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TRANSUDATION OF IMMUNOGLOBULINS INTO DUODENAL JUICE OF INFANTS WITH COW'S MILK PROTEIN SENSITIVE ENTEROPATHY

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SUMMARY

Eighteen infants clinically suspected to be intolerant of cow's milk were placed on a milk-free formula and six to eight weeks later were orally challenged with cow's milk. Following challenge three groups were recognised. Group A: Four infants tolerated oral feeds of cow's milk and lacked mucosal abnormality or clinical symptoms. Group B: Seven infants had mucosal deterioration but lacked clinical symptoms and tolerated cow's milk. Group C: Seven infants had mucosal abnormality, developed clinical symptoms and were intolerant of cow's milk.

The intestinal transudation of IgA was increased in Group A and unchanged in Group B and C : the IgM levels in the duodenal juice was increased in Group A and B but unchanged in Group C : the IgG levels in the juice were increased in all Groups following challenge. It appears that increased transmission of IgA and IgM or IgM alone in the duodenal juice is associated with lack of development of clinical symptoms. Symptoms are present in infants in whom the IgA and IgM levels in duodenal juice remained unchanged after challenge.

It is suggested that patients responding to cow's milk challenge with intestinal production of IgA and IgM (or IgM alone) are able to counter balance the deleterious mechanisms leading to clinical cow's milk intolerance whereas those who, for some unknown reason, do not mount a secretory immune response become ill.

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INTRODUCTION

Intolerance to cows milk usually becomes apparent within the first month of life in some infants. Several clinical features including skin, sinopulmonary and gastrointestinal manifestations have been recognised in association with the disorder but the majority of the infants present with diarrhoea and vomiting. [1,2] The clinical symptoms rapidly disappear when the infants are placed on an elimination diet free of cow's milk proteins. Rechallenge with cow's milk results in intestinal mucosal damage and recurrence of the symptoms. [2,4] There is now increasing evidence to suggest that several types of immune hypersensitivity reactions are involved, independently or together, in the manifestation of cow's milk protein-sensitive enteropathy (CMPSE). [5,6]

There are few studies on the immunoglobulin concentration of the intestinal juice in CMPSE. An important morphological feature in CMPSE is the damage induced by milk to the small bowel mucosa. Thus, the changes in the concentration of immunoglobulin in the duodenal juice before and after milk challenge are of interest. It would be expected that the normal immunological homeostasis maintained in the mucosa through a critical balance between various immunoglobulin components would be altered.

Savilahti [7] (1973) studied the intestinal juice and fecal extract in infants intolerant to cow's milk. He found increases of juice IgA and IgM concentration during challenge. In infants with chiefly gastro-intestinal symptoms cow's milk challenge caused a rise in IgA and IgM cells in small bowel biopsy but no consistent change in IgE cells. [7,8] Similar challenge studies, [5,9] however, demonstrated increases in IgE and IgM cells, and degranulation of mast cells in infants with typical reaginic reactions; in these patients the population of IgA-cells in the mucosa remained unchanged after challenge. The present study reports the changes in the concentration of immunoglobulins in the duodenal juice following milk challenge in three categories of infants.

MATERIALS AND METHODS

Patients

Eighteen infants clinically suspected to be intolerant to cow's milk protein were studied. At admission, the infants were placed on a formula free of cow's milk proteins e.g. Pregestimil, Prosobee and Nutramigen, and when improvement was observed, assessed clinically by lack of symptoms and satisfactory weight gain, the infants were discharged. The parents were instructed not to introduce any new food items without our knowledge. The infants maintained satisfactory weight gains on this diet and at the end of 6 to 8 weeks the infants were readmitted for further tests (Table 1).

Cow's milk challenge and jejunal biopsy

The challenge studies were carried out as previously described. [2] Briefly, the infants were challenged with 5 mls of low-lactose cow's milk. If no reactions were observed, the volume was doubled hourly for the first 4 hours and then 3-hourly until total daily fluid requirements were met. The biopsy specimens were taken with the Watson paediatric capsule at or just distal to the duodeno-jejunal junction under fluoroscopic control. Jejunal biopsy appearence was classified as

TABLE I
CLINICAL FEATURES OF 18 INFANTS WITH DIARRHOEA AT FIRST ADMISSION

Category	Group A	Group B	Group C
Number of infants	4	7	7
Age at admission, weeks	5.8 (1-12)[a]	5.2 (0.5-16)	16.7 (4-56)
Sex, males/females	3/1	7/0	4/3
Race, Chinese/Indian/Malays	2/0/2	3/1/3	4/3/0
Birth weight, kg.	3.4 (2.3 - 3.3)	3.2 (2.7 - 4)	3.0 (2.3 - 3.8)
Weight at admission, kg.	3.6 (2.7 - 9.3)	3.4 (2.7 - 4.5)	5.8 (2.7 - 9.5)
Age of onset, weeks	5.3 (1 - 8)	4.9 (0.5 - 16)	12.3 (0.5 - 53)
Total duration of diarrhoea, days	9.3 (1 - 28)	3.4 (1 - 14)	17.5 (1 - 42)
Weight 8 weeks after admission and before challenge, kg.	5.4 (4.3 - 6.79)	4.6 (4.2 - 4.9)	6.6 (4.3 - 9.6)
Clinical symptoms	None	None	Diarrhoea
Mucosal histology rating, Prechallenge/Postchallenge	6.9 (4-9)/6.9 (4-9)[b]	2.5 (0-4.5)/8.3 (7-10.5)	1 (0-3)/9.4 (8-11)

(a) Mean and parenthesis shows range of values.

(b) Mucosal histology was numerically rated as follows: Normal 0-3, mildly abnormal 4-7, moderately abnormal 8-11, and severely abnormal 11-16. Postchallenge biopsy was taken 21-24 hours after the initial (prechallenge) biopsy. See Iyngkaran *et al* 1978 for details.

TABLE II

IMMUNOGLOBULIN CONCENTRATIONS IN SERUM AND DUODENAL JUICE BEFORE AND AFTER 24 HOURS FOLLOWING CHALLENGE WITH COWS MILK

Groups	Nos. Studied		Immunoglobulin Class IU/ml[a]						Ratio[b]			
			G		A		M		G/A		G/M	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
ADULTS		Serum										
	1,489	mean	156		132		270		1.20		0.70	
INFANTS		Serum										
Group A	4	mean	72	71	18	23	79	78	5.30	4.10	1.06	1.11
		± SD[c]	18	18	14	13	34	39	2.90	2.60	0.53	0.63
		% change[d]		-1		+28		-1		-23		+5
		Duodenal juice										
		mean	0.64	1.7	4.40	6.95	4.30	5.30	0.215	0.516	0.164	0.335
		± SD	0.34	1.2	2.62	5.90	1.90	2.16	0.179	0.510	0.075	0.255
		% change		+166		+58		+23		+140		+104
		Serum										
Group B	7	mean	99	77	13	13	113	106	7.00[e]	5.30	1.08	0.81
		± SE	40	21	11	11	48	42	5.80	2.90	0.79	0.30
		% change		-22		0		-6		-24		-25
		Duodenal juice										
		mean	0.93	1.48	4.80	3.91	6.70	9.72	0.236	0.381	0.027	0.060
		± SD	0.41	1.98	4.30	2.89	7.40	9.61	0.173	0.360	0.034	0.079
		% change		+59		-19		+45		+61		+122
		Serum										
Group C	7	mean	77	71	19	19	86	82	4.30	3.80	0.96	0.95
		± SD	29	21	6	5	24	23	1.73	1.40	0.47	0.44
		% change		-8		0		-5		12		-1
		Duodenal juice										
		mean	0.42	1.33	4.37	4.65	5.04	4.74	0.113	0.351	0.140	0.228
		± SD	0.28	1.16	2.42	3.35	3.27	2.97	0.100	0.310	0.120	0.201
		% change		+217		+6.4		-6		+211		+62

[a] To convert to g/l multiply values by 0.8333 for IgG, 0.1515 for IgA and 0.0699 for IgM; Prechallenge (Pre) and postchallenge (Post) with low-lactose cows milk.

[b] The concentration ratios are calculated from the immunoglobulin values in IU/ml; multiply these values by 5.5 for IgG:IgA and 11.9 for IgG: IgM ratio to compare with data calculated from immunoglobulins presented in g/l.

[c] Standard deviation.

[d] Negative sign indicates decrease and positive sign indicates an increase of postchallenge observations over that of prechallenge observations.

[e] Two infants who had transitory IgA deficiency were excluded because they gave very high IgG:IgA concentration ratios.

previously detailed. [2]

Duodenal juice was collected at the time of the biopsy via the biopsy tube. The juice was stored at -20°C after the addition of 1 mg antitrypsin per ml juice. (Sigma, St. Louis).

Mucosal imprints were routinely taken to exclude *Giardia lamblia* infections. [10] The stools before and after challenge of the infants were routinely examined for enteropathogenic bacteria and virus. If diarrhoea occurred on milk challenge the clinitest method was used to identify secondary sugar intolerance.

Assay for immunoglobulin and complement

As previously reported [11] Immunoglobulin G, A, M and D, complement C_3 and D_4 and C_3 activator levels were each quantitated by the radial immunodiffusion technique of Mancini *et al* [12] on specific low-concentration-immunoplates which were obtained commercially (Behringwerke, Germany) : the standards used were also from Behringwerke. The duodenal juice IgA was assayed against monomeric IgA standards and the results are presented as such. However, the monomeric IgA standard gave readings which were 2.78 times greater than a standard of secretory IgA. (Kindly supplied by Professor J. P. Vaerman, Belgium).

RESULTS

Clinical symptoms following cow's milk challenge

Table I summarise the general information on the patients studied and the onset of symptoms after milk challenge. The patients were classified into 3 groups on the basis of histopathological changes in the gut mucosa and the development of clinical symptoms especially diarrhoea and vomiting following milk challenge.

Group A: Four infants had no change in mucosal abnormality or clinical symptoms. These patients clinically tolerated oral feeds of cow's milk and could be considered as the control for the present series.

Group B: Seven infants developed mucosal abnormality but lacked clinical symptoms. The patients clinically tolerated cow's milk.

Group C: Seven infants developed mucosal abnormality and also clinical symptoms. These patients were intolerant of cow's milk.

Enteropathogens were isolated from stool during illness at initial admission from 1 (*Salmonella*) of 4 infants in Group A, 3 (1 *Salmonella* 2 *E. coli 0126/B16*) of 7 infants in Group B and 2 (*Salmonella*) of 7 infants in Group C. *Rotavirus* and *Giardia* was not identified in any of the infants in this series. An analysis of the stools obtained at prechallenge and also postchallenge shows that none of the infants in Group A, 1 infant each from Group B and C had retained the *Salmonella* infection: the infection in others had cleared.

Two infants in Group B during the study had transient selective IgA deficiency (< 0.05g/l or 3.3IU/ml) but these infants 3 month later were found to have low serum levels of IgA (1.5 - 6 g/l or 10 - 40 IU/ml).

Immunoglobulins in the serum

The changes in immunoglobulin concentration in serum before and after challenge with cow's milk are summarised in Table 2. Following challenge the mean serum IgA levels increased in Group A and were unchanged in Groups B and C. On the other hand, the serum IgM and IgG levels were unchanged in Group A and slightly decreased in Groups B and C. The IgG : IgA ratio decreased in the 3 groups and the IgG : IgM ratio decreased in Group B following challenge ; in Groups A and C the IgG : IgM ratio remained after challenge.

The immunoglobulin IgD and complement C_3 and C_4 and C_3 activator were also analysed in the serum. In all the sera tested IgD was absent (sensitivity of assay 12.5 IU/ml). In Groups A, B and C, the complement C_3 level in prechallenge sera was 63.4, 68.3 and 68.4 mg/100 ml respectively and these levels decreased by 3 - 4 percent in all three groups in the postchallenge sera. In the 3 groups the complement C_4 in prechallenge sera was 21, 36.4 and 37.4 mg/100 ml respectively. These values decreased by 33 and 11 percent in Groups A and B respectively and increased by 20 percent in Group C. Similarly, serum C_3 activator levels were 11.2, 15.2 and 16 mg/100 ml in Groups A, B and C, respectively and these levels increased by 40, 7.2 and 5.6 percent, respectively.

Immunoglobulin levels in the duodenal juice

Following challenge the IgG concentration of juice was increased in all 3 groups and the IgM concentration was increased in Group A and B and decreased slightly in Group C. The IgG : IgA and IgG : IgM concentration ratios were increased in all 3 groups.

The duodenal juice was also assayed for IgD,

complement C_3 and C_4 and C_3 activator. IgD was present in prechallenge juice at the concentration of 31 to 71 IU/ml and decreased by 19, 41 and 81 percent in the juice following challenge in Groups A, B and C, respectively. The complement C_3 ranged from 8 to 67 mg/100 ml in the juice and it was unchanged in Group A, increased in Group B and decreased in Group C : complement C_4 and complement C_3 activator were absent in the juice.

DISCUSSION

The intestinal transudation of IgA was increased in Group A and unchanged in Group B and C. In serum, too, the IgA level increased in Group A but was unchanged in Group B and C. The transudation of IgM was increased in Group A and B but unchanged in Group C after milk challenge. The serum IgM levels were unaltered by milk challenge in all 3 groups. It appears that increased transmission of IgA and IgM (or IgM) into the juice is associated with lack of development of clinical symptoms following milk challenge; clinical symptoms develop in patients in whom the juice IgM and IgA remained unchanged following challenge as in Group C. These observations are consistent with those reported by Shiner *et al* [5,9] who found no change after milk challenge in the population of IgA-secreting cells in the gut mucosa of infants who reacted immediately to the challenge. On the other hand, in infants in whom the clinical symptoms were delayed, Savilahti [7] recorded an increase in IgA and IgM concentration in the intestinal fluid and an increase in IgA and IgM-cells in the gut muscosa following milk challenge.

Several workers have suggested that IgA deficiency may form the primary predisposing factor in the development of milk intolerance. [1,13] The present work shows no difference in the mean level IgA concentration in the duodenal juice following milk challenge; however, it shows that the challenge does not evoke enhanced IgA in infants who develop severe mucosal damage. Following challenge the presence of enhanced level of IgM in Group B, and not Group C, suggests that mucosal damage alone is not responsible for the absence of change in IgA concentration in the juice. It may suggest that milk proteins of the challenge do not evoke the appropriate cells in the gut mucosa for increased production of IgA. Two infants in Group B had transient IgA deficiency and surprisingly none in Group C infants who showed severe reactions to milk challenge.

The increased concentration of IgG in Group B and C may be related to the mucosal damage following milk challenge. However, subcellular changes may account for the enhanced transudation of IgG in Group A patients who showed no clinical symptoms or mucosal abnormality following milk challenge. Similarly, Morin *et al* [14] noted no histological deterioration after introduction of milk in 2 patients with significant changes in both the one-hour blood-xylose level and disaccharidase activities in gut mucosa following milk challenge. In another study we noted a decrease in three disaccharidase enzyme levels in the small bowel mucosa in the absence of microscope change in mucosal histology in 6 infants following intake of milk. [4] Thus the above examples indicate that present methods fail to diagnose enteropathy in infants who develope ultrastructural lesions in the small bowel mucosa but fail to develop clinical symptoms and mucosal deterioration following reintroduction of milk.

A typical feature of CMPSE is that it resolves spontaneously within 2 years. [1] The patients in Group A had ages ranging from 1 to 12 weeks which was similar to the range of ages for Group B and C. Followup studies show that Group A infants continued to clinically tolerate the milk and did not require special diets. It appears that subcellular changes do not pose serious problem under normal circumstances. In Group B some infants clinically tolerated the milk but others later required dietary management to control the intolerance to milk. Thus, these infants become intolerant to milk in a chronic fashion over months and the treatment with cow's milk-free diet rapidly abrogates the deterioration of the mucosa and promotes quick recovery in some and slow recovery in other infants of this group. Group C infants needed dietary management for about a year after which they clinically tolerated the milk.

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