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Effects of Nutritional Recuperation on E-Rosetting Lymphocytes and in Vitro Response to Thymosin in Malnourished Children

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Summary: The percentage of peripheral blood lymphocytes forming rosettes with sheep erythrocytes (E-rosettes) was determined at admission to the INCAP Clinical Center in eight acutely malnourished Guatemalan children, and again after 14 and 28–30 days of nutritional therapy. While the mean percentage of E-rosettes increased during therapy, the change (from 35.6 ± 10% to 43.3 ± 19%) did not reach statistical significance because of the variable response of different subjects. At each time period, however, in vitro incubation with the thymic factor, thymosin fraction 5, significantly increased the percentage of E-rosetting lymphocytes. The presence of thymosin responsive cells in circulation after 1 month of optimal nutritional support indicates that immature T-lymphocytes can persist in circulation in patients with severe malnutrition, even after clinical improvement. Thus, neither the percentage of E-rosettes in peripheral blood nor their response to in vitro incubation with thymosin correlated with anthropometric measures of nutritional status in individual patients. This suggests that other nutritional or nonnutritional factors may be important modulating influences on T-lymphocytes, and that prospective studies with thymic factor administration are warranted. Key Words: Peripheral blood lymphocytes—E-Rosettes—Thymosin—Malnourishment.

Defective cell-mediated immune (CMI) responses are frequently encountered in children with protein–energy malnutrition (PEM) (1–6). Because of the central role of the thymus in the development of T-lymphocytes involved in CMI responses (7) and the presence of thymic atrophy and decreased numbers and percentage of mature circulating T-cells that accompany PEM (1–6,8,9), it has been postulated that replacement with thymic factors may improve immune function in these patients (6). In the accompanying paper (10), we have shown that some but not all PEM patients of similar nutritional status, as defined by anthropometric and clinical asssessment, have subnormal proportions of mature T-lymphocytes in peripheral blood. In vitro exposure of peripheral blood lymphocytes from these subjects to a thymic peptide fraction, thymosin fraction 5 (f-5), increases the proportion of E-rosetting T-cells, suggesting that patients who demonstrate such a response in vitro have increased numbers of immature circulating pre-T-lymphocytes in vivo.

In this paper, we report the effects of nutritional therapy on the proportion of E-rosetting lymphocytes in circulation and the lymphocyte response in vitro to f-5. These data indicate that despite progressive improvement in nutritional status during 1 month of intensive nutritional support, the change in percentage of E-rosetting T-cells is variable in individual patients. More important, because cells responsive in vitro to f-5 remain in circulation, con-

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tinued thymic dysfunction may be present in these patients.

MATERIALS AND METHODS

Eight children, five boys and three girls aged 14–38 months, were admitted to INCAP's clinical center with severe edematous PEM by clinical, anthropometric, and biochemical criteria (Table 1). The research protocol was approved by the Committee on Human Rights at INCAP, and informed parental consent was obtained. Ten milliliters of peripheral blood was obtained in preservative-free heparin (20 U/ml) within 24 h of admission and on days 14 and 28–30 of hospitalization. Peripheral blood mononuclear cells were isolated and exposed to a final concentration of 20 μg/ml of the partially purified bovine thymic hormone, thymosin fraction 5 (f-5, lot number BPP-100, a gift of Hoffmann La-Roche, Nutley, NJ) in Hanks' balanced salt solution (HBSS) or HBSS alone, as described in the accompanying paper (10). Following incubation, T-lymphocytes were identified by the E-rosetting technique, and the number of nonrosetting, small-rosetting (1–2 firmly attached sheep erythrocytes), and E-rosetting lymphocytes (3 or more firmly attached sheep erythrocytes) were enumerated in a 200 cell count (10). Based on the variability in measurement of E-rosettes in our laboratory (mean, 54.62; SD, 4.2%; SD/mean, 0.077), we have arbitrarily designated a change equal to or greater than ± 1.5 SD (6.3%) as a response to either nutritional treatment or in vitro exposure to f-5.

Nutritional therapy consisted of a gradual increase in diet to 150 calories and 4 g protein/kg body weight/day over a 1-week period. Vitamin A (100,000 IU) was given orally on day 1. Daily supplementation with vitamins and trace minerals (including 60-mg elemental iron as $FeSO_4$) to meet requirements was started on day 8.

The results were examined statistically by analysis of variance.

TABLE 1. *Anthropometric and biochemical data*

	Admission	Day 14	Day 28–30
Weight-for height (% of standard)[a]	70.0 ± 7[b]	77.0 ± 6	87.0 ± 11
Plasma protein (g/dl)	4.3 ± 0.4	6.1 ± 1.1	7.3 ± 1.2
Serum albumin (g/dl)	1.8 ± 0.7	2.8 ± 0.7	3.6 ± 0.8
Hemoglobin (g/dl)	8.8 ± 1.2	9.0 ± 1.5	9.4 ± 0.9

[a] Based on "dry weight" calculated after disappearance of edema compared to 50th percentile of NCHS standards.
[b] Mean ± SD.

RESULTS

Clinical Evaluation

Table 1 summarizes the patient characteristics in the eight patients on admission. Nutritional therapy resulted in marked improvement in weight-for-height after 4 weeks in all but one child.

E-Rosettes

The mean percentage of E-rosetting T-lymphocytes in the first blood sample was 35.6 ± 10% (Fig. 1). Although this value increased during the 4 weeks of observation, the change was not statistically significant because of variable responses of individuals ($F = 0.2868$, $p = 0.76$). By 4 weeks of nutritional support, the mean of 43.3 ± 19% was still below the value obtained in healthy children with mild growth retardation from the same population (10). The reciprocal change in the percentage of nonrosetting cells, and the minor decrease in small rosettes with time, did not reach statistical significance ($F = 0.0457$ and 0.2554; $p = 0.96$ and 0.78, respectively).

Effects of f-5

In vitro treatment of lymphocytes obtained at admission with f-5 resulted in a mean increase of 8.0 ± 4.7% in the proportion of lymphocytes forming E-rosettes (Fig. 1; $F = 48.6$, $p < 0.0001$). An increase of similar magnitude was observed in the follow-up samples at 2 and 4 weeks of nutritional therapy. This increase in E-rosettes was entirely at the expense of nonrosetting cells ($F = 49.9$, $p < 0.0001$). A significant increase in response to f-5 as defined in this study (≥6.3%) was observed in 14/20 samples in which the baseline value in the absence of f-5 was less than the mean value observed in clinically well Guatemalan children [mean E-rosette values of 35% (20 samples) versus 52% (41 samples), respectively] (10). This can be compared to 1/4 responders among those with higher baseline percentage of E-rosettes (mean, 61.9%). The response to f-5 was independent of time ($F = 0.0407$, $p = 0.06$ for E-rosettes and $F = 0.0523$, $p = 0.95$ for nonrosetting lymphocytes).

Nutritional Status and E-Rosettes

Although nutritional status and the proportion of E-rosettes increased over the 4 weeks of observa-

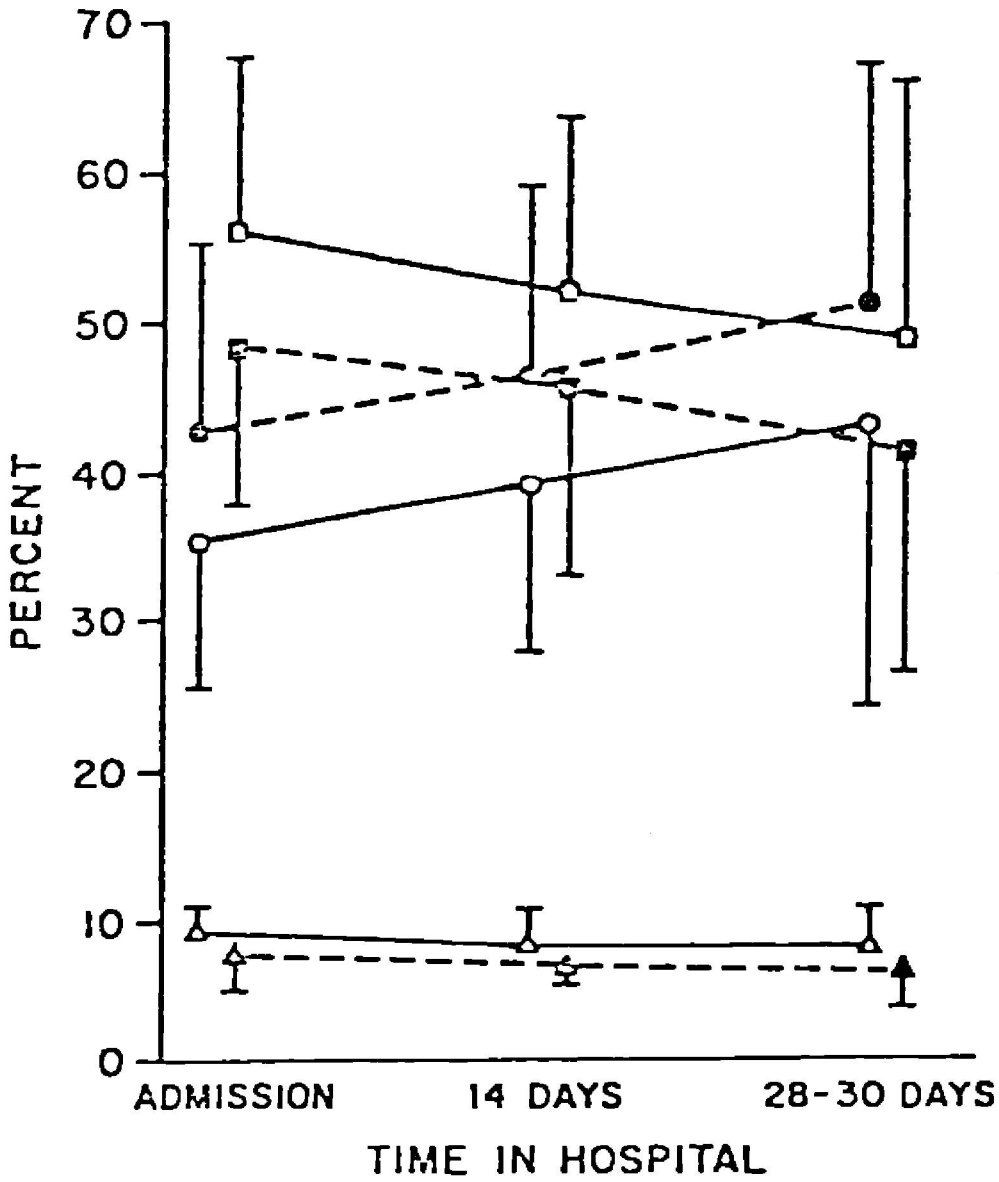

FIG. 1. Mean percentage (% 1 SD) of E-rosettes (circles), small rosettes (triangles), and nonrosetting lymphocytes (squares) among peripheral blood mononuclear cells in eight patients with protein-energy malnutrition. The open symbols represent assays in the absence of thymosin fraction 5, and the closed symbols are the results of assays following incubation in the presence of thymosin (20 μg/ml).

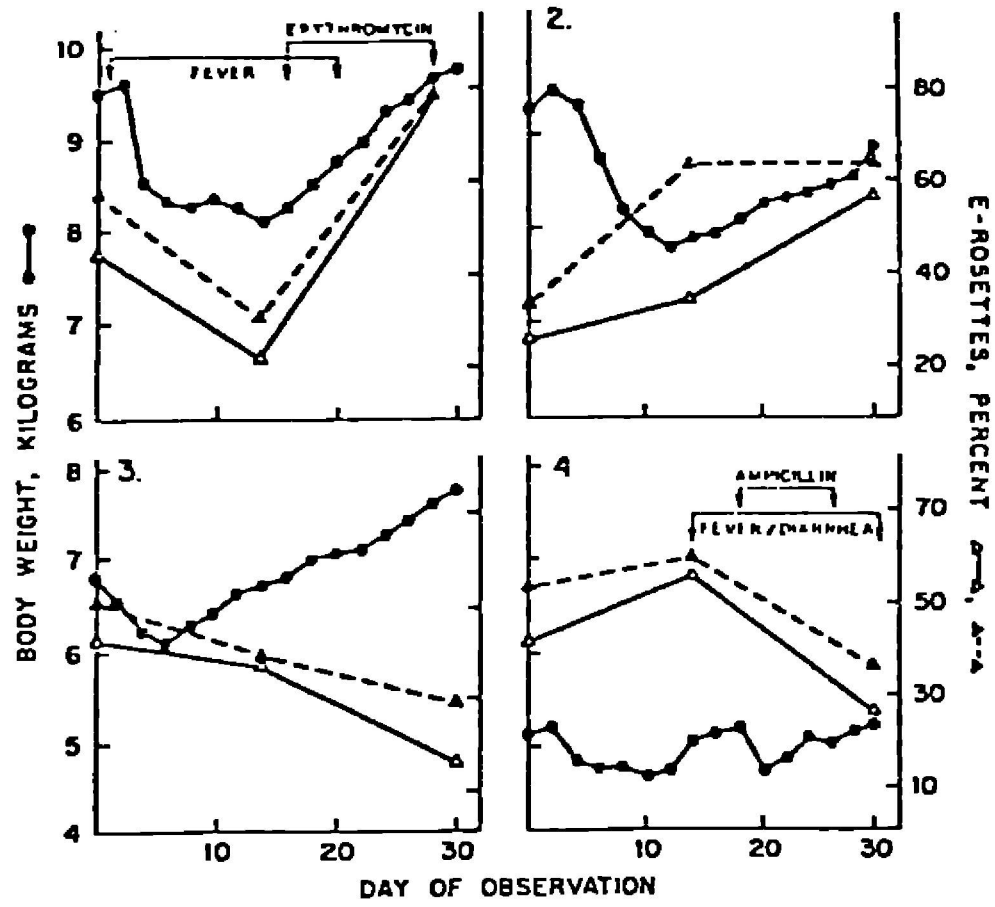

FIG. 2. Weight curve (closed circles) and the percentage of E-rosettes in the absence (open triangles) and presence (closed triangles) of 20 μg/ml of thymosin fraction 5 in four children with acute protein–energy malnutrition. Cases 563 and 567 were clinically uncomplicated and showed a good nutritional response (weight-for-height 87 and 91% of standard by 4 weeks). Percentage of E-rosettes improved in case 567 but steadily diminished in case 563. Cases 562 and 569 experienced febrile illness during the course of observation, with an associated fall in percentage of E-rosettes. In the former patient, weight-for-height failed to improve by day 30 of treatment (increase in weight-for-height from 56 to 63%) and E-rosettes dropped to 27%. In the latter child, percentage of E-rosettes improved when antibiotic treatment was initiated and was associated with improvement in nutritional status (weight-for-height, 94% by day 30).

tion in the group as a whole (Table I and Fig. 1), the response in individual patients was variable (Fig. 2). In some, clinical, nutritional, and immunological status changed in parallel; in others, there was no obvious relationship. Four cases are illustrated.

Case 1 (PC 569)

A 34-month-old female had an unexplained fever for the first 3 weeks of the study and concomitant decrease in E-rosettes from 45 to 22%. When erythromycin was given, she defervesced and rapid catch-up growth began. By day 28 the percentage of E-rosettes had increased to 80%. Weight-for-height did not change from admission to day 14 (77–81% of standard), but then reached 94% of standard by day 30.

Case 2 (PC 567)

A 20-month-old male had an uncomplicated clinical recovery and steady increase in percentage of E-rosettes to normal (56%) as weight-for-height reached 91% of standard. The effect of f-5 on day 14 was striking, increasing percent E-rosettes from 34 to 64%.

Case 3 (PC 563)

An 18-month-old female had an uncomplicated clinical recovery, and an increase in weight-for-height from the initial value of 65–87% of standard. Nevertheless, the percentage of E-rosettes steadily decreased from 43% on admission to 38% at day 14 to 17% by day 30.

Case 4 (PC 562)

A 14-month-old male had unexplained anorexia and little improvement in weight-for-height during the first 2 weeks (from 56 to only 63% of standard), but a normalization of the percentage of E-rosettes

from 42 to 57%. Fever developed on day 14. Ampicillin was begun on day 18, with defervescence, however, diarrhea began on day 20. While there was no further change in weight-for-height during this period (64% of standard at day 30), the percentage of E-rosettes decreased to 27%.

DISCUSSION

The results of the present study show that despite clinically adequate nutritional recuperation 1 month after hospitalization, PEM patients may still have subnormal proportions of E-rosetting lymphocytes in their blood. For comparison, we have used data obtained in our laboratory from other Guatemalan children who are mildly growth retarded but clinically healthy (mean value, 52%) (10) and other data reported in the literature from Africa and Bangladesh (49.9 and 53.7%, respectively) (11,12). As it is likely that the majority of these children are at least marginally malnourished as well, these figures represent a minimum estimate of the true normal proportion of E-rosetting cells in this age group.

As we and others have noted before, in vitro incubation with thymic factors results in an increase in the proportion of E-rosetting cells in subjects with initially low values (10,13,14). Of interest in the present study, a similar magnitude of response to f-5 in vitro was found after 2 weeks and 1 month of optimal nutritional rehabilitation. This indicates that despite clinically satisfactory nutritional recuperation, f-5 responsive and presumably immature cells may still be found in the circulation. Indeed, this response to f-5 may be a useful functional indicator of recovery of the immune system with nutritional treatment for severe PEM. By 4 weeks of nutritional therapy, the mean proportion of E-rosetting cells in vitro in the presence of f-5 reached the value observed in vivo in mildly malnourished controls (10). The failure to accomplish this degree of in vitro restoration earlier in the course of treatment suggests that there may be subpopulations of cells responsive to different thymic hormones and/or a prethymic lymphocyte defect in PEM, resulting in a deficit of cells able to respond to the thymic hormone signal.

Thus, in contrast to other data (4), our results show that clinically acceptable nutritional recuperation does not guarantee concomitant reversal of the T-lymphocyte abnormalities associated with PEM. Therefore, therapeutic measures are still needed to accelerate the improvement in immunocompetence. Studies in patients with primary immunodeficiencies have led to the concept that the degree of responsiveness of T-lymphocytes to in vitro treatment with thymosin represents the level of thymic factor-associated CMI deficiency in the patient (13,14). Because of the in vitro effects of f-5 on E-rosettes in PEM patients and because in vivo administration of thymic factors to some immunosuppressed patients can improve immune function (13,14), administration of f-5 to malnourished children may represent one way to accomplish more rapid restoration of CMI functions. Because it is possible that subpopulations of T-lymphocytes could respond to different thymic factors, it may be necessary to administer various thymic factors together for maximum improvement. If successful, however, this therapy might reduce both morbidity and mortality from infection during the clinically vulnerable initial period of nutritional therapy (15,16) until full recovery of the T-lymphocyte system can occur.

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