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Humoral immune response in children with iron-deficiency anaemia

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Summary and conclusions

The humoral immune response (as shown by plasma immunoglobulin concentrations and antibody response to diphtheria and tetanus toxoids) was evaluated in 14 children with iron-deficiency anaemia and in 24 normal controls. Mean concentrations of haemoglobin and serum iron and mean transferrin saturation were significantly lower in children with iron-deficiency anaemia than in controls. Serum immunoglobulin concentrations were within the normal range in both groups. Two weeks after immunisation with diphtheria and tetanus toxoids the concentrations of IgG increased significantly in both groups. Antibody titres in iron-deficient children were similar to those of controls before and after immunisation. The mean T-lymphocyte count was significantly lower in iron-deficient children than that in controls, but the mean B-lymphocyte counts were similar in the two groups.

These observations suggest that humoral immunity in children is not affected by iron deficiency and that conventional immunisation programmes would be effective in children with iron-deficiency anaemia.

Introduction

Iron-deficiency anaemia is widely prevalent in many groups of the Indian population, particularly children and pregnant

women. There is increasing evidence that iron deficiency can affect host defence mechanisms.¹⁻³ Earlier studies from these laboratories have shown that the cell-mediated immune response and bactericidal activity of leucocytes were impaired in children with haemoglobin concentrations of under 10 g/dl. After iron supplementation, haemoglobin concentrations and immune functions were restored to normal.^{4,5} We report here on the humoral immune response in children with iron-deficiency anaemia.

Subjects and methods

We studied 14 children with iron-deficiency anaemia, and 24 apparently normal children served as control subjects. Their ages ranged from 2 to 10 years. Their weights were over 80% of the appropriate standard.⁶ Fasting samples of blood were collected for the estimation of haemoglobin concentration, packed cell volume, plasma iron concentration, and transferrin saturation. Informed consent was obtained from the parents of the control subjects before the samples were taken. Children were considered anaemic if their haemoglobin concentrations were 11.0 g/dl or less, as suggested by the World Health Organisation expert group.⁷ Plasma albumin concentrations were estimated to enable us to exclude the effects of associated protein and energy malnutrition. Haemoglobin concentrations were determined by a cyanmethaemoglobin technique,⁸ plasma iron concentrations and total iron binding capacities by the method suggested by the International Committee for Standardisation in Haematology,⁹ and plasma albumin concentrations by the dye method using Bromocresol green.¹⁰ Plasma vitamin B₁₂ and red blood cell folate concentrations were assayed microbiologically using *Euglena gracilis* (Z strain)¹¹ and *Lactobacillus casei*¹² respectively.

Humoral immune response was assessed by determining the following variables: plasma IgA, IgG, and IgM concentrations (determined by the radial immunodiffusion technique¹³); and rise in titres of antibody to diphtheria and tetanus toxoid two weeks after immunisation (determined by the indirect haemagglutination method¹⁴).

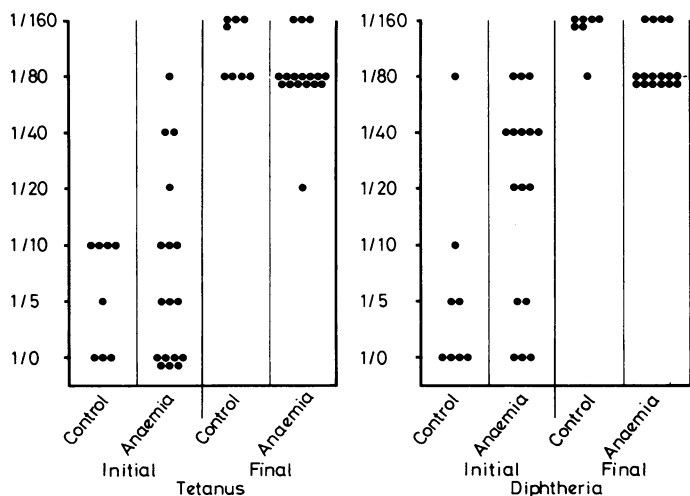
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B-lymphocyte and T-lymphocyte counts were determined by rosette formation technique¹⁵; 200 lymphocytes were examined, and cells binding three or more sheep red blood cells were counted and expressed as a percentage of the total lymphocyte population.

Results

Plasma albumin concentration was over 30 g/l (3 g/100 ml) in all the children. Mean haemoglobin concentration, packed cell volume, plasma iron concentration, and transferrin saturation were significantly lower in anaemic children than those in normal controls (table I). In controls the T-lymphocyte count ranged from 58 to 65% (mean 60.6).



Initial and final antibody titres to tetanus and diphtheria toxoids in normal control children and in children with iron-deficiency anaemia.

These values were significantly lower in anaemic children ($p < 0.001$). There were, however, no significant differences in B-lymphocyte counts between the two groups, the mean counts being 16.8% in controls and 18.7% in anaemic children. Plasma vitamin B₁₂ and red blood cell folate concentrations in the anaemic children were within the normal range.

Plasma IgA, IgG, and IgM concentrations were similar in both groups. Two weeks after immunisation with diphtheria and tetanus toxoids there was a significant increase in IgG concentration in both groups. Initially, some of the children showed antibodies against diphtheria and tetanus toxoids, the titres ranging from 1/20 to 1/40 (figure). After immunisation there was a considerable increase in

antibody concentrations in all the children, but there were no significant differences between the two groups.

Discussion

There is a widespread belief that iron-deficiency anaemia increases susceptibility to infection. Some reports have pointed out the high incidence of infections in anaemic children, which has been reduced after iron supplementation.^{16,17} Recent studies, however, do not support these initial observations. Burman¹⁸ and Fuerth¹⁹ found that the infection rates in infants were not related to their haemoglobin concentrations. A community study in South India in which two groups of preschool children were followed up for a year, one group receiving iron supplements and the other acting as controls, showed that there was no significant difference in morbidity between the two groups.²⁰ There is a clear need for further studies to settle this controversy.

In the last decade attempts have been made to study the relationship between iron deficiency and the immune response. Joynson *et al*²¹ were the first to show depressed cell-mediated immunity in iron-deficient subjects. Studies carried out by Chandra¹ and McDougall *et al*³ have also shown, by the skin test and in-vitro tests, that lymphocyte function was impaired in children with iron-deficiency anaemia. Interpretation of these results was difficult, since the presence and influence of other nutrient deficiencies and associated infection were not always excluded. In our study the T-lymphocyte count was significantly reduced in children with iron-deficiency anaemia who had no accompanying nutrient deficiencies or infection. This finding confirms observations reported earlier from this institute^{2,4} and indicates that the cell-mediated immune response is impaired in iron deficiency. In contrast, Gross *et al*²² reported that cell-mediated immunity was not altered in iron-deficient subjects. Kulapongs *et al*²³ have also found a normal cell-mediated immune response in children with iron-deficiency anaemia. The discrepancy in these findings may be due to differences in method and in the populations studied.

Although there have been several studies on cell-mediated immune response in iron deficiency anaemia, there is little information on the humoral immune response in this condition. Nalder *et al*²⁴ showed diminished antibody production in iron-deficient rats. The patients with iron-deficiency anaemia studied here, however, showed a normal humoral immune response, as shown by the concentrations of serum immunoglobulins and the antibody response to diphtheria and tetanus toxoids. These findings agree with those reported by other workers.^{1,3} We are not aware of any study suggesting an alteration in humoral immune response in people with iron deficiency.

TABLE I—Haematological variables in 24 normal control children and in 14 children with iron-deficiency anaemia. Results are given as means \pm SE

Group	Haemoglobin (g/dl)	Packed cell volume (%)	Plasma iron (μ mol/l)	Total iron binding capacity (μ mol/l)	Transferrin saturation (%)	Red blood cell folate (μ g/l)	Vitamin B ₁₂ (ng/l)
Control	12.2 \pm 0.23	33.5 \pm 0.62	15.68 \pm 1.03	71.60 \pm 2.29	23.5 \pm 1.40	104.3 \pm 20.85	252 \pm 22.9
Anaemia	8.9† \pm 0.36	28.4† \pm 1.16	10.40* \pm 0.85	95.22† \pm 4.85	11.4 \pm 1.06	123.9 \pm 8.09	295.9 \pm 21.2

Difference between means (control group *v* anaemia group) significant at: * $p < 0.01$, † $p < 0.001$.

Conversion: SI to traditional units—Plasma iron: 1 μ mol/l \approx 5.6 μ g/100 ml. Total iron binding capacity: 1 μ mol/l \approx 5.6 μ g/100 ml.

TABLE II—Plasma immunoglobulin concentrations, T-lymphocyte counts, and B-lymphocyte counts in 24 normal control children and in 14 children with iron-deficiency anaemia. Results are given as means \pm SE

Group	T cells (%)	B cells (%)	IgG (mg/100 ml)	IgM (mg/100 ml)	IgA (mg/100 ml)
Control	60.6 \pm 1.21	16.8 \pm 0.62	773 \pm 81.1	78.3 \pm 12.69	91.3 \pm 6.46
Anaemia	48.4 \pm 1.66*	18.7 \pm 1.30	1002 \pm 75.2	105.3 \pm 7.76	72.2 \pm 7.94

*Difference between means (control group *v* anaemia group) significant at $p < 0.001$.

Protein and energy malnutrition and iron-deficiency anaemia are widespread among preschool children in India. Because of a poor antibody response in severely malnourished children admitted to hospital it has been suggested that mass vaccination programmes would not be effective in populations where malnutrition is widespread. Earlier studies by this institute, however, have shown that most children in the community who suffer from mild or moderate protein and energy malnutrition respond well to conventional immunisation programmes.²⁵ Our present findings show that antibody production is also unaffected by iron deficiency. Thus conventional immunisation programmes may be effective even in undernourished communities.

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SHORT REPORTS

Human basophil degranulation test in diagnosis of hydatidosis

IgE antibodies directed against parasitic antigens in hydatidosis have been measured by the radioallergosorbent test (RAST).^{1,2} After challenge with antigen the basophils bearing specific IgE apparently disappear, and this phenomenon has been used as a diagnostic test in inhalant allergies.³⁻⁵ We report the results of the human basophil degranulation test (HBDT) performed on a suspension enriched with basophils in 12 patients with hydatidosis and in 68 control subjects.

Patients, methods, and results

The controls not suffering from hydatidosis were six newborn babies, 12 adults living in Paris (non-endemic area), and 50 black African adults with parasitic diseases other than hydatidosis. The patients were 10 adults with surgically proved hepatic cyst, one patient with only an epiploic cyst 3 cm in diameter, and one patient with a hepatic calcified cyst and positive serological reaction.

Venous blood 10 ml was drawn from patients and controls on to heparin and centrifuged for 15 min at 150 g to separate the plasma with platelets, which was discarded. The buffy-coat mixed with 5 ml HEPES-buffer⁴ was gently laid on 5 ml gradient density solution (1.080 g/ml, Institut Pasteur) in a round-bottomed plastic tube 100 mm × 18 mm. After centrifugation (400 g at the interface for 30 min) the ring containing lymphocytes and basophils was collected and washed once in HEPES-buffer (150 g, 10 min), which was then discarded except for about 0.5 ml at the bottom of the tube; 20 µl of this enriched cell suspension and 10 µl of the antigen solution (or 10 µl of buffer as control) were deposited in circles of the same surface area on a slide, which was then kept for 15 min at 37°C in a Petri dish containing wet cottonwool. After quick drying the slide was fixed in methanol before being stained with a solution containing toluidine blue (Institut Pasteur); washed successively in distilled water (30 s), ethanol (30 s), and

xylene; and mounted. The basophils were counted in equal numbers of randomly distributed microscopic fields. The degranulation, expressed as the percentage of basophils that apparently disappeared in the presence of antigen, was significant ($p < 0.001$) when higher than 35% if more than 100 basophils were counted in the control circles.⁴ The antigen was the liquid drawn from hepatic cysts during operation before injection of formalin or saline solution. It was distributed in aliquots and kept at -80°C before being used pure and diluted 1:10 and 1:100 in HEPES-buffer.

The basophils almost completely disappeared in 12 patients with pure hydatid liquid. The degranulation was highly significant at that antigen concentration and at lower concentrations except in one patient (case 9) who had an epiploic cyst (table). The degranulation was always below 35% in the 68 controls.

Number of basophils counted in 12 patients with hydatidosis

Case No	No of basophils*			
	Without antigen	With antigen		
		Pure†	1:10†	1:100†
1	484	29 (94%)	53 (89%)	126 (74%)
2	392	31 (92%)	71 (82%)	157 (60%)
3	195	52 (73%)	66 (66%)	104 (47%)
4	263	67 (75%)	75 (71%)	63 (76%)
5	147	16 (89%)	31 (79%)	63 (57%)
6	515	16 (97%)	27 (95%)	100 (81%)
7	206	29 (86%)	88 (57%)	ND
8	633	127 (80%)	316 (50%)	297 (47%)
9	404	101 (75%)	331 (18%)	421 (0%)
10	522	68 (87%)	157 (70%)	162 (69%)
11	168	24 (86%)	13 (92%)	32 (81%)
12	188	26 (86%)	55 (71%)	120 (36%)
Mean of degranulation (± SEM)		85 ± 2.2	70 ± 6.1	57 ± 7.3

*Each experiment or control was done in triplicate. Numbers represent the sum of basophils counted in 30 to 60 microscopic fields (10 to 20 fields per circle).
†Between parenthesis: % degranulation.