Absence of variation in facteur thymique sérique activity in moderately and severely malnourished Senegalese children[1,2]

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ABSTRACT Facteur thymique sérique activity was evaluated in relation to different types of malnutrition in Senegalese children aged 5 to 42 months. They were classified in four groups: controls, moderate malnutrition, marasmus, and kwashiorkor, according to anthropometric measurements and clinical examination. The two latter groups were characterized by very depressed levels of total protein, albumin, transferrin and prealbumin, and by high cortisol concentrations. Zinc status was marginal in all children. Facteur thymique sérique activity, determined by the rosette assay, was normal in the malnourished patients suggesting that moderate as well as severe malnutrition is not necessarily associated with depressed levels of circulating thymic hormone. These results are discussed in relation to zinc status and infections. *Am J Clin Nutr* 1982;36:1129–1133.

KEY WORDS Protein-energy malnutrition, facteur thymique sérique activity, zinc, cortisol

Introduction

The immune responses of children suffering from protein-energy malnutrition have been extensively investigated during the past few years. Protein-energy malnutrition often results in the impairment of functions related to cell-mediated immunity (1) but the mechanisms involved are unknown. Previous studies have indicated that thymic hormones probably play a role in the differenciation of T cell subpopulations. In Nigeria, Olusi et al (2) have observed that thymosin fraction 5 increases the low percentage of E rosettes in children suffering from severe kwashiorkor. Similarly, Jackson and Zaman (3), in Bangladesh reported an increase in T cell rosettes with thymopoietin in 10 of 16 marasmic or marasmic-kwashiorkor children. These authors have raised the possibility to use thymic hormones in the treatment of malnourished children to restore the impaired immune responses. A circulating thymic hormone, facteur thymique sérique (FTS) has been isolated by Bach et al (4). FTS activity has been found in the serum of various species including man, and its presence is clearly thymus dependent (5). Recent studies have shown a decrease in FTS activity in energy-restricted rats (6), in malnourished children (7), and in small for gestational age infants (8). However, the effect of malnutrition on the immune response depends on the severity, duration, and type of malnutrition, and on the specific nutritional deficiencies involved. Thus, a decrease in FTS activity has been reported in rats and mice fed a zinc-deficient diet and in pyridoxine-restricted rats (9, 10). Studies carried out in malnourished human subjects have not taken into account the influence of the severity and type of malnutrition on FTS activity.

The present work was undertaken to determine the FTS activity in children suffering from marginal to severe malnutrition in relation to the plasma protein pattern, the zinc level, and the concentration of cortisol which is known to be lympholytic (11).

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Received January 21, 1982.

Accepted for publication June 1, 1982.

Patients and methods

Patients

Forty-seven Senegalese children whose ages ranged from 5 to 42 months, were investigated, 33 of the children were hospitalized for various diseases including malnutrition. They were classified as moderately or severely malnourished using the Waterlow's classification (12). The severe cases were discriminated in kwashiorkor or marasmus according to a clinical examination. The controls were selected among children who belonged to the same families as the patients.

Group 1—controls. Their weight for height was more than 80% and height for age over 90% of the NCHS standard (13).

Group 2—moderately malnourished children. Their weight for height ranged from 70 to 80% of the same standard with a height for age below or more than 90% of the reference value.

Group 3—marasmic children. They showed a very reduced subcutaneous fat tissue and muscular atrophy. Their weight for height was under 70% of the NCHS standard and their height for age ranged from 85 to 95% of the reference value, indicating an acute or chronic evolution.

Group 4—kwashiorkor children with or without skin and hair changes. They were all edemic. Their weight for height ranged between 63 and 80% of the NCHS standard. Only one patient was stunted (height for age less than 90% of the standard).

No clinical signs of vitamin deficiency were noticed except a gingivitis in one child with kwashiorkor. Anemia associated with hyposideremia was present in 40% of the children, in controls as well as in malnourished groups without any difference in prevalence.

Most of the subjects, including the controls, were suffering from various infections, mainly respiratory and gastrointestinal infections. Four marasmic children died during hospitalization from grave, acute, superimposed infections (myocarditis, measles, and bronchopneumopathies).

Methods

Blood was withdrawn by venipuncture before any nutritional and anti-infectious treatment was started. Blood was collected in cooled (4°C) heparinized tubes between 9 and 10 AM to take into account the circadian rhythm of cortisol. Plasma was immediately separated and kept frozen at −20°C until analyzed.

The total protein concentration was determined using the biuret method and plasma albumin, transferrin, and prealbumin levels according to the radial immunodiffusion method of Mancini et al (14).

Plasma zinc was assayed by atomic absorption spectrophotometry.

Cortisol was measured by radioimmunoassay (Kit Cortetk 125-Oris, CEA, Gif-sur-Yvette, France).

FTS activity was quantified in plasma according to the method previously described by Dardenne and Bach (15): this assay analyzes the conversion of relatively azathioprine (Az) resistant spleen cells of adult thymectomized mice to Θ positive rosette-forming cells that are more sensitive to Az. Briefly, plasma samples were filtered by centrifugation at 4°C on CF 50 Amicon membranes (molecular weight cut off at 50,000 to remove an FTS inhibitor). The ultrafiltrates were incubated for 90 min at 37°C with spleen cells from mice thymectomized 10 to 15 days before the test and Az (10 μl/ml) was added simultaneously. At the end of the incubation time 12×10^6 sheep red blood cells were added to the cells. This cellular preparation was centrifuged (at 4°C 150 × g, for 5 min) and resuspended by low speed rotation (10 rpm) on a roller. Rosette-forming cells were counted in a hemocytometer. In the presence of FTS, Az inhibited rosette formation at a concentration of 10 μg/ml. The highest dilution of the plasma sample which induced the rosette inhibition by Az was considered as the active dilution. All the determinations were carried out in duplicate, and the results were expressed as log-2 reciprocal titer.

Statistical analysis of data

Differences in mean values between the groups were determined by analysis of variance.

Results

The average age was similar in the three groups of malnourished children: 17.5 ± 2.12, 19.1 ± 2.47, 20.1 ± 3.78 months in groups 2, 3, and 4, respectively. Compared to the control group (9.6 ± 1.09) they were significantly older ($p < 0.05$).

The various degrees of malnutrition in our subjects are reflected in the values obtained for plasma proteins (**Table 1**).

In moderately malnourished children, only the level of prealbumin was reduced, whereas the marasmic and kwashiorkor groups exhibited very depressed values for all the parameters tested, compared to the control group. The level of plasma proteins (total protein, albumin, and transferrin) was slightly higher than expected in the marasmic children. There was no difference in the mean plasma protein values between this group and the kwashiorkor group.

The plasma zinc concentrations of groups 2 (629 ± 52.1 μ/l) and 3 (700 ± 66.5) were comparable to those of the controls (655 ± 90.7). The decreased zinc level observed in group 4 (488 ± 116.3) was not statistically significant.

The cortisol concentration was higher in the severely malnourished children (groups 3 and 4) than in the other groups. However, the only significant difference was observed in group 4 compared to group 1 (**Fig 1**).

Figure 2 shows the plasma levels of FTS activity. When expressed as log-2 reciprocal titers the values recorded for normal subjects ranged between 4.5 and 7 (mean 5.7 ± 0.20). In moderate and severe malnutrition (maras-

TABLE 1
Plasma protein levels* of Senegalese children grouped according to different types of malnutrition

	Controls (n = 14)	Moderate malnutrition (n = 14)	Marasmus (n = 11)	Kwashiorkor (n = 8)
	1	2	3	4
Total protein	67.4 ± 2.15	62.9 ± 2.32	56.8 ± 2.55†,‡	51.8 ± 4.35†,‡
Albumin	33.3 ± 1.58	31.5 ± 1.58	22.2 ± 1.13†,‡	19.9 ± 1.38†,‡
Prealbumin	0.14 ± 0.015	0.11 ± 0.001†	0.08 ± 0.004†	0.09 ± 0.008†
Transferrin	3.2 ± 0.25	2.9 ± 0.23	1.5 ± 0.27†,‡	1.2 ± 0.33†,‡

* Values expressed per g/l (mean ± SEM).
† $p < 0.05$, versus the group 1.
‡ $p < 0.05$, versus the group 2.

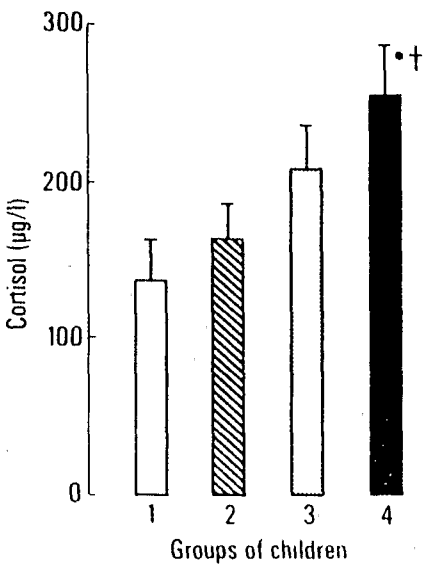

FIG. 1. Plasma cortisol levels of children with varying degrees of malnutrition. Groups of children: 1, controls; 2, moderate malnutrition; 3, marasmus; 4, kwashiorkor. Means + SEM are shown. * $p < 0.01$ versus the control group; † $p < 0.05$ versus the moderately malnourished group.

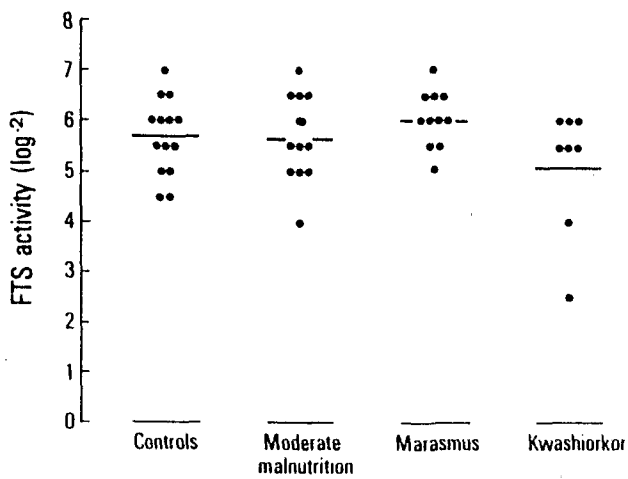

FIG. 2. Plasma FTS activity in normal and malnourished children.

mus and Kwashiorkor) FTS activity was similar to that of the control group (5.6 ± 0.22, 6.0 ± 0.17, 5.1 ± 0.44, in groups 2, 3, and 4, respectively.

Discussion

For practical and ethical reasons, well-nourished patients were selected among siblings. Reference values for some plasma proteins given by the manufacturer (Behringwerke AG Marburg, Germany) indicate slightly lower values below 1 yr of age. Our controls were younger than the malnourished groups so that the differences observed were not age dependent.

Prealbumin was the only protein that allowed a distinction between normal and moderately malnourished children. This finding is in keeping with the results of Ogunshina et al (16). However, its level was not further decreased in groups 3 and 4 in contrast with total protein, albumin, and transferrin values. The kwashiorkor cases were similar to those described by Lunn et al (17) in the Gambia where infections are precipitating factors for the appearance of edema in children who would otherwise be regarded as cases of marasmus. This explains the rather high levels of cortisol observed in these children. The fact that the levels of the various plasma proteins were lower than expected in the marasmic children (group 3) suggested a possible evolution to a marasmic-kwashiorkor state. As previously reported for normal humans, FTS found in the sera of children less than 20 yr of age was active between the concentrations of 1/16 and 1/28 (18, 19). Our control values for FTS activity paralleled this finding. In our patients, moderate and severe malnutrition were not associated with a decrease in FTS activity. In contrast, Chandra (7) reported elevated FTS titers in his controls and reduced levels of FTS activity in children suffering from protein-energy malnutrition.

It is known that protein-energy malnutrition and high cortisol (or corticosterone)

levels may induce thymic atrophy. The strict thymic dependency of FTS, already shown by its disappearance after thymectomy and its presence in thymic extracts (20) was recently confirmed using indirect immunofluorescence or immunoperoxidase binding to reticuloepithelial cells of an antibody produced against synthetic FTS (21). Similar findings have been reported for human (22) and murine thymuses, using anti-FTS monoclonal antibodies (23). The localization of FTS in thymic epithelial cells was further confirmed by immunoelectron microscopic studies (24). In malnutrition, various reports have indicated that the thymic involution involves mainly the lymphoid part; in a postmortem study of kwashiorkor, Mugerwa (25) observed well-preserved thymic epithelial cells. Therefore, a normal FTS activity would not be inconsistent with protein-energy malnutrition.

In addition to their lympholytic effect, glucocorticoid hormones might also influence the circulating thymic hormone. Steriod treatment of normal mice results in a drop of FTS activity; however, this effect is induced by high doses and is transient (26). Under physiological conditions, adrenal corticoid hormones do not seem to interfere with FTS levels since the high cortisol concentrations recorded in the marasmic and kwashiorkor children were not negatively correlated with the levels of circulating thymic hormone.

The normal FTS activity in moderately and severely malnourished children might possibly be explained by the status of their concurrent infections. Studies have shown that activated T cells secrete an *allogenic factor* which is active in the rosette assay (27, 28). Our subjects sustained many infectious diseases, therefore, we assumed that the production of this factor in these children might artificially enhance the FTS activity. This could probably explain the discrepancy between our results and those of Chandra (7) who did not mention any apparent acute infections in his patients.

However, from our point of view, the most critical factor to be considered is the possible role of a zinc deficiency in malnourished children. Rats and mice fed a zinc-deficient diet show depressed FTS activity (9, 10). In contrast to Chandra's report (7), we have determined the plasma zinc concentration in our patients. The mean zinc concentrations reached borderline values in groups 1, 2, and 3, and fell below normal levels in group 4 (29). Yet, the variations between the individual kwashiorkor children did not result in a statistically significant difference between the four groups. When we looked at FTS levels in individual patients in relation to zinc concentrations, we did not observe any correlation of ($r = 0.12$). However, Iwata et al (9) reported that a state of marginal deficiency in mice did not lower their FTS activity while a 0% zinc deficient diet markedly reduced the FTS level. The rosette assay used to evaluate the FTS levels is based on the biological activity of this hormone. Natural as well as synthetic FTS are active only when some minerals, particularly zinc, are bound to the peptide: FTS loses its biological activity after passage on a chelating agent and recovers it after addition of zinc (30). So, the possible influence of the zinc status in the maintenance of normal circulating thymic hormone must be considered although the minimum zinc level required is not yet established.

The effects of protein-energy malnutrition on immunological processes are often conflicting. According to the nutritional, sanitary, and cultural environment, numerous factors may produce heterogenous results.

Our study demonstrates that moderate as well as severe malnutrition including marasmus and kwashiorkor were not associated with a decrease in FTS activity. One may hope that further experimental studies would be useful to clarify this point and we suggest that the unaffected level of circulating hormone might be explained either by the zinc status of the children or by the stimulatory effect of their concurrent infections.

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