Use of morphometry and immunohistochemistry of small intestinal biopsy specimens in the diagnosis of food allergy

PCM ROSEKRANS¹, CJLM MEIJER², CJ CORNELISSE², AM vd WAL²,
AND J LINDEMAN²

From the ¹Department of Gastroenterology and ²Department of Pathology, University Medical Center, Leiden, The Netherlands

SUMMARY Eight babies with cow's milk protein intolerance and malabsorption, ages ranging from 2 weeks to 6 months, and five adult patients with a history of food allergy were studied. In the jejunal biopsies of the eight children with cow's milk protein intolerance we found partial villous atrophy with an increased number of interepithelial lymphocytes as determined by morphometric analysis. In the five adult patients no morphological changes of the jejunal mucosa were found. However, immunoperoxidase staining revealed a markedly increased number of IgE containing cells in the lamina propria of all jejunal biopsies studied. This proved to be specific for young and adult patients with food allergy. It is concluded that immunohistochemistry of jejunal biopsy specimens is of considerable value in the diagnosis of food allergy.

Allergic reactions, of both the immediate and delayed type, to a variety of ingested antigens have been reported by various authors.¹⁻⁴ Although intolerance to certain foodstuffs is clinically obvious, it is important to distinguish between non-immunological causes, for example, a deficiency of brush-border enzymes such as lactase, and allergic reactions. Moreover, it is a diagnostic problem to distinguish food allergy from functional gastrointestinal disorders. Therefore histological confirmation of the diagnosis of food allergy is important.

Immunological tests are not always helpful.^{5,6} Skin tests with extracts of allergen occasionally show immediate type (type I) reactions to food.⁷ However, negative results occur, often due to the instability of allergen extracts. Another problem is that the preparation of the allergen abstract is possible only from whole foodstuffs and not from products of digestion which actually cause the allergic reaction in the patient. False-positive skin tests frequently occur in atopic individuals due to non-specific histamine liberators.

It has been shown that the radioallergosorbent test (RAST) for specific IgE dietary antibodies is not reliable in the diagnosis of food allergy. For example, high titres of circulating antibodies to β lacto-

globulin or egg proteins may be found not only in some patients with food allergy but also in patients with gluten-sensitive enteropathy. In the latter patients, this finding is probably not specific for food allergy but is secondary to injury of the small intestinal mucosa.⁸⁻¹⁰

A challenge test with a suspected food allergen is easy to interpret if the reaction appears promptly after the food has been ingested, but in some cases the reaction is delayed and in others the challenge test is negative.^{7,11}

We present our study on the jejunal biopsies from eight children with cow's milk protein intolerance and from five adult patients with food allergy against foodstuffs of various origins in order to find objective morphological and immunohistochemical parameters to confirm the clinical diagnosis of food allergy.

Patients

Jejunal biopsies from two groups of patients with food allergy were studied. One group consisted of eight patients, 2 weeks to 6 months of age, with cow's milk protein intolerance. Six of the eight children came from Mediterranean countries. Five had a family history of asthma. All the children suffered from diarrhoea and vomiting shortly after

introduction of cow's milk to their diet; some patients failed to thrive. Jejunal biopsies were taken, either while the patients were still taking cow's milk or within one week of starting a cow's milk free diet. All children became free of symptoms after elimination of the cow's milk from the diet. Those children who failed to thrive showed a rapid gain of weight. Bacterial or protozoal disease was excluded. A provocation test with cow's milk was performed in all eight patients and caused an immediate recurrence of diarrhoea and vomiting in six. In two patients diarrhoea or vomiting recurred on the third and sixth days respectively after reintroduction of cow's milk. Three patients were biopsied one month after elimination of cow's milk and again two days after cow's milk provocation.

The second group consisted of five women with a history of diarrhoea. Three patients had asthma or allergic rhinitis. All five patients reported intolerance to certain kinds of food, manifested by diarrhoea, vomiting, headache, urticaria, and bronchospasm soon after ingesting the food. The allergens varied (egg, chicken, seafood, chocolate, nuts), and it was not always possible to induce symptoms by exposure to the suspected allergen. Avoidance of the allergen was successful in three patients. The other two (9 and 10, Table 1) became free of symptoms after treatment with disodium cromoglycate (Lomudal^R).

The jejunal biopsies of 10 healthy volunteers and of 30 patients with a variety of gastrointestinal diseases, such as gluten-sensitive enteropathy, Crohn's disease, irritable colon syndrome, and lactase deficiency, served as controls.

Methods

Peroral biopsies of the small intestinal mucosa were performed under radiological control using a hydraulic multiple biopsy capsule. Two biopsy specimens were taken distal to the ligament of Treitz. One was oriented under a dissecting microscope with a minimum of handling on a 'mesh' to which it adhered.¹² The second specimen was fixed without handling in a sublimate-formaldehyde mixture.¹³ After fixation for 3 hours the tissue samples were embedded in paraplast. Tissue sections were cut 4 µm thick, perpendicular to the luminal surface and mounted on glass slides. Sections were stained with haematoxylin and eosin (HE) and periodic acid Schiff (PAS), and specifically for IgA, IgM, IgG, IgD, and IgE heavy chains using indirect immunoperoxidase staining.¹³ Appropriate controls were done according to Sternberger.¹⁴ Rabbit antisera against IgA, IgM, and IgG heavy chains were purchased from Dakopatts (Denmark). The rabbit antisera against IgD and IgE heavy chains were purchased from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam. The specificity of the antisera was confirmed by immunoelectrophoresis and for IgA, IgG, IgM, and IgD by cytoplasmic immunofluorescence on monoclonal bone marrow preparations.¹⁵ The rabbit anti IgE antiserum showed no cytoplasmic immunofluorescence with bone marrow preparations monoclonal for IgA, IgM, IgG, or IgD heavy chains and their respective κ or λ light chains. Horseradish peroxidase-labelled goat anti rabbit IgG was obtained from Miles-Yedah (Israel).

Table 1 Clinical data and jejunal biopsy findings of 13 patients with food allergy

Patient	Age	Sex	Morphometry			IgE cells	Allergen	Main symptoms	Atopy
			V/C	IEL	SA				
1	½ mth	F	1.06:1	33	2.70	90	Cow's milk	Diarrhoea, vomiting	Asthma in family
2	1 "	M	0.75:1	39	2.03	69	"	Diarrhoea	—
3	3 "	M	1.71:1	38	4.22	63	"	Diarrhoea	Asthma in family
4	6 "	F	1.82:1	36	4.62	84	"	Diarrhoea, failure to thrive	Asthma in family
5	½ "	F	1.01:1	32	2.57	76	"	Diarrhoea, vomiting	Asthma in family
6	2 "	M	1.13:1	28	3.29	76	"	Vomiting, failure to thrive	—
7	5 "	M	2.04:1	28	4.91	83	"	Diarrhoea, vomiting	—
8	1 "	F	0.89:1	51	3.12	59	"	Diarrhoea, vomiting	Asthma in family
9	30 yr	F	2.22:1	22	5.79	83	Egg, chicken	Diarrhoea, urticaria	Asthma
10	26 "	F	2.50:1	32	4.68	95	Nuts, egg, chocolate	Stomatitis aphthosa, diarrhoea, urticaria	Asthma
11	53 "	F	2.23:1	28	5.32	75	Seafood	Diarrhoea	Asthma, allergic rhinitis
12	37 "	F	3.88:1	20	6.90	104	Olives?	Diarrhoea	—
13	42 "	F	3.50:1	18	5.28	82	Cow's milk	Diarrhoea	—

V/C = villous:crypt ratio; IEL = number of interepithelial lymphocytes per mm surface epithelium; SA = length of surface epithelium in mm per mm muscularis mucosae; IgE cells = number of IgE containing cells per mm² lamina propria area.

Fig. 1 Morphometry of jejunal biopsies: v/c = villous: crypt ratio; IEL = number of intraepithelial lymphocytes per mm surface epithelium; AB = length of surface epithelium per mm stretched muscularis mucosae; LP area = lamina propria cross-sectional area per mm stretched muscularis mucosae.

The HE slides were used for morphometric studies. The following parameters were measured (Fig. 1):

- mean villous to crypt ratio, that is, the linear measurement of five villous heights divided by the corresponding crypt depths (v/c);
- number of intraepithelial lymphocytes per millimetre surface epithelium counted in the upper two-thirds of the villus (IEL);
- length of surface epithelium per millimetre stretched muscularis mucosae (SA);
- lamina propria cross-sectional area per millimetre stretched muscularis mucosae (LP area).

Images of the HE and immunoperoxidase stained sections were projected with a standard magnification of $\times 200$ on graph paper, and drawings were made. Morphometric analysis of the drawn contours was made on a graphic tablet (Tektronix) interfaced to a small laboratory computer (PDP 11/10; Digital Equipment Corporation, Maynard, Mass.).¹⁶

The slides stained for IgA, IgM, IgG, IgD, and IgE were used for counting the immunoglobulin-containing cells per mm² lamina propria area in three consecutive sections.

Statistical analysis was performed using the Student *t* test.

Results

The initial proximal jejunal biopsy, performed as soon as possible after cow's milk protein allergy in the group of young children was suspected, showed in all cases partial villous atrophy. The villous:

Fig. 2 Number of IgE containing cells per mm² lamina propria area. Statistical analysis showed no significant difference between the group of healthy volunteers and patients with various gastrointestinal disorders including gluten-sensitive enteropathy ($P = 0.1$). However, the patients with food allergy showed a significant difference against both groups ($P < 0.0005$).

crypt ratio was reduced, but total villous atrophy was never found (Table 1). The number of intraepithelial lymphocytes was increased but the number per millimetre surface mucosa was not as high as we found in gluten-sensitive enteropathy (data not shown). The cellular density of the lamina propria was slightly increased and consisted of plasma cells, eosinophils, granulocytes, and mononuclear cells. In most cases there was a relative increase of eosinophils. The number of IgE-containing cells in the lamina propria was markedly increased (Fig. 2). The

absolute and relative number of IgA and IgM containing cells was slightly increased in most patients intolerant to cow's milk protein.

In the adult group, we found no morphological changes of the small intestinal mucosa. The villous: crypt ratio was normal, and the number of interepithelial lymphocytes and the cellular density in the lamina propria were indistinguishable from normal. The immunoperoxidase staining, however, showed a striking increase in the number of IgE containing cells similar to the findings in the group with cow's milk protein intolerance (Fig. 3). In the adult group, the absolute and relative number of IgA, IgG, and IgM containing cells did not differ substantially from that of the controls.

In three patients with cow's milk protein intolerance, biopsies were taken one month after elimination of cow's milk and two days after reintroduction of cow's milk in the diet. After elimination of cow's milk, the villous: crypt ratio and the length of surface epithelium outline per millimetre muscularis mucosae improved markedly and became almost normal (normal v/c > 2.0). The number of interepithelial

lymphocytes decreased but remained above normal. The number of IgE containing cells did not change (Table 2). After cow's milk challenge the results of the morphometric studies were indistinguishable from those before cow's milk elimination (Fig. 4).

Table 2 Morphometric results of serial jejunal biopsies of three patients with cow's milk protein intolerance

	v/c $\pm$ SD	IEL	SA	IgE cells
<i>Patient 1</i>				
On cow's milk	1.06 ($\pm$ 0.16)	33	2.70	90
1 month after elimination	1.67 ($\pm$ 0.15)	25	4.83	72
3 days after provocation	1.38 ($\pm$ 0.07)	28	3.48	83
<i>Patient 2 (Fig. 4)</i>				
On cow's milk	0.75 ($\pm$ 0.12)	39	2.03	69
1 month after elimination	2.17 ($\pm$ 0.31)	20	6.09	82
2 days after provocation	0.96 ($\pm$ 0.18)	31	2.63	89
<i>Patient 6</i>				
On cow's milk	1.13 ($\pm$ 0.12)	28	3.29	76
1 month after elimination	1.52 ($\pm$ 0.11)	22	3.88	76
2 days after provocation	1.37 ($\pm$ 0.17)	24	3.84	65

v/c = mean villous: crypt ratio; IEL = number of interepithelial lymphocytes per mm surface epithelium; SA = length of surface epithelium in mm muscularis mucosae; IgE cells = number of IgE containing cells per mm² lamina propria area.

Discussion

In both the juvenile and the adult group of patients with clinical symptoms of food allergy we found a marked increase in the number of IgE containing cells in the proximal jejunal lamina propria. Partial villous atrophy with an increased number of intraepithelial lymphocytes occurred in the group of young children with cow's milk protein intolerance only. We did not find increased numbers of IgE containing cells in the jejunal lamina propria in any of the healthy volunteers. In the group of patients with a variety of gastrointestinal abnormalities, only those with gluten-sensitive enteropathy showed a slight increase in the number of IgE containing cells in jejunal biopsies. This might be caused by the same mechanism that leads to the formation of circulating antibodies to food allergens in gluten-sensitive patients.

The distribution of immunoglobulin-containing cells in the intestinal lamina propria of infants suffering from the cow's milk induced malabsorption syndrome was studied by Jos *et al.*¹⁷ They found an increased number of IgA containing cells using an immunofluorescence technique. Savilahti¹¹ studied the cell counts during cow's milk challenge and observed a rise in IgA, IgM, and IgG containing cells but found no consistent change in the number of IgE containing cells. In similar challenge investigations, however, Shiner *et al.*¹⁸ showed an increase in

Fig. 3 Jejunal biopsy stained for IgE of an adult patient (11) with food allergy.

Fig. 4 Three jejunal biopsies stained for IgE from patient 2 with cow's milk protein intolerance: (a) taken during cow's milk containing diet; (b) taken one month after elimination of cow's milk from the diet; (c) taken two days after cow's milk provocation.

mucosal IgE containing cells. It is not easy to compare these results. In most immunohistochemical studies of IgE, workers have neglected to test the anti IgE reagent on positive and negative substrates. Standardised studies based on properly defined and characterised reagent are necessary.¹⁹

During the past decades food allergy has gained increased recognition as an important factor in intestinal disorders. The best defined type of food allergy is the allergy to cow's milk. Yet, in spite of many carefully performed clinical studies on the symptomatology of milk allergy, even today the diagnostic criteria are difficult to define. The final diagnosis is based mainly on the results of clinical provocation tests after a favourable response to elimination of cow's milk.⁷ Sumithran and Iyngkaran²⁰ claimed that there is no clinical justification for jejunal biopsy in infants in whom cow's milk protein intolerance is suspected. In our opinion, the clinical evidence derived from cow's milk challenge after an elimination diet is unreliable. It is important to find objective criteria on which the diagnosis of food allergy can be based, and to distinguish it from other diseases of the small intestine. A single jejunal biopsy, performed when the diagnosis of food allergy is suspected, is, in our opinion, sufficient to confirm the diagnosis.

It seems worth-while to examine small intestinal mucosa biopsies from atopic patients with clinical allergic manifestations of bronchial asthma and

without gastrointestinal symptoms because of the high incidence of a personal or family history of asthma in patients with the juvenile or adult type of food allergy. Studies now in progress, however, show that patients with bronchial asthma without a history of food allergy do not have an increased number of IgE containing cells in the jejunal lamina propria (Rosekrans, in preparation).

In conclusion, our study indicates that it is possible to differentiate patients with food allergy from patients with other kinds of gastrointestinal disorders using immunohistochemistry of proximal jejunal biopsy specimens in combination with morphometry.

The cooperation of the paediatric department of the University Medical Center Leiden and the Juliana Kinderziekenhuis, Den Haag, is gratefully acknowledged.

References

- ¹Kuitunen P, Visakorpi J K, Hallman N. Histopathology of duodenal mucosa in malabsorption syndrome induced by cow's milk. *Annales Paediatrici* 1965; **205**: 54-63.
- ²Truelove S C, Wright R. In: Gell P G H, Coombs R R A, eds. *The Intestine in Allergic Diseases in Clinical Aspects of Immunology*. Oxford: Blackwell. 1968; 1053-1062.
- ³Bleumink E. Food allergy, the chemical nature of the substances eliciting symptoms. *World Rev Nutr Diet* 1970; **12**: 505-570.
- ⁴Freier S. Paediatric gastrointestinal allergy. In: Brostoff J, ed. *Clinical Immunology-Allergy in Paediatric Medicine*. Oxford: Blackwell. 1974; 107-128.
- ⁵Dannaeus A, Johansson S G O, Foucard T, Öhman S. Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA, and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 1977; **66**: 31-37.
- ⁶Dannaeus A, Johansson S G O, Foucard T. Clinical and immunological aspects of food allergy in childhood. II. Development of allergic symptoms and humoral immune response to foods in infants of atopic mothers during the first 24 months of life. *Acta Paediatr Scand* 1978; **67**: 497-504.
- ⁷Goldman A S, Anderson D W, Jr, Sellers W A, Saperstein S, Kniker W T, Halpern S R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963; **32**: 425-443.
- ⁸Carswell F, Ferguson A. Plasma food antibodies during withdrawal and reintroduction of dietary gluten in coeliac disease. *Arch Dis Child* 1973; **48**: 583-586.
- ⁹Bleumink E. Allergens and toxic proteins in food. In: Hekkens W Th J M, Peña A S, eds. *Coeliac Disease*. Leyden: Stenfert Kroese. 1974; 46-55.
- ¹⁰Eastham E J, Walker W A. Adverse effects of milk formula ingestion on the gastrointestinal tract. *Gastroenterology* 1979; **76**: 365-374.
- ¹¹Savilahti E. Immunochemical study of the malabsorption syndrome with cow's milk intolerance. *Gut* 1973; **14**: 491-501.
- ¹²Rubin C E, Brandborg L L, Phelps P C, Taylor H C, Jr. Studies of celiac disease. I. The apparent identical and specific nature of the duodenal and proximal jejunal lesion in celiac disease and idiopathic sprue. *Gastroenterology* 1960; **38**: 28-49.
- ¹³Bosman F T, Lindeman J, Kuiper G, van der Wal A, Kreunig J. The influence of fixation on immunoperoxidase staining of plasmacells in paraffin sections of intestinal biopsy specimens. *Histochemistry* 1977; **53**: 57-62.
- ¹⁴Sternberger L A. *Immunohistochemistry*. Englewood Cliffs, NJ: Prentice Hall Inc. 1974; 53.
- ¹⁵Hijmans W, Schuit H R E, Klein F. An immunofluorescence procedure for the detection of intracellular immunoglobulins. *Clin Exp Immun* 1969; **4**: 457-472.
- ¹⁶Van de Velde C J H, Meijer C J L M, Cornelisse C J, van de Velde E A, van Putten L M, Zwaveling A. A morphometrical analysis of lymph node responses to tumors of different immunogenicity. *Cancer Res* 1978; **38**: 661-667.
- ¹⁷Jos J, Rey J, Frézal J. Etude immunohistochimique de la muqueuse intestinale chez l'enfant I. Les syndromes de malabsorption. *Arch Fr Pédiatr* 1972; **29**: 681-698.
- ¹⁸Shiner M, Ballard J, Smith M E. The small-intestinal mucosa in cow's milk allergy. *Lancet* 1975; **1**: 136-140.
- ¹⁹Brandtzaeg P, Baklien K. Inconclusive immunohistochemistry of human IgE in mucosal pathology (Letter). *Lancet* 1976; **1**: 1297-1298.
- ²⁰Sumithran E, Iyngkaran N. Is jejunal biopsy really necessary in cow's milk protein intolerance? *Lancet* 1977; **2**: 1122-1123.

Requests for reprints to: Dr PCM Rosekrans, Department of Gastroenterology, University Medical Center Rijnsburgerweg 10, Leiden, The Netherlands