Cow's Milk Allergy as a Cause of Infantile Colic: Immunofluorescent Studies on Jejunal Mucosa

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Harris, M. J., Petts, Miss V. and Penny, R. (1977). Aust. Paediatr. J. 13, 276-281. Cow's milk allergy as a cause of infantile colic: Immunofluorescent studies on jejunal mucosa. Fourteen infants and children who had suffered from infantile “colic” and related symptoms were diagnosed as being allergic to cow's milk because of their response to cow's milk exclusion and then to repeated challenge with cow's milk. The parents of these children frequently were allergic themselves and very frequently refused to drink cow's milk.

Small bowel biopsy specimens were taken on these children, but only seven had biopsies both while taking cow's milk and when on a milk-free diet. The mucosae were all histologically normal with normal disaccharidase levels. However, on immunological study it has been shown that significantly higher numbers of IgE containing plasma cells were present in the mucosal specimens when these infants were taking cow's milk, than when they were on a milk-free diet.

The diagnosis of cow's milk allergy remains an enigma. Clinical assessment is largely subjective, and the available laboratory tests inconclusive. The American medical literature has considered this disorder to be common, while the English literature has very scant references to cow's milk allergy—and then only to the rarer manifestations.

Analysis of the syndrome of cow's milk allergy has been:

(i) Clinical, including wide ranging features from vomiting, colic and diarrhoea, to the potentially fatal anaphylactic shock;
(ii) Histological, providing description of intestinal mucosa; and
(iii) Immunological, depending mainly on unreliable skin testing or detection of serum antibodies.

The aim of this study has been to apply particular sophisticated laboratory techniques to substantiate the clinical claim that infantile “colic” can be due to cow's milk allergy. The authors are not aware that this has been done previously. This study is not a review of the role of cow's milk allergy in the aetiology of infantile “colic”.

Because the allergic reaction takes place in the intestine, this study was designed to analyse the immunological mechanisms operative in the small bowel mucosa of children with cow's milk allergy by studying the changes in plasma cell populations during milk challenge. This study was limited to the small bowel mucosa of infants and children who suffered from severe infantile “colic” which was clinically thought due to cow's milk allergy.
Between January 1973 and June 1975, approximately 100 infants, with a tentative diagnosis of cow's milk allergy, were seen by one of the authors (M.J.H.) in consultation. The diagnosis was confirmed if the patient's symptoms resolved within 48 hours of changing to a milk-free diet, supplemented with "Isomil" brand of soya formula. These symptoms recurred consistently with repeated challenge with cow's milk products as required in the criteria established by Goldman et al. (1963). The mother was not forewarned of what changes to expect.

Inclusion into the series required the parents' informed consent for a small bowel mucosal biopsy to be performed while the child was "off" and then "on" milk. This permission was refused in all but 14 instances and was occasionally withdrawn before the second biopsy. There was no obvious clinical difference between the subjects biopsied and those not submitted to this procedure.

Fourteen children (9 males, including 2 brothers and 5 females) with cow's milk allergy diagnosed by the above criteria underwent small bowel mucosal biopsy; seven of these had repeat biopsies.

When an infant with suspected cow's milk allergy responded favourably to cow's milk exclusion, he was fed on "Isomil" brand soya formula for two weeks and then challenged with a small dose of cow's milk. If his symptoms recurred promptly, he was given a milk-free diet again. Then he was challenged consecutively with 5 g, 10 g and 20 g lactose and also 20 g sucrose. Clinical changes after any one administration were reported immediately by the mother. Further challenge with cow's milk was carried out and finally small bowel biopsy was arranged to coincide with the third challenge of milk, usually lasting one week so as to obtain specimens "on" and "off" milk. Clinical changes after any one administration were reported immediately by the mother. A further challenge with cow's milk was carried out and finally small bowel biopsy was arranged to coincide with the third challenge of milk, usually lasting one week so as to obtain specimens "on" and "off" milk. Routine investigations, such as a blood count and microscopy of urine, were performed as indicated.

"Isomil" brand of soya formula was initially given to all the infants in the series. Generally this was found to be a highly satisfactory form of feeding; occasionally an infant did not respond well, and if the clinical diagnosis remained tenable, a further trial with "Nutramigen" feeding was begun and the same regime followed.

Only the involved clinician (M.J.H.) was aware of which specimens were taken while "on" and "off" cow's milk. The key was broken only after all the results were available.

Controls were obtained by comparing the mucosal findings of the allergic children while "on" milk with specimens taken during the same time period on 20 children with non-specific diarrhea (which excluded coeliac disease) or with failure to thrive, and also 6 children with common variable immuno-deficiency. These children were all drinking milk at the time.

It was not felt to be ethical to do mucosal biopsies on normal, healthy infants merely to find out the changes in IgE cell count in the mucosa when cow's milk was first introduced into the diet.

Jejunal Mucosal Biopsy

Jejunal mucosal biopsy was obtained by a technique using the Watson version of the Crosby paediatric capsule described by Harris et al. (1968). The biopsy specimen was divided into three pieces (or a second piece obtained if necessary); one piece was assessed under the dissecting microscope, photographed, and fixed in formol-saline for routine light microscopy. The second piece was snap-frozen in dry ice and used in the measurement of mucosal enzyme activity. Enzyme assays were performed using the methods described by Dahlquist. The third piece, was snap-frozen in isopentane and dry ice and stored at -70°C for immunofluorescent studies.

Immunofluorescent Staining

Four µ serial sections were cut and stained unfixed by the direct immunofluorescent technique. Fluorescein conjugated anti IgG and IgA, anti IgM (Wellcome Pharmaceuticals) and anti IgE (Travenol Labs.) were used at dilutions between 1:2 and 1:10. Slides were examined by epi-illumination on a Reichert Zetopan microscope. All the immunofluorescent positive plasma cells in the villi, but excluding the crypt, were counted and the result expressed...
as the average number of cells per villus. Any abnormal staining pattern seen in the crypts was also noted.

CLINICAL RESULTS

The 14 children were all seen for the first time before their first birthday: 6 before 2 months of age and 10 before 6 months of age. Symptoms began early in all infants and in 12 before 4 months of age.

Thirteen infants presented with excessive crying or screaming, 8 with severe “colic” or “wind”, and 7 with spilling and vomiting. Most slept badly and were never happy or relaxed. Four were constipated and four had frequent bowel actions; three had recent poor weight gains but none had significantly failed to thrive (Table 1).

<table>
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<th>TABLE 1</th>
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<tr>
<td>Clinical features of 14 patients with cow’s milk allergy</td>
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<tr>
<td>Screamed excessively</td>
</tr>
<tr>
<td>“Colic”</td>
</tr>
<tr>
<td>Spilling or vomiting</td>
</tr>
<tr>
<td>Constipation</td>
</tr>
<tr>
<td>Diarrhoea</td>
</tr>
<tr>
<td>Poor weight gain</td>
</tr>
<tr>
<td>Always hungry</td>
</tr>
<tr>
<td>One allergic parent</td>
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<tr>
<td>Two allergic parents</td>
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<tr>
<td>Family history of allergy</td>
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A history of allergy in the parents occurred in over half of the series—in both parents in 3 and in one parent in 5. Reluctance or refusal to drink cow’s milk occurred in one or both parents in 9 out of 12. These latter parents were not studied to elucidate the reasons or mechanism of their milk refusal. One child had a sibling with coeliac disease.

Thirteen of these infants were fed on Isomil brand of soya formula and on milk-free solids as indicated. Two children responded inadequately to Isomil and needed Nutramigen feeding.

Sugar loading tests produced changed in the bowel function of only two of these children, one had “colic” with 5 g of lactose but not with 10 g and 20 g and one had “loose bowels” after 20 g lactose. Both of these children had normal mucosal lactase levels.

LABORATORY RESULTS

Five children were biopsied before six months of age: ten before the first birthday and the remaining 4 at various ages up to 5½ years. Most repeat biopsies (5 of the 7) were done within three months of the first, and all within six months.

1. Dissecting Microscope Findings

None showed any abnormal villous structural patterns of significance.

Figure 1: Plasma cell content of villi in 7 patients with cow’s milk allergy serially biopsied when off milk (open column) and on milk challenge (hatched column). Histograms represent mean numbers of immunofluorescent positive plasma cells/6 intestinal villi, the bars defining the standard error of the mean.

2. Light Microscopy

No gross abnormalities of villous structures were seen—the relevance of this finding in relation to the clinical material is discussed below. There were minor differences in the specimens of four paired cases when challenged with milk. These changes included mononuclear infiltration of the lamina propria and/or the epithelium, with or without epithelial oedema.

3. Mucosal Enzymes

No infants were found to be lactase, sucrase or isomaltase deficient. One child had low normal results for all enzymes while on milk and another had a low normal sucrase level while on milk.
4. Immunofluorescence

In Figure 1, the number of plasma cells staining for IgG, IgA, IgM and IgE is compared in biopsies taken both on and off milk. This is the group of seven infants who had repeat biopsies. There is no difference in the IgG staining in the two groups. The IgA and IgM show a trend towards increased numbers when on milk challenge although the differences were not statistically significant by the student t-test in the number of patients analysed (IgA: \( P < 0.15 \), IgM: \( P < 0.2 \)). There was, however, a significant increase in the number of IgE stained plasma cells after milk challenge (\( P < 0.0125 \)). The data of the matched and non-matched biopsies (13 biopsies taken off milk and 8 taken on milk) are shown together in Figure 2.

The results were quite similar to the matched group, although the tendency for an increase in IgM plasma cells on milk challenge was diminished. The difference in IgE staining between the two groups was again highly significant (\( P < 0.0025 \)). No extracellular IgE was noted. Figure 3 shows a typical pattern seen in the intestinal biopsy with immunofluorescent antisera to IgE.

The results of analysis of IgE plasma cell content in the two other groups of children are shown in Table II. All were on milk at the time of the study. The group of children with cow’s milk allergy on milk had a higher IgE

![Figure 2: Plasma cell content of villi in all patients studied with cow’s milk allergy. Hatched columns represent mean result when on milk challenge and open column when off milk, the bars defining the standard error of the mean.](image)

![Figure 3: Small intestine in patient on milk challenge seen with immunofluorescent antisera specific for IgE. IgE bearing plasma cells are prominent.](image)

### Table II

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>No. of IgE plasma cells*</th>
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<tbody>
<tr>
<td>Cow’s milk allergy —on milk</td>
<td>8</td>
<td>13.5 ± 2.5</td>
</tr>
<tr>
<td>Cow’s milk allergy —off milk</td>
<td>13</td>
<td>4 ± 1.0</td>
</tr>
<tr>
<td>Common variable immunodeficiency</td>
<td>6</td>
<td>7.5 ± 3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>20</td>
<td>9.5 ± 1.5</td>
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* Average for 6 villi ± S.E.
plasma cell content in the intestinal villi than either group, but due to small numbers, the differences were not significant ($0.1 > P > 0.05$ for both groups).

**DISCUSSION**

Certain clinical syndromes have been ascribed to cow's milk allergy, in which there are measurable changes which alter with cow's milk exclusion. These include gastrointestinal bleeding (Wilson et al., 1964) and protein-losing enteropathy (Waldman et al., 1967). In other instances there are measurable changes with strong presumptive evidence of the role of cow's milk protein, such as in failure to thrive (Goldman et al., 1963) malabsorption (Kuitunen et al., 1975) and acute dehydration from diarrhoea (Freier et al., 1969). However most instances of clinical cow's milk allergy occur in patients in whom changes of symptomatology may be the only method of assessing change. The range of symptoms in infants was listed by Clein in 1954 and includes (in order of frequency) eczema, regurgitation, spilling, colic diarrhoea, unhappiness and various respiratory tract symptoms. "Colic" occurred as a solitary symptom in 5% of the series of Rosenblum and Rosenblum, 1952 and in that of Bigler, 1955.

The series presented has concentrated on babies with "colic" and "unhappiness", where the symptoms and assessments are very subjective, but the need for proof of the diagnosis remains important to the patient and his family.

It is stressed that diarrhoea was not a significant feature in the past or presenting history of these babies. Acceptance of cow's milk allergy as a cause will allow further clinical studies.

"Isomil" brand soya formula was easily accepted by almost all infants in this series. No cases of allergy to soya formula were seen, and an inadequate response was infrequent. A trial with "Nutramigen" feeding was carried out and no cases were seen which did not respond to either Isomil or Nutramigen.

The positive family history of allergy is not unexpected, when discussing paediatric allergic disorders, but the frequency with which these parents stated their dislike or inability to tolerate cow's milk is remarkable. The frequency of 75% in this series is in excess of the 60% quoted by Gerrard et al. in 1963, and one feels that this should be considered to be of diagnostic value. However, the mechanisms of the parents' refusal to drink cow's milk has not been studied.

One child had a sibling with known coeliac disease. In view of the familial nature of this disorder and in view of the association of cow's milk allergy and coeliac disease in some patients (Visakorpi et al., 1967), it will be interesting to follow up this child further, even though there has been no evidence of gluten enteropathy to date.

Lactase deficiency was not a feature of this series. All mucosal enzyme studies were normal and the clinical loading tests, in which the mother made assessments of increased bowel activity or changes in temperament, were generally normal.

Furthermore, none of these infants had a preceding history of acute or significant diarrhoea. Lactose tolerance tests were not done because of the age of these infants, but these may have been relevant in view of the flat curves which were reported in milk sensitivity patients who did not get diarrhoea from lactose loading (Lubos et al., 1967). Nevertheless, it would seem that cow's milk allergy and lactase deficiency are unrelated clinically and aetologically.

Histological studies have only recently been included in reports on cow's milk allergy. It is stressed that the mucosal specimens in this series looked normal under the dissecting and the light microscope. Similar results were reported by Lubos et al., 1967, minor abnormalities by Silver and Douglas, 1968, and almost universal abnormality in the series of Visakorpi, 1967, in which the patients later developed coeliac disease. It may be that the severity of symptomatology reflects the extent of mucosal abnormality and "colic" is less pathological than diarrhoea or failure-to-thrive. This may explain the difference between this series and that of Fontaine and Navarro, 1975, where 31 infants had vomiting and/or diarrhoea, and 26 abnormal mucosae; but there may be other differences in the case material in view of the slow return to normal of these mucosae after milk exclusion.

Most early studies on cow's milk allergy utilized serum or skin testing in infants and children. As the allergic reaction takes place in the bowel, this is a more relevant area to
investigate. Despite the fact that this group of patients had significantly milder symptoms and gastrointestinal pathology than in other series, immunofluorescence studies of the small bowel provided support for the involvement of immune mechanisms and helped to document the benefit from milk exclusion.

In our patients a highly significant increase in the number of IgE bearing plasma cells was found in the lamina propria during milk challenge. The IgE plasma cell content is greater than in a miscellaneous control group of patients. This would strongly favour a Type 1 immediate hypersensitivity reaction to milk and patients. This would strongly favour a Type 1 immune mechanism and supported the results of smaller series of Shiner (1975) and Kilby (1975) who, in addition, were able to show degranulation of mast cells. Unlike their study no extracellular IgE was demonstrated, although this does not deny the potentially important role of IgE in cow's milk allergy.

The suggestive increase in IgA bearing plasma cells in cow's milk allergy would be out of keeping with the hypothesis of Soothill, reported by Taylor et al. (1973) that infants with cow's milk allergy were relatively IgA deficient as one may expect the plasma cell content in the bowel to be also relatively deficient in IgA bearing plasma cell.

By immunofluorescence, no IgA, IgM or IgG were found in the blood vessels of the lamina propria on milk challenge; this would tend to exclude antigen-antibody complex reaction in intestinal mucosa.

Finally, the lower numbers of IgE bearing plasma cells in patients receiving soya formula provides considerable immunological basis for the role of cow's milk exclusion in the management of these patients.

It is felt that the significance of this series lies in the fact that babies suffering from "colic" and "unhappiness" may be allergic to cow's milk, and small bowel mucosal biopsy with appropriate immunological studies offers proof of this. Although this procedure would be hard to justify in routine investigations, it has been useful in the present study in defining changes from the normal immune status in this disease.

ACKNOWLEDGEMENTS

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REFERENCES


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