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IRON DEFICIENCY, CELL-MEDIATED IMMUNITY AND INFECTION AMONG 6-36 MONTH OLD CHILDREN LIVING IN RURAL TOGO[1,2]

Jacques Berger[3], PhD; Dominique Schneider, BSc; Jean-Luc Dyck, PhD; Ange Joseph, Aristide Aplogan, MD; Pilar Galan, MD; Serge Hercberg, PhD, MD.

Institut Français de Recherche Scientifique pour le Développement en Coopération (JB, DS, JLD, AJ, AA), Paris, France; Centre de Recherche sur les Anémies Nutritionnelles (PG, SH), Paris, France.

ABSTRACT

Relationships between iron deficiency, cell-mediated immunity (CMI) and morbidity were studied in 220 children in rural south Togo. Iron deficiency was defined by abnormal values of at least two biochemical indicators of iron status viz. (i) plasmat ferritin, (ii) transferrin saturation, (iii) erythrocyte protophorphyrin. In children without any sign of inflammation, the percentage of B lymphocytes was higher in iron-deficient than in iron-sufficient children (26.7 $\pm$ 2.9 vs 18.1 $\pm$ 1.5). Mature T lymphocyte and helper-inducer T lymphocyte percentages were lower (51.6 $\pm$ 3.7 vs 62.2 $\pm$ 1.6 and 32.5 $\pm$ 2.4 vs 38.7 $\pm$ 1.4, $p<0.05$ respectively). The number of mature T and helper-inducer T lymphocytes was inversely related to iron status. No alteration of the CMI function assessed by delayed skin hypersensitivity was observed. Prevalence of diarrhea, upper respiratory tract infections and febrile episodes was increased in iron-deficient children.

Key words: Iron deficiency, Iron-deficiency anemia, Cell-mediated immunity, Infection, children, West Africa.

INTRODUCTION

Nutritional anemia is a major public health problem in developing, but also to a lesser extent in industrialized countries (1). In 1976 and 1977, a survey conducted by the Centers for Disease Control of Atlanta in 163 villages and 41 urban blocks all over Togo, found a prevalence of anemia of 58.6% for 6-36 month old children (2); among them, 10% having

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3 Correspondence and reprint requests: Dr. Jacques Berger, ORSTOM-Nutrition, 2051 Av. Val de Montferrand, BP 5045, 34032, Montpellier Cedex, France.

severe iron deficiency anemia. The etiology of anemia would be a combination of inadequate iron intake, malaria, intestinal parasites and hemoglobinopathies (2).

There are many effects of iron deficiency. In addition to anemia, cell-mediated immunity and resistance to infections are affected (3-14). According to some researchers (3-5), the infectious morbidity is higher in iron deficient subjects; the frequency of infection is decreased or infection supressed by iron supplementation. Other authors (6, 7) note less infectious episodes in iron deficient subjects than in control subjects; infectious morbidity was increased by iron supplementation (6,7). It is often argued that increased risk of infection associated with iron deficiency is related to altered immunocompetence, specially cell-mediated immunity (3).

Most authors find a decreased number of peripheral T lymphocytes in iron deficiency (8, 9) and a return to normal after iron therapy (8, 10). There is more discussion regarding T lymphocyte function assessed by the blastogenic response to several antigens or by delayed hypersensitivity skin tests. This function is decreased in some studies (9-12), but not in other (13, 14). However, in these studies iron deficiency and anemia are not always well defined; protein-energy malnutrition and infection are not excluded.

This study aimed at assessing the relationships between iron deficiency and cell-mediated immunity in children living in a tropical environment, by studying T lymphocyte subsets and function using delayed hypersensitivity skin tests.

MATERIALS AND METHODS

Subjects

In this study, 220 children aged 6-36 months, living in a village 50 km north of Lomé, Togo, were investigated. This study is the baseline for longitudinal follow-up of a cohort of children. The population was informed of the purpose of the study. The study was conducted in accordance with the Togolese Ethical Committee for Human Experimentations.

Weight, height, mid arm circumference, triceps skinfold thickness and clinical examination were performed when the blood sample was collected.

Laboratory Methods

Blood was withdrawn by femoral venipuncture, collected in cooled heparinized tubes and kept at 4°C until analysis. All blood samples were collected between 7 to 8 a.m. An aliquot of blood was used to determine the hemoglobin (Hb) concentrations (cyanmethemoglobin method) and types (by electrophoresis), hematocrit (Ht), erythrocyte and leucocyte counts, erythrocyte protoporphyrins (EPP) (AVIV Biomedical hematofluorimeter). Plasma was then immediately separated by centrifugation and stored at - 70°C in 0.5 cc aliquots for determination of iron (colorimetric method), ferritin (PF) (ELISA with NIBSC international controls, London),

transferrin, orosomucoid, C-reactive protein (CRP) by radial immunodiffusion according to Mancini (15) using radial immunodiffusion plates from Berhing.

Lymphocytes were isolated from peripheral blood by Ficoll-Hypaque gradient-density centrifugation. Monoclonal anti-T cell antibodies (Ortho Diagnostics Systems Inc, Westwood, MA) were used to evaluate the proportion of T-Lymphocytes subsets by indirect immunofluorescence: the percentage of mature T cells, helper-inducer and cytotoxic-suppressor cells were determined using OKT3, OKT4, and OKT8, respectively (16). B lymphocytes (sIg-bearing lymphocytes) were quantified by microscopy (direct immunofluorescence with a fluorescein conjugated Ig-antiserum).

The Delayed Skin Hypersensitivity (DSH) responses were assessed by the "Multitest IMC" (Institut Mérieux, France) with seven different antigens (tetanus, diphtheria, streptococcus, tuberculin, candida, trichophyton, proteus) and one control. An induration greater than 2 mm was considered a positive response. The DHS score correspond to the sum of positive indurations of the seven antigens.

Nutritional status of children was assessed by anthropometric data and determination of plasmatic albumin and total protein concentrations. Height was measured to the nearest 0.1 cm using a wooden measuring board. Weight was measured to the nearest 0.01 kg using a SECA scale (UNICEF). Mid-upper arm circumference of the left arm was measured to the nearest 0.1 cm using an unextensible fiber-glass tape. Skin-fold triceps of the same arm was measured with an Harpenden's caliper. Nutritional indices for weight-for-age, height-for-age and weight-for-height were calculated in z-scores, representing the difference of an anthropometric measure with the NCHS reference expressed in standard deviation units (SD) (17). Total protein and albumin concentrations were determined by colorimetric method (Protienes-Kit and Albumine-Kit, Bio Mérieux, Marcy-l'Etoile, France).

Statistical Analysis

Statistical analysis was carried out using the Student's t test. Since plasma ferritin approached a log normal distribution, a log transformation was used for all calculations.

RESULTS

Anemia was defined as an hemoglobin concentration of less than 100 g/L in children under 24 months and of less than 110 g/L in children aged 24 months and more (1). Anemia was found among 165 of the 220 children (75%). Hemoglobin was type AA in 73.0% of chidren, AS in 13.1% and AC in 7.9% (SS: 0.4%, SC: 3.0%, SF: 1.0%) Hemoglobin was AF in 2.0% of children. The frequency of anemia was not related to the type of hemoglobin.

Iron deficiency was defined by abnormal values of at least two of the three independent indicators of iron status: (i) plasmatic ferritin of less than 12 μg/l, (ii) transferrin saturation (TfS) lower than 0.12, (iii) erythrocyte protoporphyrin more than 80 μg/dl red blood cells (18). Forty eight children were iron deficient (21.8 %). Hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), and the mean corpuscular volume (MCV) were lower in iron-deficient children. Moreover, the iron-deficient children showed lower TfS and PF and higher EPP. B lymphocytes subsets were not different between iron-deficient and iron-sufficient children. In contrast, number of mature T lymphocytes and helper-inducer T lymphocytes was decreased in iron-deficient versus iron-sufficient children: mature T lymphocytes (109/L); 2.4 $\pm$ 0.2 vs 3.0 $\pm$ 0.1, $p<0.01$ and T helper lymphocytes (109/L); 1.5 $\pm$ 0.1 vs 1.9 $\pm$ 0.1, $p<0.01$ in iron-deficient and iron-sufficient subjects respectively. The nutritional status of iron-deficient and iron-sufficient children was similar (height-for-age, mean $\pm$ SEM: -0.82 $\pm$ 0.14 vs -0.69 $\pm$ 0.11 and weight-for-height: -0.81 $\pm$ 0.14 vs -0.72 $\pm$ 0.07 in iron-deficient and iron sufficient children respectively).

To assess the relationships between iron deficiency and immunity, we eliminated the possible effect of infection and investigated only children free from biological signs of inflammation. An inflammatory process was defined by an orosomucoïd concentration of more than 1.4 g/l and/or C-reactive protein concentration of more than 12 mg/l and/or a leucocyte count of more than 12 109/L of blood. Biological signs of inflammation were found among 140 children (63.6%). Among the 80 children free from of inflammation, 24 were iron deficient and 56 iron sufficient. The biological data of children free from inflammation are presented in table 1.

Iron-deficient children showed lower Hb, Ht, MHCC, MCV, TfS and PF but higher EPP than iron-sufficient children. A low MCV, defined as less than 70 fl below 24 months and less than 73 fl above 24 months, was always related to abnormal values of at least two iron status indicators.

Table 2 shows lymphocyte distribution from the peripheral blood into B cells, mature T cells, helper-inducer cells, cytotoxic-suppressor cells. White cells and lymphocytes counts were not significantly different in either iron-deficient or iron-sufficient children. The percentage of B lymphocytes was higher in iron deficient children. On the other hand, mature T cell and helper-inducer cell percentages were lower in iron-deficient children.

There were no significant differences for mean values between the two groups of the percentage or number of cytotoxic-suppressor cells or of the ratio of helper to cytotoxic cells. However, 43.8% of iron deficient and 57.8 % of iron sufficient children showed a ratio markedly increased (>2). Only one child from each group had a depressed ratio (<0.8).

Percentage of mature T cells and helper-inducer cells was correlated with EPP ($r = -0.34$ and $r = -0.26$, $p<0.05$), TfS ($r = 0.29$ and $r = 0.33$, $p<0.05$), MCV ($r = 0.30$ and $r = 0.31$, $p < 0.05$), Hb ($r = 0.28$ and $r = 0.30$, $p<0.05$) and plasmatic iron ($r = 0.31$ and $r = 0.28$, $p < 0.05$). The ratio of helper to cytotoxic cells was correlated with MCV ($r = 0.29$, $p < 0.05$). B lymphocytes were only correlated with TIBC ($r = 0.27$, $p<0.05$). Although these correlation coefficients were not very high, they strengthened the link between the decrease of

T cells and iron deficiency. Delayed skin hypersensitivity tests showed no differences between iron-deficient and iron-sufficient children (score: 9.17 ± 1.80 vs 9.59 ± 0.87). Number of children responding to each antigen was the same in both groups. The number of children showing anergy was however higher in the iron-deficient group.

The nutritional status of both iron-deficient and iron-sufficient children was similar (table 3). According to the classification of Waterlow (19) only five iron sufficient children had chronic protein-energy malnutrition (height-for age less than -2 SD). However, all children had normal albumin and total protein concentrations. In children, with and without biological signs of inflammation, the mean DHS score did not show any significant difference between iron-deficient and sufficient-children.

TABLE 1

Laboratory Data among Iron-deficient and Iron-sufficient Children in Absence of Biological Markers of Inflammation (mean ± SEM)

	Iron-deficient n=24	Iron-sufficient 6n=56
Hemoglobin (g/l)	92 ± 2	103 ± 2 *
Hematocrit	0.31 ± 0.01	0.32 ± 0.01 #
MCV (fl)	77 ± 1	87 ± 1 *
MCHC (g/L)	301 ± 4	318 ± 2 *
Eryhtrocyte count (1012/L)	4.1 ± 0.1	3.7 ± 0.1 $
Plasmatic iron (μmol/L)	6.4 ± 0.4	11.1 ± 0.4 *
TIBC (μmol/L)	79.3 ± 1.9	67.2 ± 2.3 *
Transferrin saturation	0.08 ± 0.01	0.17 ± 0.01 *
Erythrocyte protoporphyrin		
(μg/g Hb)	5.6 ± 0.6	2.5 ± 0.2 *
(μg/dL EC**)	165 ± 18	78 ± 3 *
Plasmatic ferritin (μg/L)	41.0 ± 9.8	85.9 ± 9 *
Geometric mean	25.4	70.3
Leucocyte count (109/L)	7.6 ± 0.3	7.7 ± 0.3
Orosomucoid (g/L)	1.04 ± 0.05	1.10 ± 0.03
CRP (mg/L)	1.9 ± 0.6	1.8 ± 0.4
Transferrin (g/L)	3.17 ± 0.08	2.69 ± 0.06 *

Significant difference between iron-deficient and iron-sufficient children: * $p < 0.001$; # $p < 0.01$; $ $p < 0.05$; ** EC: Erythrocyte Count.

TABLE 2

Peripheral Lymphocytes Distribution into Several Subsets among Iron-deficient and Iron-sufficient Children free from inflammation (mean ± SEM).

	Iron-deficient n=17	Iron-sufficient n=42
Leucocyte count (10^9/L)	7.9 ± 0.4	7.6 ± 0.3
Lymphocytes (%)	63 ± 4	67 ± 2
Lymphocyte count (10^9/L)	5.0 ± 0.4	5.0 ± 0.2
Lymphocytes subset (%)		
sIg-bearing cells	26.7 ± 2.9	18.1 ± 1.5 *
Mature T cells	51.6 ± 3.7	62.2 ± 1.6 *
Helper-inducer T cells	32.5 ± 2.9	38.7 ± 1.4 *
Cytotoxic-supressor cells	18.4 ± 1.7	19.7 ± 1.3
Thymocytes	6.2 ± 1.9	5.3 ± 1.4
NonB-NonT cells	22.4 ± 4.4	19.9 ± 2.1
T helper/T suppressor ratio	2.01 ± 0.21	2.45 ± 0.22
Lymphocyte subset count (10^9/L)		
sIg-bearing cells	1.4 ± 0.2	0.9 ± 0.1
Mature T cells	2.5 ± 0.2	3.2 ± 0.2 *
Helper-inducer T cells	1.5 ± 0.1	2.0 ± 0.1 *
Cytotoxic-supressor T cells	0.9 ± 0.1	1.0 ± 0.1
Thymocytes	0.4 ± 0.1	0.3 ± 0.1
Non B-Non T cells	1.1 ± 0.2	1.0 ± 0.1

Significant difference between iron-deficient and iron-sufficient children;
* $p < 0.05$

Morbidity was not different in the two groups (Table 4). Diarrhea or dysentery episodes, upper respiratory tract infections and febrile episodes were however more frequent in iron-deficient than in iron-sufficient group.

The proportion of T lymphocyte subsets and the response to DHS tests were not significantly different between anemic and non anemic children.

TABLE 3

Nutritional Status of Iron-deficient and Iron-sufficient Children Free From Inflammation (mean ± SEM).

	Iron-deficient n=24	Iron-sufficient n=56
Anthropometric data (z-score):		
Heigt-for-age	- 0.76 ± 0.18	-0.47 ± 0.21
Weight-for-age	- 1.08 ± 0.19	- 0.98 ± 0.14
Weight-for-height	- 0.72 ± 0.19	- 0.74 ± 0.11
Biological data:		
Total Protein g/L	72.9 ± 1.29	72.8 ± 0.73
Albumin g/L	40.1 ± 0.82	39.0 ± 0.40

TABLE 4

Frequency of Different Infections among Iron-sufficient and Iron-deficient Children

Infections	Iron deficient n=43	Iron sufficient n=177
Upper respiratory tract	39.5	20.9 *
Cuteaneous	14.0	17.5
Diarrhea/dysentery	14.0	3.4 *
Acute respiratory	16.3	18.6
Malaria episodes	14.0	8.5
Conjonctivitis	4.7	10.7
Febrile episodes	30.2	9.6 #

Significant difference between iron-deficient and iron-sufficient children:
* $p < 0.02$; # $p < 0.01$.

DISCUSSION

The number of circulating lymphocytes between iron-deficient and iron-sufficient children is not significantly different. This is in agreement with the study of Mc Dougall (12) in 13-14 month old children. The larger number of lymphocytes in this study compared to our study is due to an higher level of white cells since the percentage of lymphocytes is the same in both studies.

In our study, the distribution of B and T lymphocytes in healthy children is similar to the study of Fleischer et al (20) in 7 days-18 month children although laboratory techniques were different and to the study of Wade et al (21) in apparently healthy Senegalese children.

In children without any inflammatory process, the percentage of T mature lymphocytes and T helper-inducer lymphocytes was decreased by iron deficiency. Our results are in agreement, for T mature cells, with those of studies carried out in children (8, 11) and in adults (9). While the percentage of T helper-inducer lymphocytes was lower in iron-deficient children, the percentage of T cytotoxic-suppressor cells was not different. The decrease in mature T lymphocyte percentage was accounted by the decreased percentage of helper-inducer T lymphocytes. On the other hand, the percentage of B lymphocytes and antibodies producing cells was higher in iron-deficient children.

To our knowledge, the decreased percentage of helper-inducer cells and the increased percentage of B lymphocytes is reported here for the first time in iron-deficient children free from inflammatory process. A similar decrease of mature T cells and helper-inducer cells was observed in protein-energy malnutrition together with an increase of nonB-nonT lymphocytes (22). In our study, iron-deficient children did not show any change in nonB-nonT lymphocytes, but there was a higher B lymphocytes number which is not shown in protein-energy malnutrition (22). Unfortunately, we did not investigate the circulating immunoglobulins. In our study, no iron-deficient child had signs of severe or moderate protein-energy malnutrition. Moreover, we observed an inverse connection between EPP, TfS, CMV, plasmatic iron and mature T or helper-inducer T lymphocytes: these T lymphocyte populations decreased when the severity of iron deficiency increased. The decreased helper-inducer T lymphocyte number would be related to iron deficiency.

Differenciation and maturation of T lymphocytes take place in thymus, under dependance of thymic hormones. Iron deficiency is known to decrease the number of thymic cells (23), to depress thymus growth and function (24). The decrease of peripheral T helper lymphocyte number observed with iron deficiency may be due to a defect in differenciation or maturation process.

Skin delayed hypersensitivity reflects the proliferation capacity of T lymphocytes. Although the helper-inducer T lymphocyte percentage was decreased in iron-deficient children, the mean test scores were not different: the decrease of helper-inducer T cell percentage might not be strong enough to alter the immune response. Tetanus, diphtheria and tuberculin antigens were more often positive than other antigens and certainly related to the immunization programs.

Moreover, the number of anergic children tended to be higher, though not significantly, in iron-deficient children.

Previous studies on the relationships between iron deficiency and delayed hypersensitivity give different results. Some studies show a lower skin delayed hypersensitivity in case of iron deficiency (11, 12). In contrast, Gross et al. (14) report no abnormality of the skin delayed response in iron-deficient children. In Kemahli's study, (25) response to tuberculin do not show any difference between iron-deficient and control subjects whereas a less important response to DNCB is observed in iron-deficient children.

The studies who do not find any abnormality are those done on children free from other nutritional deficiency and infection. Iron-deficient children from our study were free from protein-energy malnutrition, and recent infections. Iron deficiency was established by a combination of biochemical indicators.

The higher frequency of febrile episodes, diarrhea or dysentery episodes and upper respiratory tract infections showed in iron-deficient children is in accordance with previous studies (3-5). However, all these studies do not prove that a lower resistance to infection is the result of decreased immunocompetence due to iron deficiency. This study does not allow us to relate the frequency of infectious episodes to the altered cell-mediated immune response.

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