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Numerical and functional deficiency in T helper cells in protein energy malnutrition

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SUMMARY

Protein energy malnutrition decreased the number of rosette forming T lymphocytes, of T4 positive cells and their ability to provide help to B cells in antibody synthesis. There was a reduction in serum thymic hormone activity and an elevation of leucocyte terminal transferase and plasma cortisol levels. The numerical and functional deficiency of $T4^+$ helper cells may be important in the pathogenesis of some of the clinical and immunological manifestations of protein energy malnutrition.

INTRODUCTION

Cell-mediated immunity (CMI) is impaired in protein energy malnutrition (PEM) (Chandra & Newberne, 1977; Katz & Stiehm, 1977; WHO Scientific Group, 1978; Chandra, 1980a, 1980b, 1982). Rosette forming thymus-dependent (T) lymphocytes are reduced (Chandra, 1974), delayed cutaneous hypersensitivity is impaired (Chandra, 1972; Neumann *et al.*, 1975; McMurray *et al.*, 1981) and lymphocyte proliferation response to phytohaemagglutinin is depressed, particularly in the presence of autologous plasma (Moore, Heyworth & Brown, 1974; Beatty & Dowdle, 1978; Chandra, 1980b). In this paper, data on T lymphocyte subsets are presented and a numerical and functional deficiency in T helper cells in PEM is reported.

SUBJECTS AND METHODS

Subjects. Eight patients with PEM, five boys and three girls, 8–24 months old, were studied. PEM was diagnosed on the basis of reduced dietary intake, weight-for-age and weight-for-height between 55 and 70% of the 50th centile of National Center for Health Statistics (1976) standard for chronological age, and clinical manifestations including irritability and/or apathy in seven, skin and hair changes in five and dependent oedema in three. Biochemical analyses (Table 1) supported the diagnosis of malnutrition. Deficits of vitamins and trace elements were excluded by normal cell indices and morphology, blood levels of folate, ferritin, vitamin B_{12} and zinc within the normal range, and normal transferrin saturation. The presence of active concurrent or recent infection was excluded by clinical examination, normal chest X-ray, negative blood and urine cultures, negative Limulus assay for endotoxemia and normal levels of C-reactive protein. Blood was drawn on first

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Table 1. Anthropometric and biochemical profile

Group	Weight-for-age* (%)	Weight-for-height* (%)	Serum concentration: Albumin (g%)	Prealbumin (mg%)	Transferrin (mg%)	Retinol binding protein (mg%)
Protein energy malnutrition						
I (BT (n=8)	62±4·9	57±4·3	2·41±0·63	12±3·7	101±29	1·7±0·5
II AT (n=8)	74±5·3	84±4·1	3·97±0·41	29±5·1	261±36	5·6±1·2
Well nourished controls						
III (n=11)	91±4·7	97±3·7	4·31±0·38	32±4·9	191±31	5·9±1·1
Significance P						
I vs II	<0·05	<0·01	<0·01	<0·02	<0·05	<0·001
I vs III	<0·01	<0·01	<0·01	<0·01	<0·05	<0·001
II vs III	<0·05	<0·05	n.s.	n.s.	n.s.	n.s.

Values are given as mean±s.e.m.

BT = before therapy; AT = after therapy; n.s. = not significant.

* Expressed as percentage of the 50th centile of standard for age (National Center for Health Statistics data).

examination and 6–8 weeks after nutritional therapy. The latter provided approximately 150 Kcal per kg body weight and 3·5 g protein per kg body weight. Clinical and biochemical recovery (Table 1) was evident at the time of the second examination.

Eleven well-nourished children, six boys and five girls, 9–24 months old, seen in the clinic for trauma or other non-medical complaints, were studied as controls.

Mononuclear cells. Mononuclear cells were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. Interface cells were collected, washed three times with Hanks' balanced salt solution (HBSS) and then re-suspended at a concentration of 2×10^6 cells/ml in RPMI 1640 medium, containing glutamine (2 mM), penicillin (100 units/ml) and streptomycin (50 μg/ml) and 10% fetal calf serum (FCS). Adherent cells were removed by overnight incubation in 60 × 15 mm Petri dishes at 37°C in an atmosphere of 95% air and 5% carbon dioxide.

Rosette forming T cells. Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells (SRBC) in 10% FCS. The mixture was incubated at 37°C for 5 min, centrifuged at 200*g* for 5 min and incubated again at 4°C for 10 min and examined for the proportion of cells forming rosettes with SRBC.

T lymphocyte subsets. Three monoclonal antibodies with specificites for T3, T4 and T8 cell subpopulations were used (Ortho). They induced complement-mediated cytolysis and/or indirect immunofluorescence to a titre from 1:100,000 to 1:500,000. Cytofluorographic analysis of lymphocytes was performed by indirect immunofluorescence with fluorescein conjugated goat anti-mouse IgG on a system 50H cytofluorograph cell sorter.

B lymphocytes. Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped fluorescence on staining with fluorescein conjugated goat IgG $f(ab')_2$ directed against human IgG, IgA and IgM heavy chains. Mononuclear cells were incubated in culture medium at 37°C for 1 hr and then incubated with the conjugated antisera. After thorough washing, the cell preparations were examined under a fluorescence microscope equipped with epi-illumination. Furthermore, duplicate sets of cell preparations were treated with trypsin, incubated in culture medium for 8 hr and restained to demonstrate newly produced immunoglobulin.

Reverse haemolytic plaque assays. Immunoglobulin producing cells were enumerated by a reverse plaque technique. Lymphocytes were cultured for 7 days in the presence or absence of pokeweed mitogen at a final concentration of 1:200. One million washed viable cells were

suspended in RPMI 1640 and 10% FCS. A 100 μl aliquot of cells was mixed with 25 μl SRBC linked with staphylococcal protein A, 50 μl of 1:5 diluted rabbit IgG directed against human γ heavy chain and 50 μl of 1:5 diluted guinea-pig complement, and the suspension transferred to 0·3 ml of 0·5% agarose kept at 45°C. The mixture was layered over plastic Petri dishes and incubated at 37°C for 16 hr. Haemolytic plaques with a central lymphocyte were counted with use of indirect illumination.

Lymphocyte co-cultures. Cells from patients and controls were mixed at a ratio of 1:1 and pokeweed mitogen stimulated IgG plaque forming cells enumerated as described above.

Plasma cortisol. The plasma concentration of cortisol was determined by radioimmunoassay.

Thymic factor activity. Thymic factor (TF) activity was quantified by the modified method of Dardenne & Bach (1973) as described earlier (Heresi & Chandra, 1980). The blood was allowed to clot at 4°C in plastic centrifuge tubes. The serum was separated in a refrigerated centrifuge and filtered immediately. TF activity was assayed either on the same day that the blood was collected or on samples kept frozen at −40°C for up to 1 week. Spleen cells were obtained from 8–12 week old male C57Bl/6J mice that had been thymectomized for 10–20 days before they were killed. The spleen cells were dissociated, washed twice in HBSS, resuspended in a concentration of 30×10^6 cells/ml, and incubated at 37°C with serial $\log_2$ dilutions of test sera in HBSS and azathioprine (10 μg/ml). After 80 min, a 1% suspension of SRBC was added. SRBC had been kept at Alsever's solution for up to 3 weeks and before use were washed twice with saline and twice with HBSS. The cell suspension was centrifuged at 4°C for 5 min and resuspended gently. Rosettes were observed in a haemocytometer. The highest dilution of test serum sample that was able to restore azathioprine sensitivity, i.e. induce 50% inhibition of SRBC rosette forming cells, was taken as the concentration of TF activity in the serum.

Terminal deoxynucleotidyl transferase (TdT). Mononuclear cells were ruptured by freezing and thawing three times. The suspension was centrifuged at 40,000*g* for 1 hr and the supernatant assayed for TdT activity by the method of Coleman *et al.* (1976). The results were expressed in units per 10^8 cells, one unit of activity being the polymerization by TdT of 1 nmol of nucleotide for 1 hr.

Statistics. Differences between groups were analysed by the Student's two-tailed *t*-test.

RESULTS

The number of lymphocytes and rosette forming T cells was lower in children with PEM and increased after nutritional therapy (Table 2). The proportion of B cells was comparable in the two groups.

Table 2. Lymphocyte count and subsets

	Absolute lymphocyte count per mm³	Rosette forming T cells (%)	SmIg bearing B cells (%)
Protein energy malnutrition			
I BT ($n=8$)	2,086±283	41±3·7	15±1·8
II AT ($n=8$)	2,878±357	68±2·8	13±1·3
Well nourished controls			
III ($n=11$)	2,908±510	75±1·9	11±0·9
Significant P			
I vs II	<0·05	<0·01	n.s.
I vs III	<0·05	<0·01	n.s.
II vs III	n.s.	n.s.	n.s.

Values are given as mean±s.e.m.

BT = before therapy; AT = after therapy; n.s. = not significant.

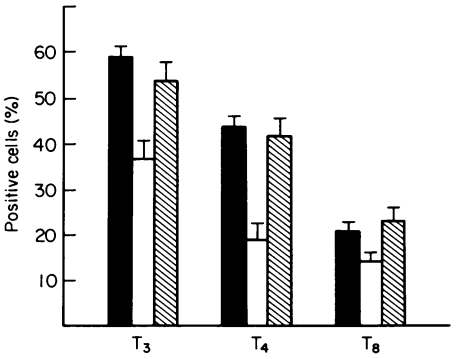

Fig. 1. Proportion of T lymphocyte subsets estimated by monoclonal antibodies and cell sorting. (■ = control; □ = malnourished; ▨ = recovered).

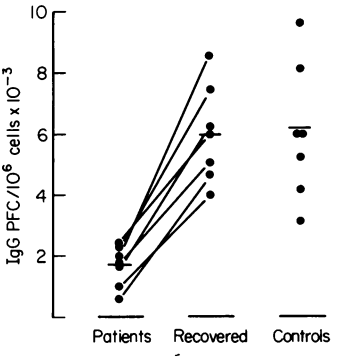

Fig. 2. Generation of IgG haemolytic plaque forming cells (PFC).

The proportion of all three subsets of T cells was decreased (Fig. 1). The most significant alteration was in the number of helper T4$^+$ cells ($P < 0{\cdot}001$). The cytotoxic/suppressor cells also were reduced but to a lesser extent ($P < 0{\cdot}05$). Following nutritional rehabilitation, complete recovery in T cell subset numbers was observed. The number of pokeweed mitogen stimulated IgG haemolytic plaque forming cells was significantly decreased on first examination and increased on nutritional recovery (Fig. 2). Co-culture experiments confirmed a marked functional deficiency in T helper cell activity (Fig. 3). B lymphocytes functioned normally in the presence of control T cells from the well nourished group.

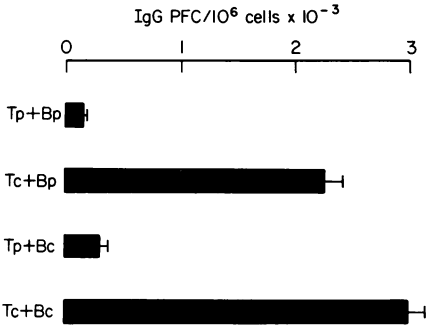

Fig. 3. Co-culture experiments in which T cells of malnourished patients (Tp) or control subjects (Tc) were mixed with B cells of patients (Bp) or controls (Bc). Results are expressed as number PFC generated.

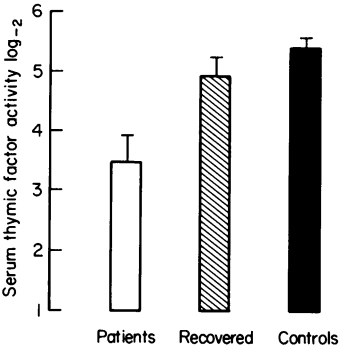

Fig. 4. Thymic factor activity.

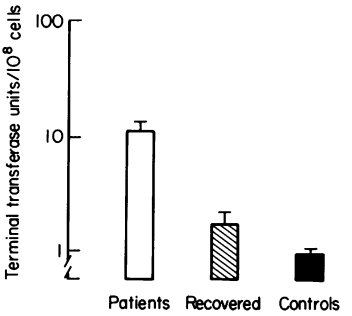

Fig. 5. Terminal deoxynucleotidyl transferase activity.

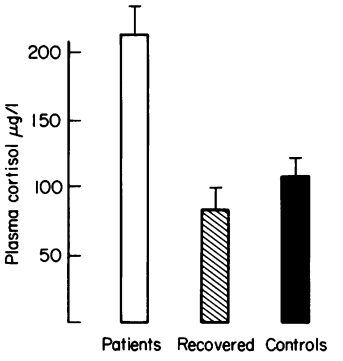

Fig. 6. Plasma cortisol concentration.

Thymic factor activity was significantly lower in the malnourished group ($P < 0{\cdot}01$) and rose to the control range after a few weeks of nutritional supplements (Fig. 4). Reciprocal changes were observed in TdT activity ($P < 0{\cdot}02$) (Fig. 5). Malnourished children showed a moderate elevation in plasma cortisol concentration ($P < 0{\cdot}01$) (Fig. 6).

DISCUSSION

The results of this study demonstrate that PEM results in numerical and functional deficiency in T helper cells which may be linked to incomplete differentiation of T lymphocyte precursors and steroid-induced lympholysis.

We confirmed earlier observations that the proportion and absolute number of rosette forming T lymphocytes is reduced in PEM. This is an easily reversible abnormality. About 15% of malnourished children show lymphopenia and T cells are invariably decreased. On providing supplements to correct nutritional deficiency, there is a rapid increase in these cells. The quick and dramatic recovery in T cell number had led to the suggestion that this index could be used as a sensitive and functional measure of nutritional recovery (Chandra, 1981a, 1981b).

This study also demonstrates that PEM results in a marked and almost selective reduction in the proportion and absolute number of $T4^+$ cells. The number of 'null' cells not detected by the rosette formation assay or by the presence of surface membrane immunoglobulin was increased. It is possible that such cells possess a low density of surface antigens not picked up by the fluorescence activated cell sorting method. It is recognized that PEM, particularly if it is associated with vitamin A deficiency, can alter cell surface glycoproteins. However, two alternative hypotheses are more likely. PEM results in reduced thymic factor activity, which may impair the normal differentiation and maturation of T cell precursors. Similar data have been recorded in undernourished children (Chandra, 1979; Jackson & Zaman, 1980) and in laboratory animals deprived of calories, zinc or pyridoxine (Iwata *et al.*, 1979; Chandra, Heresi & Au, 1980; Heresi & Chandra, 1980). Trace elements may play a critical role in the activity of facteur thymique serique (Dardenne *et al.*, 1981). The elevated TdT activity in the children with PEM may reflect such abnormal differentiation. Alternatively, the elevated cortisol levels in PEM may exert a selective lympholytic effect on $T4^+$ cells. Glucocorticoid hormones are known to influence circulating thymic factor activity; pharmacological doses cause a drop in thymic factor activity (Bach, Duval & Dardenne, 1975).

We also found a profound depression of *in vitro* helper cell function in PEM. IgG plaque forming cells, reduced in PEM, were improved almost to control values when B cells of malnourished patients were co-cultured with T cells of well nourished controls. These functional changes were again completely reversed after nutritional recovery.

The changes in the proportion and function of T cell subsets may explain some of the clinical and immunological features of PEM. The reduced ability of malnourished individuals to deal with many common infections may be due to inefficient antibody synthesis. PEM results in decreased antibody response to those antigens which require T cell help (Chandra, Chakraburty & Chandra, 1976; Chandra, 1980a, 1980b), but response to these antigens improves if they are given in adjuvant or in repeated large doses. It is interesting to note that young infants malnourished from early life, fetal or immediate post-natal, often show hypoimmunoglobulinaemia G, which corrects itself after the first few months of life (El-Ghomy *et al.*, 1970; Chandra, 1972, 1975). These features are reminiscent of transient hypogammaglobulinaemia of infancy in which there is a $T4^+$ defect (Siegel *et al.*, 1981).

However, human malnutrition is usually a composite and variable, modulated further by the contaminated environments in which malnutrition evolves. Vitamin and mineral deficiencies commonly occur with energy and protein deficiency; each interacts with the others. Infection, a common accompaniment of malnutrition, may also interfere both with immune responses and body metabolism (Chandra, 1981c, 1982). The clinical significance of the reduction in $T4^+$ cells is uncertain.

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