Chapter 14

Wheat as an Allergen: Baker's Asthma, Food and Wheat Pollen Allergy

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Abstract

Food incompatibilities affect approximately 20% of the population and can be caused by allergy. Many plant proteins act as sensitizing agents in humans upon repeated exposure. Wheat is a prominent allergen source and is one of the causes of baker's asthma, food and pollen allergy. On the basis of differential solubility, wheat grain proteins have been classified as salt-soluble albumins and gluten fraction or prolamins, which include gliadins and glutenins. Both proteins sources have been implicated in the development of wheat hypersensitivity.

During the past years we have purified and characterized several proteins from wheat, barley and rye, which are associated with flour allergy. These allergens have a potential role as a biological defense against the insect infestation of the grain.

Until recently gluten intolerance has been has been considered to be typical of celiac disease and wheat allergy. In the last the last few years, new digestive syndromes has been described. A new syndrome has been named non-celiac gluten sensitivity (NCGS) and eosinophilic esophagitis can be also due to wheat ingestion.

The introduction of microarray techniques featuring a large panel of purified allergens has been a major advance in the diagnosis of allergic diseases. However, this technique has been hardly applied to the diagnosis and characterization of patients with occupational asthma due to wheat allergy.

Here, we described these investigations, the most important pathologies associated with wheat, their prevention and treatment.

Keywords

Baker's asthma, wheat allergy, wheat pollen allergy, non-celiac gluten sensitivity, Eosinophilic esophagitis, lipid transfer protein, Wheat alpha amylase inhibitors, microarrays, component resolved diagnosis.

Abbreviations

IgE: antibodies E,

IgG_4: antibodies IgG_4,

IL10: Interleukin 10,

ATG: Anti-transglutaminase antibody,

AGA: Anti-gliadin antibody,

ARA: Antireticulin antibody,

AEA: anti-endomisyal antibody,

LTP: Non-specific lipid transfer protein,

CM3: Wheat tetrameric alpha amylase inhibitor subunit,

ELISA: Enzymo-linked-immuno-assay,

NCGS: non-celiac gluten sensitivity,

EoE: Eosinophilic oesophagitis,

LTP: Lipid transfer proteins,

Tri a a 19: Wheat LTP.

This manuscript is dedicated to professor Raphaël Panzani, in memoriam.

Raphaël Panzani was born August 1921 in Marseille. He received his MD degree at the age of 25. He became interested in allergy at the beginning of his long career. In 1966 he received a Fullbright scholarship award in recognition for his distinguished work on asthma. Raphaël pursued his research investigations, in close collaboration with many of his colleagues, until the very end of his life with great devotion and boundless enthusiasm. Raphaël was fond of nature, expert on Roman culture and in history and literature. He identified with Shakespeare and Dante and embraced the philosophy of Seneca. He was a great sportsman and an exemplary family man. For all of us who knew him, we will miss him immensely and will always remember him as a friend and a scientific role model.

1. Introduction

1.1. Allergy to Wheat and Related Diseases

Wheat is a potent allergen source and is one of the causes of baker's asthma, food and pollen allergy[1]. The prevalence of wheat flour allergy is increasing, ranging from 2 to 3.6%, depending on diagnostic methods and geographic areas[2]. Wheat is also one of the most frequent allergenic foods associated with food-dependent exercise-induced anaphylaxis[3]. Another type of wheat IgE-mediated hypersensitivity is baker's asthma, an important occupational disease, caused by inhalation of wheat and other cereals flours[4] (Figure 1). In our data base of 22.726 allergic patients, baker's asthma caused 8.20% of allergic asthma and the 52.5% of the occupational asthma diagnosed, but we works in an area were the jobs related with cereals are very important. Nowadays, the prevalence of wheat pollen allergy is not known although the sensitization to grass pollen is estimated in 38.6% in United Kingdom and 33.6% in EEUU[1].

Common symptoms of food wheat allergy can begin within a few minutes after eating, or they can start a few hours after. Symptoms often involve the skin and include reactions such as rashes, swelling around the mouth, hives, and eczema. Also, symptoms can typically involve the intestines and might include diarrhea, vomiting, nausea, indigestion, and stomach and abdominal cramps.

Figure 1. Baker's asthma is an important occupational disease, caused by inhalation of wheat and other cereals flours.

The most severe allergy response, anaphylaxis, is a severe reaction involving major body systems.

Conventional medical advice in dealing with food related allergy is to avoid the substance people are sensitive to (Table 1). But since wheat is as important element in all diets, avoidance becomes a major ordeal. This type of avoidance diet severely limits the selection of foods. Wheat is quite difficult to avoid. Aside from being an ingredient in many foods, it is a substance that is also present in medical tablets. It is also used as a glaze and thickener, a stabilizer, a bulking agent, an emulsifier, a binder, and a starch. Is very useful to have information on wheat substitutes (Table 2), and ever read labels (Table 3).

Table 1. General guidelines for wheat allergy.

Foods	Allowed	Not allowed
Beverages	Coffee, tea, fruit juices, decaffeinated coffee, carbonated beverages, all milks, cocoa	Cereal beverages, coffee substitutes Beverages made from wheat products: beer, ale, root beer Instant chocolate drink mixes
Breads & Cereals	Ry-Krisp, rice wafers Pure corn, rice, arrowroot, barley, potato, or rye bread made without wheat flour or wheat products Cornmeal, cornstarch, soybean flour, barley flour, oat flour, rice flour, potato starch, arrowroot flour Oatmeal, cream of rice, puffed rice, or other cereals made from pure corn, oats, or rice to which no wheat has been added	Whole wheat, enriched, or white bread, rolls, or bread crumbs Graham or gluten bread Donuts, sweet rolls, muffins, french toast, waffles, pancakes, dumplings, bread stuffing, rusk, popovers Prepared mixes for pancakes, waffles, biscuits, breads, and rolls Cornbread, potato, or soybean bread unless made without wheat flour or wheat products Cereals made from farina, wheat, or those with wheat products or malt added Pretzels, crackers
Desserts	Custards, Bavarian creams Oatmeal, arrowroot, rice, or rye cookies made without wheat products Cornstarch, tapioca, or rice puddings Water or fruit ices, meringues, gelatin	Cakes, pastries, commercial frosting, icing, ice cream, sherbet, ice cream cones Cookies, prepared mixes, or packaged pudding containing wheat flour Graham crackers, donuts

Foods	Allowed	Not allowed
Eggs	Eggs prepared any way without wheat products	Souffles or creamed eggs made with wheat products
Fats	Butter, margarine, animal, or vegetable fats and oils, cream Salad dressings or gravy prepared without wheat flour or products	Any salad dressing thickened or gravy with wheat flour or products
Fruit	All fresh, canned, dried, or frozen fruits and fruit juices	Strained fruits with added cereals
Meat, Fish, Poultry	Baked, broiled, boiled, roasted or fried: beef, veal, pork, ham, chicken, turkey, lamb, or fish "All meat" wieners or luncheon meats prepared without wheat flour fillers or wheat products	All breaded or floured meats, meats containing filler such as meatloaf, frankfurters, sausage, luncheon meats, bologna, or prepared meat patties
Milk & Milk Products	Milk, buttermilk, yogurt, cheese, some cottage cheese	Malted milk, milk drink containing powdered wheat cereal or products Cottage cheese with modified starch or other wheat containing ingredients
Potatoes & Substitutes	White and sweet potatoes Rice	Scalloped potatoes Noodles, spaghetti, macaroni, and other pasta products at semolina
Soup	Clear bouillon, consommé, or broth Homemade soups made without wheat products	Cream soups unless made without wheat flour Soups with noodles, alphabets, dumplings, or spaghetti Soup thickened with wheat flour
Sweets	Corn syrup, honey, jams, jellies, molasses, sugar	Chocolates, chocolate candy containing malt, candy with cereal extract
Vegetables	All fresh, frozen, or canned vegetables, and vegetable juices	Vegetables combined with wheat products Breaded or floured vegetables
Miscellaneous	Salt, chili powder, condiments, flavoring extracts, herbs, nuts, olives, pickles, popcorn, peanut butter	Malt products, Worcestershire sauce, gravies thickened with wheat flour Monosodium glutamate (MSG), meat tenderizers containing MSG, prepared oriental food seasoned with MSG, soy sauce

A wheat allergy is an abnormal response of the body to the protein found in wheat. Wheat products are found in many foods. In order to avoid foods that contain wheat, it is important to read food labels.

Table 2. Information for using wheat substitutes.

1-cup wheat flour equals:
• 1 cup rye meal • 1 to 1 1/4 cups rye flour • 1 cup potato flour • 1 1/3 cups rolled oats or oat flour • 1/2 cup potato four plus 1/2 cup rye flour • 5/8 cup potato starch • 5/8 cup rice flour plus 1/3 cup rye flour

Table 3. Other possible sources of wheat or wheat products.

How to read a label for a wheat-free diet	
Be sure to avoid foods that contain any of the following ingredients:	
• bread crumbs • bran • cereal extract • couscous • cracker meal • enriched flour • farina • gluten • graham flour • high gluten flour	• high protein flour • spelt • vital gluten • wheat bran • wheat germ • wheat gluten • wheat malt • wheat starch • whole wheat flour
Ingredients that may indicate the presence of wheat protein include the following:	
• gelatinized starch • hydrolyzed vegetable protein • kamut • modified food starch • modified starch	• natural flavoring • soy sauce • starch vegetable • gum • vegetable starch

In this chapter we review the allergenic power among wheat proteins, the changes in allergenic properties of wheat induce by heat and industrial processing, the allergenic cross-reactivity between cereals, pollens and other vegetal foods, the possible reason why patients with baker's asthma due to wheat tolerate wheat flour ingestion, the relationship between wheat allergy and grass pollen asthma, the relationship between cereals in diet and allergic digestive symptoms and the mechanisms of immune tolerance to cereals.

Finally we summarized the new trends in diagnosis (component resolved diagnosis) and therapy of wheat allergy.

2. Allergenic Power Of Wheat Proteins

On the basis of differential solubility, wheat grain proteins have been classified[5]:

Water/salt-soluble albumins and globulins, mainly structural proteins and metabolically enzymes, such as α-amylases and their inhibitors and they are implicated in allergy respiratory symptoms.

The water/salt insoluble gliadins and glutenins, together known as prolamins or gluten, are the major storage proteins of the wheat grain, associated to other clinical expression of allergy.

Among salt soluble proteins, members of the α-amylase inhibitor family seem to be the most important allergens responsible for baker's asthma[6,7]. These allergens have a potential role as a biological defense against the insect infestation of the grain (Figure 2). They have also been described as wheat food allergens[8,9]. Other salt-soluble proteins, such as peroxidase and non-specific lipid transfer protein (LTP), have been implicated in allergy to wheat, both by inhalation and ingestion[9-11]. Gliadins are involved mainly in IgE-mediated reactions to ingested wheat[9-12] and recently in baker's asthma as well[13]. Wheat allergy can cause only digestive symptoms in children and adults although the real prevalence has not been published yet. These patients may be misdiagnosed as suffering from irritable bowel syndrome (IBS).

In celiac disease, limited information is available regarding cereal allergens responsible for allergic reactions, although both diseases can affect at the same patient[14,15]. Celiac disease is a lifelong intolerance to the gluten found in wheat, barley and rye, genetically determined as in allergic diseases. Of the patients with celiac disease 95% are human leucocyte antigen (HLA-DQ2 or HLA-DQ8 positive). Characteristically, the jejunal mucosa becomes damaged by a T-cell-mediated autoimmune response that is thought to be initiated by a 33-mer peptide fragment in A2 gliadin, and patients with this disorder have raised levels of anti-endomysium (AEA) and tissue transglutaminase antibodies (tTG) in blood samples. This disease is the major diagnosable food intolerance and, with the event of a simple blood test for case finding, prevalence rates are thought to be approximately 1:100[15].

Figure 2. Members of the α-inhibitor family of wheat are proteases with have a potential role as a biological defence against the insect infestation of the grain. In the figure Eurygaster austriaca *(left) and* Tenebrio mollitor *(right). The first is a frequent pest of wheat and the second parasite the barley.*

The allergenic reactivity of ingested and inhaled cereal allergens in allergic and celiac people was recently studied by our group. Allergic sensitisation IgE mediated to cereals may be observed in celiac children. Inhalation and ingestion routes causing cereal allergy seem to involve similar allergens, but,

in celiac disease specific response to the α-amylase inhibitor CM3 may be important[15].

There is no doubt that the intestinal mucosa can be involved in food allergy. However, food allergy-induce ulcerative colitis has been questioned[16]. Specific IgEs to foods are more frequent in patients with inflammatory bowel disease than in healthy subjects, but this is probably due to a greater absorption of antigens. Patients with positive colic intramucosal provocation tests with food, have been cured by exclusion of the offending food. Moreover, the specific treatment of ulcerative colitis was suspended[17].

Until gluten intolerance has been believed to be typical of celiac disease and wheat allergy the last few years, new digestive syndromes has been described. Two new syndromes have been named non-celiac gluten sensitivity (NCGS) and eosinophilic esophagitis (EE) due to wheat ingestion. NCGS has been included in the new list of gluten-related disorders published in 2012[18-21]. In other chapter of this book Molina-Infante and cols. review different aspects of epidemiology, diagnosis and dietary interventions in NCGS.

Recent evidence shows that a personal history of food allergy in infancy, coexistent atopy, positive for immunoglobulin G (IgG) antigliadin antibodies and flow cytometric basophil activation test, with wheat and duodenal and/or ileum-colon intraepithelial and lamina propria eosinophil counts, could be useful to identify a subgroup of NCGS patients with characteristics pointing to food allergy[20]. Nevertheless, we require a better understanding of the clinical presentation of NCGS as well as on its pathogenesis, epidemiology, and management.

On the other hand, the role of wheat in conditions like IBS is not clear. A paradigm shift has led to focus on the relationship between diet, that restricts a group of short-chain carbohydrates known collectively as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs), and the pathophysiological mechanisms in IBS such as effects on intestinal microbiota, inflammation, motility, permeability and visceral hypersensitivity. Carroccio et al carried out in vitro basophil activation tests (BAT) for gluten- and cow's milk protein sensitivity in IBS-like patients. The BAT based on

CD63 detection on whole blood samples did not work in the diagnosis of food hypersensitivity diagnosis and showed a significant lower sensitivity, specificity and diagnostic accuracy that the assay based on separated leukocytes[21].

EE is a disorder characterized by esophageal dysfunction and, histologically, by eosinophilic inflammation. Although treatment, which include dilatation, oral corticosteroids and restrictive diets, is often effective, choosing the foods to be eliminated from the diet is difficult. Actual treatment includes proton pump inhibitor therapy and the Six-Food- Elimination Diet (SFDE) that include wheat. This empirical elimination sometimes is effective, but requires multiple control endoscopies and can significantly hinder quality of life. A definitive aetiological diagnosis would be fundamental in determining the specific allergens which cause eosinophilic inflammation of the oesophageal mucosa and which foods should be avoided[22]. Very recently, component resolved diagnostic by microarray allergen assay have been applied in detecting allergens that might be involved in the inflammatory process. The predominant allergens in EE patients were pollen enzymes and among foods allergens lipid transfer proteins (LTP) of peach and nuts. LTP from wheat Tria 19, were detected in only few patients[23].

3. Changes in Allergenic Properties of Wheat Induced By Heat and Industrial Processing

As we comment before, salt-soluble proteins from wheat flour have been described as main allergens associated with both baker's asthma and food allergy. However, most studies have used raw flour as starting material, thus not considering potential changes in allergenic properties induced by the heat treatment and other industrial processing to produce wheat-derived foodstuffs. Salt extracts from different commercial wheat-derived products were obtained and their allergenic properties investigated by IgE-immunedetection, ELISA assays, and skin prick test (Figure 3)[24]. The IgE-binding capacity of salt-soluble proteins from commercial breads and

cooked pastas was reduced around 50% compared with that of raw flour, the reduction being less dramatic in no-ncooked pastas and biscuits. Several wheat-derived foodstuffs showed major IgE-binding components of 20 and 35 kDa, identified as avenin-like and globulin proteins, respectively. These proteins, as well as most flour and bread salt-soluble proteins, were hydrolyzed when subjected to simulated gastrointestinal digestion. However, the digested products still exhibited a residual IgE-binding capacity in SDS-PAGE- immunedetection.

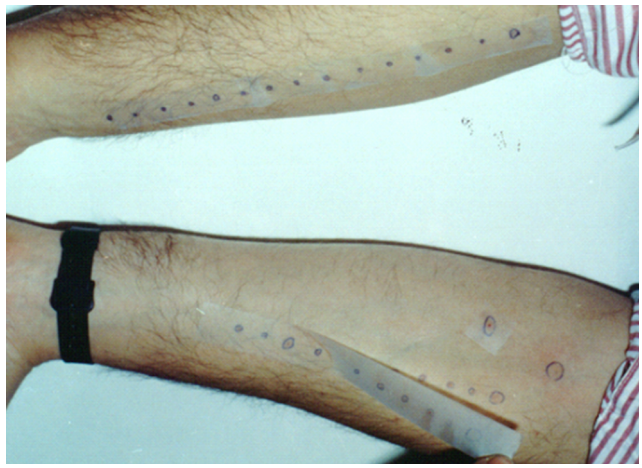


Figure 3. Prick tests with purified proteins from wheat.

Therefore, processing of wheat flour to obtain derived foodstuffs decreases the IgE binding-capacity of the major salt-soluble wheat proteins. Moreover, simulated gastric fluid digestion further inactivates some heat-resistant IgE-binding proteins[25].

4. Allergen Cross-Reactivity Between Cereals, Pollen and Other Vegetal Foods

Many plant proteins, particularly those found in foods and pollen, are known to act as sensitizing agents in humans upon repeated exposure. Among the cereal flour proteins involved in asthmatic reactions, those members of the alpha-amylase inhibitor family which are glycosylated, polypeptides, BMAI-1, BTAI-CMb*, and WTAI-CM16* are particularly reactive both in vivo and in vitro. These major glycoprotein allergens carry a single asparagine-linked complex glycan that contains both beta 1-->2 xylose and alpha 1-->3 fucose. These residues (xilose and fucose) are key IgE-binding epitopes and largely responsible for the allergenicity of these and unrelated proteins from plants and insects (Figure 3). Our results[26], suggested that the involvement of xylose- and fucose-containing complex glycans in allergenic responses may have been underestimated previously; these glycans provide a structural basis to help explain the cross-reactivities often observed between pollen, vegetable food, and insect allergens.

Baker's asthma is a frequent occupational allergic disorder mainly caused by inhalation of cereal flours. Lipid transfer proteins (LTPs) constitute a family of plant food panallergens, but their role as inhalant and wheat allergens is still unclear. We sought to explore the involvement of wheat LTPs in baker's asthma caused by wheat flour sensitization[27]: Forty patients with occupational asthma caused by wheat flour inhalation were studied. Wheat LTP, Tri a 14, was purified by using a 2-step chromatographic protocol and characterized by N-terminal amino acid sequencing and 3-dimensional modeling. Its reactivity was confirmed by means of IgE immunedetection, ELISA and ELISA-inhibition assays, and skin prick tests. Specific IgE to Tri a 14 was found in 60% of 40 individual sera from patients with baker's asthma, and the purified allergen elicited positive skin prick test reactions in 62% of 24 of these patients. Tri a 14 and peach LTP, Pru p 3, showed a sequence identity of 45%, but the low cross-reactivity between both allergens detected in several individual sera reflected great differences in their 3-dimensional IgE-binding regions.

Wheat LTP is a major inhalant allergen associated with baker's asthma caused by wheat flour sensitization. Poor cross-reactivity with its peach homolog was found in some patients. LTPs can be considered relevant inhalant allergens linked to respiratory disorders. LTP from wheat (Tri a 14) can be used as a helpful tool for the diagnosis of baker's asthma.

Peach non-specific lipid transfer protein (Pru p 3; nsLTP) has been characterized as the major food allergen in the adult Mediterranean population. Its wheat homologous protein, Tri a 14 has a relevant inhalant allergen in occupational baker's asthma. Different sensitization patterns to these allergens have been found in patients with this latter disorder[28]. Cross-reactivity between grass-pollen, cereals flours and fruits belong to Rosaceae family (Figure 4) are very common in patients allergic to pollen. Sensitization only to a LTP from peach can be associated with more severe symptoms like anaphylaxis.

Figure 4. Cross-reactivity between grass-pollen, cereals flours and fruits belong to Rosaceae family are very common in patients allergic to pollen.

5. Why Patients With Baker's Asthma Due to Wheat Tolerate Wheat Flour Ingestion?

Wheat is a potent allergen source and is one of the causes of baker's asthma, food and pollen allergy. Recently, we have performed a study on pollen sensitization in our area, where cereal crops are very important[30]. The clinical data from 19718 patients reviewed showed that grass pollen was the main source of clinical symptoms (6369 patients, 32.30% of asthmatics). However, wheat and cereal crop pollen showed very low prevalence. On the other hand, patients with wheat flour allergy after ingestion and/or with baker's asthma were not sensitized to wheat pollen, despite it containing some common allergens. In the same way, all our asthmatic bakers (135 patients) tolerated the ingestion of bread. The reason of these surprising observations was difficult to explain.

The most of patients with baker's asthma in different surveys[4,31] did not present wheat food allergy. A different via of sensitization (inhalation versus ingestion) and allergenic source (wheat flour versus wheat processed foodstuffs) could explain this fact, despite some wheat allergens, like α-amylase inhibitors or lipid transfer proteins are implicated in both types of allergy[7,8] and in some cases of celiac disease as we have demonstrated before[15].

We have shown already that the allergenicity of foods could be modified by heat and other treatment. Most of studies on wheat food allergy have been performed with raw flour, although raw wheat flour is rarely consumed. Thus, the effect of heat treatment during processing or cooking did not be taken into account on the IgE-binding capacity of potential wheat allergens. Recently, using extracts from wheat-derived foodstuffs (French bread, wholemeal tin loaf bread, toasted bread, pasta, biscuits, pizza, baby cereal food and breakfast cereals), we have found that this processing of these foods seems to decrease strongly the IgE binding capacity of the major salt-soluble proteins. Moreover, the simulated gastric fluid digestion could further inactivate some heat-resistant potential allergens[24]

On the other hand, bakers can usually eat bread and wheat-derived foodstuffs during all their life without problems. Their symptoms begin with the inhalation of wheat flour probably due to a change in target immune receptors. IgE sensitization to soy and wheat are classified as "primary" when it is generated by food ingestion and as "secondary" when it is a consequence of primary sensitization to cross-reacting pollen antigens via inhalation. In a German multi-centre longitudinal study, in which 1314 children were followed from birth to age 13, IgE sensitization to wheat and soy were so uncommon. In the early infancy, the type of sensitization was mostly primary, while they were secondary at school age[9]. In our patients, wheat flour sensitization did not seem secondary to wheat pollen inhalation. Perhaps, the cereal ingestion may be acting like an oral mechanism of tolerance, similarly to oral immunotherapy. In a study on tolerance mechanisms in response to antigens responsible for baker's, we found that the presence of higher levels of IgG_4, IL10 and sub-clinic grass-pollen sensitization may have helped to develop a kind of natural hyposensitization[32].

High pollen exposure is not always associated with more severe allergic conditions. Researches working on the relationship between diet and allergic asthma showed conflictive results[33-37]. Serological studies using micro-arrayed wheat seed and grass pollen allergens for the discrimination of baker's asthma, wheat-induced food allergy and grass pollen would be very useful[33]. We will explain the usefulness of component resolved diagnostic later.

6. Relationship Between Wheat Allergy and Asthma

We have previously demonstrated that allergy after ingestion or inhalation of cereals involves similar allergens in different ages[29]. The aim of a new study was to evaluate the allergenic reactivity of ingested and inhaled cereal allergens in different ages, in order to investigate if the response to different allergens would depend on the sensitization route.

We included 66 patients in three groups. Group 1: 40 children aged 3 to 6 months who suffered from diarrhea, vomiting, eczema or weight loss after the

introduction of cereal formula in their diet and in which a possibility of celiac disease was discarded. Group 2: 18 adults with food allergy due to cereals by prick tests, specific IgE and food challenge. Group 3: eight patients previously diagnosed as having baker's asthma. Sera pool samples were collected from each group of patients and IgE immunoblotting was performed.

We found an important sensitization to cereal in the 40 children. The most important allergens were wheat followed by barley and rye. Among the adults with cereal allergy, sensitization to other allergens was common, especially to *Lolium perenne* (rye grass) pollen. Immunodetection showed similar allergenic detection in the three groups.

In summary, clinically significant reactivity to cereal may be observed in early life. Inhalation and ingestion routes causing cereal allergy seem to involve similar allergens. Therefore, the possibility of cereal allergy after the introduction of cereal formula during the lactation period should not be underestimated. In order to investigate this possible risk factor, we performed another study.

The prevalence of asthma has increased from the last 30 years. The relationship between diet and asthma is an area of controversy that has never been fully evaluated. Attempts at dietary prevention of asthma have produced conflicting results.

We identified allergens from cereals that show cross-reactivity with proteins in grass pollen[7]. An early intake of cereals in the diet during early life might cause IgE sensitization to cereals. It was not known whether such sensitization predisposes the development of allergy to pollen. To test this hypothesis, a cross-sectional study and an observational case-control analysis of reviewed data were carried out on 16381 patients who had been admitted to our Allergy Unit during ten years. All the patients underwent allergy tests to identify asthma risk-factor. We demonstrated that grass-pollen asthma was associated with sensitization to cereals. The early introduction of cereals in the diet of children was found to be a risk factor for grass-pollen asthma (OR = 5.95; 95% CI 3.89-9.10). These findings documented the progression of allergic asthma during a decade in a large sample of people who were

influenced by similar environmental conditions and studied with the same diagnostic methods. This study represented the largest database of patients in which a common food is shown to be a risk factor for asthma.

7. Cereal Tolerance Mechanism and Treatment Possibilities

Although baker's asthma (BA) is among the most common occupational disease, the risk factors and immune features that may be important as predictors of tolerance or development of the disease are not completely understood. We try to study the evolution over time of antigenic reactivity on baker's asthma in Spain and in France (BA is the second most common reported cause of occupational asthma in France), in order to find differences in their allergenic response and evidence of protective or risks mechanisms against this disease[32].

Two groups of subjects were randomly selected. A group of bakers with asthma from Spain and a second group from France whose blood was taken 30 years ago: bakers with asthma, bakers without occupational respiratory allergy, and wives and children living close to the bakery. In all subjects skin tests were carried out with cereals, insects and the most common allergens in their area. Serum levels of specific IgE, IgG_4 and IL10 (implicated in tolerance mechanism) measurement were also determined. Spanish patients were mainly sensitized to cereal allergens and presented higher levels of IgE ($p < 0.001$). French patients are more often sensitized to insects and cereal pests than the Spanish ones: 5.26% versus 80% ($p < 0.005$). Symptom free or without aggravation at work subjects have higher specific IgG_4 and IL 10 levels than the others ($p < 0.01$). Antigens implicated in baker's asthma may change with time. The presence of higher levels of IgG_4, IL10 and diversity of sources of sensitization in French patients may have helped them to develop a kind of natural hypo-sensitization[32].

8. Medical Treatment for Wheat Related Allergy

Medical treatment for wheat related allergy as well as food allergy in general can include the following: Epinephrine - given for severe allergic reactions (anaphylaxis), antihistamine and corticosteroid.

Although treatment of wheat allergy is focused in avoidance measures, in bakers' asthma disease is possible specific immunotherapy. One hundred thirty-nine bakers and pastry cooks were included in a prevalence study of IgE-mediated hypersensitivity to wheat flour demonstrated by skin tests, specific IgE to wheat flour (RAST), and inhalation challenge[38]. From the sensitized workers, we selected 30 asthmatic patients. Twenty patients were treated with a standardized wheat flour extract, and ten with a placebo in a double-blind clinical trial. Before and after immunotherapy we performed tests in vivo (skin tests with wheat flour and methacholine tests), and in vitro (total IgE and specific IgE to wheat flour). We found substantial prevalence of wheat flour allergy (25.17% of workers), and a significant decrease ($p < 0.001$) in hyperresponsiveness to methacholine, skin sensitivity ($p = .002$), and specific IgE ($p < 0.005$) to wheat flour after 20 months of immunotherapy. There was also significant subjective improvement ($p < 0.001$). The placebo group showed no changes in these variables.

9. Diagnostic Usefulness of Component Resolved Diagnosis (Microarrays) in Wheat Hypersensitivity

The exposure to wheat proteins through different routes can trigger IgE-mediated allergic reactions affecting several populations and age groups worldwide. However, the current diagnosis of wheat allergy has several limitations. Regarding in vitro diagnosis (specific IgE assays), all known diagnostic approaches have shown poor predictability and specificity[1,2], which may be associated with insufficient purity of wheat extracts used or with the lack of inclusion of all major allergens in these extracts. In contrast, basophil

activation test are considered to be a reliable in vitro diagnostic technique although its use is not widely used in routine practice[21].

The introduction of microarray techniques featuring a large panel of purified allergens has been a major advance in the diagnosis of allergic diseases. However, this technique has been hardly applied to the diagnosis and characterization of patients with occupational asthma due to wheat allergy or BA.

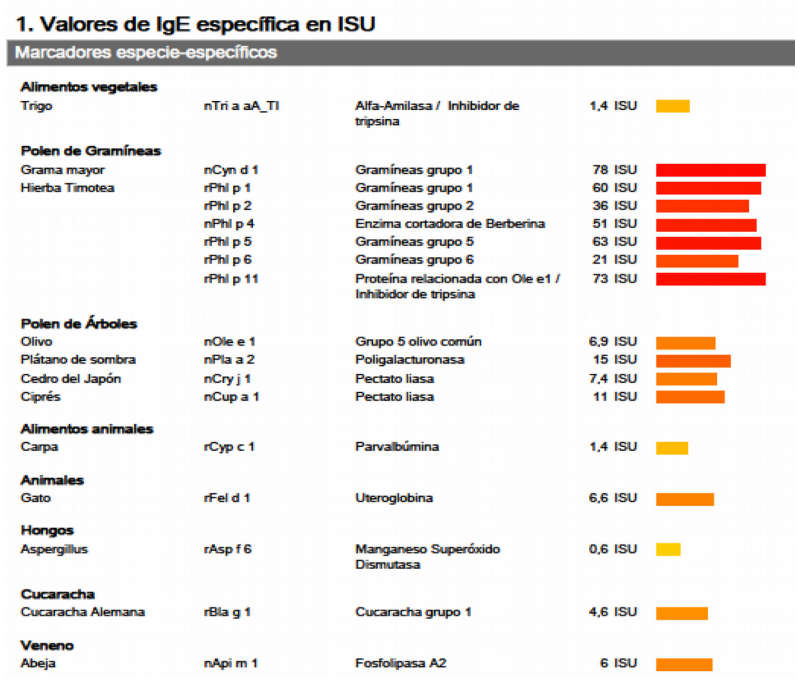
Recently we tested the usefulness of microarrays in diagnosis of wheat allergy[39]. The aim of our study was to characterize the allergenic profiles of BA patients from three different regions in Spain. The pattern of recognition will be compared with subjects allergic to wheat by ingestion and with seasonal rhinitis patients. To this end, a panel of wheat allergens purified and pollen allergens from natural sources and printed on a protein microarray is used. Forty five patients from 3 regions in Spain (Madrid n = 17, Malaga n = 10, Valladolid n = 18) with confirmed diagnosis of BA, positive results to skin-prick test and bronchial challenge with wheat flour were recruited. Twelve wheat allergens (WDAI-0.19 and WDAI-0.53, WTAI-CM1, WTAI-CM2, WTAI-CM3, WTAI-CM16,WTAI-CM17, Tri a 14, profilin, ω-5-gliadin, Tri a Bd 36 and Tri a TLP) were purified. A group of subjects with seasonal rhinitis (SR, n = 41) and allergy to wheat through ingestion (wheat food allergy patients or WFA, n = 9) were also analyzed for comparison.

As results, WTAI-CM16 and Tri 14 were defined as the most prevalent allergens (54 and 45% on average, respectively) covering a total of 64% of the baker's asthma population. On the other hand, ω-5-gliadin and Tri a Bd36 were recognized by less than 10% of the baker´s population. Tri a 14 (wheat LTP) was exclusively recognized by BA patients only (45%, p = 0.0379) and not for WFA or SR patients.

We concluded that the highest prevalence of IgE binding was observed for WTAI-CM16 (54%) and Tri a 14 (45%), with 64% of patients with baker's asthma that recognized at least one of these markers. Tri a 14 seems to be exclusively recognized by BA patients.

In summary, the diagnosis in patients sensitized to wheat is difficult by the relationship between pollen and this allergens and the different expression of

the disease. The misdiagnosis is often a cause for unsuccessful specific immunotherapy and no necessary food avoidance. Epidemiological analysis by molecular component-resolved diagnosis is a new method which may elucidate the interaction between allergen exposure gradient and patient sensitization (Figure 5).



1. Valores de IgE específica en ISU

Marcadores especie-específicos

Alimentos vegetales			
Trigo	nTri a aA_TI	Alfa-Amilasa / Inhibidor de tripsina	1,4 ISU
Polen de Gramíneas			
Grama mayor	nCyn d 1	Gramíneas grupo 1	78 ISU
Hierba Timotea	rPhl p 1	Gramíneas grupo 1	60 ISU
	rPhl p 2	Gramíneas grupo 2	36 ISU
	nPhl p 4	Enzima cortadora de Berberina	51 ISU
	rPhl p 5	Gramíneas grupo 5	63 ISU
	rPhl p 6	Gramíneas grupo 6	21 ISU
	rPhl p 11	Proteína relacionada con Ole e1 / Inhibidor de tripsina	73 ISU
Polen de Árboles			
Olivo	nOle e 1	Grupo 5 olivo común	6,9 ISU
Plátano de sombra	nPla a 2	Poligalacturonasa	15 ISU
Cedro del Japón	nCry j 1	Pectato liasa	7,4 ISU
Ciprés	nCup a 1	Pectato liasa	11 ISU
Alimentos animales			
Carpa	rCyp c 1	Parvalbúmina	1,4 ISU
Animales			
Gato	rFel d 1	Uteroglobina	6,6 ISU
Hongos			
Aspergillus	rAsp f 6	Manganeso Superóxido Dismutasa	0,6 ISU
Cucaracha			
Cucaracha Alemana	rBla g 1	Cucaracha grupo 1	4,6 ISU
Veneno			
Abeja	nApi m 1	Fosfolipasa A2	6 ISU

Figure 5. Array in a patient suffering from celiac disease.

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