commercial serologic and skin tests for wheat allergy. In our view, especially in the case of a history suggesting wheat allergy associated with negative allergologic test results, IgE testing for gliadin is recommended to avoid false-negative test results and a false-negative diagnosis in terms of occupational asthma, which could lead to personal and economic consequences for the individual, even if just a small number of patients is concerned. In addition, a bronchial challenge test with fine-grained gliadin might be required for the identification of a bronchial reaction caused by gliadin allergy in asthmatic bakers.

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Clinical implications: Gliadins can be considered possible allergens associated with baker's asthma. In the case of negative results on routine IgE testing with wheat extract, diagnostic tests with water-insoluble allergens, such as gliadins, should be considered.

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