Iron deficiency anaemia with hypoproteinaemia

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SUMMARY Forty two children were admitted to this hospital between 1975 and 1980 with severe iron deficiency anaemia and 8 of them also had oedema caused by a low concentration of serum proteins. These 8 patients, aged 8-24 months, and 13 age matched controls were investigated. The patients had excessive faecal loss of $^{59}\text{FeCl}$ or ^{51}Cr -albumin, or both; their jejunal biopsy specimens showed little decrease in the ratio of villous height to crypt depth; and they had fewer intraepithelial lymphocytes and cells containing IgA than controls. They were all treated with an oral ferrous iron preparation and the oedema, hypoproteinaemia, and low haemoglobin concentrations rapidly resolved. These results show that immunologically mediated hypersensitivity is not implicated in iron deficiency anaemia associated with hypoproteinaemia.

Iron deficiency anaemia in infants and small children is often associated with ingestion of cows' milk. Low concentrations of iron in the milk is one reason, if milk is the main food. Wilson *et al*¹ reported that gastrointestinal bleeding was often caused by cows' milk and that this could be prevented by eliminating the milk or boiling it. Milk proteins may act as antigens and cause immunological reactions in the intestinal tract. We studied a group of 8 infants to determine whether iron deficiency anaemia and hypoproteinaemia were caused by an allergic reaction in the intestine.

Subjects

We studied all children with a blood haemoglobin concentration less than 8 g/dl, evidence of iron

deficiency, and a serum protein concentration less than 50 g/l, admitted to this hospital between 1975 and 1980. Of 42 children with severe anaemia (haemoglobin less than 8 g/dl) only 8 met all criteria (Table). The feeding pattern of these children varied: 6 had been breast fed for 3 months or less, 1 had been breast fed for 5 months, and 1 had been given breast milk exclusively for 9 months (Fig. 1).

In 2 patients anaemia had developed over a short period of time. Two months before the study their haemoglobin concentrations had been 10.9 and 11.4 g/dl but these had dropped to 4.1 and 6.3 g/dl respectively. The children's growth before admission to hospital was normal. Laboratory studies showed that case 2 had a high number of eosinophils in peripheral blood. Cases 4 and 6 had precipitins for cows' milk protein. None had gluten antibodies.

Table Clinical details of patients: their haemoglobin values (Hb), mean corpuscular volume (MCV), serum concentrations of iron, total iron binding capacity (TIBC), serum ferritin, and serum protein IgA

Case No	Age (mths)	Sex	Weight (SD)	Height (SD)	Hb g/dl	MCV fl (μ^3)	Serum iron ($\mu\text{mol/l}$)	TIBC ($\mu\text{mol/l}$)	Serum ferritin (ng/l)	Serum protein (g/l)	IgA (g/l)	% of normal for the age
1	8	M	-0.6	-0.5	5.3	56	3.7	64	1	47	0.19	136
2	9	M	-0.3	+1.5	5.7	67	<1.0	54	2	42	0.13	87
3	10	M	+0.4	+0.4	6.3	63	7.7	72	3	44	0.30	189
4	10	F	-0.5	+0.1	5.3	57	2.0	54	2	35	0.09	56
5	12	F	-0.3	+0.7	4.1	54	6.6	91	1	42	0.05	27
6	15	F	-0.8	-1.0	7.6	56	2.8	29	6	30	0.09	42
7	15	M	-1.5	-1.2	5.2	58	4.6	74	7	48	0.30	142
8	24	F	-1.8	-1.8	2.0	48	5.8	53	3	40	0.41	128
Mean	12.9		-0.7	-0.3	5.8	57	4.2	61	3.1	41		101
Normal limit					>11.0	>70	>10	<70	>10	>52		

Conversion: SI to traditional units—serum iron 1 $\mu\text{mol/l} \approx 0.179 \mu\text{g/100 ml}$; total iron binding capacity 1 $\mu\text{mol/l} \approx 0.179 \mu\text{g/100 ml}$.

Fig. 1 Milk diet of patients before admission to hospital.

Each child presented with clinical oedema, indicated by transient weight gains (Table). The average degree of oedema was 6.8% of body weight, with an individual range of 2.7–20.5%. These figures were based on the difference between weight at the time of admission and the lowest weight during the subsequent 2 weeks. All the patients were treated with a ferrous iron preparation, 3 mg/kg/day. Three were transfused with 4–7 ml/kg red cells at the time of diagnosis. They were all given cows' milk during treatment and follow up. There were 13 age matched controls.

Methods

Biopsy specimens of the small intestine were taken

from the proximal jejunum with a paediatric Crosby-Kugler biopsy capsule. The morphometric parameters—villous height, crypt depth, and surface epithelial cell height—were measured as described by Kuitunen,² and the number of epithelial lymphocytes was counted by the method of Ferguson and Murray.³ The number of cells containing immunoglobulin was counted in paraffin sections stained by direct immunoperoxidase technique.⁴ The biopsy specimens of the jejunum were taken to exclude any intestinal cause of symptoms (changed growth pattern or vomiting) or because the cytotoxic test to wheat gluten was positive. No child had intestinal disease or symptoms after prolonged observation with no treatment.

In 6 patients loss of iron in the stools was measured by 5 day stool collections 2 days after 0.1 μ Ci, $^{59}\text{FeCl}$ had been given intravenously. The stools were counted on a whole body counter.⁵ The mean radioactivity in the stools was 0.97 nCi over 5 days, range 0.5–1.9 nCi. These values are greater than those considered normal using this technique, showing that each of the patients was bleeding from the intestinal tract.

Protein loss into the faeces was studied in 3 children. ^{51}Cr -albumin was injected intravenously and radioactivity was measured by 5 day stool collections. In the 3 children radiolabelled albumin in the stools was 1.9%, 4.7%, and 5% respectively—

Fig. 2 The ratio of villous height to crypt length (VH:CL), the numbers of intraepithelial lymphocytes (IEL) per 100 epithelial cells, and the numbers of cells containing IgA and IgM per mm² in the lamina propria of the jejunum of controls (C) and patients (P). Mean values of groups are shown as well as the difference between the groups tested with Student's *t*-test. NS=not significant.

the normal upper limit of protein loss is 1.0%. The dose of radioactive isotopes given conformed with the Scandinavian research regulations of a dose of less than the annual endogenous exposure.

Results

Morphometric study of the jejunal biopsy specimens showed minimal changes only: a reduced ratio of villous height to crypt depth because of elongation of the crypts (Fig. 2). Average villous height of the jejunum was (mean $\pm$ SD) $349 \pm 104 \mu\text{m}$ in the 8 patients, compared with $337 \pm 57 \mu\text{m}$ among controls and the range of values was wide. Reduced villous height was seen in 2 patients: 250 and $280 \mu\text{m}$, respectively. The surface epithelial cell height in the patients was (mean $\pm$ SD) $27.8 \pm 5.0 \mu\text{m}$ and in the control group was (mean $\pm$ SD) $29.7 \pm 2.4 \mu\text{m}$.

The number of intraepithelial lymphocytes (mean $\pm$ SD) 13.6 ± 2.3 per 100 epithelial cells, and the number of cells containing IgA (mean $\pm$ SD) 354 ± 262 per mm^2 , were lower than the controls (mean $\pm$ SD) 27.2 ± 7.5 and 663 ± 278 , respectively (Fig. 2). There was no difference in the number of IgM and IgG containing cells in patients and controls, but there were few IgE containing cells in either group.

Oedema and hypoproteinaemia responded rapidly to ferrous iron treatment. In all patients the oedema resolved during the first week and the serum protein concentration improved from a mean value of 42 g/l to 58 g/l within 2 weeks. By this time half of the 8 patients had a serum protein concentration that did not increase further during the follow up. On the other hand, in only 1 of the 8 children was the haemoglobin concentration normal (Fig. 3). The

haemoglobin and serum iron values were normal in all patients by 6 weeks. The improvement in the serum ferritin concentration was slow; the mean value after 4 months of continuous iron medication was $19 \mu\text{g/l}$, which is lower than that seen in healthy children of this age.

Discussion

Although only rough estimates of incidence are available, it is rare to find an infant with nutritional iron deficiency anaemia in Finland. In the Helsinki well baby clinics, where over 99% of the infants are seen, it is estimated that only 10 infants out of 8860 screened during 1979 at age 1 were anaemic. It seems that cases of severe iron deficiency anaemia are not representative of the lowest values of Gaussian distribution, but are independent disorders. We estimate that the incidence of severe iron deficiency anaemia with a haemoglobin concentration of less than 1 g/dl in children aged 8–24 months is roughly 1:700. Severe iron deficiency anaemia is occasionally accompanied by hypoproteinaemia and oedema.⁶ According to our findings the incidence of this in the 8–24 months age group would be about 1:7000.

There is evidence that the hypoproteinaemia is caused by increased intestinal losses of serum protein. The observations in some cases in our study agree with this.^{1,6} The aetiological and pathogenetic events in this disorder are controversial: some believe that nutritional iron deficiency leads to jejunal damage and then protein leakage,⁶ whereas others suspect that fresh cows' milk causes an adverse reaction in the intestinal epithelium which results in loss of iron and protein.^{1,7}

Intestinal cows' milk allergy and coeliac disease may lead to secondary malabsorption of iron and intestinal loss of iron.^{5,8,9} The infant with active coeliac disease or intestinal cows' milk allergy may therefore present with severe iron deficiency anaemia and hypoproteinaemia. These 2 disorders are, however, usually seen together with moderate or severe morphological lesions of the intestinal mucosa and an appreciable increase in the number of intraepithelial lymphocytes. The pathogenesis is believed to be immune destruction of the surface epithelial cells of the jejunum,^{10,11} and there is a considerable increase in the number of cells containing IgA and IgM in the lamina propria of the jejunum.^{12,13} The IgA and IgM containing cells synthesise antibodies¹⁴ against damaging foreign protein and they are increased even in infants with cows' milk allergy without evident intestinal symptoms (Fig. 4). In contrast to coeliac disease and cows' milk allergy our patients had slight or no mucosal changes and fewer cells containing IgA and IgM

Fig. 3 Serum protein and haemoglobin concentrations of patients on admission and after 2 weeks' iron treatment.

Fig. 4 The ratios of villous height to crypt length (VH:CL) and the numbers of IgA-containing cells of patients; of eczematous children with cows' milk allergy;²¹ of children with cows' milk induced enteropathy;¹³ of children with coeliac disease,¹² and of controls.²¹ Mean values ± 1 S.D.

than the controls. In our patients immunologically mediated hypersensitivity was not the cause of the iron deficiency anaemia with hypoproteinaemia and oedema.

Opinions on the influence of iron deficiency on immune functions differ. Many investigators have found evidence of low cellular immunity^{15 16} even if these functions have been normal in some studies.¹⁷ The numbers of intraepithelial lymphocytes, predominantly T cells,¹⁸ in the intestine of children who are iron deficient are low. The results of laboratory investigations indicate that humoral immunity, including the concentration of salivary IgA, is normal in man^{16 19} and impaired in some experimental animals.²⁰ We found appreciably fewer cells containing IgA in the jejunum of our patients, and this may be associated with an impairment in the mucosal defences.

The excellent response of our patients to treatment with oral iron suggests that iron deficiency anaemia triggers off intestinal damage, altered intestinal function with iron and protein loss, and finally anaemia and oedema. It seems that this process can be stopped by iron treatment, even before the anaemia is completely cured: in our patients serum protein values returned to normal well before blood haemoglobin concentration. Severe iron deficiency anaemia with hypoproteinaemia can be cured rapidly by iron treatment, whereas cows' milk allergy and coeliac disease require the elimination from the diet of cows' milk and gluten, respectively.

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References

- Wilson JF, Heiner DC, Lahey ME. Milk induced gastrointestinal bleeding in infants with hypochromic microcytic anemia. *JAMA* 1964; **189**: 568-72.
- Kuitunen P. Duodeno-jejunal histology in malabsorption syndrome in infants. *Ann Paediatr Fenn* 1966; **12**: 101-32.
- Ferguson A, Murray D. Quantitation of intraepithelial lymphocytes in human jejunum. *Gut* 1971; **12**: 988-94.
- Perkkiö M, Savilahti E. Time of appearance of immunoglobulin-containing cells in the mucosa of the neonatal intestine. *Pediatr Res* 1980; **14**: 953-5.
- Kosnai I, Kuitunen P, Siimes MA. Iron deficiency in children with coeliac disease on treatment with gluten-free diet. Role of intestinal blood loss. *Arch Dis Child* 1979; **54**: 375-8.
- Naiman JL, Oski FA, Diamond LK, Vawter GF, Schwachman H. The gastrointestinal effects of iron-deficiency anemia. *Pediatrics* 1964; **33**: 83-99.
- Woodruff CW, Clark JL. The role of fresh cows' milk in iron deficiency. I. Albumin turnover in infants with iron deficiency anemia. *Am J Dis Child* 1972; **124**: 18-23.
- Kuitunen P, Visakorpi JK, Savilahti E, Pelkonen P. Malabsorption syndrome with cows' milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 1975; **50**: 351-6.
- Young WF, Pringle EM. 110 children with coeliac disease, 1950-1969. *Arch Dis Child* 1971; **46**: 421-36.
- Booth CC, Peters TJ, Doe WF. Immunopathology of coeliac disease. In: Porter R, Knight J, eds. *Immunology of the gut*. Ciba Foundation Symposium 46 New Series. Amsterdam: Elsevier, 1977. 329-46.
- Savilahti E. Cows' milk allergy. *Allergy* 1981; **36**: 73-88.
- Savilahti E. Intestinal immunoglobulins in children with coeliac disease. *Gut* 1972; **13**: 958-64.
- Savilahti E. Immunochemical study of the malabsorption syndrome with cows' milk intolerance. *Gut* 1973; **14**: 491-501.
- Falchuk ZM, Strober W. Gluten-sensitive enteropathy. Synthesis of antigliadin antibody *in vitro*. *Gut* 1974; **15**: 947-52.
- Chandra RK, Saraya AK. Impaired immunocompetence associated with iron deficiency. *J Pediatr* 1975; **86**: 899-902.
- Maddougall LG, Anderson R, McNab GM, Katz J. The immune response in iron-deficient children: Impaired cellular defense mechanisms with altered humoral components. *J Pediatr* 1975; **86**: 833-43.
- Kulapongs P, Suskind R, Vithayasai V, Olson RE. Cell-mediated immunity and phagocytosis and killing function in children with severe iron-deficiency anaemia. *Lancet* 1974; **ii**: 689-91.
- Ferguson A. Intraepithelial lymphocytes of the small intestine. *Gut* 1977; **18**: 921-37.
- Van Heerden C, Oosthuizen R, Van Wyk H, Prinsloo P, Anderson R. Evaluation of neutrophil and lymphocyte function in subjects with iron deficiency. *S Afr Med J* 1981; **60**: 111-3.
- Nalder BN, Mahoney AW, Ramakrishnan R, Handricks DG. Sensitivity of the immunological response to the nutritional status of rats. *J Nutr* 1972; **102**: 535-42.
- Perkkiö M. Immunohistochemical study of intestinal biopsies from children with atopic eczema due to food allergy. *Allergy* 1980; **35**: 573-80.

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