

Occupational asthma from reactivity to an alkaline hydrolysis derivative of gluten

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A 29-year-old female subject had been working for 13 years in a company producing biscuits. She was exposed intermittently to an alkaline hydrolysis wheat gluten derivative (AHGD) that was incorporated into marshmallows. Five years before being referred, she started reporting rhinoconjunctivitis and asthmatic symptoms immediately after handling AHGD. When she was first assessed, she had avoided contact with AHGD for 1 year, and she was asymptomatic. Normal spirometry and bronchial responsiveness to histamine (provocative concentration causing a 20% fall in FEV₁, 22 mg/ml) were obtained. There was a positive allergy skin test to ragweed pollen and to AHGD at 0.01 mg/ml. Skin tests with individual extracts of cereals, including wheat, were negative. Inhalation challenge with AHGD for 15 seconds caused immediate rhinoconjunctivitis and bronchoconstriction (maximum fall of 40% in FEV₁ 30 minutes after the exposure) with complete recovery 2 hours later. There was no reaction thereafter, and the provocative concentration of histamine causing a 20% fall in FEV₁ was not significantly changed (13.1 mg/ml) 9 hours after the challenge. Inhalation challenge with wheat flour containing native gluten for 30 minutes did not cause any symptoms or significant change in FEV₁. IgE RAST counts were greater with the AHGD than with gluten and the whole wheat extract; the opposite was generally found with sera from individuals with baker's asthma and wheat food allergy. With the serum of the affected worker, AHGD completely inhibited the corresponding RAST, but gluten did not. With wheat food allergy, AHGD was relatively inactive in inhibition of the wheat and gluten RAST, but gluten was effective. This is the first account of occupational asthma and immunologic reactivity caused by AHGD. This article illustrates the principle of immunologic reactivity dependent on alteration of a food component (gluten) with resulting emergence or expression of new antigenic determinants. (J ALLERGY CLIN IMMUNOL 1988;81:385-90.)

Food allergy and occupational inhalant allergy to wheat products are well documented.¹⁻⁵ In these conditions, there is typically an IgE antibody response to numerous components of wheat products, including gluten.³⁻⁷ In contrast, in the gluten-induced enteropathy of celiac disease, the immune response to gluten includes antibody of the IgG, IgA, and even IgD, but not the IgE class.^{4, 8-10}

Studies of alterations in the antigenic composition of substances to which people are naturally exposed

Abbreviations used

AHGD: Alkaline hydrolysis gluten derivative
HSA: Human serum albumin

have taken many forms but have commonly involved the antigens in foods. Some food components, such as bovine serum albumin and gamma globulin of cow's milk, are heat labile.¹¹ This has practical relevance for individuals otherwise allergic to milk who may tolerate it when it has been heated. The spontaneous lability of some antigens of fruits is evident from the loss of reactivity (false negative results) of the allergen extracts in allergy skin tests.¹² Alteration of antigens in vitro could also lead to the emergence of new antigenic determinants. It is of historic interest that Dr. Küstner of the classic Prausnitz-Küstner ex-

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periments was allergic to cooked fish but not uncooked fish.¹³ An additional possibility is that intestinal digestion processes could lead to the generation of new antigenic reactivity. This could explain the occurrence of clinical allergic reactions to foods that elude diagnosis by immunologic tests when intact native food that does not contain the reactive epitope is the source of the test antigen.

Allergy to a food antigen that depends on prior in vitro processing of the food is not the same as a novel reactivity caused by in vivo digestion but does illustrate the principle of reactivity generated by alteration of a food component. In a food-industry worker with occupational exposure to AHGD, there was the development of occupational asthma, sensitization to the gluten derivative, and evidence for antibodies with specificity for the AHGD without comparable reactivity with native gluten.

CASE REPORT

A 29-year-old female subject had been working for 13 years in a food company where biscuits with marshmallows were manufactured. A commercial ingredient that was received as a calcium hydroxide hydrolysis derivative of gluten was used. This treatment of the gluten yields a product with physical properties desirable for the commercial production of foods, such as marshmallows, pudding, and candy filling. Seven years after starting the exposure to this product, she noticed the onset of sneezing, runny and itchy eyes, as well as shortness of breath with wheezing. These symptoms were present only at work and disappeared during the evenings after work exposure or at weekends. She was transferred to another section where the gluten derivative was used. On every occasion of exposure, she noticed the onset of the nasal, eye, and chest symptoms within a few minutes after exposure.

When she was first assessed, she had not been exposed to AHGD for 1 year, and she was asymptomatic. The personal and familial atopic history was negative. Skin tests were done by the prick method with 15 common inhaled allergens. These included the following extracts provided by Bencard Allergy Service, Mississauga, Ontario: mixed tree pollens, mixed grass pollens, ragweed pollens, each at 2.5% wt/vol, house dust (75% wt/vol), *Dermatophagoides farinae* (1% wt/vol), *Alternaria*, *Aspergillus*, and *Cladosporium* (*Hormodendrum*), each at 5% wt/vol, as well as feathers and horse danders, each at 10% wt/vol. Cat and dog danders (10% wt/vol) as well as house dust (10% wt/vol) and *D. farinae* (2% wt/vol) obtained from Omega Laboratories, Montreal, Quebec, and Hollister-Stier, Mississauga, Ontario, respectively, were also used. In addition, skin tests were carried out with individual extracts of cereals (10% wt/vol) (oat, wheat, soya, corn, barley, and rye) obtained from Bencard Allergy Service, Mississauga. All these tests were negative except for a mean wheal diameter of 10 mm by 10 mm with pseudopods observed with ragweed pollen.

METHODS

Baseline spirometry was performed on a Collins 9-L water spirometer (W. E. Collins, Braintree, Mass.) according to the criteria of the American Thoracic Society.¹⁴ A histamine inhalation test was performed according to the method of Cockcroft et al.¹⁵ Specific inhalation challenges were carried out in a challenge room as described by Pepys and Hutchcroft.¹⁶ FEV₁ was monitored before the exposure, immediately after the exposure, and every 10 minutes for 1 hour, every 30 minutes for the subsequent hour, and hourly thereafter until 9, 7, and 5 hours after the end of the challenge for the gluten derivative, lactose, and wheat flour, respectively. In addition, maximum peak expiratory flow rates were performed with the mini-Wright peak flow meter (Clement Clarke Int., London, England) by asking the subject to give three blows at each hour before and after the challenges until going to bed. The maximum of two reproducible (± 20 L/min) values was kept for analysis.¹⁷

The serum of the affected worker was compared with serum from the following patients: (1) three patients with baker's asthma from wheat flour exposure and positive skin tests with a whole wheat aqueous extract, (2) five patients with food allergy to wheat, diagnosed on the basis of a history of acute allergic symptoms from wheat ingestion and positive allergy skin prick tests with a water-soluble wheat extract, (3) six ragweed-allergic subjects whose sera were known to contain high IgE concentrations, and (4) six people known to be allergic to grass-pollen extract.

HSA and gluten were purchased from Sigma Chemical Co., St. Louis, Mo., and gliadin from Vega Biochemicals, Tucson, Ariz. Whole wheat flour was obtained from a local miller. The calcium hydroxide wheat AHGD termed Hyfoama 88 was obtained from PPF International, Toronto, Ontario, and was the product to which the worker was exposed. Nitrocellulose paper (0.45 μ m pore size) for antigen disk preparation was obtained from Fisher Scientific, Toronto, Ontario. ¹²⁵I-labeled anti-IgE with approximate specific activity of 12 μ Ci/ μ g of protein and commercially prepared gluten antigen disks were purchased from Pharmacia Canada Ltd., Montreal, Canada.

The antigen extracts used to coat all nitrocellular disks for RAST were prepared as 1.75 mg/ml (wt/vol) solutions in 1.0% potassium hydroxide. Nitrocellulose disks were coated with the different antigen preparations, including AHGD, gluten, gliadin, or water-soluble whole wheat flour extract, or with HSA by the method of Walsh et al.⁷ Results reported are for tests with these disks unless it is otherwise stated. CNBr-treated cellulose disks were also used

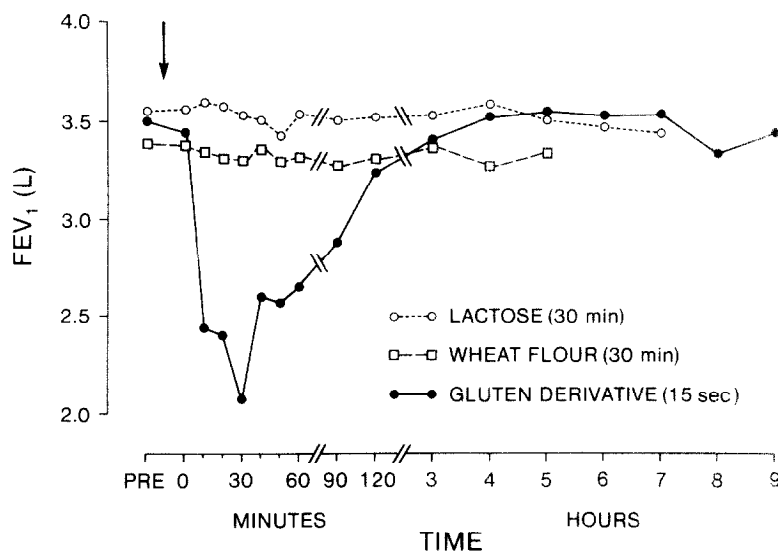


FIG. 1. Specific inhalation challenges. The arrow illustrates the exposure period that varied with the substance inhaled. The time intervals are indicated in parentheses.

separately to prepare the solid antigen phase for RAST.²⁰ The disks were coated with pollen extracts by incubation of 500 mg of disks with 20 ml of ragweed extract, 10.5 mg/ml, and 1000 mg of disks with 20 ml of mixed grass-pollen extract, 4.2 mg/ml. In addition, gluten-coated disks purchased commercially were used. RAST was performed by standard methods.¹⁸

For RAST inhibition, the AHGD and a water-soluble wheat antigen were extracted in phosphate buffered saline, pH 7.2. The protein content of these extracts and all others reported was determined by the Lowry method unless it is otherwise specified.¹⁹ These extracts, at a protein concentration of 1.0, 0.2, 0.1, or 0.01 mg/ml were incubated overnight with an equal volume of test serum, and the RAST was then performed. Serum, incubated in phosphate buffered saline, served as the noninhibited reference control (buffer control). An exception to the above conditions was an inhibition procedure wherein either 25 or 50 mg of gluten, as an insoluble slurry, or buffer, to provide a noninhibited reference control, was added to 200 μ l of serum. In the inhibition experiments, mean counts obtained with four or more sera from nonallergic individuals were considered to be background. The formula for calculation of percent inhibition of test sera was (buffer control-background) minus (antigen inhibition-background) divided by (buffer control-background) times 100.

RESULTS

Baseline spirometry demonstrated an FEV₁ of 3.23 L or 108% predicted²⁰ and an FEV₁/FVC ratio of 83%

or 97% predicted.¹⁸ The PC₂₀ was 22 mg/ml, which corresponds to the absence of significant bronchial hyperresponsiveness.²¹ Skin prick tests with AHGD at increasing concentrations of 0.01, 0.1, 1, and 10 mg/ml wt/vol in order to get a dose-response curve elicited a significant wheal reaction that was of 4 by 5 mm and 1 cm with pseudopods, respectively, at the lowest and highest concentrations. Similar tests with commercial extracts of cereals, including wheat, were entirely negative.

Results of specific inhalation challenges are illustrated in Fig. 1. Exposure of the affected worker to lactose for 30 minutes was accomplished by tipping this powder from one tray to another. There was no significant change in FEV₁ in the following minutes and hours. On the next day, a short exposure for 15 seconds to the AHGD caused immediate rhinoconjunctivitis and a significant (40%) fall in FEV₁, which was maximal 30 minutes after exposure with functional recovery (FEV₁ $\pm$ 10% baseline) 2 hours after the challenge. There was no significant change in FEV₁ thereafter. Maximal peak expiratory flow rates measured during the rest of the day until going to bed demonstrated no significant changes (<10%). On the next day, exposure to wheat flour for 30 minutes did not cause any symptoms or significant change in FEV₁.

The serum of the affected worker in an IgE RAST performed with nitrocellulose disks with the AHGD as antigen yielded 5.3% binding. The corresponding result for an IgE HSA RAST was 1.1%. The calculated antigen/HSA ratio was 4.8 (Table I). With this worker's serum, the binding was greater in the IgE

TABLE I. IgE RAST (percent binding)*

Subject	Serum: total IgE (U/ml)	AHGD	Gluten	Gluten: commercial disks	Gliadin	Water-soluble whole wheat extract
Affected worker	88	5.3 (4.8)†	2.8 (2.5)	1.5 (1.4)	3.6 (3.3)	4.6 (4.2)
Baker's asthma						
1	40	3.5 (3.2)	4.0 (3.6)	4.8 (4.4)	4.0 (3.6)	2.8 (2.5)
2	59	3.2 (2.1)	2.9 (1.9)	1.6 (1.1)	4.7 (3.1)	3.1 (2.1)
3	>1000	3.3 (2.5)	16.0 (12.3)	13.5 (10.4)	13.1 (10.1)	15.4 (11.8)
Food allergic						
4	>1000	2.5 (1.6)	14.3 (8.9)	3.5 (2.2)	4.5 (2.8)	7.9 (4.9)
5	726	3.1 (2.4)	4.7 (3.6)	2.3 (1.8)	3.8 (2.9)	4.3 (3.3)
6	>1000	5.8 (1.2)	28.6 (6.0)	28.0 (5.8)	24.5 (5.1)	24.4 (5.1)
7	>1000	1.7 (0.9)	2.4 (1.2)	3.7 (1.8)	1.7 (0.9)	1.9 (1.0)
8	<5	2.2 (2.8)	1.6 (2.0)	2.0 (2.5)	3.1 (3.9)	1.6 (2.0)
9		3.5 (1.6)	5.4 (2.5)		5.8 (2.6)	3.3 (1.5)
Ragweed allergic						
10	568	3.3 (1.8)	6.0 (3.3)	1.7 (0.9)	4.0 (2.2)	3.6 (2.0)
11	549	8.2 (4.3)	25.7 (13.5)	3.1 (1.6)	18.0 (9.5)	22.3 (11.7)
12	956	2.7 (1.4)	3.9 (2.0)	2.5 (1.2)	3.4 (1.7)	3.2 (1.6)
13	1444	2.9 (1.5)	8.5 (4.5)	3.6 (1.9)	5.9 (3.1)	5.1 (2.7)
14	766	2.9 (1.7)	7.0 (4.1)	2.9 (1.7)	4.8 (2.8)	3.8 (2.2)
15	592	2.6 (1.8)	3.5 (2.5)	2.4 (1.7)	2.6 (1.8)	3.2 (2.3)

*All performed with nitrocellulose disks except the commercial gluten disks.

†Numbers in parentheses represent antigen/albumin ratio.

RAST with the AHGD than with gluten or gliadin, but binding to whole wheat extract was almost as high as with AHGD (Table I). One ragweed-allergic subject¹¹ had an AHGD binding ratio comparable to the affected worker.

The AHGD, gliadin, and water-soluble wheat antigen are soluble and could be also used to coat conventional cellulose disks that had been prepared with cyanogen bromide. In the IgE RAST with these disks, comparison of serum of the affected worker with serum from patients with baker's asthma, wheat food allergy, and ragweed allergy yielded results (not presented) that were entirely comparable to those with nitrocellulose disks.

With the serum of the affected worker, the AHGD completely inhibited the corresponding RAST (Table II). In contrast, the AHGD was relatively inactive in inhibition of the IgE antibody binding in the gluten or water-soluble wheat extract RAST (Table II). With the serum of subject No. 3, insoluble gluten inhibited the gluten RAST but did not inhibit the AHGD RAST. The water-soluble wheat extract inhibited the AHGD RAST less than the IgE RAST with native gluten (Table II). Neither ragweed- nor mixed grass-pollen extract (1.0, 0.1, and 0.01 mg/ml) blocked the AHGD RAST with serum of the affected worker; values for ragweed were 10.9%, 6.5%, and -7.2%, and 2.6%,

22.3%, and -2.1% inhibition for grass, respectively. Similarly, AHGD, 1 mg/ml, failed to block a ragweed RAST with sera of six ragweed-allergic subjects or grass pollen-extract RAST with sera of five grass pollen-allergic subjects (<10% for both). There was 61% inhibition with one grass-allergic serum.

DISCUSSION

Baker's asthma from flour-protein allergens is a well characterized and frequent form of occupational asthma.^{1-4, 6, 7} Gluten is a viscous complex protein-containing component of cereal that persists after the elimination of starch from cereal flours. The functional and immunologic investigation of our subject did not suggest sensitization to gluten. Specific inhalation challenge with wheat flour that contains gluten was negative. There was no evidence of allergy to native gluten. However, both the functional and allergy skin tests demonstrated sensitization to the AHGD. The subject experienced rhinoconjunctivitis and a significant fall in FEV₁ immediately after the exposure. Results of the RAST binding and RAST-inhibition studies confirmed immunologic sensitization to the gluten derivative.

The IgE AHGD RAST with serum of the affected worker was considered to be positive, even though the level of IgE antibody was not exceptionally high.

TABLE II. IgE RAST: Inhibition experiment (percent inhibition)

Subject	Inhibiting reagent	AHGD	Wheat: water-soluble	Gluten	Gliadin
Affected worker	Wheat: water-soluble	1.0 mg/ml	(20)*		
		0.1 mg/ml	(40)		
		0.01 mg/ml	(30)		
	Gluten: alkaline hydrolysis	1.0 mg/ml	(109)		
		0.1 mg/ml	(106)		
		0.01 mg/ml	(92)		
1	Gluten†	50 mg	4		
		25 mg	15		
	Wheat: water-soluble	1.0 mg/ml			45
		0.2			55
	Gluten: alkaline hydrolysis	1.0 mg/ml			37
3		0.2 mg/ml			6
	Wheat: water-soluble	1.0 mg/ml	(68)	(91)	(88)
		0.1 mg/ml	(70)	(84)	(73)
		0.01 mg/ml	(50)	(72)	(67)
	Gluten: alkaline hydrolysis	1.0 mg/ml	(6)	(16)	(10)
		0.1 mg/ml	(4)	(7)	(-5)
		0.01 mg/ml	(9)	(28)	(6)
11	Gluten	50 mg		80	
		25 mg		89	
	Wheat: water-soluble	1.0 mg/ml	48		55
		0.1 mg/ml	9		29
		0.01 mg/ml	4		8
	Gluten: alkaline hydrolysis	1.0 mg/ml	50		58
		0.1 mg/ml	12		32
		0.01 mg/ml	8		1

*Numbers in parentheses represent mean results of two separate inhibition experiments.
†Gluten suspension (weight per volume).

Despite the low total IgE of the serum of the affected worker, the total binding in the IgE AHGD RAST (5.3%) was higher than with 13/15 of the other sera and the antigen (AHGD)/HSA ratio (4.8) was higher than with 15/15 of the other sera. One serum of a ragweed-allergic control subject (subject 11) had markedly elevated binding in IgE RAST with the various wheat antigens, and this was inhibitable and therefore is considered to represent IgE antibody activity. This serum, intended as a high IgE, antibody-negative control, turned out to be inappropriate. If this serum is excluded, the remaining five control sera yielded a mean $\pm$ 2 SD value for the IgE AHGD RAST that was 3.42 and an antigen (AHGD)/HSA ratio of 2.0; both these values are considerably below values obtained in the AHGD RAST with the serum of the affected worker. Further confirmation of the demonstration of antibody by the IgE AHGD RAST in serum of the affected worker was the selective complete inhibition of this test by prior incubation of the serum with the corresponding antigen.

A potential alternative explanation of the reactivity

of the serum of the affected worker with AHGD would be sensitivity to grass pollen or ragweed pollen and a cross-reactivity with the corresponding antigens. The patient had a positive IgE ragweed pollen-extract RAST but negative grass pollen-extract RAST (not presented). It was demonstrated that AHGD did not inhibit a ragweed pollen-extract RAST or (with one exception) a grass pollen-extract RAST. In any case, both the ragweed- and grass-pollen extracts failed to inhibit the IgE AHGD RAST performed with the serum of the affected worker.

The RAST-inhibition studies suggest that the alkaline hydrolysis treatment of gluten results in the loss of antigenic reactivity as well as the generation of novel epitopes. Reduced antigenic activity is indicated by the IgE RAST performed with the other antigenic preparations, including native ("nonderivative") gluten; the AHGD was relatively ineffective in inhibition of binding in these tests. Native insoluble gluten was demonstrated to absorb antibodies from the serum of a patient with baker's asthma with resulting inhibition of the gluten and the water-soluble

whole wheat RAST. In contrast, native insoluble gluten failed to absorb antibodies reactive in the AHGD RAST with serum of the affected worker; this is taken to indicate that the latter antibodies have a selectivity for epitopes in the modified gluten (AHGD) that are not present or available to react in the native gluten.

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