

A CONSISTENT PATTERN OF MINOR IMMUNODEFICIENCY AND SUBTLE ENTEROPATHY IN CHILDREN WITH MULTIPLE FOOD ALLERGY

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Objective Although immunoglobulin (Ig)E-mediated allergies are readily identifiable, non-IgE-mediated allergies present more diagnostic difficulty. We performed a formal retrospective analysis to determine whether there is a recognizable clinical pattern in children.

Methods We studied 121 children (mean age, 17.3 months) with multiple food allergies who were recruited on the basis of adequate immunological assessment by using case notes and parental questionnaire.

Results Group 1 (n = 44) had rapid reactions to dietary antigens, of whom 41 also showed delayed reactions. Group 2 (n = 77) had delayed reactions only. Mean IgE was increased in group 1 but both groups otherwise shared a pattern of increased IgG1, decreased IgG2/4, and low-normal IgA. Lymphocyte subsets were skewed, with an increased percentage of CD4 and CD19 and decreased CD8 and natural killer cells. Gastroesophageal reflux, esophagitis, subtle enteropathy, and constipation were frequent in both groups. Of 55 exclusively breast-fed infants, 44 sensitized before weaning. Twenty-one of the mothers suffered from autoimmunity.

Conclusions There appears to be a recognizable pattern of immune deviation and minor enteropathy in children with multiple food allergy, irrespective of the speed of reactions. Disturbed gut motility is particularly common, as is a maternal history of autoimmunity. (*J Pediatr* 2003;143:39-47)

There has been both a recent increase and a change in the presentation of childhood food allergy.¹⁻⁶ Sensitization to milk classically occurred in formula-fed infants, often after gastroenteritis,^{7,8} representing lost oral tolerance after epithelial disruption.⁹ This also occurs in infants in developing countries.^{10,11} Recently there have been increasing reports of multiply-sensitized infants^{1-3,6,9} often despite exclusive breast-feeding. Such infants may be intolerant of the trace proteins in hydrolysates and require amino acid-based formulae.^{4-6,12,13} Associated problems include eczema, gastrointestinal dysmotility, and enteropathy.^{1,3-6,9,12,13} Those with IgE-mediated rapid responses usually present little diagnostic difficulty.¹ Conversely, delayed non-IgE-mediated responses may be missed because of a lack of specific diagnostic tests.^{1,3-6,9,12}

Although genetic predisposition to allergy is clearly important, early environmental exposures are also critical; thus, allergies increase as material conditions improve.^{3,9,14,15} Oral tolerance requires an inflammatory response by innate immunity to enteric bacteria.^{9,16} Animals maintained in germ-free environments do not establish oral tolerance and show increased an energy requirement for growth,^{17,18} whereas the blockade of innate immune response to gut bacteria abrogates oral tolerance in mice.¹⁹ The relevance of innate immune responses in childhood immune tolerance has been shown by the prevention of eczema with probiotics²⁰ and evidence that gut infections protect against allergy.²¹ Although excess IgE production is associated with allergy, other mechanisms appear important in determining sensitisation.^{3,9} Links have been established with low-quartile

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CMSE	Cow's-milk sensitive enteropathy
MFA	Multiple food allergy
NK	Natural killer
RASTs	Radioallergosorbent tests

IgA,²² reduced IgG subclasses, and CD8 cells.^{23,24} Serum IgA levels are higher in infants in developing countries,²⁵ which supports a link with environmental exposures.

Having noted apparently stereotyped immunodysregulation in the increasing numbers of multiply allergic children we now see, notably skewing of IgG subclasses and lymphocyte subsets, we examined a cohort of affected children to determine whether there may be sufficiently consistent clinicopathological features to allow pattern recognition, even in the absence of immediate responses.

PATIENTS AND METHODS

This was a retrospective analysis of clinical, histologic, and immunologic characteristics of infants and children with multiple food allergy (MFA) referred by consultant pediatricians or general practitioners during 1997 through 1999 to the Paediatric Food Allergy Clinic of the Centre for Paediatric Gastroenterology at the Royal Free Hospital, a British Society of Allergy and Clinical Immunology-recognized tertiary referral center. The inclusion criteria used were a secure diagnosis of MFA by currently accepted criteria,¹ that is, reproducible adverse responses to 2 or more dietary antigens, with clinical response to exclusion diet and recurrence of symptoms on challenge, and the availability of blood test results for circulating immunoglobulin concentrations and/or lymphocyte subsets. We identified 121 children who fit the initial criteria (mean age, 17.3 months; range, 1-93 months). The children were divided into groups on the basis of time of onset of symptoms: group 1 (*n* = 44 children), who showed rapid hypersensitive responses (within 1 hour), and group 2 (*n* = 77 children), who showed delayed responses only (Table I). The mean age at referral in group 1 was 33 months and in group 2 was 19 months. The study protocol was approved by the local research ethics committee, and analysis was based on review of case notes, dietetic records, mucosal biopsies, and a parental questionnaire distributed at follow-up clinic assessment.

Family History

An extensive family history was taken for atopy (hay fever/allergic rhinitis, asthma, eczema), allergies, and organ-specific autoimmunity. Autoimmune diseases specifically enquired for included thyroid disease, insulin-dependent diabetes mellitus, rheumatoid arthritis, psoriasis, and multiple sclerosis. Immediate hypersensitivity included reactions to foods, animals, and bee and wasp stings.

Food Challenges

All patients underwent food challenges supervised by pediatricians, clinical nurse specialists, and/or dietitians, although these were deferred in infants <18 months of age who made had a clear response to antigen exclusion after a prolonged period of ill health. Peanut and whole-nut challenges are also routinely deferred in our clinic until children at >6 years of age. In 18 subjects, cow's milk, soya, or hydrolysate challenges were performed before referral by local

pediatricians, and in 3 subjects inadvertent challenges at home induced immediate reactions requiring medical attendance. Challenges were either double-blind placebo-controlled or open-label depending on clinical circumstances and conducted in our ward in all cases with a history of immediate reaction. In those with such a history, the antigen was administered in increasing quantities over a continuously supervised period of 4 hours. A positive immediate reaction was defined by the development of skin rash, urticaria, lip swelling, vomiting, or wheezing.^{1,7} In those patients with delayed reactions only, most of the challenges were commenced in clinic and continued at home under the supervision of our dietitians. The parents were thus instructed to increase the child's intake of the antigen, making no other change in the diet, and to phone for guidance if symptoms occurred. A positive delayed response to challenge was defined as previously reported,¹² with induction of blood in stools, diarrhea, vomiting, eczema, rhinitis, or weight loss. In the absence of such symptoms, the challenge was considered negative. In circumstances of doubt, the parents were advised to discontinue the challenge for 1 to 2 weeks and then to repeat the challenge.

Immunologic Assessment

Serum IgA, IgG, and IgM assessments were performed routinely using an immunoturbidimetric assay. Total IgE levels and specific IgE antibodies were measured by immunofluorescence (Unicap 100, Pharmacia Diagnostics UK, Milton Keynes). Levels were compared with age-specific normal values (Protein Reference Unit, Sheffield). T-cell subsets were measured by flow cytometry by using whole blood and direct staining with isotype controls. Results were plotted against age-specific normal values derived from 2 large published series.^{26,27} Skin prick tests were performed using Soluprick Allergen extracts (Solumed, UK, 1:20), classed positive if a wheal 3 mm greater than the negative control was elicited. Skin prick tests included a variety of antigens, most commonly cow's milk, wheat, soya, peanut, nuts, egg, fish, fruits, and environmental antigens. Specific IgE testing (radioallergosorbent tests; RASTs) included, as appropriate, milk, soya, egg, peanuts, tree nuts (hazel, almond, brazil, walnut, cashew, pistachio), sesame, wheat, fish, tuna, tomatoes, banana, chick pea, spinach, mixed grass, house dust mite, and animal dander.

Morphometric Assessment of Small Intestinal Biopsies

The villous height and crypt depth were measured under 160× magnification by two of the investigators using Imagan 2 software (Kompira, Glasgow, UK). Villous height was measured from the tip of the villous epithelium to the crypt-villous junction, and the crypt depth from the crypt-villous junction to the muscularis mucosae. An average was obtained from a minimum of 5 to a maximum of 16 units. The results were compared to 47 histologically normal specimens from children investigated to exclude enteropathy in whom no final diagnosis was made (median age, 13 months; range, 3-23), 46 archival cases from the 1980s of classic cow's-milk sensitive

Table I. Patients divided into groups according to mode of onset

Total (n = 121)	Type of symptoms	Percentage of all patients	Mean age at referral (range)
Group 1 (n = 41)	Immediate symptoms (within 1 h) + delayed symptoms	34	33 mos (4–96 mos)
Group 1a (n = 3)	Immediate symptoms only	2.5	43 mos (16–96 mos)
Group 2 (n = 77)	Delayed symptoms only	63.5	19 mos (0.5–85 mos)

Table II. Clinical manifestations at onset of symptoms and at time of first assessment in group 1 (n = 41) and group 2 (n = 77)

	Symptoms at onset		Symptoms at referral	
	Group 1 n (%)	Group 2 n (%)	Group 1 n (%)	Group 2 n (%)
Urticaria/edema/anaphylaxis	41 (100)	0 (0)	17 (42)	0 (0)
Eczema	28 (68)	34 (44)	3 (7)	5 (7)
Vomiting	13 (32)	32 (42)	6 (15)	27 (35)
Failure-to-thrive	8 (20)	30 (39)	14 (34)	37 (48)
Diarrhea	11 (27)	38 (49)	7 (17)	37 (48)
Wheezing	19 (46)	21 (27)		
Colic/pain/persistent crying	8 (20)	21 (27)	3 (7)	18 (23)
Rectal bleeding	0 (0)	7 (9)	1 (2)	6 (8)
Constipation	1 (2)	7 (9)	7 (17)	10 (13)

enteropathy (CMSE; median age, 8 months; range, 1–18) and 17 cases of untreated coeliac disease (median age, 13 months; range, 1–22), whose clinical details have been previously reported.² The measurements were performed twice in 35 biopsies and 3 times in 8 biopsies, with intraobserver and interobserver variation <5%.

Statistical Analysis

Comparison of serum total IgE, IgA, and IgG subclasses between the groups, as well as crypt-villous morphometric measurements between the children with food allergy and normal controls, was made using the Kruskal-Wallis test, with $P < .05$ considered significant.

RESULTS

Initial Presentation

Of the 121 children studied, symptoms had begun in the first year of life in 43 of 44 children from group 1 and 69 of 77 children from group 2, commencing after gastroenteritis in 1 patient from group 1 and 9 patients from Group 2. Twenty-two of these children were reported by parents to show their first symptoms (irritability, vomiting) in the first week of life, and 42 of the infants within one month. Fifty-five mothers (47%) had exclusively breast-fed until weaning, and 41 of their children (14 in group 1 and 27 in group 2) developed gastrointestinal or skin symptoms while solely breast-fed. Of these, 48% developed eczema, 78% excessive crying or colic, 35% vomiting, 28% diarrhoea, 23% poor weight gain, 15% constipation, and 8% rectal bleeding. At referral (Table II),

different clinical patterns were seen between the groups, with classic atopic responses, such as wheezing, more common in group 1 whereas diarrhea, excessive crying, colic, and failure-to-thrive were more apparent in Group 2. Eczema was common to both groups. Prolonged or recurrent viral infections were reported by parents of 38 children (31%).

Response to Food Antigens

GROUP 1: IMMEDIATE RESPONDERS. The most common antigens inducing immediate responses in these 44 children were milk, eggs, and nuts (Table III). Symptoms included urticaria, lip swelling, or skin rash (86%); immediate vomiting (29.5%); and anaphylaxis (7%). Of these children, only 3 (7%) had no delayed symptoms at all. By contrast, 41 of the 44 (93%) manifested both immediate reactions (rash/urticaria in 38, anaphylaxis in 3) and additional delayed reactions (predominantly to milk, soya, wheat, or egg). These included diet-responsive eczema (68%), diarrhea or vomiting (59%), wheezing (46%), or failure-to-thrive (18%). Of this group, 50% had IgE > 100 ku/L, 70% positive skin prick tests, and 75% positive RASTs. Skin prick tests for egg, milk, and nuts were predictive in almost all cases of positive immediate reactions, whereas soya and wheat tests were negative in several infants who made delayed clinical responses on challenge.

GROUP 2: DELAYED RESPONDERS. Symptoms shown by these 77 children included 1 or more of diet-responsive eczema (44%), failure-to-thrive (39%), vomiting (42%), loose stools (49%), constipation (11%), rectal bleeding (11%), and eosinophilic colitis (7%). Symptoms were induced most com-

Table III. Responses to individual antigens in groups 1 (n = 41) and 2 (n = 77)

	Group 1	Group 2
Cow's milk	Rash/urticaria: 27 cases, Vomiting: 15 cases, Angioedema 6 cases, Wheezing: 11 cases, Eczema flare: 11 cases, Loose stools: 10 cases Constipation: 5 cases Hypotension: 1 case	Eczema flare: 22 cases, Non-eczema rash: 6 cases, Vomiting: 15 cases, Abdominal pain: 20 cases, Loose stools: 24 cases Blood in stools: 3 cases, Constipation: 14 cases Wheezing: 3 cases, Rhinitis: 6 cases
Soya	Rash/Urticaria: 7 cases Vomiting: 9 cases Wheezing: 7 cases, Eczema flare: 9 cases Loose stools: 11 cases, Constipation: 4 cases	Eczema flare: 10 cases, Non-eczema rash: 6 cases Vomiting: 7 cases, Abdominal pain: 4 cases Loose stools: 11 cases, Constipation: 4 cases Wheezing: 2 cases, Rhinitis: 2 cases
Egg	Rash/Urticaria: 11 cases, Vomiting: 4 cases Angioedema: 2 cases, Wheezing: 5 cases Eczema flare: 8 cases	Eczema flare: 7 cases, Non-eczema rash: 4 cases Vomiting: 6 cases, Constipation: 1 case
Wheat	Rash/Urticaria: 1 case, Vomiting: 5 cases Wheezing: 2 cases, Eczema flare: 7 cases Loose stools: 6 cases, Constipation: 6 cases	Eczema flare: 12 cases, Non-eczema rash: 4 cases Vomiting: 7 cases, Abdominal pain: 7 cases Blood in stools: 2 cases, Loose stools: 11 cases Constipation: 9 cases, Rhinitis: 3 cases
Peanut	Rash/Urticaria: 5 cases, Vomiting: 1 case Angioedema: 3 cases, Hypotension: 1 case	
Tree nuts	Rash/Urticaria: 2 cases Angioedema: 1 case	
Hydrolysates	Rash/Urticaria: 5 cases Vomiting: 3 cases, Loose stools: 5 cases Eczema flare: 4 cases	Eczema flare: 10 cases, Non-eczema rash: 6 cases Vomiting: 9 cases, Loose stools: 10 cases Blood in stools: 1 case
Meats/Chicken	Rash/Urticaria: 2 cases Eczema flare: 2 cases, Loose stools: 3 cases	Non-eczema rash: 3 cases, Eczema flare: 3 cases Wheezing: 1 case, Loose stools: 4 cases
Others	Rash/Urticaria: 5 cases, Vomiting: 6 cases Angioedema: 2 cases, Loose stools: 5 cases	Non-eczema rash: 6 cases, Eczema flare: 9 cases Vomiting: 8 cases, Loose stools: 7 cases, Constipation: 9 cases

Challenges for probable peanut or whole-nut allergies are deferred in our clinic until patients are ≥ 6 years of age and have not yet been performed in 5 patients (all with positive skin prick tests and/or RASTs).

monly in response to milk, soya, wheat, hydrolysates, egg, meats, and rice (Table III). In contrast with group 1, only 18% had IgE >100 ku/L, 27% positive skin prick tests, and 25% positive RASTs. Atopic symptoms were apparent in 52 of 77 children (68%); of these, 34 with eczema, 24 asthma, and 4 hayfever. In 5 subjects, there had been previous concerns about Munchausen syndrome by proxy, despite findings of eczema or diarrhea, because skin prick tests were negative and thus reported symptoms of the patients not believed. In each case, extensive in-patient assessment was undertaken and positive delayed responses seen to food antigens on double-blind placebo-controlled food challenge.

Clinical Response

In group 1, 5 of 44 infants settled on maternal elimination diets while breast-feeding, 6 tolerated soya and 15 hydrolysates, but 7 children required an amino acid formula (Neocate; SHS International, Gaithersburg, Md). In group 2, 12 of 77 settled on maternal elimination diets, 7 tolerated soya milk and 13 hydrolysates, but 29 of 77 children required amino acid formula. Thus overall, 36 of 121 children (29.7%) were

intolerant of hydrolysed cow's milk formula. It was noticeable that all infants of currently breast-feeding mothers were intolerant of hydrolysates and required an amino acid formula. By age 2 years, only 15 children had regained tolerance of cow's milk, and 43 continued on a substitute formulae. Seven children had regained tolerance to soya, whereas others were maintained on oat or rice milk or hydrolysates, and 10 children still required an amino acid formula. During episodes of viral illness, which appeared particularly prominent and prolonged during the childrens' second winter, symptoms of gastro-esophageal reflux worsened substantially despite strict dietary exclusions, and prokinetic agents, H2 receptor antagonist, or proton pump inhibitor therapy had to be reintroduced during these episodes. One child in group 1 and 3 in group 2 required treatment with intravenous immunoglobulins because of bronchiectasis or persistent enteropathy or colitis.

Family History

A family history of classic atopy was reported for 90% of the children. This was particularly strong on the maternal side, as 65% of mothers and 33% of fathers had a history of atopy.

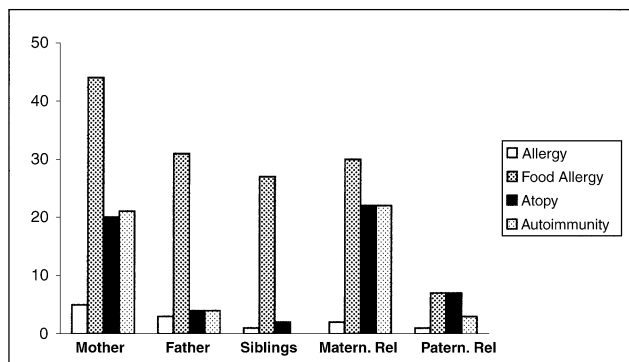


Fig 1. Family history of reported immediate hypersensitivity (allergy), food allergies, atopic disorders, and autoimmunity in 110 patients from whom full details were available. *Matern Rel*, maternal relative; *Patern Rel*, paternal relative.

There was a strong family history of atopy and organ-specific autoimmunity among mothers and maternal grandparents, most often grandmothers (Fig 1). There was no difference between groups.

Immunological Assessment

IgE concentrations were increased in group 1 (median, 97 kIU/L; range, <2-2096) compared with group 2 (median, 9 kIU/L; range, <2-19170, $P < .001$; (Fig 2, A). IgA tended to the low-normal range or below, and 45% of children had serum IgA ≤ 0.3 g/L (Fig 2, B). The median IgA g/L in group 1 was 0.6 g/L and in Group 2 was 0.4 g/L ($P < .01$), reflecting the younger age in group 2. By contrast, other immunoglobulins were similar in the 2 groups. IgM levels were normal (not shown). There was evidence of skewing of IgG subclasses: in contrast with increased IgG1, both IgG2 and IgG4 were low, and 40% of children had undetectable IgG4 (Fig 2, C-E). Lymphocyte subsets also showed evidence of immune deviation (Fig 3, A-E). CD4 cell percentages were increased above the upper quartile, in contrast with reduction in CD8 cells below the lower quartile, leading to elevated CD4:CD8 ratio. As previously reported, the percentage of CD19 B cells was increased.²⁴ By contrast, natural killer (NK) cell numbers were reduced. These findings were seen in both groups, and no differences detected between the 2 groups for any parameter. Multi-Test cell-mediated immunity (Merieux, Lyon, France) prick tests were performed on 10 patients (1 patient from group 1). This test measures delayed hypersensitivity response to various antigens, including tetanus, diphtheria, streptococcus, candida, tuberculin, trichopyton, and proteus and a glycerin control. All 10 patients showed uniformly negative responses.

Gastroenterological Assessment

Gastrointestinal symptoms occurred in 28 of 41 in group 1 and 75 of 77 in group 2. Of those with a history suggestive of reflux, 24-hour pH probe analysis was abnormal (reflux index $>10\%$) in 18 of 24 tested, whereas 29 of 56 undergoing endoscopy had histologic oesophagitis, with papillary lengthen-

ing and lymphocytic or eosinophilic infiltration. No difference was seen between groups. In addition, 29 children required treatment for chronic constipation, confirmed by radiography.

Forty-five duodenal biopsies were suitable for morphometric analysis. Although many were initially reported within normal limits, there was evidence of subtle enteropathy in most, with focal lymphocytic or eosinophilic infiltration and villous blunting and reduced crypt-villus ratio. Morphometric analysis of duodenal biopsies (Fig 4) confirmed subtle but significant abnormality, with a reduced median villous height compared with normal control subjects (282 vs 345 μm , $P < .0001$), modest increase in crypt depth (188 vs 164 μm , $P < .001$), and marked reduction in crypt:villus ratio (1.43 vs 2.1, $P < .0001$). There was overlap with the 1980s cases of CMSE, but the features were less severe in the children with multiple food allergy than in the classic lesion (Fig 4).

DISCUSSION

We initiated this study because we had noticed a substantial recent increase in the numbers of multiply-sensitized children referred to our clinic, which has provided a regional tertiary service in food allergy for over two decades. We had noted apparent overlap in the clinical presentation of children with multiple food allergy in clinical areas not classically viewed as allergy related. The relative predominance of non-IgE-mediated symptoms in our patients may reflect referral bias towards a pediatric gastroenterology center. Indeed, we encountered several cases in which the possibility of food allergy had been dismissed because skin pricks and RASTs were negative.

It is important to recognize that this study represents a retrospective analysis of a large cohort assessed in actual clinical practice in a busy tertiary center rather than a formal prospective study. We acknowledge difficulties imposed by geography in our assessment after delayed reaction and often have had to rely on telephone discussion between dietician and parent to determine whether genuine reaction is occurring. Although this represents a common means of assessment of non-IgE-mediated allergy²⁹ and is almost unavoidable in the real-life management of multiply-sensitized children because each challenge takes several days, it does mean that diagnosis may be less clear-cut than in the relatively straightforward immediate food allergy. We defer challenges in younger infants who had been unwell for a prolonged period before exclusion diet and did not challenge our peanut- or nut-allergic children before the age of 6 years. Although we acknowledge these reservations, we found evidence to support and extend previous reports of disturbed gut motility, minor immunodeficiency, and enteropathy in children with food allergies^{1-6,12,13,22,24} and thus suggest that pattern recognition may be helpful in suggesting the possibility of non-IgE-mediated food allergy.

The diagnosis of food allergy is easier in the child with rapid responses and positive skin prick tests, and such cases are rarely ascribed to maternal over-reporting. By contrast, in children without rapid responses, the diagnosis may not be consid-

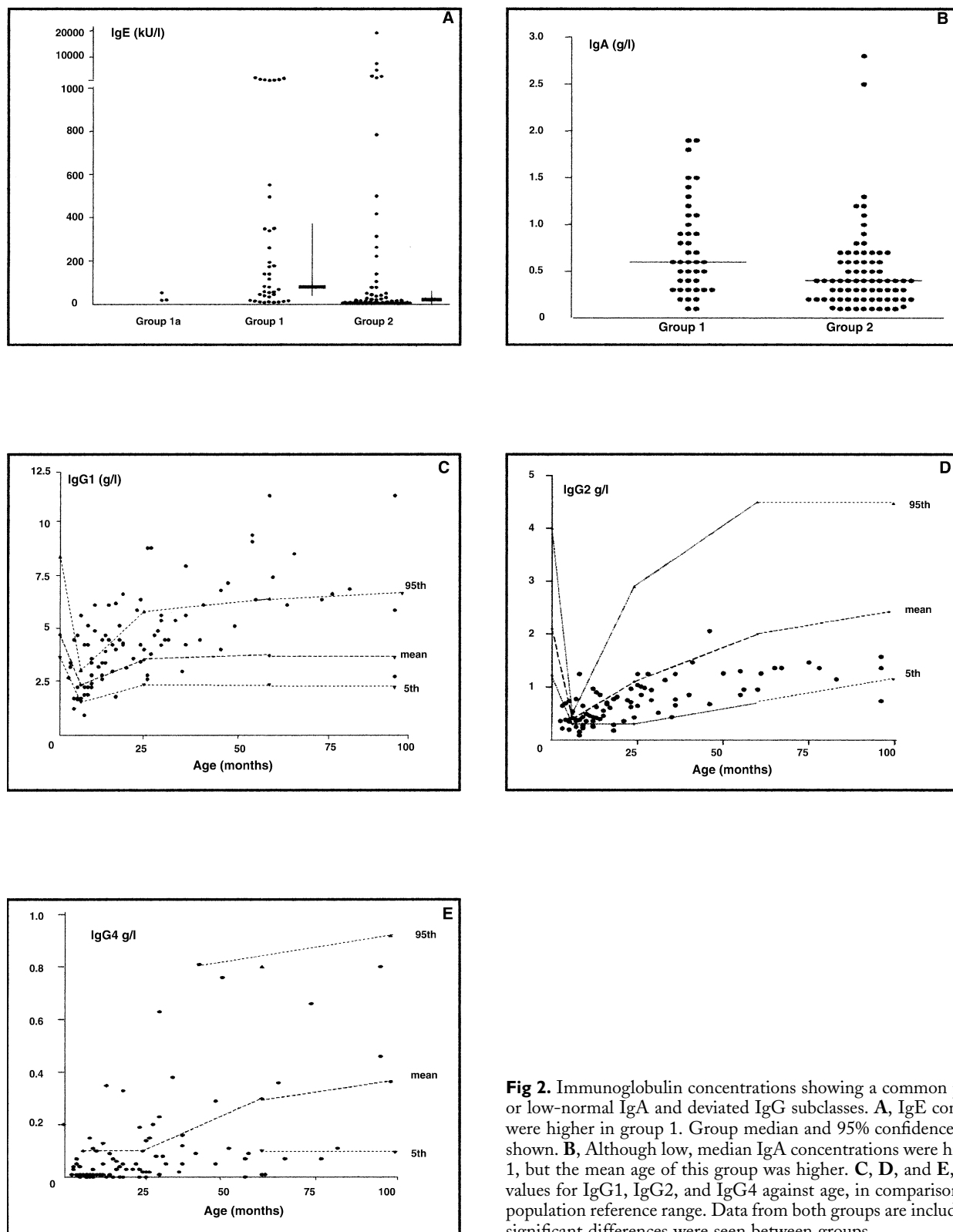


Fig 2. Immunoglobulin concentrations showing a common pattern of low or low-normal IgA and deviated IgG subclasses. **A**, IgE concentrations were higher in group 1. Group median and 95% confidence intervals are shown. **B**, Although low, median IgA concentrations were higher in group 1, but the mean age of this group was higher. **C**, **D**, and **E**, Individual values for IgG1, IgG2, and IgG4 against age, in comparison with UK population reference range. Data from both groups are included because no significant differences were seen between groups.

ered or conflict may arise between parents and pediatricians.¹ Our data suggest a recognizable constellation of findings, none diagnostic in itself, that may increase suspicion of genuine delayed food allergies. These include a family history of atopy or autoimmunity and a personal history of eczema, infant colic,

and sleep disturbance. In our study and others, findings of low IgA, IgG2, and IgG4 and deviation of peripheral lymphocyte subsets with high CD4 and CD19 and low CD8 and NK cell percentages appear associated with food allergy.^{9,22-24} Supportive clinical findings may include gastrointestinal dys-

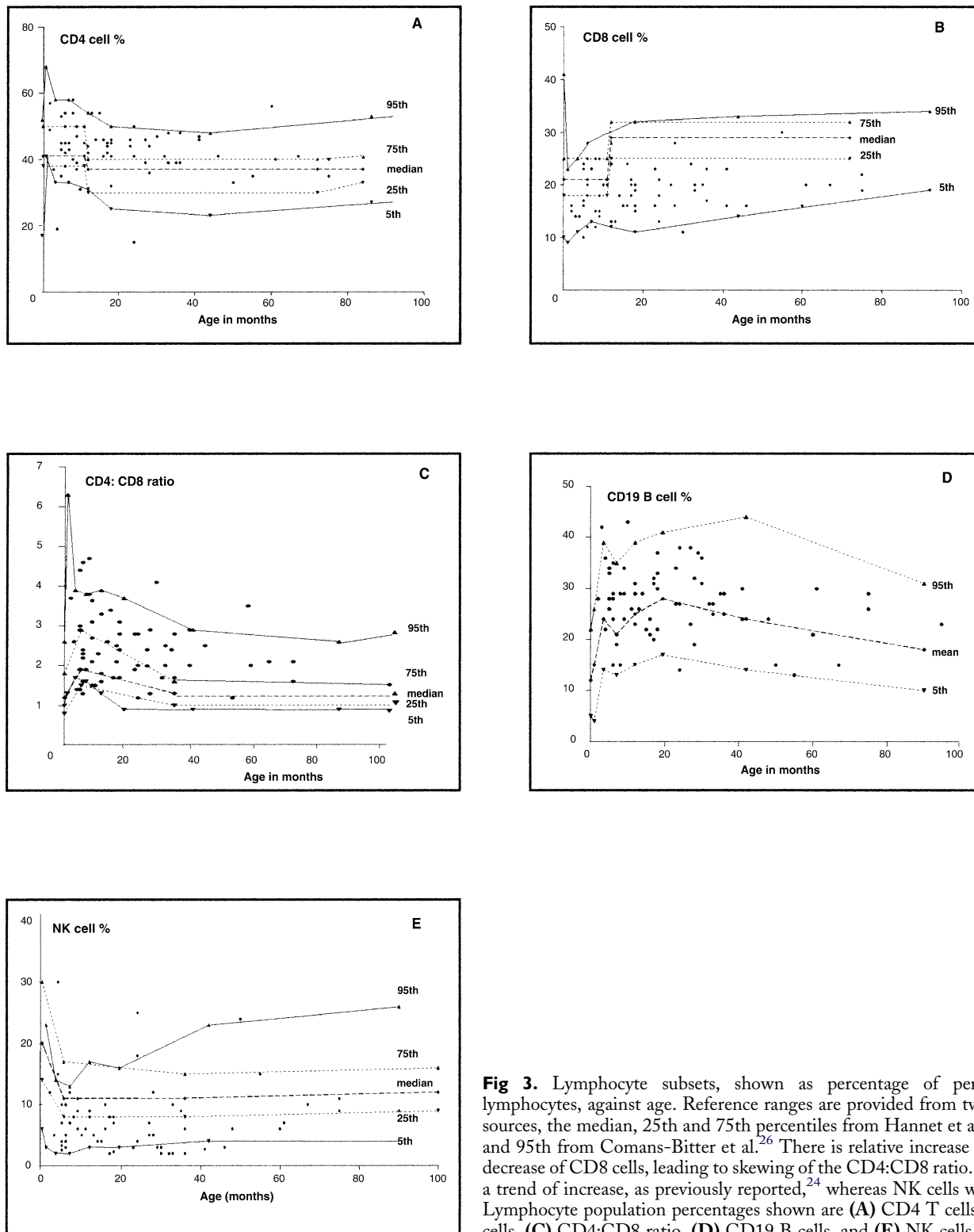


Fig 3. Lymphocyte subsets, shown as percentage of peripheral blood lymphocytes, against age. Reference ranges are provided from two recent sources, the median, 25th and 75th percentiles from Hannet et al.²⁷ and the 5th and 95th from Comans-Bitter et al.²⁶ There is relative increase of CD4 and decrease of CD8 cells, leading to skewing of the CD4:CD8 ratio. B cells showed a trend of increase, as previously reported,²⁴ whereas NK cells were reduced. Lymphocyte population percentages shown are (A) CD4 T cells, (B) CD8 T cells, (C) CD4:CD8 ratio, (D) CD19 B cells, and (E) NK cells.

motility with gastroesophageal reflux, constipation, or both, together with endoscopic evidence of esophagitis or low-grade enteropathy, biopsies showing infiltration of lymphocytes or eosinophils, and reduced crypt:villus ratios.^{1,3-6,12-14,22,24,30} As in previous reports,^{1-6,9,12-14} several of these children sensitized during exclusive breast-feeding. We speculate that this relates to failed induction of low-dose oral tolerance.^{9,31}

Family history is a highly important risk factor for childhood atopy, and maternal history has classically predominated.^{1,9} This study has also demonstrated a maternal dominance of autoimmunity. Links between immunodeficiency and autoimmunity have been recognized previously,³² and it is notable that the incidence of organ-specific autoimmunity in these mothers is already high. This raises the

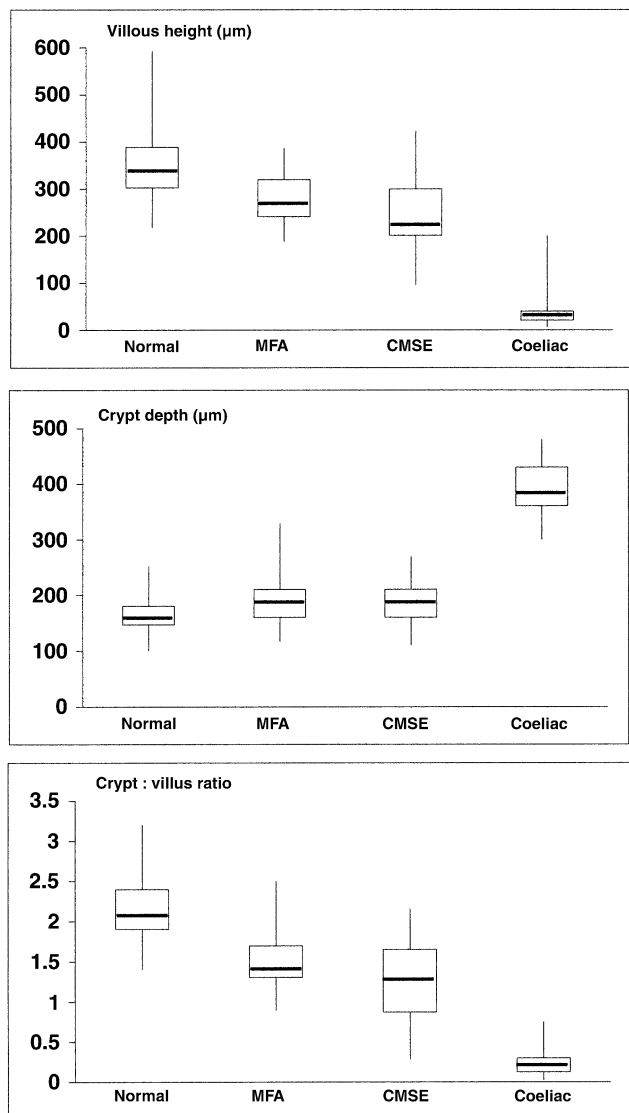


Fig 4. Results of morphometric analysis of duodenal biopsies, showing overall reduction of villous height and reduction of the crypt-villous ratio in the children with MFA compared with 47 normal controls, 46 children with classic CMSE and 17 children with celiac disease. Data are shown for (A) villous height, (B) crypt depth, and (C) crypt-villous ratio.

questions whether such children are also at increased risk of later autoimmunity and whether early introduction of appropriate exclusion diets may reduce that risk. Dietary exclusion reduces the incidence of autoimmunity in mice genetically predisposed to diabetes,³³ and there are ongoing prospective studies of milk exclusion in infants of diabetic mothers.³⁴ However further study is clearly necessary to clarify any potential links to later autoimmunity.

Although our children with immediate responses had higher mean IgE and a higher chance of positive food-specific IgE and skin prick tests, the other findings were common to both groups. IgE hyperresponsiveness is largely genetically determined, and increased responses relate to its excess production or enhanced signaling.^{9,35,36} Although the increased IgE in the group with immediate responses presumably accounted

for these reactions, over 90% of these children had additional delayed, likely non-IgE-mediated, symptoms that were identical to those seen in the group with delayed responses only. The immunological parameters shared by both groups may thus be more directly associated than is IgE with the predisposition to sensitization. This has previously been suggested at the whole-population level for low quartile IgA,²² which is linked with both infant food allergy and atopy.^{8,9,22} It is notable that shift of B cell isotype towards IgA is mediated by transforming growth factor- β , a cytokine pivotal in oral tolerance that is induced in the gut through innate immune responses to the flora^{9,19} and is reduced in the mucosa of infants with food allergy.³¹ The IgG subclass pattern in these children is characteristic of reduced T_H1 responses, which are also dependent on innate immune responses, through nuclear factor- κB .^{9,16} Innate immune cells promote survival of CD8 and NK cells by interleukin-15 production³⁷ and mediate antiviral responses by production of type-1 interferons.³⁸ We thus suggest that these apparently disparate findings can be explained by a common mechanism of an inadequately driven innate immune response, occurring as early life infectious exposures change.¹⁶ The concept that such a mechanism may apply in non-IgE-mediated infant allergy is supported by findings of reduction in eczema, without alteration of IgE responses, upon stimulation of innate immune responses through neonatal administration of probiotics.²⁰ Because there is evidence of important heterogeneity of innate immune responses to gut bacteria, with genetically determined reduction of nuclear factor- κB responses to the flora promoting the development of inflammatory bowel disease,³⁸⁻⁴⁰ we postulate that further study of the innate immune responses to enteric bacteria in multiply-sensitized children may give insights into the rising incidence of pediatric food allergies.

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