ABSENCE OF MILK-SPECIFIC IgE IN INFANTS WITH COW'S MILK PROTEIN–SENSITIVE ENTEROPATHY

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SUMMARY

Infants, one to 56-weeks-old, presenting with persistent diarrhoea were placed on a diet free of cow's milk protein which improved their clinical condition. Six weeks later, 67 infants were challenged with a low-lactose cow's milk formula and jejunal biopsy was taken before and 24-hours after challenge. On the basis of histological changes in the intestinal mucosa and development of clinical symptoms the infants were categorised into three groups: Group 1 (n = 16) with no clinical or mucosal abnormality, Group 2 (n = 20) with mucosal abnormality but lacking clinical symptoms, and Group 3 (n = 31) with manifestation of mucosal abnormality and clinical symptoms.

In addition to the total IgE the radioallergosorbent test (RAST) was performed on sera from the infants taken before and after milk provocation. The mean total serum IgE level ranged from 288 to 560 IU/ml. In Groups 2 and 3 the prechallenge serum IgE levels were significantly higher than the postchallenge levels but in Group 1 the levels remained unchanged on challenge. A positive RAST to milk proteins was observed in five infants (7.4%), that is, one in Group 2 and four in Group 3, of 67 infants studied.

In a survey of 405 consecutive paediatric-age patients admitted for a variety of symptoms, 90 were positive for RAST specific for milk proteins. Interestingly the majority of the patients positive for RAST presented with gastrointestinal ailments.

The measurement of specific IgE appears not to be a useful adjunct in the diagnosis of CMPSE in Malaysian children.

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INTRODUCTION

Gastrointestinal symptoms during early infancy provoked by cow's milk protein have attracted increasing attention in recent years. Cow's milk protein-sensitive enteropathy (CMPSE) is a common cause of persistent diarrhoea, malabsorption and failure to thrive in infancy. [1-5] The exclusion of cow's milk or milk-proteins especially β-lactoglobulin [6-8] from diet results in marked improvement but relapse may be induced by reintroduction of the offending proteins in the diet. The etiopathogenesis of cow's milk-induced mucosal atrophy remains uncertain but factors which cause malabsorption of macromolecules are contributory to development of hypersensitivity to the proteins. [9] In addition, recent evidence suggests that gastroenteritis in early infancy may predispose susceptible infants towards the disease. [10]

The diagnostic criteria for milk-protein intolerance proposed by Goldman *et al* [1] needed repeated positive provocations with improvement

after elimination of the milk from diet. The method was considered time-consuming and subject to anaphylactic risk. Present methods for diagnosis of milk intolerance require jejunal biopsy obtained before and after 24-hours after provocation with lactose-free milk, the degree of villious atrophy is indicative of the positive condition. Iyngkaran *et al*, [4,5] combined clinical observations with the patterns of change in mucosal histology to improve diagnosis. The method is invasive and may be hazardous and therefore, there is general interest in developing alternative ways of reaching the diagnosis.

Reaginic antibodies of the IgE class can now be assayed by extremely sensitive radioallergosorbent test. This method is increasingly employed to support the clinical diagnosis of food-antigen sensitivity in infants with eczema and other atopic syndromes. [11-13] The frequent occurrence of reaginic IgE antibodies in serum, [11,13] the presence of IgE-containing cells in jejunal submucosa, [14] the degranulation of mast cells on exposure to the provoking antigen [15] and the development of acute anaphylactic shock-like symptoms strongly suggest that immediate type allergic (Type I) reaction are taking place in some infants with milk-protein intolerance.

Thus, the present study was carried out to analyse the relevance of the IgE in facilitating the diagnosis of CMPSE in Malaysian infants.

MATERIALS AND METHODS

Patients

The series consisted of 67 infants who were clinically suspected to be intolerant of cow's milk protein. At admission cow's milk was eliminated from the diet and a formula free of cow's milk was substituted (Pregestimil or Nutramigen). If satisfactory response was obtained, judged by lack of symptoms and satisfactory weight gain, the infant was discharged and the parents instructed not to introduce any new food without prior permission. The weight gains were maintained satisfactorily on this diet and at the end of six to eight weeks the infants were readmitted for further tests including milk challenge studies. The study was also continued in a further 338 infants and children who were admitted for a variety of disorders. Blood samples collected for routine tests was used in this study.

Cow's mik challenge and jejunal biopsy

The challenge studies were performed as described previously. [5] The provocation test was carried out with low-lactose cow's milk (Lactolac V; Co-operative Condensfabriek Friesland, Holland). If no reaction occurred after an initial feeding of 5 ml, the volume was doubled hourly for the first four hours and subsequently three hours until the daily fluid requirements was met. After challenge the infants were closely monitored for clinical symptoms.

The biopsy specimens were taken with the Watson paediatric capsule at or just distal to the duodenojejunal junction under fluroscopic control. Jejunal biopsies were routinely taken before and 23-24 hours after milk challenge. Examination of the stools and jejunal biopsy before and after milk provocation was carried out as described previously. [4,5] The majority of the infants were found to be intolerant to cow's milk and the oral challenge caused substantial villous atrophy. The histological appearance of the biopsies were graded as previously reported. [4]

Serum IgE and milk IgE levels

Total IgE level determinations in the serum were performed by the competitive binding radio-immunoassay using commercial Phadebas kits (Pharmacia Diagnostics, Sweden).

The radioallergosorbent assay was carried out with milk allergen discs for use with Phadebas RAST on commercial kits (Pharmacia Diagnostics, Sweden).

RESULTS

Nature of clinical symptoms

The clinical data and the onset of symptoms after milk challenge in the 67 infants are summarised (Table I). The patients were classified into three major groups on the basis of histopathological changes in the gut mucosa and the development of clinical symptoms, especially diarrhoea, after oral mik provocation.

Group 1 (n = 16)

These infants did not develop mucosal abnormality or clinical symptoms on challenge, and may effectively be considered as the control for the present series. It is possible that the original diarrhoea and other clinical symptoms presented

TABLE I
CLINICAL FEATURES OF INFANTS WITH DIARRHOEA AT FIRST ADMISSION

	Group 1 (n = 16)	Group 2 (n = 30)	Group 3 (n = 31)
Age at admission (weeks)	23.5 (1-54*)	6.0 (0.5-20)	8.8 (1-56)
Ratio of Boys : Girls	11 : 5	13 : 7	12 : 19
Chinese/Indian/Malays/Orang Asli/ Mixed	7/2/5/1/1 +	11/3/5/1/0	15/8/6/1/1‡
Birth weight (kg)	3.04 (2.3-4.2)	3.0 (1.4-4.1)	2.9 (2-3.9)
Weight at admission (kg)	5.9 (3.1-8.7)	3.6 (1.5-4.5)	3.6 (1.9-7.4)
Age at onset of symptoms (days)	22.8 (1-52)	5.1 (0.5-20)	34.8 (2-3.75)
Total duration of diarrhoea (days)	8.1 (1-30)	8.3 (1-35)	17.2 (1-75)
Weight 8 weeks after admission and before challenge (kg)	7.1 (4.3-10)	5.2 (4-10)	5.6 (3.6-8.4)
Clinical symptoms on challenge.	None	None	Diarrhoea §
Mucosal histology rating, prechallenge/post challenge	2.6 (0.6)/ 2.3 (0-6) @	2.3 (0.7)/ 8.3 (5-11)	2.2 (0.6)/ 10.1 (7.5-14)

* Mean range is shown in parenthesis, + Mixed race: Malay father and Chinese mother.

‡ Indian father and Chinese mother.

§ Reintroduction of milk caused diarrhoea within four hours in 15 infants and 5-24 hours in 9 infants; clinical symptoms (including diarrhoea) were delayed (1-14 days) in seven infants.

@ Mucosal histology was numerically rated as follows:
normal 0-3.9, slightly abnormal 4-7.9, moderately abnormal 8-11.9, and severely abnormal 12-16.
Postchallenge biopsy was taken 23-24 hours after initial (prechallenge) biopsy (Iyngkaran *et al* 1978).

might have been due to causes other than cow's milk, but the short period of nutritional management appeared to have alleviated the causation factors.

Group 2 (n = 20)

These infants developed moderate villous atrophy on challenge with milk but developed no clinical symptoms. Follow-up studies showed that majority of these infants tolerated the milk but some became intolerant to milk in a chronic fashion over months and these few required brief dietary management later.

Group 3 (n = 31)

These infants developed severe atrophy on challenge and had various clinical symptoms (Table II). The milk provocation produced diarrhoea within four hours in 15 infants and 5-24 hours in nine infants; the symptoms were delayed in appearance by 1-14 days in seven infants.

Although the range of age at admission was similar for the three Groups, the mean age was similar for Group 2 and 3 but higher in Group 1. More males than females were involved in Group 1 and 2 and the sex ratio was reversed in Group 3. In general the Chinese and Malays were more susceptible, but the Indians also formed a significant number in Group 3.

TABLE II
CLINICAL SYMPTOMS OBSERVED AT ADMISSION AND WHEN CHALLENGED WITH MILK

Symptoms	Group 1 (n = 16)	Group 2 (n = 20)	Group 3 (n = 31)	
	At admission	At admission	At admission	Post challenge
Diarrhoea	16	20	31	31
Vomiting	8	10	6	11
Urticaria	0	0	5	3
Allergic rhinitis	0	1	2	1
Perianal excoriation	5	11	6	1

In Groups 1 and 2, no overt clinical symptoms were seen after challenge.

Serum total IgE

A surprising observation was the high IgE levels which in some infants attained levels of 20,000 IU/ml (Table III). This appears to be a normal

TABLE III
TOTAL SERUM IgE AND MILK-SPECIFIC IgE IN 67 INFANTS

Group	N	Total IgE*		Percent change	Milk-IgE : No positive	
		Pre-challenge	Post-challenge		Pre-challenge	Post-challenge
1	16	295 ± 5 (± 21) [30 – 2200]	288 ± 5 (± 22) [30 – 3800]	−2.4	0	0
2	20	357 ± 6 (± 39) [30 – 20,000]	304 ± 7 (± 49) [30 – 7000]	−14.9	1 (Grade 1)	1 (Grade 1)
3	31	560 ± 6 (± 31) [30 – 20,000]	299 ± 6 (± 37) [30 – 5200]	−46.6	1 (Grade 1) 3 (Grade 2)	1 (Grade 1) 1 (Grade 2)

* Geometric mean ± ; standard deviation (±); range in parenthesis [ ].

feature of infants in the tropics. The post-challenge mean serum IgE levels decreased in Groups 2 and 3 but were unaffected in Group 1. In Group 3 the decrease occurred in 15 of 31 infants (48 %), in Group 2 it occurred in six of 20 infants (30 %) and in Group 1 it occurred in four of 16 infants (20%). The mean IgE level before and after challenge decreased by 14.9% in Group 2 and 46.6% in Group 3.

Anti-milk allergen RAST test in CMPSE

Of 67 infants tested only five infants gave a positive result which included one in Group 2 and four in Group 3 (Table III). During challenge the positive result became negative in two infants. The RAST class in the positive infants was indicated at low or moderate level of IgE concentration.

Anti-milk IgE in infants and children

A total of 405 serum samples from infants and children were analysed for anti-milk IgE by the RAST assay. Ninety serum samples (22.2 %) were positive for milk allergens. Table IV summarises the clinical diagnosis of the 90 patients. It is interesting that IgE-antibodies to milk proteins may be present in a wide variety of diseases. Children with milk intolerance represent 7.2% of the total sample. In general, the milk specific Ig-E antibodies were common in patients with intestinal problems including acute gastroenteritis, Giardia infection, helminthic infestation and malnutrition.

TABLE IV
SUMMARY OF 90 SERUM POSITIVE FOR ANTI-MILK IgE IN 405 PATIENTS ANALYSED USING THE RAST TEST

Diagnosis	Number positive	Percent of Total positive
Normal	12	13.3
Viral Diarrhoea	2	2.2
Acute Gastroenteritis	15	16.7
Milk Intolerance	29	32.2
Malnutrition/Helminths	10	11.1
Giardia	9	10.0
Intestinal pneumatosis	2	2.2
Immunodeficiency	2	2.2
Thalassemia	2	2.2
Anaemia	1	1.1
Lipid dystrophy	1	1.1
Systemic lupus erythromatosis	1	1.1
Bronchiostasis	1	1.1
Liver cirrhosis	1	1.1
Immunoblastic tumour	1	1.1
Epilepsy	1	1.1

DISCUSSION

The involvement of IgE-mediated immediate type I hypersensitivity reactions in milk intolerance have been previously recognised. [11,15] In our study only five out of 67 patients tested (7.5%) gave a positive RAST test to anti-milk IgE and in all these cases the reaction was of a moderate or low grade type. Surprisingly only two of 15 infants who developed symptoms immediately after oral milk provocation gave a positive result on the RAST test. Although high serum total IgE was present in majority of the infants, it clearly was not associated with milk allergy. The presence of high IgE levels in the tropics is a recognised fact in both adults and infants [16] and in general is associated with intestinal parasitic infection (unpublished). The reason for the very high IgE in some of the infants is not clear since obvious intestinal infection was not noticed. The total serum IgE level was unchanged in Group

1 but decreased to 14.9% in Group 2 and 46.6% in Group 3 after challenge. The consumption of IgE on milk challenge was also reflected in the RAST where two patients in Group 3 became negative after challenge. How the non-specific and specific IgE is used up remains unclear but it is possible that challenge may result in recruitment of new mast cells thus making available new IgE receptors.

In the Malaysian infants with CMPSE the RAST test does not appear to be useful in diagnosis. In the experience of Hoffman and Haddard, [13] the test was useful in identifying 50% of the cases especially those cases which developed acute clinical symptoms. The difference in the proportion of infants responding to the test in Malaysia and in countries located in temperate regions is not clear. The absence of milk-specific IgE in serum does not preclude the probability of locally produced IgE acting at the mucosal level. However, this appears unlikely since the high serum IgE would have interfered by saturating mast cell IgE receptors. [17] The most striking observation from this study was that those infants with clinical histories of acute symptoms did not respond to the RAST test following ingestion of the offending milk protein. Our data is in agreement with that of Kletter *et al* [11] but the reason for the noted low frequency of milk specific IgE in patients with acute reaction are obscure. It is possible that at this age of development of the infant, the reagins are bound to mast cells in the tissue, more completely than later in life. However, we have no evidence for this and furthermore, the presence of high levels of circulating serum IgE argues against this concept. However, it seems likely that the high level of non-specific IgE interferes with the binding of milk-specific IgE to mast cells and its synthesis.

The present observations raise important basic considerations in regards to the immunological mechanisms associated with the development of villious atrophy in infants intolerant to milk proteins. In many infants the Arthus-type allergic reaction (Type III) have been implicated by the demonstration of antigen-antibody complexes which fix complement. Thus, on challenge with milk the level of complement C_3 decreased [18] and electrophoretically altered fractions of C_3 appeared in serum. [19] Bock *et al* [20] used peroxidase conjugated antisera to milk proteins to demonstrate the presence of phologistic immune complex deposits in the basement membrane of the bowel lamina propria and goblet cells. This suggests the involvement of IgE-mediated complement-consuming immune reactions in the development of the damage in the intestinal mucosa. [18] However, the presence of anti-milk protein antibodies in serum are unreliable signs of milk intolerance, because Gunther *et al* [21] found that 98% of 286 sera from normal infants between seven and 97 weeks of age (average 1.9 years) had measurable antibodies against the one or more cow's milk proteins. Furthermore, our observations show the presence of anti-milk protein IgE antibodies in infants diagnosed for a wide variety of diseases especially those involving the intestine; significantly not one of these infants had or developed milk intolerance.

Other types of hypersensitivity reactions have also been recognised in CMPSE. There is objective evidence for the presence of specific cell-mediated immunity in infants with milk intolerance and therefore, various techniques have been designed to provide a useful assay. For instance, Neutrophil chemotaxis is depressed in infants with cow's milk and/or soy protein intolerance [22] and the leucocyte migration inhibition factor is significantly increased in CMPSE. [23,24] The incorporation of triatied thymidine is increased *in vitro* in the presence of milk proteins. [6, 25.]

The above discussion leads to the inescapable suggestion that at least three types of immunological mechanisms are associated with the villious atrophy in infants intolerant to milk proteins. In the Malaysian context, and certainly in the present series, IgE-mediated Type 1 reactions are of minor importance. We have previously [18] reported that the Type III immune-complex mediated responses are observed in 60-70% of the infants with mucosal damage. We have unpublished data which strongly suggests the involvement of Type IV cell-mediated responses in majority of the infants studied. Thus, Type III and IV immune hypersensitivity should be looked for in Malaysian infants with CMPSE.

The fundamental question of why some infants develop CMPSE and others do not remains an enigma. It is apparent that although factors like transient or selective IgA deficiency, acute gastroenteritis, damaged mucosal barrier, lysosomal dysfunction, abnormal intraluminal digestion or T-cell deficiency [9,10,26] may predispose towards CMPSE yet the pathological absorption of macromolecules by itself is insufficient to evoke the

disease. It appears certain that other contributing factors are necessary but the nature of these has yet to be specifically identified.

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