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ORIGINAL ARTICLE

Tolerance Mechanisms in Response to Antigens Responsible for Baker's Asthma in Different Exposed People

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Background. Baker's asthma 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. **Objective.** To study the evolution over time of antigenic reactivity on baker's asthma in Spain and in France, in order to find differences in their allergenic response and evidence of protective or risks mechanisms against this disease. **Methods.** 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. Specific IgE, IgG₄ and IL 10 measurement were also determined. **Results.** Spanish patients were mainly sensitized to cereal allergens and presented higher levels of IgE ($p < 0.001$). French patients are more often sensitised to insects than the Spanish ones: 5.26% versus 80% ($p < 0.005$). Symptom free or without aggravation at work subjects have higher specific IgG₄ and IL 10 levels than the others ($p < 0.01$). **Conclusions.** Antigens implicated in baker's asthma may change with time. The presence of higher levels of IgG₄, IL10 and diversity of sources of sensitisation in French patients may have helped them to develop a kind of natural hyposensitization.

Keywords Baker's asthma, pests, IgG, IgG₄, IL10

INTRODUCTION

Baker's asthma is a type I immunoglobulin-E (IgE)-mediated allergic response to the inhalation of cereal flour, mostly wheat. It is among the most frequent occupational respiratory disorders and represents an important clinical problem requiring compensation in European countries (1, 2). Bakers, millers, pastry factory workers, and farmers are the main occupational groups affected.

The prevalence of asthma in these risk groups is approximately 15% to 18%. Despite this high prevalence, the risk factors and immune features that may be important as predictors of the disease in different exposed people are not completely understood and specific treatment trials are very rarely attempted (3).

The difficulty is the diversity of allergens associated with baker's asthma (4). The major cause of the baker's asthma condition is the inhalation of wheat, barley, and rye flour proteins (5). Salt-soluble proteins (albumins and globulins) show the strongest IgE reactivity, at least in wheat (6, 7), but prolamines (typical storage proteins, not extractable by salt solutions) have also been implicated as potential allergens (7, 8). In addition to cereal proteins, a wide array of other allergenic

components has been associated with baker's asthma. The most important ones include several additives used to enhance wheat flour quality for baking, such as *Aspergillus*-derived enzymes (mainly α -amylase) (5) and soybean flour proteins (9). In addition, cereal flour contaminants, including mites, pests, molds, and pollens, have also been recorded (10, 11).

However, water/salt soluble wheat flour proteins, i.e., albumins and globulins, are the most relevant allergens, and most studies have shown that 12–16 kDa components corresponding to members of the α -amylase/trypsin inhibitor family are major salt-soluble allergens in cereals (12). IgE antibodies to a large number of these cereal proteins have been detected in the allergic patient's sera but not in other symptom-free exposed people. No only bakers but their wives, children, and other relatives might have been exposed to these allergens. Why only some of the exposed people have the disease whereas others can tolerate exposure to cereal dust is not known.

Natural specific IgG₄ is more likely one of the mechanisms with which the organism defends itself against offending allergens. We already know that this is the case in castor bean allergy (13). Like castor bean respiratory allergy, baker's asthma is a kind of "experiment of nature" but more complicated because many allergens are involved and not only one as in the case of castor bean allergy. The role of interleukin 10 in specific immunotherapy and its inhibitor effects on the allergy response may be important in tolerance mechanisms

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(14, 15). It would be interesting to see whether any bakers who are often very heavily exposed to wheat dust do not develop asthma or allergic rhinitis and why.

The aim of our investigation was to study the antibody IgE, IgG₄, and IL-10 specific response to antigens responsible for baker's asthma in different exposed healthy and affected people from two different countries at two different times (30 years ago and nowadays) to find differences and similarities in their allergenic response and to find evidence of protective mechanisms against this very prevalent disease.

METHODS

Selection of Patients

We selected patients from France and Spain to have a wide range of patients and to study different particularities in their sensitization pattern to cereal dust.

French Patients. Clinical data and serum samples were obtained from bakers and their relatives who were studied 20 to 30 years ago by one of us. All sera were lyophilized and kept at about 4°C; these samples were reconstituted to their original volume, adding distilled water. We know by experience that after freeze-drying, specific IgE remain unaltered even after 30 years or more (Prof. R.A.A. Coombs, personal communication).

There were 11 patients who had baker's asthma, 7 baker patients without any symptoms at work, 3 bakers with asthma due also to grass-pollen, and 14 baker's wives and their children who lived very close to the bakery.

The Spanish people were selected from a database of patients who had previously participated in an epidemiologic survey (12). We selected 10 asthmatics workers exposed to cereal dust in which sensitization to cereal allergens was demonstrated by positive prick, bronchial challenges, and specific IgE to cereal flours. We also selected 9 patients who had anaphylaxis after ingestion of cereals in which the responsible cereal allergen was detected by oral challenge and 6 patients who worked as bakers with the asthmatic patients but who had not developed the disease.

All participants gave their consent and the procedure was approved by the local ethics committee.

All participants underwent the following tests:

In Vivo Test

Skin Prick Tests. Skin prick tests were performed with a standard panel of allergenic extracts, including putative suspected occupational allergens in the participant under study, such as flour and their additives (wheat, barley, rye, oat, gluten, alpha-gliadin, alpha-amylase, soy-bean lecithin), pollens (Grasses, *Parietaria judaica*, *Cupressus sempervirens*), molds (*Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarum*, *Ustilago* spp), animal danders (dog, cat, horse, cow, rat, pig), mites (family Pyroglyphidae: *D. pteronyssinus* and *D. farinae*, subfamily Pyroglyphinae: *Euroglyphus maineyi*; family Acaridae: *Tyrophagus putrescentiae*, *Acarus siro*; family Glycyphagidae: *Glycyphagus domesticus*, *Lepidoglyphus destructor*, *Gohieria fusca*; family Echimyopidae: *Blomia kulagini*; family Chortoglyphidae: *Chortoglyphus arquatus* and extracts of *Eurygaster*, *Tenebrio molitor*, *Blatta orientalis*, household insect, house fly and *Ephestia* spp. Extracts were supplied by Aristegui Laboratories (Bilbao, Spain). Wheals were measured after

15 minutes and wheal obtained was outlined by tracing on adhesive paper to obtain measurements of diameters. The diluent of prick solution was used as a negative control and histamine (10 mg/mL) as a positive control. The reaction was considered positive if the wheal diameter was at least 3 mm once subtracted from the value of control solution. The average diameter of the wheal induced by each dilution was calculated according to the formula $(D+d)/2$, where D is the maximum diameter and d the perpendicular diameter of the midpoint.

Bronchial Challenge Tests

These tests were performed following a technique previously described (3). The initial dose was a dilution that produced a 3-mm-diameter wheal by prick testing.

In Vitro Tests

IgE, IgG, and IgG₄ Antibody Measurements. Total and specific IgE antibodies to the allergens proved in prick and specific IgG₄ to wheat were measured with the ImmunoCAP technology using the ImmunoCAP 100 System (Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions. Results greater than 0.35 were considered positive.

IL-10 Measurements. IL10 was measured with the Immulite 2000 (DCP Dipsa, Diagnostic Products Corporation, Los Angeles, California, USA) in pg/mL until reaching 5.4% for a mean concentration of 813 pg/mL. A pattern curve was studied with different dilutions and different concentrations between 101% and 119%.

Statistical Analysis

Database and statistical analysis were performed using the SPSS for Windows v. 11.5 (SPSS Inc., Chicago, Illinois, USA) program. Continuous variables are presented as mean $\pm$ SD. Discrete variables are expressed as frequencies and percentages. The Kolmogorov-Smirnov test was used to assess normality of the data. To study statistical difference between groups, the following tests were performed where needed: Student's *t*, Mann-Whitney's U, two-way analysis of variance (ANOVA), H de Kruskal-Wallis, Chi square, and Fisher's exact test. Significance was set at $p \leq 0.05$.

RESULTS

Clinical data and results of antibodies and IL10 determinations can be seen in Tables 1 and 2. Analytic studies can be seen in Tables 3 and 4.

The two groups of patients (Spanish and French) were homogeneous considering age and time of exposure to cereal flour.

Spanish patients who have baker's asthma (BA) were mainly sensitized to cereal allergens wheat, barley, and rye and presented higher levels of IgE to these cereals than French patients (Table 3). Two patients presented sensitization to alpha-amylase and only one to the storage mite *Lepidoglyphus destructor* (Table 1). In contrast, French bakers and their relatives were sensitized to cereals but also to cereal pests: *Sitophilus*, *Ephestia*, *Tenebrio molitor*, house fly, and *Lepidoglyphus*. (Table 2). IgE levels in pests were significantly higher to *Lepidoglyphus* ($p < 0.005$), *Sitophilus*, and house fly ($p < 0.05$) (Table 3). The total levels of specific IgE to cereals were lower than among Spanish bakers ($p < 0.001$)

TABLE 1.—Patients who suffered from baker's asthma (1–10) and patients who had anaphylaxis after ingestion of cereals (11–19).

SPANISH PATIENTS:

No. sample	Sex	Age	Clin	Year exp	IgE wheat	Prick wheat	IgE barley	IgE rye	Others	IgG wheat	IgG4 wheat	IL10
1	F	30	Asthma	4	>100	20	>100	>100	Lolium 86	105	0.65	—
2	M	42	Asthma	10	3.70	10	2.04	2.32	DPt >100 Lep 53	8.36	1.62	—
3	F	51	Asthma	20	9.21	6	6.28	2.81		22.3	1.61	—
4	F	38	Asthma	12	7.04	5	9.83	32.8	Lol 12.1 Pt 0.76	3.6	3.42	—
5	M	35	Asthma	5	0.54	5	1.31	1.29	Lol 33	5.37	0.25	—
6	M	29	Asthma	6	0.64	5	0.67	0.74	Pea nut 1.12	45.2	4.21	—
7	F	52	Asthma	19	1.58	6	1.86	1.64	Alpha-amylase 5.4	13.1	0.57	—
8	M	71	Asthma	40	3.78	7	5.34	3.29	Anisakis: 5.50	21.4	6.95	—
9	F	32	Asthma	5	3.73	7	3.24	4.04	Alpha-amylase 0.6	15.5	2.46	—
10	F	30	Asthma	4	2.7	6	3.9	4.04		7.62	0.20	—
11	F	20	Food allergy	9	>100	18	>100	>100		13	3.5	—
12	M	42	Food allergy	10	3.2	6	1.6	1.4	Lentil 0.45	7.47	0.35	—
13	F	18	Food allergy	10	>100	29	>100	>100	Soy bean 2.23	18.4	3.95	—
14	F	17	Food allergy	2	1.9	5	5.82	1.3		9.31	0.40	—
15	M	51	Food allergy	30	1.97	5	2.03	2.62	Lolium>100 Lentil: 33.2	7.07	2.06	—
16	F	62	Food allergy	30	2.1	5	6.47	1.08	DPt 5.84	3.79	0.21	—
17	M	15	Food allergy	17	Nd	Nd	3.74		Almond 1.7	2.18	3.61	—
18	F	30	Food allergy	4	4.7	6			Soy 3, corn 4.5	2.9	0.1	—
19	M	28	Food allergy	3	6.46	8				4.45	0.24	—

CONTROLS: Bakers without symptoms:

Patient	Sample	Sex	Age	Year exposure	IgE wheat	IgE barley	IgE rye	IgE others	IgG wheat	IgG4 wheat	IL10
BAO1	20	F	15*	1	0	0	0	Lolium 0.37	12.8	0.28	—
BAO2	21	M	35	5	0	0	0		13.6	0.58	—
BAO3	22	M	50	32	0	0	0		12.5	5.63	—
BAO4	23	M	28	12	0	0	0		9.74	2.19	—
BAO5	24	M	30	2	0	0	0		4.51	0.36	—
BAO6	25	M	45	15	0	0	0		4.59	29.27	—

*This patient is a baker's apprentice.

Clin: Clinical symptoms, IgE: KU/L, IL 10: <5 (-);

Lolium: Lolium perenne (rye grass pollen);

Lep: Lepidolophus destructor;

DPt: Dermatophagoides pteronyssinus;

Soy: soy bean.

(Table 3). The highest levels of specific IgG to wheat were found in bakers with sensation to grasses (BAP) French patients and the highest levels of IgG₄ to wheat were found in French bakers patients exposed but without symptoms ($p < 0.01$) (Table 4). Significant levels of IL10 were found in only three French patients (BA4, BA11, and BAP1). These three patients presented also the highest level of IgG₄. The number of years in the profession was higher than in the other bakers and they have presented a spontaneous resolution of their symptoms, whereas Spanish bakers were more affected with more time of exposure and no remission was found. The minor levels of IgG and IgG₄ to cereals were found in Spanish patients with cereal food allergy ($p < 0.05$). None of control-exposed patients without symptoms had significant levels of IgE and IgG₄ to cereals.

DISCUSSION

Baker's asthma is among the most frequent occupational respiratory disorders. Despite the high incidence, the risk factors and immune features that may be important as predictors of tolerance or development of the disease in different exposed people are not completely understood.

The aim of our investigation was to study the antibody specific response to antigens responsible for baker's asthma in different exposed healthy and affected people from two different countries and in two different times to find differences and similarity in their allergenic response and to find

evidence of protective or risk mechanisms against this very prevalent disease. IgE antibodies to a large number of these cereal proteins have been detected in the allergic subject's sera but not in other exposed people who do not have the disease. Not only bakers but also their relatives can have been exposed to these allergens.

The fact that in the sera of French patients the incidence to allergy to insects is much higher than among the Spanish probably means that 20 to 30 years ago wheat was not treated by products to kill insects and their larva as it is today.

There is an extensive cross-reactivity between the different cereals, i.e., in our case barley and rye. We found also some positive tests regarding legumes. As it has been mentioned above, the most relevant protein fraction from wheat flour for baker's asthma is the water/salt soluble fraction and the IgE binding capacity of purified inhibitor subunits, almost all of which belong to different subfamilies, using sera from Spanish patients (15–22). Members of the α -amylase/trypsin inhibitor family have also been identified as major allergens associated with wheat-induced asthma in patients from other countries such as Germany (23) and Japan (24). Spanish patients who have baker's asthma, diagnosed by positive prick-tests, specific IgE, and positive bronchial challenge tests with wheat flour, did not have symptoms after eating cereal-made foods. Processing of wheat flour to obtain wheat-derived foodstuffs may decrease the IgE-binding capacity of the major salt-soluble wheat proteins, which is because

TABLE 2.—Patients who suffered from baker's asthma, occupational asthma, or sensitization to grasses.

FRENCH PATIENTS:

Pat	S	Age	Clin	Year exp	IgE wheat	Prick wheat	IgE barley	IgE rye	IgE Others	IgG wheat	IgG4 wheat	IL10
BA1	M	40	A	2	3.13	5	1.66	4.31	Anisakis 0.50 Sitophilus 0.50, Lep 1.3	16.8	2.56	—
BA2	M	27	RA	12	0.94	4	0.77	1.57	Lep 3.20 Blatta 0.40	8.82	1.63	—
BA3	M	36	RA	8	1.04	2	1.16	2.66	HF: 2.7, Dpt 0.5 Lep 3.2	25.1	1.84	—
BA4	M	37	A U	18	21.5	6	58.7	38.7	Dpt 1.2 Lep 1.3 Pea 1.4	11.5	20.90	36.9
BA5	M	38	A	4	2.15	5	1.06	1.62	Dpt 0.6, Fava bean 1.3	15.2	1.95	—
BA6	M	41	A	4	1.83	6	0.42	1.19		9.13	1.76	—
BA7	M	32	A. Ag	4	0.35	4	0.35	0.35	Lep1.94 Anisakis 0.56 Sitophilus1.03 Ephesia 0.60 Blatta 1.90	12.4	0.33	—
BA8	M	65	RA	10	0.62	Nd	1.13	0.48		34.5	5.79	—
BA9	M	40	RA	9	0.49	3	0.39	0.38	Lep 10.6 Sitopyilus 0.38 Ephestia 10.9 Blatta 0.70 Tenebrio 11.3	17.1	4.66	—
BA10	M	52	A	12	1.32	3	0.54	1.46	Aspergillus: 0.4	11.2	1.80	—
BA11	M	34	RA Ad	20	43.1	3	23.8	25.3	Dpt 0.5 Lep 3.4 HI 2.3	35.4	18.67	14.7
BAS1	M	30		18	0	—	0	0	Dpt 1.4	34.6	1.98	—
BAS2	M	41		3	0	—	0	0	HF 4.4	5.28	0.34	—
BAS3	M	35		10	0	—	0	0	Dpt 2.4	19.4	3.77	—
BAS4	M	36		2	0	—	0	0	Dpt 3.8	25.7	15.50	—
BAS5	M	22		2	0	—	0	0	Candida	23.6	7.15	—
BAS6	M	50		6	0	—	0	0	ASA	7.25	0.68	—
BAS7	M	33		5	0	—	0	0	Dpt 2.3 HF 1.8	13	2.63	—
BAP1	M	26	A	5	8.30	4	7.18	11.0	Lolium: 22.4 Dpt 1.2	6.68	14.22	18
BAP2	M	41	A	6	0.66	4	0.44	0.64	Lolium: 8.2 Sitophilus 0.60 Ephestia 0.56	23.5	0.45	—
BAP3	M	35	A	7	0	5	0	0	Lolium 23	30.8	2.84	—
BAC1	M	27	RA	1	0	—	0	0	Anisakis 0.96 HI 1.2 Alt 2.3 Lep 12	31.8	0.25	—
BAC2	F	14	RA	7	0	—	0	0	Dfa 2.1	17.5	0.62	—
BAC3	M	10	A	4	0	—	0	0	Lep 1.6	11.7	0.23	—
BAC4	M	10	A	4	11.6	3	8.39	6.84	Lep 12.8 Anisakis 7.76 Sitophylus 8.21 Ephestia 11.8 Blatta 10.9 Tenebrio 9.74	20.4	0.41	—
BAC5	F	40	A	26	0	—	0	0	Dfa 3.2	14.8	0.25	—
BAC6	F	59	A	16	0	—	0	0	Lep 3.69 HF 1.7	37.6	0.33	—
BAC7	F	9		1,5	0	—	0	0		10.5	0.30	—
BAC8	F	65	A	5	0	—	0	0	Dfa 1.6 Lep 4.2	21.3	3.53	—
BAC9	M	36	AU	10	0	—	0	0	Lep1 0.56	13.2	0.27	—
BAC10	F	26	A	6	0	—	0	0	Lep 0.6	9.60	0.36	—
BAC11	M	4	A	2	0	—	0	0	Lep 1.4 Dfa 0.5	28.4	0.48	—
BAC12	F	22	A	10	0	—	0	0	HF 3.4	32	0.24	—
BAC13	M	2	A	1	1.14	—	1.10	1.14	Sitophylus 0.97 Ephestia 1.05 Blatta 1.03 Tenebrio 1.21	23	11.13	—
BAC14	M	18	A	4	0	—	0	0	Alternaria 2.3 Dfa 0.7 Lep 1.3	0	2.11	—

11 bakers had baker's asthma (BA).

7 bakers without occupational asthma (BAS).

3 bakers had symptoms due to sensitization to grasses (BAP).

14 baker's wives (BAC).

Sitophilus: Sitophilus granaries;

Lep: Lepidoglyphus destructor;

Blatta: Blatta orientalis;

Dpt: Dermatophagoides pteronyssinus;

Tenebrio: Tenebrio molitor;

HF: House fly;

Dfa: Dermatophagides farinae;

ASA: Aspirin.

TABLE 3.—Differences between Spanish and French bakers.

	Spanish (n = 19)	French (n = 35)	Sig.
Age (years)	36.5 ± 15.5	32.4 ± 15.6	NS
Sex	Female: 11 (64.7%) Male: 8 (22.2%)	Female: 6 (35.3%) Male: 28 (77.8%)	0.003
Exposure time (years)	12.6 ± 10.8	7.6 ± 6.0	NS
Wheat IgE (KU/L)*	19.6 ± 37.1	2.8 ± 8.2	<0.001
Barley IgE (KU/L)*	20.8 ± 37.9	3.1 ± 10.6	<0.001
Rye IgE (KU/L)*	22.5 ± 39.2	2.8 ± 7.8	<0.001
Il-10 (pg/mL)*	0.0 ± 0.0	2.0 ± 7.2	NS
IgE Lepidoglyphus (KU/L)*	2.8 ± 12.2	1.8 ± 3.4	0.005
IgE Sitophilus (KU/L)*	0.0 ± 0.0	0.3 ± 1.4	0.050
IgE Ephestia (KU/L)*	0.0 ± 0.0	0.7 ± 2.7	NS
IgE Tenebrio (KU/L)*	0.0 ± 0.0	0.6 ± 2.5	NS
IgE House fly (KU/L)*	0.0 ± 0.0	0.4 ± 1.0	0.050
IgE Horse fly (KU/L)*	0.0 ± 0.0	0.1 ± 0.6	NS
IgG (mg/L)	16.6 ± 23.7	18.8 ± 9.9	NS
IgG4 (mg/L)	1.9 ± 1.9	3.5 ± 5.2	NS

*: Normal distribution; NS: not significant.

patients who have baker's asthma do not have symptoms after eating these foods. In addition, bakers were mainly sensitized to raw wheat flour. Patients who had anaphylaxis after ingestion of cereals had not been exposed to inhaled raw wheat flour. Then, an immune response after digestive absorption to processed wheat derived foodstuffs proteins was the cause of their symptoms. Bronchial challenges with a raw wheat flour extract were negative in these patients.

The response pattern of French patients seemed to be different than the Spanish patients. Allergy to storage mites and insects that used to widely act as parasite cereals played an important role regarding French patients. Conversely, Spanish patients were mainly sensitized to cereal allergens and enzymes whereas sensitization to insects was not found. In the patients allergic by ingestion to cereal, legumes sensitization, (lentil and soy bean) was also important. It is well known that the insect cause every year in the USA a loss

TABLE 4.—Differences among clinical groups of French bakers.

	BA (n = 11)	BAS (n = 7)	BAP (n = 3)	BAC (n = 14)	Sig.
Age (years)	40.2 ± 10.3	35.3 ± 8.8	34.0 ± 7.6	24.4 ± 19.5	0.050
Sex	F: 0 (0%) M: 11 (39.3%)	F: 0 (0%) M: 7 (25.0%)	F: 0 (0%) M: 3 (10.7%)	F: 6 (100%) M: 7 (25.0%)	0.003
Time of exposition (years)	9.4 ± 5.9	6.6 ± 5.8	6.0 ± 1.0	7.0 ± 7.0	NS
Wheat IgE (KU/L)*	7.0 ± 13.5	0.0 ± 0.0	3.0 ± 4.6	0.9 ± 3.1	<0.001
Barley IgE (KU/L)*	8.2 ± 18.1	0.0 ± 0.0	2.5 ± 4.0	0.7 ± 2.2	<0.001
Rye IgE (KU/L)*	7.1 ± 12.7	0.0 ± 0.0	3.9 ± 6.2	0.6 ± 1.8	<0.001
IL-10 (pg/mL)*	4.7 ± 11.6	0.0 ± 0.0	6.0 ± 10.4	0.0 ± 0.0	NS
IgE Lepidoglyphus (KU/L)*	2.3 ± 3.1	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 4.3	0.017
IgE Sitophilus (KU/L)*	0.2 ± 0.3	0.0 ± 0.0	0.2 ± 0.3	0.7 ± 2.2	NS
IgE Ephestia (KU/L)*	1.0 ± 3.3	0.0 ± 0.0	0.2 ± 0.3	0.9 ± 3.1	NS
IgE Tenebrio (KU/L)*	1.0 ± 3.4	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 2.6	NS
IgE House fly (KU/L)*	0.5 ± 1.0	0.6 ± 1.7	0.0 ± 0.0	0.4 ± 0.8	NS
IgE a Horse fly (KU/L)*	0.0 ± 0.0	0.3 ± 0.7	0.0 ± 0.0	0.2 ± 0.9	NS
IgG (mg/L)	17.9 ± 9.6	18.4 ± 10.6	20.3 ± 12.4	19.4 ± 10.4	NS
IgG4 (mg/L)	5.6 ± 7.2	6.6 ± 5.3	2.5 ± 1.9	1.5 ± 2.9	0.016

French bakers patients without occupational asthma (BAS) have higher levels of IgG4 to wheat when comparing with groups BAP and BAC. Bakers had baker's asthma (BA) and their relatives (BAC) have higher levels of specific IgE to *Lepidoglyphus* than groups BAS and BAP.

of millions of dollars. The detection of insects during grain storage is a major problem. The majority of insects in commercial wheat consist of hidden insects, i.e., immature insects that are developing within individual wheat kernels. So there is no wonder that many insects are found in cereal flours (25). Storage mites and arthropods have been implicated in baker's asthma. *Ephestia Kühniella Zelles* and *Eurygaster austriaca* are the commonest offenders (26). Another species, *Ephestia elutella*, lives in homes and attacks bread. Other grain infesting arthropods include storage mites, cockroaches, lepidoptera (*lepisma saccharina*), *Sitophilus granarius*, *Ryzopertha dominica*, lesser grain borer, *Trogoderma granarium*, *Tribolium confusum* (flour beetle), and *Liposcelis bostrychophylus*, which is also an important pest of the poultry industry. In addition to these, Kleine-Tebbe et al. (27) reported cases of respiratory allergy by *Sitophilus orizae*, which are also known as grain insects, such as *Sitophilus zeamais* (maize weevil), *Calandra granarius*, *orizae* *Sitotroga cerealella*, and the mealworm *Tenebrio molitor*. Many other household insects that we also tested could pollute cereals: cockroaches (*blatella germanica*), *blatta orientalis*, house fly (*mosca domestica*), and spider (*tegenaria domestica*). The higher incidence of arthropods allergy among French patients is more likely due to the fact that sera were collected years ago and that at this time wheat flour was less clean and more contaminated by insects. It is quite amazing that after many pioneering works (28–33) we found almost no echo on this subject in more recent publications (34), in which there is no mention of insects, and only few papers (10, 26, 27, 35, 36) had been recently published on the clinical relevance of cereal pests in baker's asthma.

Undoubtedly there had been a change in flour composition and baking techniques. In the last decades, more and more lethal pesticides had been used in cereal crops and toxic contaminants such as dioxins, biphenyl-chlorides, aromatics hydrocarbons, cadmium, mercury, and lead have been found in wheat flour. The industrial manufacturing flour methods include addition of other seeds than wheat and fungal and synthetic enzymes. All of these substances may have increased the allergenic power of flour allergens, which is the reason why the analysis of new sources of allergens implicated in baker asthma is very important. Conversely, it would be interesting to have good purified extracts at least of the common

insects: *Ephestia*, *Tenebrio*, *Sitophilus*, and *Eurygaster*. The cross-reactivity among these species is quite possible and another aim would be to find one or several pan-allergens to facilitate the diagnosis and treatment of baker's asthma (37). The interaction of allergens from house-dust mite and cereals flours also deserves further analysis (38).

Baker's asthma clinical incidence has increased and despite the new drugs and environmental measures, baker's asthma is still the most frequent occupational respiratory disorder (2). An increase in risk factors together with a decrease in tolerance mechanism may be possible causes. French patients with more response to cereals and more time of exposure had the highest values of IL10, IgG4, and also the lowest symptoms. In general, if IgG is an antibody of exposure and IgG4 is a blocking antibody symbolizing tolerance, one would expect higher levels overall in control patients (exposed bakers without symptoms). One control subject had very high IgG4, two had moderate levels, and three had very low levels. The cause may be the duration of the exposure to wheat flour. We measured the strength of the association between IgG4 level and years of exposure, and the correlation coefficient using the Tau-b of Kendall was 0.727, $p < 0.001$.

The role of interleukin 10 in specific immunotherapy and their inhibitor effects on the allergy responses may be important in tolerance mechanisms (14, 15). IL10 can suppress adhesion molecules and T-cell proliferation and can induce specific anergy in TCD4+ (15, 39). Their increase in immune response is delayed, according to the time of exposure, and in this way may downregulate the allergic response to flour antigens. Conversely, statistically significant increases of specific IgG4 values have been found in patients treated with specific hyposensitization (40). In occupational asthma caused by castor beans, IgG4 has been related to a protective role (13). The presence of higher levels of IgG4, IL10, and diversity of sources of sensitization in French baker's patients may have helped them in reducing disease expression but further study may be desirable because the small size of the sample-made difficult statistical analysis.

Baker's asthma is nowadays the most important source of occupational respiratory disease. All the responsible antigens have to be tested to assess a reliable diagnosis and a possible immunotherapy.

Developing a selection of low allergenic plant culture, removing allergens by genetic modification, and new food processing strategies are possibilities to lessen cases of baker's asthma but we think that a sound specific immunotherapy is very useful in selected cases.

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