

Diagnosis of food allergy by counting IgE-positive duodenal cells

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RÉSUMÉ

Diagnostic de l'allergie alimentaire par dénombrement des cellules duodénales IgE-positives. – Le dénombrement des cellules contenant des immunoglobulines a été effectué par immunofluorescence au sein des muqueuses gastrique, duodénale et jéjunale chez 120 sujets sains et 200 malades chez lesquels une sensibilisation alimentaire avait été mise en évidence par la détection des IgE spécifiques plasmatiques et la réactivité intestinale par une épreuve de provocation orale. Aucun sujet sain et aucun malade n'était porteur de parasite. Les nombres de cellules contenant de l'IgA, de l'IgM, de l'IgG ou de l'IgD étaient semblables chez les malades et chez les témoins. Chez les allergiques les cellules renfermant de l'IgE présentes dans la lamina propria de l'intestin étaient systématiquement en nombre très accru. Ceci a été observé en ce qui concerne la muqueuse gastrique chez seulement 118 des 200 malades. Cinquante autres malades suspectés de présenter une allergie alimentaire et présentant un accroissement de la densité des cellules duodénales contenant de l'IgE ont été traités par le cromoglycate disodique oral pendant un minimum de trois mois. Trente-trois malades ont observé une guérison ou une amélioration marquée de leurs symptômes. Le dénombrement des cellules duodénales contenant de l'IgE, mais non celui des cellules de l'estomac, représente un moyen efficace de diagnostiquer une allergie alimentaire chez des sujets exempts de parasites intestinaux.

SUMMARY

The quantitative distribution of immunoglobulin-containing cells in the gastric, duodenal and jejunal mucosae of 120 healthy controls and of 200 food-allergic patients, in which sensitization had been demonstrated by the detection of IgE plasma antibodies and intestinal reactivity by oral provocation test, has been studied after immunofluorescence staining. No patient or control subject was bearing gut parasites. The numbers of IgA-, IgM-, IgG- and IgD- containing cells were similar in patients and in controls. In food-allergic patients, the numbers of IgE-positive cells in the lamina propria was markedly increased in all small intestinal biopsy specimens studied. This was observed only in 118 out of 200 gastric biopsy specimens. Fifty other patients suspected of food allergy and presenting an increased number of duodenal IgE-positive cells were treated with oral sodium cromoglycate for a minimum of three months. Thirty-three patients reported healing or marked improvement. Enumerating IgE-positive cells from the duodenum, but not from the stomach, may be an efficient method for the diagnosis of food allergy in patients without gut parasites.

Food allergy is one of the most controversial topics in medicine [16, 9]. One explanation for this situation is the confusion which often exists between food allergy and food intolerance. Food allergy is caused by intestinal IgE mediated hypersensitivity reactions. Food intolerance may mimic this immunological hypersensitivity but is caused by some psychiatric disorders, certain enzyme deficiencies, bacterial contamination of the small

bowel [12] and some pharmacologically active substances, like histamine [14] and tyramine, present in certain food. The object of this study was to investigate whether the production of IgE is always present in the small intestine for patients who are well characterized in terms of exhibiting a food allergy, and then to check whether this observation would form a diagnostic test for other patients.

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Reçu le 25 janvier 1993. Accepté le 12 février 1993.

ANDRÉ F., ANDRÉ C., DESCOS L., COLIN L., CAVAGNA S. –
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Rev. fr. Allergol., 1993, 33 (2), 119-123.

PATIENTS AND METHODS

Two groups of allergic patients were studied. The first group consisted of two hundred patients, comprising 39 females and 21 males presenting with atopic dermatitis, with an average age of 31 (the ages varying from 3 to 64); 26 females and 41 males exhibiting chronic urticaria with an average age of 35 (the ages varying from 13 to 60); and 38 females and 35 males with an average age of 35 (the ages varying from 4 to 75) for whom food allergy had appeared 22 times as asthma, 7 times as rhinitis, 26 times as Quincke's oedema, 14 times as diarrhoea and abdominal pains (irritable bowel syndrome), twice as anaphylactic shock and once as migraine or buccal aphthosis. In all cases, accountability due to one or more often a large number of foodstuffs had been strongly suggested on questioning the patients, and the implication of IgE-dependent mechanisms had been demonstrated by the positive results obtained from skin prick tests performed on Stallergènes extracts (Fresnes, France) or on fresh extracts prepared as required in the laboratory, and by the subsequent demonstration of the presence of antibodies specific to the IgE class by radioimmunological dosing (RAST, Pharmacia, Sweden). The list of sensitizations identified in the 200 patients is given in Table I. A demonstration of the responsibility of sensitizing antibodies in the genesis of the diseases was effected for all the patients by an oral provocation test for one foodstuff. The reactivity was placed on an objective basis by associating it with a measurement of the intestinal permeability according to a procedure which has been described previously [1, 2]. Briefly, gastrointestinal permeability was evaluated by oral administration of 5 g mannitol, a marker for the absorption of small molecules, and of 5 g lactulose, a marker for the abnormal absorption of large molecules, and subsequent measurement of urinary excretion of mannitol and lactulose by gas-liquid chromatography. In the fasting state, the patients exhibited a mean 5-hour urinary recovery of mannitol of 14.46% (SD 4.32) and a mean recovery of lactulose of 0.43% (SD 0.32). The mean mannitol/lactulose ratio was 0.032 (SD 0.026). After ingestion of a food allergen by the patients, mean mannitol recovery fell to 13.25% (SD 5.19) and mean recovery of lactulose rose to 1.05% (SD 0.84) with a ratio of clearances of 0.093 (SD 0.094). On challenging the patients with the same allergen after they had taken 300 mg sodium cromoglycate orally, a drug that prevents mast cells degranulation, mean mannitol and lactulose recoveries were 13.32% (SD 4.76) and 0.62% (SD 0.43) respectively with a ratio of clearances of 0.051 (SD 0.040).

TABLE I. – Food sensitizations identified by RAST in 200 patients with food allergy

<i>Foods</i>	<i>Numbers</i>	<i>Foods</i>	<i>Numbers</i>
Celery	86	Tuna	25
Peanut	79	Soy	21
Crab	78	Kiwi	20
Milk	59	Mustard	15
Shrimp	58	Beef	14
Parsley	56	Melon	14
Egg white	55	Oat	13
Tomato	53	Barley	12
Carrot	44	Mango	12
Pea	42	Rye	12
Wheat	42	Walnut	11
Orange	41	Maize	10
Yeast	38	Mussel	10
Onion	36	Rice	10
Pork	36	White Bean	9
Cod	32	Peach	8
Potato	32	Banana	8
Strawberry	31	Chicken	7
Hazel-nut	28	Avocado	6
Apple	28	Almond	5
Garlic	25	Lamb	4

A second group of patients was studied; this comprised 28 females with an average age of 28 (the ages ranging from 17 to 65), and 22 males with an average age of 37 (the ages ranging from 12 to 58). Their symptomatology comprised 24 cases of urticaria, 9 cases of atopic dermatitis, 6 cases of abdominal pains of the irritable bowel syndrome type, 6 cases of Quincke's oedema, 2 cases of migraine and one case each of rhinitis, aphthosis or anaphylactic shock. In these patients the symptoms were caused or exacerbated by certain types of meals, but all the techniques employed to identify the sensitizations, particularly skin tests and testing for anti-food plasmatic IgEs by radioimmunological dosing (RAST, Pharmacia), proved negative. These patients were selected from a larger group of patients, based on the number of times an abnormally high density of IgE-positive cells was found in their duodenal mucosae.

These patients were compared with a group of 120 healthy controls. They comprised 50 males and 50 females with an average age of 34 (the ages ranging from 12 to 80). These were either volunteers who were members of the hospital staff or medical students, or patients presenting with functional digestive problems, the evaluation of which had excluded any allergy and any organic lesion. No patient and no control was a carrier of intestinal parasites, as established by parasitological examination of their faeces. All the subjects, or their parents in the case of minors, had given their informed consent, and the study had received the endorsement of the hospital's ethical committee.

Tissue specimens

Biopsies were obtained during endoscopic examination from jejunum, third portion of duodenum, second portion of duodenum, first portion of duodenum, gastric antrum and corpus mucosae in the 120 control subjects and in the 200 food-allergic patients from group one. One biopsy from the second portion of duodenum was obtained from the 50 patients from group two.

Fluorescence microscopy and cell counting

Tissue specimens were washed for 24 h in phosphate-buffered saline at 4 °C to remove extracellular immunoglobulin before ethanol fixation and paraffin embedding. Serial sections were cut at 4 µm. The distribution of IgA-, IgM-, IgG-, IgD- and IgE-positive cells was studied. Polyclonal anti-Ig antibody reagents (Beringwerke AG, Germany) were used following specificity validation using myeloma proteins. Counting of immunoglobulin-positive cells was performed with an X 25 objective in tissue units as defined by the outer frame of an ocular grid representing a section area of 0.07 mm². Three tissue units were evaluated in each section to obtain representative counts. They were chosen at random. Counting was done by the same investigator on coded slides. A check of the cell counting using different observers had previously shown that this was reproducible. An abnormally high local production of IgE was confirmed each time the IgE plasmocyte count was equal to or greater than 3 cells per three tissue units for the gastric samples, and 6 cells for the duodenal or jejunal samples.

The effect of an anti-allergy treatment on the 50 patients from Group 2.

In the absence of sensitizing foods, these patients were treated with a medication in the form of oral cromoglycate (Nalcron, Fisons, France), which prevented the release of allergy mediators. The treatment was administered a quarter of an hour before each meal. The effect of the treatment was assessed by monitoring for a minimum of 3 months and a maximum of 48 months, with the average being 10 months. The mean daily dose of cromoglycate was 700 mg; this varied from 400 to 1200 mg.

RESULTS

The results of the cell counts performed on the 120 healthy subjects and on the 200 patients exhibiting a food allergy, where the sensitizing foods

were also identified, are given in Table II. Both for the control subjects and for the allergic subjects, these showed a predominance of IgA plasmocytes over other classes of plasmocytes. Moreover, none of these subjects exhibited a deficit of IgA. IgD plasmocytes were rare in all subjects, and IgE-positive cells were rare in the control subjects. The density of plasmocytes was clearly less in the stomach than in the small intestine. Both in the control subjects and in the allergic subjects, there was no difference in plasmocyte density between the jejunal or duodenal intestine segments studied. The only notable differences concerned the density of the IgE-positive cells. None of the healthy subjects exhibited an abnormally high density of IgE-positive cells, in any of the biopsies examined. In contrast to this, a clear increase was observed in all the small intestine samples taken from the allergic subjects. However, for the gastric biopsies an increased density of IgE-positive cells was only recorded for 118 of the 200 patients.

For the 50 patients for whom it was not possible to demonstrate the nature of the food sensitization, the density of the cells counted for 3 tissue units from the second part of the duodenum was as follows: 134 positive cells (SD 23), distributed as 91 IgA (SD 15), 19 IgM (7), 5 IgG (2), 1 IgD (1), and 18 IgE (6). Regarding the IgE positive cells, the range varied between 10 and 39 cells. The treatment of 50 patients with oral cromoglycate gave the following results: 21 patients were pronounced cured (their symptomatology comprised 10 cases of urticaria, 3 cases of atopic dermatitis, 4 cases of Quincke's oedema, one case each of anaphylactic shock, aphtosis, irritable bowel syndrome or migraine), and a clear improvement in symptomatology was noted for 12 others (their symptomatology comprised 5 cases of urticaria, 4 cases of atopic dermatitis, one case of each of Quincke's oedema, irritable bowel syndrome or migraine). No improvement in symptomatology was noted for 17 patients. Their symptomatology comprised 9 cases of urticaria, 4 cases of irritable bowel syndrome, 2 cases of atopic dermatitis and one of each of Quincke's oedema or rhinitis). In 7 of the latter cases, side effects appeared which were associated with the treatment, leading to its discontinuation. These comprised 4 cases of diarrhoea, 2 cases of urticaria and 1 case of arthralgia.

DISCUSSION

This study has shown that the mucosa of the small intestine of all the patients exhibiting a food allergy, as authenticated by the appearance of anti-food IgE in the plasma and by an intestinal

TABLE II. – Average numbers of Ig-positive cells in three tissue units of gastric antrum, gastric corpus, duodenum and jejunum, in 100 healthy subjects and in 200 patients with food allergy

	Gastric antrum	Gastric corpus	Duodenum 1	Duodenum 2	Duodenum 3	Jejunum
Total plasma cells						
Controls	43 (30)*	30 (20)	125 (32)	118 (25)	125 (25)	117 (31)
Allergy	39 (26)	30 (18)	126 (24)	132 (30)	129 (26)	132 (28)
IgA cells						
Controls	31 (22)	25 (15)	100 (27)	94 (20)	98 (19)	94 (25)
Allergy	27 (18)	20 (14)	88 (16)	89 (17)	88 (16)	91 (20)
IgM cells						
Controls	5 (5)	3 (3)	18 (10)	17 (8)	20 (8)	16 (7)
Allergy	4 (4)	3 (3)	17 (8)	19 (9)	18 (9)	18 (8)
IgG cells						
Controls	6 (6)	4 (4)	5 (2)	5 (2)	5 (2)	5 (2)
Allergy	5 (5)	4 (4)	5 (2)	5 (2)	5 (2)	5 (2)
IgD cells						
Controls	0 (-)	0 (-)	1 (1)	1 (1)	1 (1)	1 (1)
Allergy	0 (-)	0 (-)	1 (1)	1 (1)	1 (1)	1 (1)
IgE cells						
Controls	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)
Allergy	3 (4)	3 (4)	15 (6)	18 (10)	17 (8)	17 (8)

* = Standard deviation

reactivity which was objectively recorded in oral provocation tests, contained IgE-positive cells. The results obtained were the same as those described more than ten years ago for the jejunal mucosae of 8 children allergic to the proteins in cow's milk and on 5 adults with a history of food allergy [18]. The nature of the cells identified remains the subject of a discussion which is not yet closed [7]. When observations were made on rodents it was stated that the cells recognized by anti-epsilon heavy chain antibodies were mast cells recovered from IgE, whilst the antibody-producing plasmocytes were located in the mesenteric ganglions [13]. In humans, however, electron microscopy has shown that the IgE plasmocytes are even present in the digestive mucosae [15]. In reality, IgE-producing plasmocytes and mast cells activated by IgE antibodies coexist in the mucosae, as has been shown for varioliform or erosive gastritis [3]. A density of IgE-positive cells as high as that recorded for the patients studied has only been observed in cases of intestinal parasitosis [8, 10]. This comprises the only recorded immunity anomaly, in contradiction to what had formerly been asserted, namely the development of a food allergy in IgA deficient patients [19]. The plasmocyte densities observed were very similar for all the intestinal segments studied. It was therefore of no value to extend the sampling outside the Treitz angle in the duodenal mucosa. This is why the biopsies performed on the second group of patients with suspected food allergies only involved the second part of the duodenum. These 50 patients with a clinical history of food allergy

showed exactly the same plasmocyte formation as that recorded for the subjects with food allergies where the sensitizing allergens were identified. Sixty-six percent of these patients exhibited a favourable therapeutic response on treatment with oral cromoglycate. This level of effectiveness is what was observed in a similar report on the treatment of a food allergy in patients who were included in studies carried out in placebo-controlled double blind trials [6]. Moreover, the inconvenience of the treatment, which requires medication to be taken fifteen to twenty minutes before each meal, and the duration of the clinical monitoring, lead to the consideration that the therapeutic effectiveness cannot be explained in terms of a placebo effect.

The study also shows that counting the number of IgE-positive cells in the gastric mucosa is not sufficiently sensitive to be adopted as a diagnostic method. In conclusion, it appears that the antibodies in food allergy are produced locally. The situation is quite similar to that which has already been demonstrated for nasal allergies [17, 11]. The discovery of an increased density of IgE-positive cells (amounting in practice to more than 24 plasmocytes per mm² of intestinal chorion) in the absence of digestive parasitosis is an excellent test for the allergic origin of a particular symptomatology. On the contrary, the absence of local production of IgE refutes the hypothesis of a food allergy. This was also the conclusion of studies conducted on patients with food-related diarrhea [18] or migraine [4].

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