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Interleukin 2 Production in Iron-Deficient Children

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ABSTRACT

The relationship between iron status and capacity for IL-2 production by lymphocytes was assessed in 81 children from 6 mo to 3 yr of age selected at random from a population with low socioeconomic status, undergoing free systematic examination in four children's health centers in the Paris area. Iron deficiency was defined by the existence of at least two abnormal values among the three indicators of iron status: serum ferritin level $\leq$ 12 μg/L, transferrin saturation <12%, and erythrocyte protoporphyrin concentration >3 μg/g hemoglobin. According to this definition, 53 children were classified as iron deficient and 28 as iron sufficient. No differences were observed between the iron-deficient and iron-sufficient groups in terms of the IL-2 concentration without stimulation by PHA. IL-2 production by lymphocytes stimulated with PHA, as well as the stimulation index (ratio of IL-2 concentration following stimulation by PHA to that of IL-2 concentration without stimulation by PHA) were significantly lower in iron-deficient children. The reduction in IL-2 production by activated lymphocytes observed in our study of iron-deficient children may be responsible for impairments in immunity found by other authors, particularly in cell-mediated immunity.

Index Entries: Iron deficiency; interleukin production; iron status and immunity; T-lymphocyte subsets.

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INTRODUCTION

Iron deficiency is considered as the most prevalent nutritional deficiency worldwide (*1*). Unlike other nutritional inadequacies, iron deficiency is not restricted to developing countries; indeed, it is also the most common specific nutritional deficiency observed in developed countries. In industrialized countries, as in developing countries, infants and children are at high risk for iron deficiency. The deleterious effects of iron deficiency were classically defined in terms of anemia, which accompanies a marked reduction in body iron. However, recent studies have pointed out the possible consequences of iron deficiency in itself upon various functions (*2*).

Epidemiological, clinical, and experimental studies provide conflicting evidence for the relationship among iron deficiency, infection, and immunocompetence. Despite these controversies, several laboratory studies indicate that iron deficiency adversely affects immunocompetence. Phagocytosis from polymorphonuclear cells has rarely been shown to decrease, but this was not the case for bactericidal activity. In humans, humoral immunity appears to be far less affected in terms of iron deficiency. However, animal studies in which experimental conditions are closely controlled suggest that nutritional iron deficiency impairs humoral immunity. In contrast to humoral immunity, cell-mediated immunity was found to be markedly impaired in anemic and latent iron deficiency, as indicated by a smaller proportion of circulating T lymphocytes, decreased in vitro lymphocyte responses to a variety of antigens and to PHA, and impaired cutaneous delayed-type hypersensitivity reactions to several test antigens (*3*).

Until relatively recently, little has been known concerning modifications in ratios between lymphocyte subsets in human models (*4*), and no data have been available concerning the effects of iron deficiency on intrinsic functional lymphocytes, such as interleukin 2 production, which is necessary for cell cooperation. In the present study, our objective was to evaluate the relationship between iron deficiency and the latter two factors in young children.

MATERIALS AND METHODS

Subjects

The sample study was constituted without preliminary selection in children between the ages of 6 mo and 3 yr undergoing free systematic examination in four children's health centers in a Paris area known for its low socioeconomic level. The study protocol was approved by the Ethical Committee of the Medical University of Xavier Bichat, Paris. Informed consent was obtained from parents prior to participation in the study. Excluded from enrollment in the study were subjects with recent infec-

tion or chronic disease, and subjects who had been taking iron or folic acid supplements within the 3 mo prior to study. Data collected included sociofamilial, medical history, and anthropometric parameters.

Blood Collection and Analysis

Seven milliliters of whole blood were withdrawn by venipuncture for analyses. Blood iron status, inflammatory, and immunoglobulin indicators were determined as follows: Hemoglobin (Hb), hematocrit, red blood cell count, mean corpuscular volume (MCV), and white cell count (WBC) were measured on a Coulter Counter S 560 (Coultronics, France). White cell differential was assessed by microscopic examination of blood smears after Wright-Giemsa staining. Erythrocyte protoporphyrin (EP) measurement was performed on a hematofluorometer (model ZPP Metter, Aviv Analis, Namur, Belgium). Serum iron was assayed colorimetrically using ferrozine as the chromogen and carried out on a bichromatic analyzer (model ABA-100, Abbot, France). Serum ferritin was determined by an enzyme-linked immunoabsorbent assay standardized using the international reference (National Institute for Biological Standards and Control, London, UK). Serum transferrin, protein-C-reactive (CRP), orosomucoid (Oroso), IgG, IgA, and IgM were measured by nephelometry using a laser nephelometer (Behring, France). Total iron-binding capacity (TIBC) was deduced from serum transferrin concentration. Percent transferrin saturation was calculated by multiplying the ratio of serum to TIBC by 100. Monoclonal antibodies used for the identification of T lymphocyte subsets (Becton and Dickinson) were fluorescein-conjugated Leu-4 (Leu4-FITC), fluorescein-conjugated Leu3a (Leu3a-FITC), and phycoerythrin-conjugated Leu2a (Leu2a-PE), which recognized total T cells (anti-CD3+), helper/inducer (anti-CD4+), and cytotoxic/suppressor cells (anti-CD8+), respectively. Fluorescence analysis was performed with a cytofluorograph (FACS star$^+$ Becton and Dickinson). Lymphocyte proliferation was measured by [^{3}H]thymidine incorporation after stimulation with T-cell mitogen, phytohemagglutinin (PHA, Welcome), and tetanous toxoid (Merieux). Interleukin 2 (IL2) production by PHA-activated and nonactivated T lymphocytes was measured by RIA (IL2 RIA kit, Medgenix, Belgium).

RESULTS

A total of 81 apparently healthy children were recruited; 49 of them (60.5%) had hemoglobin levels <11 g/dL and could be considered as anemic according to WHO criteria. Iron deficiency was defined by abnormal values of at least two biochemical indicators of iron status: serum ferritin level ≤12 μg/L and/or erythrocyte protoporphyrin concentration >3 μg/g Hb and/or transferrin saturation <12%. According to this definition, the prevalence of iron deficiency in our sample was 65% (53 chil-

dren). An inflammatory process was considered to be present when CRP was >12 mg/L and/or the orosomucoid level was >1.4 g/L. Statistical analysis was carried out to compare the iron-deficient group (n = 53) to the non iron-deficient group (n = 28). There were no differences in average age, weight, birthweight, and other anthropometric parameters between the two groups, except for a significant increase in mothers' iron-deficient-children parity (1.8 ± 0.9 vs 2.7 ± 1.4, $p < 0.02$). In Table 1, mean values ± SD for iron parameters are presented. Except for serum ferritin levels, mean values for all iron parameters were significantly different between the two groups. No significant differences were observed for inflammatory markers (CRP, orosomucoid), number and distribution of white blood cell, and IgA, IgG, and IgM serum concentrations between the two groups.

No significant difference was observed in T-lymphocyte subsets (results expressed in total number or in percentage) between the iron-deficient group and the non iron-deficient group. No significant change was observed in the response to PHA or tetanous toxoid between the two groups. IL2 formation, expressed in U/mL or stimulation index, was significantly lower ($p < 0.05$) in the iron-deficient group than in the normal group (Table 2). The difference in IL2 production between the two groups increased ($p < 0.02$) when children with biological inflammatory parameters were excluded from statistical analysis.

DISCUSSION

Comparison of immune functions between the iron-deficient group and the non iron-deficient group suggests that iron deficiency may impair in vitro production of IL2 in response to PHA. No significant change was observed in any other immunological functions, but results must be interpreted taking into account that, in the group of children considered as non iron-deficient, there was a high frequency of isolated depletion of iron stores (serum ferritin ≤12 μg/L) without other abnormalities in iron parameters. The possible deleterious consequences of this depletion in terms of the immune response could mask possible differences compared to an optimal iron status.

The decrease in IL2 production could explain results found by various investigators concerning the role of iron deficiency in impairment of immune response and, especially, alterations in cell-mediated immunity. IL2 serves to increase specific immune responses and to amplify selectively ongoing cell-mediated and humoral immune reactions. Available results on iron deficiency suggest the following mechanisms may be involved in the decrease in IL2 production: (i) the existence of a qualitative alteration in the functions of T lymphocytes by decreased IL2 production, and (ii) the interaction between IL2 receptors and transferrin receptors, the expression of which follows activation of T lymphocytes by mitogens or antigens (5). Normal transferrin saturation is necessary for

Table 1
Biochemical Parameters of Iron Status (Mean ± SD)

	Non iron-deficient n = 28, 35%	Iron-deficient n = 53, 65%	p
Hemoglobin (g/dL)	11.4 ± 0.9	10.5 ± 1.1	<0.001
MCV[c] (fl)	78.1 ± 5.6	72.4 ± 6.4	<0.001
Serum iron (μmol/L)	13.4 ± 4.9	6.5 ± 2.7	<0.001
Serum transferrin (g/L)	3.5 ± 0.5	3.9 ± 0.6	<0.05
Transferrin sat. (%)	15.4 ± 6.4	6.9 ± 3.1	<0.001
EP[d] (μg/gHb)	2.4 ± 0.4	5.0 ± 3.7	<0.001
Serum ferritin (μg/L)	10.0 ± 9.9 5.1[a]	6.9 ± 10.9 4.4[a]	NS
Serum ferritin[b] (μg/L)	3.7[a]	2.2[a]	<0.05

[a]Geometric mean.
[b]When children with biological inflammatory parameters were excluded.
[c]Mean corpuscular volume.
[d]Erythrocyte protoporphyrin.

Table 2
Interleukin 2 Data (mean ± SD)

Interleukin 2 assay	Non iron-deficient n = 28, 35%	Iron-deficient n = 53, 65%	p
IL2 without stim.[a] (U/mL)	0.7 ± 0.7	0.8 ± 0.9	NS
IL2 without stim.[a] (cpm)	6731 ± 289	6734 ± 401	NS
IL2 after stim.[a] (U/mL)	11.3 ± 6.6	8.3 ± 5.6	<0.05
IL2 after stim.[a] (cpm)	4128 ± 1009	4678 ± 1165	<0.05
IL2 stim. index (1/cpm)	1.7 ± 0.5	1.5 ± 0.4	<0.05
IL2 after stim.[a,b] (U/mL)	13.1 ± 6.3	9.0 ± 5.8	<0.02

[a]Stimulation.
[b]When children with biological inflammatory parameters were excluded.

induction of IL2 receptors in mitogen-stimulated cells (*6*), and blocking of surface receptors for transferrin inhibits growth of numerous cell lines and of ADN synthesis.

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