

Chronic excretion of enteropathogenic bacteria in the faeces—a possible association with allergy to cows' milk

R. H. WITHRINGTON, J. M. BAYLIS and D. N. CHALLACOMBE

Somerset Children's Research Unit, Taunton and Somerset Hospital, Musgrove Park, Taunton, Somerset

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Summary

Two children with protracted gastrointestinal symptoms were found to be chronically excreting enteropathogenic bacteria in their faeces. Withdrawal of cows' milk protein from their diets resulted in clinical recovery and stool cultures became negative, suggesting that allergy to cows' milk may have contributed to their bacterial carrier state.

Introduction

Children who chronically excrete enteropathogenic bacteria in their faeces constitute a public health hazard and are often excluded from nurseries or schools until three consecutive stool cultures become negative. We report two children with protracted gastrointestinal symptoms who each excreted a bacterial enteropathogen in their faeces (*Campylobacter jejuni*, *Salmonella agona*) for 1 and 8 months respectively. Withdrawal of cows' milk and foods containing cows' milk protein from their diets led to clinical recovery and stool cultures became negative.

Case reports

Patient 1, a male, presented to hospital at 16 months of age with a history of vomiting, intermittently loose stools and colic which began in early infancy. He weighed 3.75 kg at birth, was initially bottle fed with cows' milk formula (Ostermilk) and cereals were introduced into his diet at 4 months. He gained weight slowly during infancy and at 9 months developed acute gastroenteritis which was followed by persistently loose stools. At 15 months *Campylobacter jejuni* was isolated from his faeces and one month later he was admitted to hospital for further investigation of his loose stools. On examination, he was pale with eczema over his limbs and napkin area, his height was on the 10th centile and his weight was below the 3rd centile for age. *Campylobacter jejuni* was again isolated from his faeces. As the clinical history suggested cows' milk allergy

Correspondence: Dr R. H. Withrington, Somerset Children's Research Unit, Taunton and Somerset Hospital, Musgrove Park, Taunton, Somerset TA1 6DA.

and a family history of atopy was also present in first degree relatives, he was given a cows' milk-protein free diet, using Prosobee (soy bean protein) as a cows' milk substitute. Consecutive stool cultures for *Campylobacter jejuni* became negative, his stools returned to normal and over the following 3 months his weight increased to the 10th centile.

Patient 2, a female, first presented to hospital at 7 months of age with bronchitis. She weighed 2.75 kg at birth, was initially bottle fed with cows' milk formula (Cow and Gate) and cereals were introduced into her diet at 4 months of age. Soon after birth she developed projectile vomiting with intermittently loose stools and feeding difficulties persisted throughout infancy. Treatment of her bronchitis in hospital at 7 months with ampicillin caused profuse diarrhoea, which ceased when this antibiotic was withheld. Following her discharge from hospital she continued to pass up to 7 loose stools a day, but gained weight along the 25th centile. At 2 years 1 month she was re-admitted to hospital for further investigation of her diarrhoea. A family history of both atopy and coeliac disease was present in first degree relatives and, apart from flexural eczema on her limbs, physical examination was normal. Enteropathogenic bacteria were not isolated from her faeces. As the history suggested an allergy to cows' milk, a cows' milk-protein free diet was started using Prosobee as a milk substitute. The frequency and consistency of her stools rapidly returned to normal and she was discharged home. After 2 months she failed to comply with the diet, developed colicky abdominal pain and diarrhoea and *Escherichia coli* O 125 was isolated from her faeces. Reinforcing the dietary advice resulted in normal stools and stool cultures for *E. Coli* O 125 became negative. At 2 years 9 months, after again failing to keep to her diet, she developed diarrhoea which contained visible blood. On this occasion *Salmonella agona* was isolated from her faeces and her loose stools persisted for a further 8 months. She was then re-admitted to hospital to implement a strict cows' milk-protein free diet, when *Salmonella agona* was again isolated from her faeces. After 1 week on the diet her diarrhoea ceased and consecutive stool cultures became negative. Following discharge from hospital she continued on her diet and remained free of symptoms.

Methods

Investigations routinely performed on each patient included a haemoglobin, total and differential white cell count and stool cultures. Fresh stools were also tested for the presence of reducing sugars using the Clinitest method (Kerry & Anderson, 1964). Serum immunoglobulins were estimated by single immunodiffusion techniques, IgE by radioimmunoassay and the radioallergosorbent test (Phadebas RAST) was used to detect the presence of IgE cows' milk specific antibodies in the serum. Samples of the distal duodenal mucosa were obtained by peroral biopsy under fluoroscopic control, using a Watson paediatric biopsy capsule. Tissue samples were fixed in 10% formol saline for 2 hr, processed conventionally and embedded in paraffin wax. Sections for light microscopy were then cut at a thickness of 5 μ m and stained with haemotoxylin and eosin. Eosinophils in the duodenal lamina propria were counted using a 1 cm square graticule inserted into the eyepiece of a light microscope, at a final image magnification of $\times 625$ (Withrington & Challacombe, 1979).

Results

Results of the laboratory investigations are shown in Table 1. Haemoglobins and total white cell counts were normal but patient 2 had increased numbers of eosinophils in the

Table 1. Laboratory investigations

Patient no.	Sex	Age	Hb (g/dl)	Total WBC count	Blood eosinophils	IgG (g/l)	IgM (g/l)	IgA (g/l)	IgE (iu/ml)	RAST	Duodenal histology	Faecal bacterial enteropathogens	Lamina propria eosinophils (counts/1000 hits)
1	M	1 yr 3 mo.	13.2	$11.8 \times 10^9/l$	$0.118 \times 10^9/l$	7.9	1.71	0.44	25	-ve	Normal villi with increased numbers of lamina propria eosinophils	<i>Campylobacter jejuni</i>	52
2	F	2 yr 2 mo.	13.1	$8.7 \times 10^9/l$	$0.610 \times 10^9/l$	7.6	3.36	0.92	36	-ve	Normal villi with increased numbers of lamina propria eosinophils	<i>Escherichia coli</i> O 125 <i>Salmonella agona</i>	59
Normal values			11-13	$6.2-17 \times 10^9/l$	$0.04-0.44 \times 10^9/l$	4.2-14.5	0.32-1.45	0.21-1.89	1.1-49.0				Mean = 30 s.d. = 9

peripheral blood. Raised serum levels of IgM were found in both patients but IgG and IgA levels were normal. Serum levels of IgE were also normal and the RASTs in both patients were negative. Light microscopic examination of the duodenal mucosa in both patients showed normal villi with an apparent increase of eosinophils in the lamina propria. Eosinophils were counted using an eyepiece graticule and were within the range of values found in children with cows' milk allergy (mean = 70/1000 hits, s.d. = 22) and above that found in children with normal duodenal biopsies (mean = 30/1000, s.d. = 9) (Withrington & Challacombe, 1979). Stool specimens were repeatedly tested for reducing sugars and were consistently negative. *Campylobacter jejuni* was isolated from the faeces of patient 1 on two occasions separated by an interval of 1 month, and *Salmonella agona* from the faeces of patient 2 on two occasions separated by an 8-month interval. *Escherichia coli* O 125 was also isolated from patient 2 on one occasion. Following the elimination of cows' milk protein from the diets of both patients, three consecutive stool cultures for enteropathogenic bacteria became negative. Neither patient had been given antibiotics while they were excreting enteropathogenic bacteria.

Discussion

In the absence of a definitive laboratory test, the diagnosis of cows' milk allergy in children still relies on clinical criteria. However criteria such as those proposed by Goldman *et al.* (1963) may be too rigid and impractical, leading to underdiagnosis of this disorder. Morphological changes which occur in the jejunal mucosa on light microscopy after challenge with cows' milk may be of greater diagnostic value (Shiner *et al.*, 1975), but the need for serial jejunal biopsies in a transient disorder of childhood has also been questioned (Sumithran & Lyngkarar, 1977). In the present study a diagnosis of cows' milk allergy was made on the patient's history of protracted gastrointestinal symptoms, which followed the introduction of cows' milk formula after birth, on their history of atopy in first degree relatives and on their eczema and clinical recovery which followed dietary elimination of cows' milk protein. Subsequent challenges with cows' milk were not performed in accordance with the parents wishes.

Several mechanisms have been proposed to explain the gastrointestinal symptoms which occur when children become sensitized to cows' milk protein. In some patients diarrhoea and vomiting occur immediately after the introduction of cows' milk, suggesting an IgE mediated reaction. In others the slow development of symptoms in response to cows' milk suggests an immune complex-complement mediated type of delayed hypersensitivity. In our patients normal serum levels of IgE and negative RASTs did not suggest an IgE mediated reaction. However serum IgM levels were raised in both patients suggesting involvement of IgM in the immune response, a finding that has previously been reported in cows' milk allergy (Freier, 1973). The upper small intestinal mucosa in patients with cows' milk protein intolerance may be normal on light microscopy or may show varying degrees of villous flattening. The duodenal mucosa in our patients showed normal villi on light microscopy, and an apparent increase in lamina propria eosinophils was confirmed by using a cell-counting technique. Raised eosinophil counts have been previously reported in the duodenal lamina propria of children with cows' milk protein intolerance (Withrington & Challacombe, 1979). Treatment of our patients with a cows' milk-protein free diet led to their clinical recovery and to negative stool cultures for enteropathogenic bacteria. This association could have been coincidental and will need to be confirmed in similar

patients by withdrawing cows' milk from their diets. Alternately the allergic response of the gastrointestinal mucosa to cows' milk protein may impair tissue mechanisms that normally prevent colonization of the bowel with enteropathogenic bacteria and lead to persistent excretion of these organisms in the faeces.

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