



## IMPAIRED INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR PRODUCTION IN PROTEIN-CALORIE MALNUTRITION

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### ABSTRACT

The capacity of malnourished infants to produce interleukin-1 beta (IL-1 $\beta$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ) was evaluated before and after nutritional rehabilitation. Ten marasmic patients without infectious diseases 2 to 8 months of age with  $-2.48 \pm 0.54$  weight for age Z score (mean  $\pm$  SD) were studied on admission and after 4 month of nutritional therapy in a Closed Nutritional Recovery Center. Cytokines were induced by in vitro stimulation of blood mononuclear cells with lipopolysaccharide. IL-1 $\beta$  and TNF- $\alpha$  were determined in supernatants and cell lysates using an specific immunoassay. Low levels of released and cell-associated IL-1 $\beta$  were found on admission; mean  $\pm$  SEM values were  $2.0 \pm 0.5$  and  $1.3 \pm 0.5$  ng/ml, respectively. After nutritional rehabilitation, a significant increase of released and cell-associated IL-1 $\beta$  were observed:  $5.4 \pm 0.8$  and  $3.7 \pm 0.3$  ng/ml, (mean  $\pm$  SEM) respectively,  $p < 0.01$ , paired t test. A significant increase in TNF- $\alpha$  production was observed in 8 of 10 infants after nutritional rehabilitation ( $p < 0.02$ , paired t test). The diminished production of IL-1 $\beta$  and TNF- $\alpha$  may be one of the mechanism by which protein calorie malnutrition leads to impaired immunocompetence and increased susceptibility to infectious diseases.

KEY WORDS: Interleukin-1, Tumor necrosis factor, Protein-calorie malnutrition, Marasmus.

### INTRODUCTION

It is well recognized that an impaired immune response is present in protein-calorie malnutrition (PCM) (1-4), fact contributing to diminished ability to fight infectious agents. Recently, the interaction between malnutrition and immunity has been focused on the possible participation of soluble mediators such as interleukin-1 beta (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ), two related macrophage cytokines which share multiple biological properties and participate in both normal and pathological conditions (5-7). Decreased ability to produce IL-1 has been reported in adults (8-10) and pre-school children (11) with malnutrition. However, most of these studies have been performed in adult patients with malnutrition secondary to other diseases such as diabetes, cirrhosis, ulcer, pancreatitis, etc, (8-10). Besides, IL-1 has been determined indirectly by measuring its biological effects such as induction of fever and/or leukocytosis (8-10). There is only one report in which IL-1 activity was measured by employing the thymocyte proliferation bioassay in malnourished children (11).

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We are not aware of studies of TNF- $\alpha$  production in children primary malnutrition, however increased spontaneous activity of this cytokine has been reported in young females suffering from anorexia nervosa (12).

The main purpose of this study was to determine the capacity of marasmic infants to produce IL-1 $\beta$  and TNF- $\alpha$  before and after nutritional rehabilitation by using a specific and sensitive immunoassay.

## **MATERIAL AND METHODS**

### **Patients**

Ten marasmic infants 2 to 8 months of age with a weight/age (W/A) Z score (mean  $\pm$  SD) of  $-2.48 \pm 0.54$  were studied on admission to a Closed Nutritional Recovery Center. Selected infants had a birth weight over 2,500 g and were free of acute infections on admission. Total serum protein, albumin and hemoglobin concentrations were within normal levels in all infants. Cytokines were measured before and after nutritional rehabilitation with an adapted cow's milk formula. Subjects were enrolled in the study after parent written informed consent was obtained. The study protocol was approved by the Institute of Nutrition and Food Technology Review Board on Ethics in Human Research.

### **Induction of IL-1 $\beta$ and TNF- $\alpha$ by Blood Mononuclear Cells**

Blood mononuclear cells (BMNC) were isolated by Histopaque gradient (Sigma Diagnostic, ST. Louis, MO), washed twice with phosphate buffered saline, and  $2.5 \times 10^6$ /ml cells were resuspended in RPMI 1640 culture medium supplemented with gentamicin (Elkins-Sinn, Inc. Cherry Hill, NJ), 0.2% heat-inactivated normal AB plasma and 1  $\mu$ g/ml of indomethacin (Sigma, St. Louis, MO). Two hundred  $\mu$ l of cell suspension were dispensed in 96-well flat-bottom microtiter plates in the absence ("spontaneous cytokine release") or in the presence of endotoxin (lipopolysaccharide (LPS: 1  $\mu$ g/ml) Escherichia coli O55:B5, Sigma L-2880). Cultures were incubated for 18 hours at 37°C in 5% CO<sub>2</sub> and 95% air. Supernatants and cell lysates obtained after three freeze and thaw cycles were collected and kept at -70°C until assay for release and cell-associated cytokine. In vitro production of IL-1 $\beta$  and TNF- $\alpha$  were determined by using a specific commercial enzyme-linked immunoabsorbent assays (ELISA : R&D System, Minneapolis, MN). Cytokine determinations before and after supplementation were performed at once to avoid inter-assay variations.

### **Plasma Cytokines Levels**

Plasma was collected, treated according to the method of Cannon et al. (13) and kept at -20°C until assay for IL-1 $\beta$  and TNF- $\alpha$ . Circulating cytokines were determined using the same ELISA procedure.

### **Statistic Analysis**

Statistic analysis was performed by using paired Student's t test.

**RESULTS****IL-1 $\beta$  Production by LPS-stimulated BMNC**

Released and cell associated IL-1 $\beta$  (mean  $\pm$  SEM) of malnourished infants were 2.0  $\pm$  0.5 and 1.3  $\pm$  0.5 ng/ml on admission, respectively (Fig. 1A and 1B). A significant increase in the levels of both IL-1 $\beta$  forms were observed after nutritional rehabilitation; mean  $\pm$  SEM values: 5.4  $\pm$  0.8 and 3.7  $\pm$  0.3 ng/ml, respectively ( $p < 0.01$ , paired t test).

No spontaneous release of IL-1 $\beta$  was detected at any time of study.

**TNF- $\alpha$  Production by LPS-stimulated BMNC**

Levels of TNF- $\alpha$  (mean  $\pm$  SEM) of malnourished infants were 1.6  $\pm$  0.5 and 3.4  $\pm$  0.2 ng/ml before and after nutritional rehabilitation, respectively (Fig. 2). A significant increase of this cytokine was observed in 8 of 10 marasmic infants after nutritional therapy ( $p < 0.02$ , paired t test).

No spontaneous release of TNF- $\alpha$  was detected in any of the study periods.

**Plasma Cytokines**

IL-1 $\beta$  and TNF- $\alpha$  could not be detected in plasma and their concentrations are likely to be below the detection limit of the respective assays ( $< 50$  pg/ml).

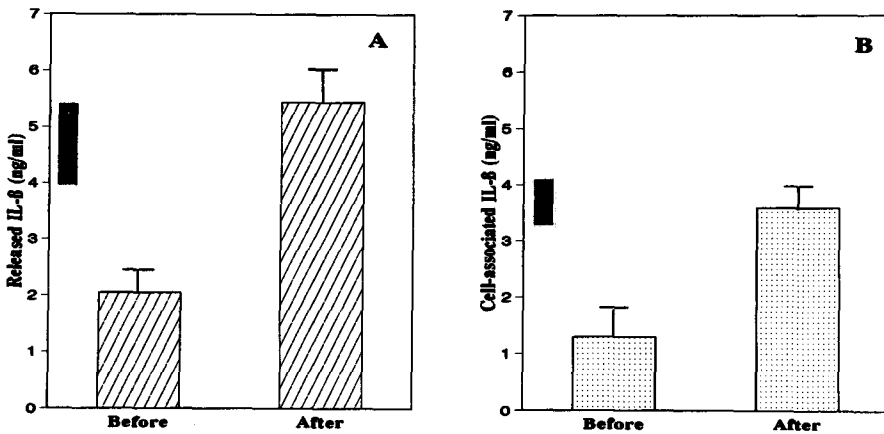


FIG. 1

IL-1 $\beta$  production by LPS-stimulated BMNC in marasmic infants before and after nutritional rehabilitation. (A) Released IL-1 $\beta$ , (B) cell-associated IL-1 $\beta$ . Data are expressed as mean  $\pm$  SEM. Released and cell-associated IL-1 $\beta$  are significantly different from admission ( $p < 0.01$ , paired t test). Left dark bars represent mean  $\pm$  2 SEM for healthy adult controls (released IL-1 $\beta$  = 4.7  $\pm$  0.7 ng/ml; cell-associated IL-1 $\beta$  = 3.7  $\pm$  0.4 ng/ml).

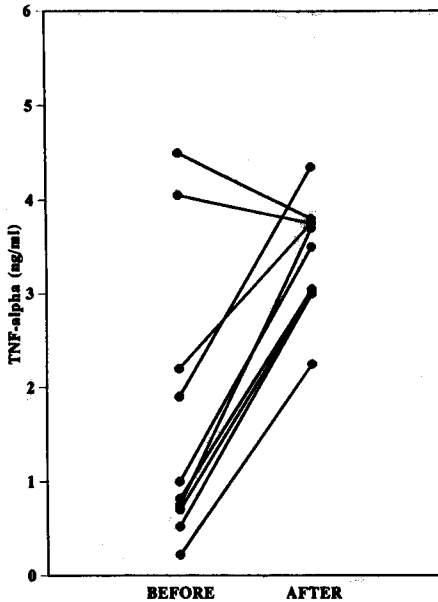


FIG. 2  
TNF- $\alpha$  production by LPS-stimulated BMNC in marasmic infants before and after nutritional rehabilitation. Significantly different from admission ( $p < 0.02$ , paired t test).

## DISCUSSION

This study demonstrates a decreased production of IL-1 $\beta$  and TNF- $\alpha$  by BMNC from infants with primary protein calorie malnutrition. Most studies on cytokines in PCM has been performed by measuring their biological effects (6-8). Since IL-1, TNF and IL-6 are all capable of producing leukocytosis and fever, it is not possible to say which of this cytokines show defective production when detected by non-specific methods.

Diminished levels of extracellular and intracellular IL-1 $\beta$  suggests the existence of an impaired ability of BMNC to release and synthesize this cytokine. Similar findings have been reported by Bhaskaram et al. (11) in children suffering from marasmus and kwashiorkor, although the decrease was more pronounced in kwashiorkor patients. On the other hand, leukocyte endogenous pyrogen (EP) production was not affected in hospitalized marasmic adults, however, a reduction in "in vitro" synthesis of EP by stimulated peripheral leukocytes (8) was reported in adult kwashiorkor patients. Our study confirms the finding that the impairment of IL-1 $\beta$  production is not only related to kwashiorkor but it is also present in marasmus.

Low IL-1 activity could explain the impaired capacity of malnourished children to mount a febrile reaction in response to infectious agents, a fact that frequently produce a delay in the diagnosis of infectious processes in these patients.

We also demonstrated a diminution of TNF- $\alpha$  production in marasmic infants, mediator measured in supernatants of LPS stimulated cell cultures, since this cytokine is secreted mainly to extracellular compartment (14). Vaisman et al. (15) reported an enhanced production of TNF- $\alpha$  in 8 healthy adult subjects after 6 days of medically supervised acute caloric deprivation. Schattner et al. (12) also found decreased TNF- $\alpha$  production by LPS-stimulated BMNC from young female with anorexia nervosa, but the cells of these patients had

significantly increased spontaneous release, suggesting that cells were already activated and responded poorly to further stimulation as a result of both caloric deprivation and neuroendocrine abnormalities associated with this disease. It seems that protein and energy modulates cytokine production by blood monocytes.

Most of our marasmic patients recovered their capacity to produce cytokines after nutritional rehabilitation. Interestingly, *in vitro* IL-1 release by BMNC obtained from protein deficient patients cannot be increased by amino acid supplementation of the culture medium in which leukocytes are incubated (16).

The diminished release and response to IL-1 $\beta$  and TNF- $\alpha$  may be one of the mechanism by which malnutrition leads to impaired immunocompetence and increased susceptibility to infectious disease.

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