Effect of continued feeding of cows' milk on asymptomatic infants with milk protein sensitive enteropathy

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SUMMARY The clinical response and the histological changes in the mucosa of the small bowel in response to continued feeding with cows' milk protein were assessed over a period of 2-6 weeks in 24 infants who had shown histological changes without immediate clinical symptoms after challenge with a diet containing cows' milk protein. Twenty of the 24 infants (83%) thrived well on cows' milk protein. Jejunal biopsy specimens taken six to eight weeks after the initial biopsy showed histological improvement in all 20 infants compared with biopsy specimens taken soon after the challenge, which had shown mucosal damage. The mucosa had returned to normal in 12, was mildly abnormal in seven, and moderately abnormal in one. Corresponding improvements in the activities of mucosal enzymes were seen. In four of the 24 infants (17%) symptoms developed between three and six weeks. Histological examination of the jejunal biopsy specimens showed that mucosal damage had progressed in two, and remained the same in two; moreover, the disaccharidase activities remained depressed. The present study shows that most infants with enteropathy caused by sensitivity to cows' milk protein but without clinical symptoms develop tolerance to the protein and the mucosa returns to normal despite continued feeding with cows' milk protein.

Villous atrophy of the proximal jejunum is a consistent histological finding in infants with enteropathy caused by sensitivity to cows' milk protein after they have been challenged with the protein.¹⁻⁴ Some infants with damaged mucosa, however, develop symptoms, and others do not. The symptoms, especially vomiting and diarrhoea, may develop either rapidly (within 48 hours) or slowly (over a period of 2-30 days). Some infants remain asymptomatic despite mucosal damage and continued feeding with cows' milk protein.³⁻⁴ In practice this group is not recognised as being intolerant to cows' milk protein. Hence if one of these infants develops diarrhoea weeks later there is confusion as to the cause of the symptoms and precious time is wasted by exploratory laboratory investigations.

Infants with symptoms following challenge with cows' milk protein need to have the protein eliminated from their diet before they will improve. The dietary management of infants with mucosal damage but without immediate symptoms, however, poses a

problem; it is not clear whether they should be maintained on an exclusion diet or should receive a conventional cows' milk formula.

The present study was undertaken to determine the effect of continued feeding of cows' milk protein on the clinical course and the histological state of the jejunal mucosa of infants who sustained small bowel mucosal damage but who did not have early (within 48 hours) clinical symptoms after challenge with cows' milk protein.

Patients and methods

We used the histological criteria for the diagnosis of enteropathy caused by cows' milk protein that we have described previously.⁴ A total of 35 infants with diarrhoeal disease thought to be caused by intolerance to cows' milk protein were studied. After appropriate correction of fluid and electrolyte imbalances⁵⁻⁶ the infants were given a predigested formula feed that did not contain cows' milk protein.⁷ The infants were kept under observation

until they were feeding well, gaining weight normally, and had normal stools. They were then discharged home, the mothers having been supplied with the appropriate prehydrolysed formulas and instructed not to offer any other milk.

The infants were readmitted six weeks later for challenge studies as previously described.⁴⁻⁸ The infant was offered 5 ml of the low lactose cows' milk (AL 110) and if no reaction occurred the volume was doubled hourly for the first four hours, then three hourly until the total fluid requirements had been met. If the infants developed symptoms the challenge was stopped and the infants were given an oral rehydrating solution after which they were given a protein hydrolysate formula.

Jejunal biopsy specimens were taken only after informed consent was obtained from the parents. The biopsy specimens were taken through the mouth just distal to the duodenojejunal flexure with the Watson paediatric capsule immediately before the challenge with cows' milk protein and then 20-24 hours later whether or not symptoms developed. The morphological changes in the mucosa were scored as previously described.⁴⁻⁸

Infants who had histological reactions but no symptoms (group 2), and infants without clinical and

histological reactions to the challenge (group 3) were studied further. They were maintained on cows' milk (for example, S-26 (Wyeth)) and two to six weeks later were readmitted for complete clinical assessment and a third biopsy. Four infants in group 2 who had relapsed were given a prehydrolysed formula and then had a further challenge about six weeks later.

Routine laboratory investigations performed on initial admission, before and after the challenge after six weeks of diet free of cows' milk protein, and at the second readmission for the third biopsy two to six weeks later included measurement of haemoglobin concentration, total and differential white cell counts, culture of the stools for bacterial and viral enteropathogens, and microscopic examination of the stool for parasitic infection. The mucosal imprint described by Kamath and Murugasu for the diagnosis of infection by *Giardia lamblia* was also carried out.⁹ Mucosal disaccharidases and alkaline phosphatase activities were estimated in the jejunal biopsy specimens as previously described.¹⁰⁻¹¹ If diarrhoea occurred during the initial illness or after the challenge the Clinitest method was used to detect secondary sugar intolerance.¹²

Figure Flow diagram showing design of study.

Results

The figure summarises the plan and the results of the studies. Thirty five infants were challenged with cows' milk protein. From the clinical responses and histological changes in the intestinal mucosa three groups were defined. Group 1 comprised seven infants who developed both clinical and histological signs. These infants were not investigated further. Group 2 comprised 24 infants whose biopsy specimens showed histological changes but who had no symptoms, and group 3 comprised four infants who showed no clinical or histological reaction to cows' milk protein. According to the diagnostic criteria groups 1 and 2 had enteropathy caused by cows' milk protein but group 3 did not, and so may be considered as a control group. Follow up studies were based on groups 2 and 3, and the clinical features of these infants at the time of their initial disease are shown in the table.

The infants in groups 2 and 3 were maintained on conventional cows' milk and during follow up 20 (83%) of the 24 infants in group 2 remained well, but four (17%) developed symptoms (figure). The 20 infants who thrived on the formula containing cows' milk protein had a mean (SD) daily weight gain of 28.8 (23.8) g during this period compared with 26.2 (15.3) g during the period when they were having the exclusion diet. The third biopsy specimen of these 20 infants showed varying degrees of

histological recovery of the mucosa with normal mucosa in 12, mildly abnormal mucosa in seven, and moderately abnormal mucosa in one.

The four infants in group 2 who failed to tolerate cows' milk developed diarrhoea, eczema, and persistent rhinitis, and failed to thrive. The histological appearance of the third biopsy in these infants showed that the mucosal damage had progressed in two, and remained unchanged in the other two. Interestingly, one of the four infants thrived on the formula containing cows' milk protein for six weeks with a mean daily weight gain of 10 g before developing diarrhoea and vomiting. The four infants required protein hydrolysate substitutes before they improved clinically. A fourth biopsy about six weeks later of these four infants showed normal histological appearances of the intestinal mucosa, but a further challenge with cows' milk protein at this time produced symptoms immediately and these were associated with severe mucosal damage unlike that seen after the first challenge when the onset of the symptoms had been delayed.

The infants in group 3 remained well and gained weight on conventional cows' milk with a mean (SD) daily weight gain of 29 (1.7) g, which compared favourably with the daily gain of 29.3 (4) g during the period when they received the exclusion diet following their initial illnesses.

The mucosal disaccharidase activities were estimated in biopsy specimens obtained before and 24 hours after challenge with cows' milk protein, and two to six weeks after the challenge on a conventional milk diet in 13 infants in group 2. Compared with the prechallenge estimations the postchallenge values of lactase, sucrase, and maltase decreased by about 37%. These values increased, however, by 37–109% after the second challenge two to six weeks later when the infants were on a conventional milk diet compared with the initial values.

Discussion

There is no satisfactory reason why infants with enteropathy caused by cows' milk protein, even those with moderately severe villous atrophy, remained asymptomatic and thrived well on cows' milk protein while others developed symptoms and required exclusion diets. There is also no satisfactory reason why some infants with mucosal damage reacted rapidly to challenge with cows' milk protein while others reacted only slowly. Similar observations have been recorded in studies on enteropathy caused by cows' milk protein, soy protein, and gluten.^{3 4 8 13}

Factors such as intercurrent infection, secondary intolerance to sugar, antigenicity of cows' milk

Table Demographic and clinical features of 24 infants with positive (group 2) and four infants with negative (group 3) reactions to challenge with cows' milk protein

	Group 2 (n=24)	Group 3 (n=4)
Mean (SD) age (days)	46 (21.3)	83 (61)
Sex: male	14	1
female	10	3
Ethnic group: Malay	4	0
Chinese	15	1
Indian	5	3
Mean (SD) age at onset of diarrhoea (days)	19.9 (16.3)	79 (62.2)
Mean (SD) duration of diarrhoea (days)	24.3 (20.4)	3.3 (1.5)
Symptoms:		
Diarrhoea	24	4
Vomiting	12	1
Dehydration	6	1
Fever	7	1
Anal excoriation	20	3
Thrush	8	0
Abdominal distension	8	0
Eczema	3	0
Anaphylaxis	1	0
Bloody diarrhoea	3	0

protein, and the extent and severity of mucosal damage, certainly influence the nature, severity, and onset of symptoms in infants with enteropathy caused by sensitivity to food.¹⁴ The current study not only confirms earlier observations of depressed mucosal enzyme activity in damaged mucosa^{8 10 11} but in addition, shows unexpected and interesting increased enzyme activity in the second follow up biopsy specimen taken about one month after the challenge compared with the enzyme activity in the mucosa before the challenge. It is possible that the increased enzyme activities were associated with induction of these enzymes by the conventional infant formula feeds containing lactose that they were receiving during this period compared with the prehydrolysed formula that they received during the period before the first biopsy. There is strong evidence that sucrase is induced by diet^{15 16} but lactase appears not to be in humans^{17 18} although it is in rats.^{19 20} On the other hand, the raised enzyme activities may merely reflect the repair of the structural damage after challenge with cows' milk protein.

Our observations suggest that both the digestive potential and the immunological integrity of the mucosa have important roles in excluding antigens from susceptible tissues. Naturally the capacity of the villi to recover rapidly would enhance the immunodigestive functions and therefore directly assist in excluding the offending antigens by rapid proteolytic breakdown to non-antigenic peptides. This physiological measurement is difficult to assess and probably relates to the functional maturity of the small bowel mucosa. Even so, it is difficult to explain why an infant given as little as 5 ml of cows' milk showed within 30 minutes of ingestion both violent clinical and histological reactions, whereas another infant showed only a histological reaction after consuming more than a litre of the milk. One explanation may lie in the nature and type of the immune mechanisms in the pathogenesis of mucosal damage induced by cows' milk protein.^{14 21-26} Moreover, it is possible that with the strengthening of the suppressor pathway the immune responses to the food proteins are modified. Another possible explanation may be the recovery potential of the mucosa under adverse conditions.

The development of clinical tolerance and histological repair despite the continued feeding of cows' milk protein to infants with enteropathy caused by cows' milk protein but without symptoms suggests that mechanisms for spontaneous self repair of the small bowel mucosa in these infants remained effective even under adverse conditions. The factors that promote or impair healing of the mucosa, however, remain unclear. Further studies on the

complementary roles of the digestive and the immune functions in the gut in excluding antigens are necessary.

The present study showed that most infants with enteropathy caused by cows' milk protein but without symptoms thrive well with satisfactory mucosal recovery when maintained on cows' milk. Furthermore, though the jejunal biopsy was useful in diagnosing the enteropathy, it had limited prognostic value, particularly in infants with histological damage without symptoms after challenge with cows' milk protein.

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References

- Kuitunen P, Rapola J, Savilahti E, Visakorpi JK. Response of the jejunal mucosa to cow's milk in the malabsorption syndrome with cow's milk intolerance. *Acta Paediatr Scand* 1973;**62**: 585-95.
- Walker-Smith J. Cow's milk protein intolerance. *Arch Dis Child* 1975;**50**:347-50.
- Shiner M, Ballard J, Brook CGD, Herman S. Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet* 1975;ii:1060-3.
- Iyngkaran N, Robinson MJ, Prathap K, Sumithran E, Yadav M. Cow's milk protein-sensitive enteropathy: combined clinical and histological criteria for diagnosis. *Arch Dis Child* 1978;**53**:20-6.
- Iyngkaran N, Zainal Abidin, Lam SK, Puthuchery SD. Acute gastroenteritis in Malaysian children: aetiological and therapeutic considerations. *Med J Malaysia* 1980;**34**:403-8.
- Zainal Abidin, Iyngkaran N, Geethan Royan. Oral rehydration in infantile diarrhoea. The optimum carbohydrates-electrolyte composition. *J Singapore Paediatr Soc* 1980;**22**:100-3.
- Iyngkaran N, Zainal Abidin, Robinson MJ, Yadav M, Boey CG, Chai KH. Nutritional rehabilitation of infants with protracted diarrhoea comparative study of three therapeutic regimens. *Asian Journal of Clinical Sciences* 1982;**3**:160-6.
- Iyngkaran N, Yadav M, LL Meng, Boey CG, Lam KL, Balabaskaran K, Puthuchery SD. Effect of soy protein on the small bowel mucosa of young infants recovering from gastroenteritis. *J Pediatr Gastroenterol Nutr* 1987;**7**:68-75.
- Kamath KR, Murugasu R. A comparative study of four methods for detecting *Giardia lamblia* in children with diarrhoeal disease and malabsorption. *Gastroenterology* 1974;**66**:16-21.
- Iyngkaran N, Abidin Z, Davis K *et al*. Acquired carbohydrate intolerance and cow milk protein-sensitive enteropathy in young infants. *J Pediatr* 1979;**95**:373-8.
- Iyngkaran N, Yadav M, Balabaskaran S, Sumithran E. In vitro diagnosis of cow's milk protein sensitive enteropathy by organ culture method. *Gut* 1981;**22**:199-202.
- Kerry ER, Anderson CM. A ward test for sugar in the faeces. *Lancet* 1964;i:981.
- MacDonald W, Dobbins W, Rubin CE. Studies of the familial nature of celiac sprue using biopsy of small intestine. *N Engl J Med* 1965;**272**:448-6.
- Iyngkaran N, Yadav M. Food allergy. In: Marsh MN, ed. *Immunopathology of the small intestine*. New York: John Wiley & Sons 1987:415-49.
- Rosenweig NS, Herman RH. Control of jejunal sucrase and maltase activity by dieting sucrose or fructose in man. *J Clin Invest* 1968;**47**:2253-62.
- Riby JE, Kretchmer N. Effect of dietary sucrose on synthesis

- and degradation of intestinal sucrase. *Am J Physiol* 1984;**246**:6757–63.
- ¹⁷ Flatz G, Rotthauwe HW. Evidence against nutritional adaption of tolerance to lactose. *Hum Genet* 1971;**13**:118–25.
- ¹⁸ Gilat T, Russo S, Gelman-Melachi E, Aldor TAM. Lactase in man—a nonadaptable enzyme. *Gastroenterology* 1972;**62**:1125–7.
- ¹⁹ Lebenthal E, Sunshine P, Kretchmer N. Effect of prolonged nursing on the activity of intestinal lactase in rats. *Gastroenterology* 1973;**64**:1136–41.
- ²⁰ Leichter J, Goda T, Bhandari SD, Bustamante S, Koldovsky O. Relation between dietary-induced increase of intestinal lactase activity and lactose digestion and absorption in adult rats. *Am J Physiol* 1984;**247**:729–35.
- ²¹ Matthews TS, Soothill JF. Complement activation after milk feeding in children with cows' milk allergy. *Lancet* 1970;ii:893–5.
- ²² Fontaine JL, Navarro J. Small intestinal biopsy in cow's milk protein allergy in infancy. *Arch Dis Child* 1975;**50**:357–62.
- ²³ Kilby A, Walker-Smith JA, Wood CBS. Intestinal mucosa in cow's milk allergy. *Lancet* 1975;i:53.
- ²⁴ Shiner M, Ballard J, Smith ME. The small-intestinal mucosa in cow's milk allergy. *Lancet* 1975;i:136–40.
- ²⁵ Yadav M, Iyngkaran N. Immunological studies in cow's milk protein-sensitive enteropathy. *Arch Dis Child* 1981;**56**:24–30.
- ²⁶ Yadav M, Iyngkaran N, Seow IKG. Absence of milk-specific IgE in infants with cow's milk protein-sensitive enteropathy. *Med J Malaysia* 1983;**38**:266–71.

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