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Leucocyte migration inhibition factor (L–MIF) in malnourished Nigerian children

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Summary
Leucocyte Migration Inhibition Factor (L–MIF) was measured in 41 children with marasmus, 19 with kwashiorkor, 5 with marasmic-kwashiorkor and 35 well-fed healthy children serving as controls. For L-MIF assay, two different antigens (live attenuated measles virus vaccine and diptheria pertussis tetanus (DPT) vaccine were used. Percentage migration indices obtained with the two antigens were significantly higher in the malnourished than in the well-fed healthy sex and age-matched controls *($P<0.01$)*. The total serum protein and albumin concentrations were significantly reduced in the malnourished children compared with the controls *($P<0.01$)*. Mean total leucocyte numbers were not significantly different in marasmic and marasmic-kwashiorkor children compared with the controls *($P> 0.21$)*

Keywords: *L-MIF, Marasmus, Kwashiokor, Marasmic-Kwashiorkor, Protein, Leucocyte*

Résumé
Le facteur d'inhibition de migration de leucocytes (L.MIF) avait été mesuré chez 41 enfants avec du marasme, 19 avec le kwashiorkor, 5 avec le kwashiorkor marasmique et 35 enfants bien nourrient et en bonne sante qui avaient servient de controle. Pour l'assay du L-MIF, deux antigenes differents (de vaccin de la rougeole fait de virus attenue vioant et celui du tetanus Dipthenique (DPT) avaient eter utilises. Le percentage des indices de migrations obtenuent avec les 2 antigenes ont été significativement plus élevé chez les enfants malnourrient par rapport aux enfants bien nourrient de meme age et de meme sexe. (P 0.01). Les concentrations total es de protein et d'albumine du serum etaient significativement reduit chez les enfants malnourrient compare aux controles ($P < 0.01$). La moyenne totale du nombre de leucocytes etait significativement different chez les enfants avec le marasme et le kwashiorkor marasmique comparé à ceux des controles ($P > 0.21$)

Introduction

Protein energy malnutrition (PEM) has been associated with cell-mediated and humoral immunodeficiencies in animals [1,2] and humans [3]. Disorders of T-cell numbers [4], complement factors [5,6] and neutrophil activities [7] have been implicated as the underlying factors responsible for the decreased immunity usually observed in malnourished children.

There have been very few studies on the production of soluble mediators of immune response by antigen stimulated-lymphocytes [8]. Leucocyte migration inhibition factor [L-MIF] a protein of 23,000-70,000 molecular weight [9] was the first lymphokine to be identified [10] which retards macrophage migration [9].

Lymphocyte stimulation by phytohaemagglutinin (PHA) and *Candida albicans* in PEM have been documented [8,10,11,12]. In the present study, cellular immunologic capacity was assessed during malnutrition in humans using live attenuated measles virus vaccine and DPT vaccine as antigens. The aim of the study is to determine the level of L-MIF in malnourished children following these antigenic challenges.

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Subjects and methods

In all, 100 subjects were assessed for L-MIF production. These included 41 with marasmus, 19 with kwashiorkor, 5 with marasmic-kwashiorkor and 35 well-fed healthy children serving as controls. The subjects were Nigerian children whose identifications were based on the criteria of Wellcome classification [13]. Apart from the clinical evaluation of the subjects, nutritional status of each subject was assessed by measuring total serum protein colorimetrically by the Biuret method [14] and albumin concentrations by the bromocresol green method [15].

L-MIF assay

In the assay of L-MIF, the method described by Hudson and Hay [16] was employed. Leucocytes were separated from 6 ml of heparinised blood using 3% dextran solution (BDH Chemicals Ltd, Poole England) alongside with a drop of heparin. Cells were washed thrice using Krebs Ringer (K.R.) solution. The cells were resuspended in 1 ml of K.R. solution, counted in a haemacytometer and adjusted to 1×10^8 cells/L. Haematocrit capillary tubes were filled to two-thirds level (at least four capillary tubes per sample) with the level cell suspension. One end of each tube was sealed with plasticine and spun at 600 rpm for 10 minutes at room temperature. The capillary tubes were then cut at the packed cell-medium interface and placed in a migration chamber held in place by anchoring the sealed ends in silicone grease (Edward High Vaccum Ltd., Sussex). The wells were immediately topped up with appropriate antigen dilutions. Controls for each sample had no antigen (i.e., only 15% Fetal Calf Serum). A drop of streptomycin was added to all the well. The chambers were covered with sterile culture plate sealing tapes avoiding air bubbles and incubated for 24 hours at 37 °C. The image of the migration field was projected on the screen of an immunodiffusion plate reader (Osram 64425; Behring Institute, Germany) and traced on a piece of tracing paper.

Assessment of the area traced was done by counting the number of small squares enclosed. Since both tests and controls were in duplicate, the mean of their areas was taken. Migration index due to the presence of the antigen was calculated thus:

$$\% \text{ Migration Index (\% M.I.)} = \frac{T}{C} \times \frac{100}{1},$$

where T = mean area of migration for test (in antigen solution), C = mean of area of migration for control (15% FCS). A percentage migration index of less than 80% was considered a significant inhibition [17].

Enumeration of leucocytes

Total leucocytes were counted by the use of haemocytometer. Whole blood was diluted 1:20 in Tuerk solution (2% acetic acid tinted with gentian violet). Using a capillary tube, this was then applied on a charge haemocytometer and counted under a light microscope at 40x objective. Cells found in all the five chambers were counted and final results multiplied by a factor of 40. The result was expressed in cubic millimeters.

Results

Table 1 shows the results of mean percentage migration index of malnourished children compared with healthy children using DPT and live attenuated measles virus vaccines.

Table 1: Mean percentage migration index* of malnourished children and controls

	Measles antigen (% M.I. values)	DPT vaccine antigen % M.I. values
Control	63.3 ± 5.1	67.8 ± 4
	n = 17	n = 18
Marasmus	71.1 ± 5.5	71.7 ± 3.2
Kwashiorkor	75.3 ± 6.7	73.6 ± 2.4
	n = 7	n = 12
Marasmic-Kwashiorkor	74.2 ± 2.1	-
	n = 5	

Using the measles virus as antigen, the mean percentage migration index of children with marasmus, kwashiorkor and marasmic-kwashiorkor, were significantly higher than in controls (marasmus, $P < 0.01$; kwashiorkor, $P < 0.01$; marasmic-kwashiorkor, $P < 0.01$; marasmic-kwashiorkor, $(P<0.01)$. This trend in mean percentage migration index obtained for marasmus, kwashiorkor and marasmic-kwashiorkor children using measles virus vaccine, was similar to mean percentage migration indices of children with marasmus were not significantly different when compared with kwashiorkor children (measles virus vaccine, $P > 0.2$; DPT vaccine $P > 0.1$).

Mean total protein and albumin concentrations were highest in the well-fed (controls) and lowest in the kwashiorkor group (Table 2).

Table 2: Mean serum total protein and albumin concentrations* of both healthy and malnourished children.

	Total protein X ± S.D.	Albumin X ± S.D.
Control	7.1 ± 0.5	3.1 ± 0.5
	n = 16	
Marasmus	6.3 ± 0.6	2.6 ± 0.4
	n = 20	
Kwashiorkor	4.6 ± 0.9	2.0 ± 0.3
	n = 9	

Total protein and albumin concentrations in controls were significantly higher than that of Malnourished children (P 0.01)
Note: P values as calculated by Student's t – test.
**Values are presented as x ± S.D.*

The differences were significant when the total protein and albumin concentrations of controls were compared with marasmus (total protein, $P < 0.01$; albumin, $P < 0.01$); or kwashiorkor (total protein, $P < 0.01$ albumin, $P<0.01$).

Table 3 presents the results of total white blood cells (TWBC) in controls and malnourished children.

Table 3: Mean total white blood cell counts (TWBC) in both well-fed and malnourished children.

	Controls	Marasmus	Kwashorkor	Marasmic-Kwashiorkor
TWBC (x ± S D X 10^9 cells/L)	9.28 ± 2.8	9.49 ± 4.2	8.33 ± 3.6	10.28 ± 3.0

The mean of the TWBC in the controls and malnourished children was comparable ($P > 0.2$).
Note: P values calculated using Student's t-test

There was no significant difference in TWBC of controls compared with each of the malnourished groups of children. $(P > 0.02)$

Discussion

Several reports have associated undernutrition with infection. It is well established that inadequate dietary intake predisposes man and animals to infection by a wide variety of organisms [18]. Protein calorie malnutrition (PCM) has been associated with cell-mediated and/or humoral immunodeficiencies in humans [19,20]. The present study employs leucocyte migration inhibition factor in the presence of commonly encountered antigens (measles virus and DPT vaccines) in the Nigerian environment to demonstrate cell-mediated immune status of malnourished children. This offered an opportunity to assess the in vitro responses of lymphocytes at the effector functions level during malnutrition. Leucocyte migration inhibition factor retards leucocyte migration from areas of inflammation. It also increase adhesiveness [9]. The mechanism by which this takes place has not been clarified, although L-MIF appears to interact with macrophage cell surface receptor.

The limited number of reports are equivocal on the effects of nutrient deficiencies on the production and/or action of L-MIF in animals or humans. Kramer and Good [12] reported that L-MIF activity of lymph node cells (LNC) from guinea pigs fed protein deficient diets was normal or augmented. Similarly, Carlomangno et al. [10] had reported normal LMIF activity of splenic lymphocytes from rats fed protein deficient diets. In contrast, decreased L-MIF production by splenic lymphocytes from mice fed protein deficient diets was documented by Hambor et al. [11]. In the present study, a significant decrease in L-MIF production was observed in malnourished Nigerian children compared to this well-fed healthy counterparts. However, few clinical reports of decreased L-MIF function [21], normal or slightly decreased L-MIF production from lymphocytes of protein-energy malnourished children have been documented [8]. Ford et al. [21] however attributed the decrease in L-MIF production to recurrent fungal infections in Australian aboriginal children with moderate PCM.

The decreased L-MIF production in the present study may be a result of decreased white blood cell function since TWBC numbers were observed to be statistically equal in both malnourished and well-fed children. It could also be as a result of diminished number of thymus-derived lymphocytes as demonstrated by other workers [20,22]. In addition, Chandra reported the occurrence of immature T-cells in malnourished children [23]. This immaturity could be a possible contributing factor to the decrease in L-MIF production. The decrease in T-cell number leading to decreased L-MIF production could be as a result of raised levels of plasma cortisol reported during PEM [24]. Cortisol is known to induce inhibition of T-cell growth factor, a mediator of T-cell proliferation [25]. Cortisol also has an anti-mitotic effect [26].

Zinc supplementation has been implicated to increase L-MIF production [27]. The decrease in L-MIF production in this study might have been as a result of mild zinc deficiency in the malnourished children. Certain plasma inhibitory factors observed by other workers [20,22,28] may affect L-MIF production. These factors bind the surface receptors of T-lymphocytes *in vivo* inhibiting *in vitro* E-rosette formation [28]. There is the possibility that these factors must have strictly hindered the surfaces of some T-cells, rendering them insensitive to antigen stimulation both *in vivo* and *in vitro* leading to decreased lymphokine production. This speculation of steric hindrance by inhibitory substances in the sera of malnourished children resulting in low L-MIF production is also supported by the findings of other workers (29, 30, 31].

The decrease in L-MIF production observed during this work may be related to the decreased protein concentration found in malnourished children. In support of this, low protein levels have been associated with impaired T-lymphocyte activities [32,33,34]. Hypoalbuminaemia could also have been a contributory factor to the decreased L-MIF production since L-MIF has been concluded to have albumin and pre-albumin electophoretic mobility [35].

This study suggests that the cause of lower L-MIF production in PEM may be multifactorial and that the capacity of cell-mediated immunity in malnourished children to combat infections like diphtheria pertusis tetanus and measles is depressed.

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