

PRELIMINARY COMMUNICATION

Rosette-forming T Lymphocytes and Cell-mediated Immunity in Malnutrition

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Summary

The frequency of rosette-forming thymus-dependent lymphocytes was reduced in the peripheral blood of malnourished infants and children. The reduced frequency paralleled the impairment of delayed hypersensitivity response to 2, 4-dinitrochlorobenzene and decreased DNA synthesis by lymphocytes challenged with phytohaemagglutinin. The abnormalities were reversed on nutritional improvement.

Introduction

Malnourished children are believed to have an increased susceptibility to infection. Among those who die infection is a causal or associated factor. Malnourished children have impaired immunocompetence (Smythe, *et al.*, 1971; Chandra, 1972; Seth and Chandra, 1972; Chandra, 1973 a). Cell-mediated immunity is consistently depressed. Lymphoreticular tissues are involuted, with depletion of the thymus (Smythe *et al.*, 1971; Chandra, 1973 b). Lymphopenia in the peripheral blood may occur (Chandra, 1972).

We have looked at the proportion of peripheral blood lymphocytes which form rosettes spontaneously with sheep red cells and apparently constitute the thymus-processed or T cell subpopulation, and correlated it with cellular immunity—measured by delayed hypersensitivity to 2, 4 dinitrochlorobenzene (DNCB)—and lymphocyte response to phytohaemagglutinin (PHA).

Patients and Methods

The diagnosis of protein-calorie malnutrition was made in 15 children on a history of failure to thrive and inadequate nutrient intake, weight and height less than 80% of the 50th percentile for age on Boston growth standards, and clinical features such as loss of subcutaneous fat, hair and skin changes, and pallor. Two children had pedal oedema. The patients were tested on first attendance at the clinic and 6-16 weeks later after correction of nutritional deficit. Ten healthy children matched for age and sex served as controls.

Rosette-forming Cells.—Spontaneously rosetting thymus-dependent or T cells were detected by the method of Jondal *et al.* (1972) except that the leucocyte sheep red cell

pellet was fixed with glutaraldehyde before being counted in a cover slip preparation. Lymphocytes were isolated by dextran sedimentation followed by Ficoll-Hypaque gradient centrifugation. Two-hundred lymphocytes were examined, and all cells binding three or more than three sheep red cells were considered to be both lymphocytes and T cells and expressed as a percentage of the total lymphocyte proportion.

Lymphocyte Response to Mitogen.—Lymphocytes were separated as described above. DNA synthesis induced by phytohaemagglutinin was measured by means of ³H-thymidine incorporation and a scintillation counter (Bullock and Fasal, 1971). Cells were washed twice and duplicate cultures containing 2×10^6 lymphocytes per ml in medium 199 and 15% pooled AB serum established. The cells were harvested at 96 hours. Stimulation indices were derived by dividing counts per minute of PHA-stimulated cultures by those of unstimulated controls and expressed as a percentage.

Delayed Hypersensitivity.—The capacity to develop sensitization to DNCB was investigated by applying a 10% solution of the chemical in acetone to one forearm, and one month later a challenge dose of 0.2 ml of 0.5% DNCB solution was used on the opposite forearm. The challenge site was examined after 48 hours and the reaction graded as positive (vesiculation or induration or both) or negative (absence of vesiculation and induration with or without mild erythema).

Results

There was a significant decrease ($P < 0.01$) in the proportion of peripheral blood lymphocytes which formed spontaneous rosettes with sheep erythrocytes (see table). There was a significant correlation between degree of weight deficit and the reduction in rosette-forming cells ($r = + 0.8295$). Cellular immunity as judged by delayed hypersensitivity to DNCB ($P < 0.01$) and lymphocyte DNA synthesis in response to PHA ($P < 0.05$) was impaired. On nutritional rehabilitation and gain in weight the number of rosette-forming cells increased, as did the response to mitogen.

Discussion

The restriction of nutrient intake imposes a severe limitation on protein synthesis and cell division in the host. This affects reparative processes and the ability to mount an adequate immune response. Lymphoid tissues undergo involution (Smythe, *et al.*, 1971; Chandra, 1973 b). The tonsils are shrunken, and there is lymphopenia in a few (Chandra, 1972). It seemed relevant, therefore, to look at the subpopulation of lymphocytes which have been processed by or are dependent on the thymus. The recent development of the sheep erythrocyte rosette technique has permitted such an investigation.

Our study has shown that the frequency of T cells in the peripheral blood is much reduced in malnutrition and that this reduction parallels the degree of weight loss. Since lymphocyte response to a mitogen such as PHA depends on the quantity, as of course on the quality, of T cells in the culture it seems that the low numbers of rosette-forming cells explain the impairment of DNA synthesis as also the failure to develop delayed hypersensitivity reaction to DNCB. This has also been seen in states of primary immunity deficiency in which the thymus is absent or functionally de-

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Proportion of Rosette-forming T Lymphocytes in Peripheral Blood and Cellular Immunity in 15 Children with Malnutrition

Case No.	Weight as % of Reference Standard for Age	T Lymphocytes (%)		Sensitization to DNCB on Admission	Lymphocyte Stimulation Index in Response to PHA (%)	
		On Admission	After Therapy		On Admission	After Therapy
1	73	36	58	Negative	68	113
2	70	40	69	Positive	49	92
3	65	31	70	Negative	50	95
4	65	22	64	Negative	33	110
5	65	25	68	Positive	50	90
6	60	24	59	Negative	23	87
7	60	15	48	Negative	29	97
8	60	22	68	Negative	11	134
9	58	31	59	Negative	44	88
10	56	20	72	Negative	21	113
11	50	18	62	Negative	40	128
12	50	15	56	Negative	47	87
13	46	19	55	Negative	33	93
14	44	11	34	Negative	5	23
15	40	12	61	Negative	17	110
Mean for patients	57	23	60	2 positive	35	97
Mean (and range) for controls		71 (58-79)		All positive	111 (87-228)	

DNCB = 2, 4-Dinitrochlorobenzene. PHA = Phytohaemagglutinin.

ficient (Wybran and Fudenbergh, 1973) and in retardation of intrauterine growth, which is a state of fetal malnutrition of diverse aetiology (Chandra, 1974 a). Together with other indicators of immune defence which are adversely affected by malnutrition (Antia, *et al.*, 1968; Sellmeyer, *et al.*, 1972; Chandra, 1972; Seth and Chandra, 1972; Sirisinha, *et al.*, 1973) the reduction in T cells in the peripheral blood, and presumably in the tissues, may account for the increased susceptibility of patients with these disorders to frequent and severe infections. The reversal of the abnormality on feeding for 6 to 16 weeks rules out any basic primary defect of the thymus. It remains to be seen whether the reduction in T cells is the result of any specific nutrient deficiency, since some cellular functions may be dramatically and severely knocked out by the lack of a single nutrient, such as iron (Chandra, 1973 a).

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MEDICAL MEMORANDA

Epidermolysis in a Case of Severe Cytomegalovirus Infection

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During the last decade an increasing number of publications have shown that cytomegalovirus (C.M.V.) is a widespread infectious agent (Stern and Elek, 1965; Hanshaw, 1966; Krech *et al.*, 1971) producing symptoms of amazing variability. The differential diagnosis is often difficult, especially in patients with other underlying or concomitant disease (Müller-Stamou *et al.*, 1973).

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Case Report

A 40-year-old male nurse complained of fatigue, nausea, and a slight cough at the beginning of January 1972. On 5 January he had severe pain in the left side of the chest, and three days later a maculopapular rash appeared at the level of the 8th thoracic segment, which developed within a few days into a vesicular stage. The diagnosis of herpes zoster thoracalis was made.

On admission on 31 January he had an enlarged spleen (3 cm below costal margin) and several firm, mobile, and slightly tender lymph nodes up to the size of a cherry were found, mainly in the axillae, on the right side of the neck, and in both inguinal regions. The liver was clinically normal. There were no other physical signs. Investigations: E.S.R. 25 to 54 mm/hr; eosinophilia 6%. All other haematological and chemical values were normal (see table).

A systemic lymphoreticular disease was first suspected. Lymph node biopsy showed non-specific inflammation however. A bone marrow examination and lymphography showed no signs of a systemic disease. Serological examinations for mononucleosis, toxoplasmosis, syphilis, brucellosis, and viruses such as ornithosis, adenovirus, Q fever, *Mycoplasma pneumoniae*, and influenza A and B as well as the anti-streptolysin titre gave negative results.

Complement fixing antibodies against C.M.V. rose from 1:40 up to more than 1:120 within two weeks. During this time lymph nodes and spleen regressed considerably and the patient felt much better. The pre-existing herpes zoster also had completely disappeared, and on 17 February he was discharged from hospital. On 29 February, 2 March, and 7 March C.M.V. was isolated from the urine.

On 1 April the patient became ill again with a temperature of 40°C, weakness, poor appetite, nausea, and increasing icterus, with