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Effect of daily iron supplementation on iron status, cell-mediated immunity, and incidence of infections in 6–36 month old Togolese children

J Berger[1]*, JL Dyck†, P Galan[2], A Aplogan[1], D Schneider[1], P Traissac[1] and S Hercberg[2]

[1]*IRD (Institut de Recherche pour le Développement), Nutrition Unit, 911 Avenue Agropolis, BP 5045, 34032 Montpellier Cedex, France; and* [2]*Centre National des Arts et Métiers, Centre de Recherche sur les Anémies Nutritionnelles, Paris, France*

Objective: To assess the impact of a daily oral iron supplementation on hematological status, cell-mediated immunity and susceptibility to infections in children living in an environment where iron deficiency, malaria and other infections are frequent.
Design: Randomized, double-blind iron supplementation including a placebo group.
Setting: A village in Togo, West Africa.
Subjects: Of the 229 6–36-month-old children of both sexes recruited, 197 with hemoglobin concentration ≥ 80 g/l were included and 163 completed the study.
Intervention: Children received daily a placebo ($n=79$) or a dose of 2–3 mg of elemental iron per kg of body weight ($n=84$) for 3 months. Hematological, nutritional and immune status were assessed at the beginning and at the end of the supplementation period, and 6 months later. Morbidity was recorded throughout the study.
Results: Iron supplementation had a significant and positive effect on iron status of children and no impact on the incidence of infections, especially malaria. Its probable effect on immune status was masked by interference of infections and their treatment, which contributed to improve hematological and immune status in both groups.
Conclusion: According to the negative consequences of anemia and iron deficiency on global child development, control of iron deficiency by oral iron supplementation in young children has to be conducted, associated with prophylaxis and treatment of malaria and repeated deworming.
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Descriptors: anemia; iron deficiency; iron supplementation; cell-mediated immunity; infection; malaria; children; developing country; Africa
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Introduction

Iron deficiency anemia is a major worldwide health problem, especially in developing countries where 2 billion individuals are concerned, particularly infants and children (CIN, 1992).

The negative consequences of iron deficiency anemia on behavior (Johnson *et al*, 1992), psychomotor development (Lozoff *et al*, 1991), and growth rate (Fairweather-Tait, 1992) are well established and underline the need to control iron deficiency anemia. Iron supplementation has proven to be useful, particularly in infants and children, when iron deficiency is important and has to be corrected rapidly (Hercberg, 1988).

However, several studies have indicated that iron deficiency protects against infection and iron supplementation increases morbidity (Harvey *et al*, 1989; Oppenheimer *et al*, 1986). Other studies show the opposite, i.e. morbidity is higher in iron deficient children and is reduced by iron supplementation (McKay, 1928; Andelman & Sered, 1966; Beisel, 1982), whereas some studies do not show any effect of iron supplementation or fortification on incidence of infections (Harvey *et al*, 1989; Heresi *et al*, 1995).

Iron deficiency would decrease the resistance to infection through impairment of immune competence of the individual, particularly cell-mediated immunity (Dallman, 1987). However, while some studies conclude alteration of cell-mediated immunity (Chandra and Saraya, 1975; Krantman *et al*, 1982; Swarup-Mitra and Sinha, 1984; Berger *et al*, 1992), other studies do not show any effect (Kulapongs *et al*, 1974; Gupta *et al*, 1982; Grosch-Worner *et al*, 1984; Thibault *et al*, 1993).

The evaluation of balance between the risks and benefits of an oral iron supplementation is of particular importance before implementing an intervention. The object of this study was therefore to assess, through a longitudinal double-blind randomized and placebo-controlled study,

*Correspondence: J Berger, IRD, Service Culturel, Ambassade de France, 57 Tran Hung Dao, Hanoi, Vietnam.
† JL Dyck died in February 1999.
Guarantor: Dr Jacques Berger, IRD, Servic Culturel, Ambassade de France, 57 Tran Hung Dao, Hanoi, Viêt Nam Tél: 84-4-971 48 26, Fax: 84-4-971 78 85, E-mail: j.berger@fpt.vn
Contributors: Jacques Berger, Jean Luc Dyck, Pilar Galan, Serge Hercberg and Dominique Schneider participated to the study design. Jacques Berger and Jean Luc Dyck had primary responsibility for implementation, personnel supervision and data collection and entry. Biological and biochemical analysis were carried out by Jacques Berger, Dominique Schneider, Jean Luc Dyck and Pilar Galan. Aristide Aplogan was in charge with the medical management of subjects and collection of morbidity data. Jacques Berger and Pierre Traissac carried out the data analysis. The paper was written by Jacques Berger with contributions of Pilar Galan and Serge Hercberg.
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the impact of an oral iron supplementation on the hematological status, the cell-mediated immunity and the susceptibility to infections in 6–36-month-old children living in an environment where iron deficiency, but also malaria and other types of infections are frequent.

Subjects and methods

Design

The effect of the iron supplementation on iron status, incidence of infection and cell-mediated immunity was tested by random assignment of children aged 6–36 months in an intervention group and a placebo group. The study was double blind and had a total duration of 9 months. The supplementation period started at the beginning of the study and lasted for 3 months. Iron status, cell-mediated immunity, and malaria-related parameters were measured at the beginning of the study (T0), 3 months after, the day following the last dose of iron (T3) and again 6 months after, i.e. 9 months after the start of the study (T9). Morbidity was recorded throughout the study period from T0 to T9.

Setting

The study was carried out in a village of the sea region of Togo, starting at the beginning of the rainy season when malaria transmission occurs and continued through the following dry season.

Subjects

Subjects were all the 6–36-month-old children of both sexes living in the selected village who had, at inclusion, a hemoglobin concentration equal to or higher than 80 g/l. Sample size calculation indicated that at least 87 subjects were required in order to distinguish a difference in hemoglobin equal or greater than 5 g/l between groups, with a type I error of 5% and a power of 95% with a one-tailed *t*-test, assuming a standard deviation of 10 g/l. Because of anticipated dropouts, the sample size was established at 100 subjects by groups. Of the 229 children measured, 32 (14%) had a hemoglobin concentration lower than 80 g/l. They were not included in the study and were given daily iron supplementation for 3 months. Included children (197) were randomly assigned to two groups: Group 1 ($n=100$) received a daily dose of iron; Group 2 ($n=97$) received, also daily, a placebo.

Supplementation

The iron dosage was calculated to provide 2–3 mg of elemental iron per kg of body weight per day. The supplement consisted of tablets containing 15 mg of iron in the form of iron betainate, used in combination to adjust the dosage for the child's weight. The placebo consisted of the tablets without iron. Tablets were given every day directly to children, at home, with clean boiled water, in the morning by trained field assistants under the supervision of a member of the research team.

Methods

Seven milliliters of blood were drawn from the femoral artery at T0, T3 and T9 between 7 and 8 a.m. for immediate determination of hemoglobin concentration (cyanmethemoglobin method), erythrocyte (RBC) and leucocyte counts, zinc erythrocyte protoporphyrin (AVIV hematofluorometer).

Lymphocytes were isolated from blood by Ficoll-Hypaque gradient-density centrifugation. Monoclonal anti-T cell antibodies (Ortho-Diagnostic System, Westwood, MA, USA) were used to evaluate the proportion of T-lymphocyte subsets by indirect immunofluorescence. Percentage of mature T cells, helper-inducer T cells and cytotoxic-suppressor T cells were determined using OKT3, OKT4 and OKT8, respectively. B lymphocytes were quantified by direct immunofluorescence with a fluorescein-conjugated Ig-antiserum. Then cell numbers were calculated.

Malaria parasite density was measured in a smear stained with May-Grünwald Giemsa stain, by counting parasitized red blood cells (PRBC), asexual forms of *Plasmodium falciparum*, and all forms of *P. malariae* and *P. ovale* in 75 microscopic fields, with a mean detection threshold of 150 parasitized red blood cells per cubic millimeter (PRBC/mm^3). Titer of antimalarial antibodies was determined by immunofluorescence (Falciparum SpotR, BioMerieux, France). Analysis was performed at the same time on all samples from the three time points of the study.

Plasmatic iron was determined by colorimetric method, ferritin by ELISA (references NIBSC, Londres, UK), and transferrin, orosomucoid and C-reactive protein (CRP) by radial immunodiffusion according to Mancini (Radial immunodiffusion plates, Berhing). Delayed-hypersensitivity skin test (DHST) response was assessed at T0, T3 and T9 with the Merieux Multitest CMI skin test (Pasteur-Merieux, Lyon, France) with seven antigens and a control. Reaction was assessed 48 ± 2 h after injection by measuring the mean induration diameter. Induration ≥ 2 mm was considered a positive response.

Anthropometry was assessed at T0, T3 and T9. Body height was taken with a precision of 1 mm by using an accurate handcrafted infantometer. Body weight was measured to the nearest 10 g with a SECA scale. The anthropometric indicators, height-for-age and weight-for-height were expressed in *Z* scores according to the National Center for Health Statistics (NCHS) reference using EPI-INFO 6.03 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Morbid episodes were recorded every day by a pediatrician. Before the beginning of the study parents were taught to bring their children to the pediatrician present each morning in the village when they suspected a sign of illness. Children were specifically treated for the illness diagnosed at any time in the course of the study. All isolated fevers were considered a symptom of malaria and treated with antimalaria drugs (Chloroquine^R).

Before inclusion, stool samples of children were analyzed for intestinal parasites by direct microscopic examination and with Bailenger's method (Bailenger, 1963). All children with positive results were treated before the start of the supplementation with Pyrantel pamoate and/or thiabendazole and/or metronidazole, according to the parasites found (Aplogan *et al* 1990).

Anemia was defined by a hemoglobin concentration of less than 110 g/l (INACG, 1985) and iron deficiency by a combination of at least two abnormal values for the three following indicators: serum ferritin ≤ 50 μg/l to take into consideration the effect of inflammation (Fleming, 1982), transferrin saturation $\leq 12\%$ and zinc erythrocyte protoporphyrin concentration ≥ 80 μg/dl RBC (INACG, 1985). Inflammation was characterized by a C-reactive protein (CRP) concentration above 12 mg/l or orosomucoid level

above 1.4 g/l or leukocyte count of more than 12,000/mm^3 of blood.

The study protocol was approved by the Pediatrics Service of Tokoin Hospital, Lomé and by the head of the nutrition antenna of OCCGE, Lomé, Togo. Only children with parental consent were included in the study.

Statistical analysis

The statistical analysis of data sets were performed using STATISTIX 4.1 (Analytical Software, Tallahassee, FL, USA) and SAS (SAS Institute, Cary, NC, USA). Differences between the two groups, before supplementation, were tested by Student's t-test and the χ^2 test. Differences between the two groups after supplementation and at the end of the study were tested by analysis of variance with repeated design measures and by analysis of covariance, adjusting for the pre-treatment value of the dependent variable. Since serum ferritin and CRP approach a log–normal distribution, a log transformation of these variables was used for all calculations. The transformation log $(\times + 1)$ was carried out on individual malaria parasite densities. Difference of prevalence of anemia, iron deficiency and infections, and incidence of infections between groups were tested by the χ^2 test, while changes of prevalence of anemia and iron deficiency in every group were tested by McNemar's symmetry test. Because a mean number of positive responses and induration of the DHST are not normally distributed, differences in such data between groups were evaluated by the rank sum test and changes in every group by the non-parametric Wilcoxon signed-rank test.

Results

Among the 197 children included, 163 (82.7%) completed the study, 84 in group 1 and 79 in group 2. The 34 dropouts (17.3%), 16 from group 1 and 18 from group 2, were mainly due to migration of the family out of the village or to the refusal of parents to wait each morning at home for the iron tablets. Iron and nutritional status of dropouts were not statistically different at T0 from those of children who completed the study.

At T0, groups were comparable for age, nutritional indices, and iron- and inflammation-related parameters (Tables 1 and 2), Hematological values at the three time points of the study are reported in Table 2. Initial mean hemoglobin concentration was low in both groups. Mean values of Zinc erythrocyte protoporphyrin (EPP) and of plasmatic ferritin (PF) were high, while mean values of transferrin saturation (TfS) were in the normal range. The high values of PF could be attributed to an inflammatory process diagnosed in 54.8% of children from group 1 and in 63.3% from group 2.

Table 1 Nutritional status of children at the beginning of the study (mean±s.d.)

	Group 1 (n=84)	*Group 2 (n=79)*	P^a
Age (months)	22.8±8.42	24.9±8.3	0.11
Height (cm)	82.4±8.1	83.7±7.5	0.28
Weight (kg)	10.7±2.1	11.0±1.9	0.34
Height-for-age (Z scores)	−0.61±1.47	−0.74±1.29	0.57
Weight-for-height (Z scores)	−0.69±0.91	−0.67±0.92	0.95

Group 1: iron supplemented children; group 2: placebo.
[a]P value between group 1 and group 2 (Student's t-test).

Table 2 Iron and inflammation-related parameters in groups 1 and 2 at the three time points of the study (mean±s.d.)

	Group 1(n=84)	*Group 2(n=79)*	P^b	P^c
Hemoglobin (g/l)				
T0[a]	98.9±11.6^{Ad}	100.4±10.6^A	0.38	—
T3[a]	107.7±12.8^B	104.1±11.7^B	0.07	0.02
T9[a]	115.3±10.5^C	114.8±10.8^C	0.77	0.54
Transferrin saturation (%)				
T0	18.3±10.1^A	17.0±7.78^A	0.38	—
T3	20.8±10.8^A	13.9±6.51^B	0.0001	0.0001
T9	19.2±7.6^A	17.7±12.61^A	0.35	0.48
Erythrocyte protoporphyrin (μg/dl red blood cells)				
T0	105±63^A	101±62^A	0.73	—
T3	104±59^A	133±66^B	0.005	0.0003
T9	67±45^C	71±50^C	0.64	0.43
Ferritin (μg/l)				
T0	109.2±110.6^A	109.7±138.6^A	0.78	—
T3	126.2±52.6^A	75.4±59.9^B	0.0003	0.0003
T9	59.4±56.9^B	39.5±39.4^C	0.03	0.003

[a]T0: before supplementation, T3: at the end of supplementation period, T9: 6 months after the end of the supplementation period.
[b]P value between groups (analysis of variance).
[c]P value between groups (analysis of covariance, adjusted for the pre-treatment value of the dependent variable).
[d]Significant difference ($P<0.05$) in the same group when letters differ.

At T3 (at the end of the supplementation period) and at T9 (6 months after), mean hemoglobin concentration was not significantly different between groups. However, after adjustment for the initial hemoglobin, mean hemoglobin concentration was significantly higher in group 1 at T3 ($P=0.02$), but not different between the two groups at T9 $P=0.54$). Between T0 and T3, hemoglobin increased significantly by 8.8±14.2 g/l ($P<0.0001$) in group 1 and by 2.7±1.2 g/l ($P=0.007$) in group 2; the increase was significantly higher in the iron group ($P=0.02$). Between T3 and T9, hemoglobin concentration increased again significantly by 7.6±12.5 g/l ($P<0.0001$) in group 1 and by 10.7±12.6 g/l ($P<0.0001$) in group 2. At the end of the study, the hemoglobin distributions of both groups were gaussian (Wilks-Shapiro test: 0.99 and 0.98 for groups 1 and 2, respectively).

All three biochemical indicators of iron status were significantly different between groups at T3, indicating that iron status was significantly better in group 1 than in group 2. Between T0 and T3, TfS increased by 2.5±12.5% ($P=0.06$) in group 1, whereas it decreased by 3.1±7.5% ($P=0.01$) in group 2. Other indicators of iron status were significantly modified only in group 2: EPP increased by 32±56 μg/dl RBC ($P<0.0001$) and PF decreased by 34.3±131.2 μg/l ($P=0.04$). Between T3 and T9, iron status improved, especially in group 2. At T9, indicators of iron status were not significantly different between both groups except for PF, which was higher in group 1.

Figure 1 presents the evolution of prevalence of anemia with or without iron deficiency and iron deficiency without anemia. Prevalence of anemia was not significantly different between groups at any time point of the study. Prevalence of anemia decreased significantly in group 1 from 84.5% at T0 to 54.8% at T3 and 26.2% at T9, and in group 2 from 79.8% at T0 to 63.3% at T3 and 31.7% at T9. A T0, prevalence of iron deficiency, with or without anemia, was not different between groups but lower in group 1 at T3 ($P<0.0001$) and T9 ($P=0.0001$). Between T0 and T3, prevalence of iron deficiency decreased significantly in

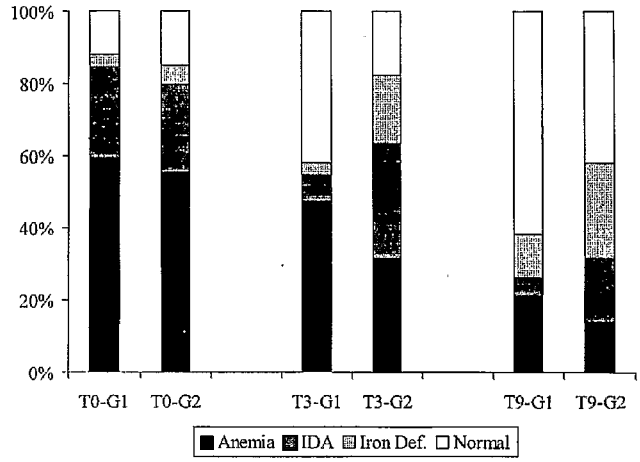

Figure 1 Proportion of children with iron deficiency (ID), iron deficiency anemia (IDA) and anemia without iron deficiency at the beginning of the study (T0), at the end of the supplementation period (T6) and six months after (T9). G1: group 1; G2: group 2.

Table 3 Prevalence and incidence of infections (%)

Infections	*Group 1*	*Group 2*
Lower respiratory tract infections		
T0[a]	3.0	5.3
T0–T3[b]	10.1	12.3
T3–T9[b]	5.1	7.6
Upper respiratory tract infections		
T0	30.3	37.9
T0–T3	40.1	41.8
T3–T9	13.9	16.1
Cutaneous infections		
T0	17.2	16.8
T0–T3	14.4	11.7
T3–T9	6.7	4.6
Diarrhea		
T0	6.1	5.3
T0–T3	13.8	9.6
T3–T9	9.6	4.9
Fever		
T0	5.1	5.3
T0–T3	26.0	25.1
T3–T9	7.8	7.2

[a]Prevalence at T0.
[b]Incidence density rate: new cases per 100 children-month between time points T0 and T3 (T0–T3), or between time points T3 and T9 (T3–T9). No significant differences for any of the variables between groups 1 and 2 at the three time points of the study.

group 1 from 28.6% to 10.7% and increased significantly in group 2 from 29.2% to 50.6%. Between T3 and T9, prevalence of iron deficiency did not change significantly in both groups and at T9 prevalence remained significantly lower at T9 in group 1 (16.9%) than in group 2 (44.3%).

Table 3 shows the prevalence of infections at T0 and their incidence during the 9-month follow-up. At T0, the prevalence of all infections was comparable in both groups, with a predominance of respiratory infections. The incidence density rate of infections (new cases per 100 children-month) during the supplementation period and the follow-up was not statistically different between both groups. The incidence density rate of all infections was higher between T0 and T3 than between T3 and T9. The respiratory tract infections were always more frequent (concerning approximately one-half of the children) and associated with half of the febrile episodes.

Data related to malaria are presented in Table 4. At T0 the mean parasite index was 0.62, mainly *Plasmodium falciparum* (more than 97%) and was not different between groups. The mean parasite density, not different between the two groups at the three time points of the study, decreased during the study. The frequency of high parasite densities, higher than either 3000 or 10,000 PRBC/mm^3, not different between both groups at any phase, also decreased throughout the study. The mean titer of antimalaria antibodies did not show any difference between the two groups at the three time points of the study.

At T0, 42% of the children had intestinal parasites. The more frequent were *Giardia intestinalis* (in 20.8% of children), *Necator americanus* (12.9%), and *Ascaris lumbricoides* (12.5%). The proportion of children with inflammatory processes was not different between groups at T0 (54.8 *vs* 63.3%) and T9 (44.0 *vs* 39.2%) but was different at T3 (61.9 *vs* 46.8%, $P=0.054$) and was lower at the end than at the beginning of the study.

The effect of iron supplementation on cell-mediated immunity is presented in Tables 5 and 6. At the three time points of the study, percentages of B and T lymphocytes and of T-lymphocyte subsets were not significantly different between groups, whereas mature and helper-inducer T cell numbers were higher in group 1 than in group 2 at T9. The percentage of helper-inducer T lymphocytes increased significantly between T0 and T3 by 6.0 ± 13.0 ($P=0.0003$) in group 1 and by 5.6 ± 12.0 ($P=0.0003$) in group 2. The same figure was observed for absolute numbers. Only group 1 showed a significant increase of helper-inducer/suppressor-cytotoxic T lymphocyte ratio by 0.43 ± 1.59 ($P=0.03$) and of mature T lymphocyte percentage by 5.2 ± 13.2 ($P=0.002$). At T9, mean percentages of mature and helper-inducer T cells and mean values of helper-inducer/suppressor-cytotoxic T cell ratio were not significantly different compared with T3, but were higher

Table 4 Malaria status at the three time points of the study

	Group 1	*Group 2*
Plasmodic indice (%)		
T0[a]	59.3^{A}	63.6^{A}
T3[a]	58.2^{A}	49.3^{A}
T9[a]	47.3^{A}	53.5^{A}
Parasitic density (parasitized red blood cells/mm^3, geometric mean)		
T0	89.8^{Ab}	143.8^{A}
T3	61.2^{B}	25.7^{B}
T9	23.9^{B}	33.2^{B}
Proportion of children with parasitic density > 3000		
T0	27.2^{Ac}	27.3^{A}
T3	11.9^{B}	4.4^{B}
T9	3.6^{B}	0
Proportion of children with parasitic density > 10,000		
T0	8.6^{Ac}	16.9^{A}
T3	3.0^{A}	1.5^{B}
T9	3.6^{A}	0
Antimalaria antibodies titer (geometric mean)		
T0	474^{A}	594^{AB}
T3	564^{A}	669^{A}
T9	502^{A}	461^{B}

[a]T0:before supplementation; T3: at the end of supplementation period; T9: 6 months after the end of the supplementation period.
No significant differences for any of the variables between iron and placebo groups at the three time points of the study.
[b]Wilcoxon signed rank test. And [c]McNemar's test. Significant difference in the same group when letters differ ($P<0.05$).

Table 5 Distribution of B and T lymphocyte populations and of T lymphocyte subsets (mean ± s.d.) at the three time points of the study (percentage of lymphocytes)

	Group 1(n = 84)	*Group 2(n = 79)*	P^b	P^c
Lymphocytes				
T0[a]	60.7 ± 19.6$^{AB\ d}$	55.5 ± 22.7^{A}	0.12	—
T3[a]	58.6 ± 18.0^{A}	61.4 ± 13.5^{B}	0.26	0.21
T9[a]	63.2 ± 10.3^{B}	59.4 ± 11.7^{AB}	0.03	0.03
B cells				
T0	22.4 ± 11.7^{A}	20.9 ± 10.2^{A}	0.40	—
T3	23.1 ± 9.4^{A}	22.0 ± 10.5^{A}	0.52	0.65
T9	18.5 ± 10.0^{B}	20.1 ± 9.7^{A}	0.30	0.46
Mature T cells (OKT3)				
T0	57.5 ± 12.7^{A}	59.3 ± 12.1^{A}	0.39	—
T3	62.5 ± 10.4^{B}	62.1 ± 9.6^{AB}	0.82	0.30
T9	63.6 ± 10.1^{B}	63.6 ± 10.1^{B}	1.00	0.68
Helper-inducer T cells (OKT4)				
T0	38.0 ± 10.7^{A}	38.1 ± 8.3^{A}	0.98	—
T3	44.7 ± 10.0^{B}	44.4 ± 9.1^{B}	0.81	0.79
T9	45.6 ± 9.0^{B}	45.3 ± 9.9^{B}	0.81	0.69
Supressor-cytotoxic T cells (OKT8)				
T0	18.3 ± 7.4^{A}	19.5 ± 8.1^{A}	0.36	—
T3	17.3 ± 7.9^{AB}	17.7 ± 6.9^{A}	0.75	0.71
T9	16.2 ± 0.5^{B}	17.7 ± 6.6^{A}	0.12	0.15
T4/T8 ratio				
T0	2.45 ± 1.27^{A}	2.42 ± 1.76^{A}	0.90	—
T3	3.10 ± 1.65^{B}	2.89 ± 1.30^{A}	0.38	0.87
T9	3.33 ± 1.88^{B}	2.98 ± 1.66^{A}	0.21	0.18

[a]T0:before supplementation; T3: at the end of supplementation period; T9: 6 months after the end of the supplementation period.
[b]*P* value between groups (analysis of variance).
[c]*P* value between groups (analysis of covariance, adjusted for the pre-treatment value of the dependent variable).
[d]Significant difference ($P < 0.05$) in the same group when letters differ.

Table 6 Distribution of B and T lymphocyte populations and of T lymphocyte subsets (mean ± s.d.) at the three time points of the study (cells/mm^3)

	Group 1(n = 84)	*Group 2(n = 79)*	P^b	P^c
Lymphocytes				
T0[a]	4986 ± 2282^{A}	4348 ± 2316^{A}	0.08	—
T3[a]	5688 ± 2744^{A}	5562 ± 2328^{B}	0.75	0.79
T9[a]	6299 ± 2615^{B}	5321 ± 2006^{B}	0.009	0.02
B cells				
T0	1143 ± 760^{A}	1001 ± 781^{A}	0.27	—
T3	1364 ± 1059^{A}	1320 ± 1060^{B}	0.80	0.72
T9	1209 ± 954^{A}	1035 ± 624^{A}	0.17	0.20
Mature T cells (OKT3)				
T0	2843 ± 1302^{A}	2616 ± 1522^{A}	0.35	—
T3	3480 ± 1610^{B}	3389 ± 1369^{B}	0.71	0.40
T9	3934 ± 1483^{C}	3285 ± 1127^{B}	0.003	0.002
Helper-inducer T cells (OKT4)				
T0	1857 ± 933^{A}	1647 ± 980^{A}	0.20	—
T3	2523 ± 1236^{B}	2456 ± 1082^{B}	0.72	0.63
T9	3934 ± 1483^{B}	2321 ± 806^{B}	0.002	0.003
Supressor-cytotoxic T cells (OKT8)				
T0	906 ± 549^{A}	860 ± 667^{A}	0.66	—
T3	975 ± 662^{A}	959 ± 517^{A}	0.86	0.44
T9	1007 ± 481^{A}	918 ± 483^{A}	0.24	0.20

[a]T0:before supplementation; T3: at the end of supplementation period; T9: 6 months after the end of the supplementation period.
[b]*P* value between groups (analysis of variance).
[c]*P* value between groups (analysis of covariance, adjusted for the pre-treatment value of the dependent variable).
[d]Significant difference ($P < 0.05$) in the same group when letters differ.

than at the beginning of the study in both groups. Between T3 and T9, the number of mature T cells increased significantly only in group 1.

Concerning the DHST responses, the mean number of positive responses (9.14 ± 7.47 in group 1 and 8.99 ± 5.80 in group 2 at T0) and the mean sum of indurations (2.25 ± 1.66 in group 1 *vs* 2.30 ± 1.46 in group 2) did not differ significantly between groups at the three time points of the study. When subjects were assigned to two categories on the basis of positive responses—anergic (no positive response), and reactive (one or more positive responses)—neither difference between groups at the three time points of measurement nor significant changes in each group with time were observed (15.5% of anergic children in group 1 and 11.4% in group 2 at T0).

Discussion

The 3-month daily iron supplementation improved significantly the iron status and decreased prevalence of iron deficiency in supplemented children. During the same period, in control children, iron status worsened and prevalence of iron deficiency increased. By the end of the supplementation period, hemoglobin concentration was higher in supplemented children, indicating that iron deficiency was one of the causes of anemia. The higher ferritin concentration and the lower prevalence of iron deficiency in supplemented children at the end of the study period underlined the beneficial long-term effect of the 3-month iron supplementation on iron status.

The increase of hemoglobin concentration during the study and the improvement of iron status during the follow-up period after the end of supplementation in control children indicated that anemia had other additional causes, likely intestinal parasites and malaria. Before intervention, 62% of children hosted malarial parasites, mainly *Plasmodium falciparum*, and one-third had a high parasitemia rate known to affect hemoglobin concentration (Brabin, 1992; Cornet *et al*, 1998). Forty-two percent of the children had intestinal parasites such as *Necator americanus* and *Ascaris lumbricoides*, leading to iron deficiency anemia (Stoltzfus *et al*, 1997). Deworming, which was conducted in all infested children before the start of supplementation, and administration of anti-malarial-drugs, given in our study systematically to all febrile children, would explain the improvement of hematological status in control children and would have had an additional effect on iron supplementation in supplemented children.

Deworming requires several months to have an impact on iron status (Stoltzfus *et al*, 1997). It increases hemoglobin (Hathirat *et al*, 1992; Stoltzfus *et al*, 1997) and iron plasma concentration (Karayadi *et al*, 1996). Administration of anti-malarial-drugs is associated with a rise in homoglobin concentration (Boele van Hensbroek *et al*, 1995; Menendez *et al*, 1997), which is higher when concomitant iron supplementation is provided (Boele van Hensbroeck *et al*, 1995). In addition to sustained administration of antimalarials, the decline of malaria parasitic density and the proportion of children with high parasitemia along the study might be attributed to lower malaria transmission related to seasonal variations. Indeed, whereas the iron supplementation trial took place during the rainy season when the malarial transmission is at its maximum, the follow-up period took place during the dry period when malaria infection is low (Chippaux *et al*, 1991). A better

food supply, common in rural Africa during the dry period succeeding the rainy period (Koppert *et al*, 1990), might also have contributed to the improvement of iron status in children of both groups.

Iron supplements had no impact on the incidence of infections. This result is in agreement with several studies showing no effect of oral iron on diarrhea and respiratory illness (Heresi *et al*, 1995; Mitra *et al*, 1997) or on malaria susceptibility (Harvey *et al*, 1989; McGregor, 1982; Boele van Hensbroeck *et al*, 1995; Menedez *et al*, 1997). Malaria, plasmodium density, and frequency of children with high parasitemia and incidence of fever episodes decreased in the same way in both groups throughout the study. Steadiness of antimalarial antibodies during the study period could be attributed to the early acquisition of antibodies among children submitted to a high exposition in a holoendemic zone of malaria where subjects may experience one or two infected mosquito bites each week (Brabin, 1992). Our results confirm preview studies where oral iron supplementation improves iron status but does not increase the parasite index, the parasite density or the titer of antimalaria antibodies (Harvey *et al*, 1989; McGregor, 1982), or the treatment failure rate when malaria is, like here, treated with antimalarial drugs (Boele van Hensbroeck *et al*, 1995). In rats the development of *Plasmodium bergei* is neither suppressed by pure iron deficiency nor enhanced by iron supplementation (Cardoso *et al*, 1996).

Other studies who concluded a negative impact of iron supplementation on infections (Oppenheimer *et al*, 1984; Murray *et al*, 1978), particularly on malaria (Murray *et al*, 1978; Oppenheimer *et al*, 1986) are subject to discussion. For instance, the 2-month-old infants included in the later study (Oppenheimer *et al*, 1986) had probably not developed immunity to malaria, whereas our children had antimalarial antibodies before the iron supplementation. The form of iron supply in the study of Oppenheimer, intramuscular injection of iron, the deficient nutritional status of their infants, or the high doses of oral iron supplements in other studies (Murray *et al*, 1978) may also account for the negative effect of iron on infections.

In the iron-deficient child, increased susceptibility to infection is associated with impaired immunity, particularly cell-mediated immunity (Chandra and Saraya, 1975; Krantman *et al*, 1982; Swarup-Mitra & Sinha, 1984; Berger *et al*, 1992). By the end of the supplementation period, only supplemented children showed a significant increase in the percentage of mature T cells and of helper-inducer/cytotoxic-suppressor T cell ratio, which would suggest a positive effect of the iron supplementation on cell-mediated immunity. However, the percentage and number of helper-inducer T cells increased in both groups to reach the values of healthy children (Thibault *et al*, 1993; Parent *et al*, 1995). Moreover, the percentage and number of mature and helper-inducer T cells as well as helper-inducer/cytotoxic-suppressor T cell ratio did not differ between groups.

Several confounding factors might have accounted for these results. First, the 3-month iron supplementation was not effective enough to correct iron deficiency in all supplemented children. Moreover, each group contained iron-sufficient children, who were expected not to respond, or to respond to a lesser degree to the iron supplementation than iron-deficient children (Chwang *et al*, 1988).

Second, the mean initial hemoglobin concentration (99.7 g/l) and proportion of T lymphocytes (58.4%) were not severely depressed. Studies showing a positive effect of iron supplementation on cell-mediated immunity were conducted in children with severe iron-deficiency anemia and low initial T lymphocyte percentage (42–49%; Chandra & Saraya, 1975; Krantman *et al*, 1982; Swarup-Mitra & Sinha, 1984). It is worthwhile noting that these studies did not include a placebo group and conclude to a positive effect of iron supplementation by before/after treatment comparison. In a more recent study, which concluded a lack of effect of iron supplementation on cell-mediated immunity by comparing iron supplemented children with children receiving a placebo, the mean initial hemoglobin concentration was 104.7 g/l and the initial proportion of T lymphocytes was comparable to the final proportion attained after iron supplementation in the former studies (Thibault *et al*, 1993).

Third, treatment of intestinal parasites and illnesses, especially malaria, had a positive impact on hemoglobin concentration and iron status, which might have contributed to increase T lymphocyte proportion in both groups. Treatment of malaria presumably might have also directly improved cell-mediated immunity because malaria produces a pronounced deficit of cell-mediated immunity in humans (Walsh *et al*, 1995) and in animals (Yadav, 1989). *Plasmodium falciparum* malaria is associated with a deficit of helper-inducer T cells (Peyron *et al*, 1989; Kremsner *et al*, 1989), whereas suppressor-cytotoxic T cells are reported to be decreased (Chougnet *et al*, 1990) or normal (Kremsner *et al*, 1989).

The higher number of mature T cells and of helper-inducer T cells at the end of the study period in the supplemented group, which had higher ferritin concentration and lower prevalence of iron deficiency, would support a positive long-term effect on cell-mediated immunity of the 3-month daily iron supplementation.

In conclusion, the 3 month-daily iron supplementation significantly improved the iron status of children living in a tropical contaminated environment, without a negative impact on the incidence of infections, especially malaria. However, the iron supplementation was not effective in overcoming iron deficiency in all children and a longer period of supplementation has to be considered in such a context of recurrent infections. The iron supplementation might have a positive effect on cell-mediated immunity that would have been masked by interference of infections and their treatment, which contributed to improve the hematological and immune status in children of both groups.

Nevertheless, improvement of iron status in control children took a longer time to occur and was not as effective as in supplemented children. Even improved, the iron status of control children remained poor and prevalence of iron deficiency unacceptably high.

These results are important in terms of public health. Indeed, according to the negative effect of anemia and iron deficiency on child development, control of iron deficiency by oral iron supplementation in infants and children is urgently needed. Malaria prophylaxis, as well as sustained treatment of malaria and periodic deworming, has to be associated.

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