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T and B Lymphocyte Rosetting in Undernourished Children (38772)

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Thymus-processed human lymphocytes (T cells) function in cell-mediated immune responses (1) and bind sheep erythrocytes (E) on their surfaces to form 'rosettes' (2). Lymphocytes processed in an unidentified mammalian system equivalent to the avian bursa of Fabricius (B cells) are precursors of plasma cells which produce immunoglobulins; B cells bind E only when the E are first treated with anti-sheep hemolysin (A) and subsequently coated with complement (C). These EAC sheep cells will then bind to complement receptor sites on the B cells and also form rosettes (3). Differential T and B lymphocyte rosetting in normal human subjects varies around general averages of 65–70% T and 30–35% B cells. Thus, significant differences in these ranges presumably indicate changes in the immune capability of the subject tested.

Children with protein-calorie malnutrition (PCM) are known to have depressed cellular immune responses as measured by reduced rates of lymphocyte transformation (4), depression in tuberculin reaction (5), and defective delayed hypersensitivity reactions (6). There are apparently no published data on T cell-rosetting values as a measure of cell-mediated immune response in children with PCM. We have tested these values in peripheral blood lymphocytes of 18 children with moderate to severe PCM: eight with clinical signs of kwashiorkor, four with marasmus, and five with nutritional edema. Since responses to skin tests with DNCB are generally considered to be a measure of cell-mediated immune responses, five children were sensitized before refeeding therapy was initiated while six children were sensitized only after clinical recovery from PCM; both groups were subsequently given challenge doses of the antigen.

Method. From among outpatients in the Institute of Child Health hospital in Calcutta, children between 4 mo and 5 yr of age were identified as clinically malnourished by the pediatrician (D.M.). Kwashiorkor was diagnosed in those who had general edema of the body, especially "moon face," puffiness of the extremities, growth retardation for age, and one or more secondary signs such as changes in skin pigmentation and in the color or texture of the hair. Children with "florid kwashiorkor" usually showed all of these symptoms. Marasmus was diagnosed in very wasted growth-retarded children with no subcutaneous fat, "old man's" or simian facies, and heads and eyes which appeared disproportionally large. The signs of nutritional edema (7), while somewhat similar to those of marasmus, were differentiated by presence of some degree of pitting edema of ankles and wrists, usually some moon face, and less loss of subcutaneous fat.

Since for cultural reasons it was not possible to test well-nourished age-matched children as controls, seven adults associated with the study served as normal controls; Wybran *et al.* (8) found no difference in rosetting values in normal infants and adults which could be attributed to age or sex. All children were tested at the time of admission, before refeeding therapy began.

For rosetting tests, 1–2 ml of heparinized blood were diluted 1:2 in Hanks' balanced salt solution (HBSS) and the diluted blood was layered onto 4 ml of cold Ficoll-Hypaque and centrifuged for 30 min at 400*g* at room temperature. Lymphocytes at the interface between blood and gradient were pipetted off and washed three times in HBSS. Final suspensions were adjusted to contain about 4×10^6 cells in 0.5 ml of HBSS containing 20% fetal calf serum. Macrophages were identified by incubating the lymphocyte suspension with equal volumes of

Lymphocyte Separating Reagent (Technicon Instrument Corp., Tarrytown, NY) for 30 min in a 37° water bath, with occasional shaking to resuspend the iron particles.

For T cell tests, 0.25 ml of lymphocyte suspension were combined with 0.25 ml of thrice-washed E, incubated 5 min at 37°, centrifuged 5 min at 200*g*, and put in a 4° icebath overnight. For counting rosettes, 1 drop of a 0.02% solution of toluidine blue was added to assure that only living lymphocytes would be counted, cells were very gently resuspended, and the percentage of rosetting lymphocytes was determined in a hemocytometer.

For B cell tests, EAC was prepared by the method of Pincus *et al.* (3); 0.25 ml of thrice-washed EAC was added to 0.25 ml of lymphocyte suspension, centrifuged 2 min at 200*g* and incubated 30 min at 37°. The preparation was then whirl-mixed, toluidine blue was added, and the percentage of rosetting lymphocytes was determined. All lymphocytes to which one or more sheep cells was firmly adherent were counted as rosette-forming cells, though in most preparations 5–10 or more sheep cells were bound to the lymphocytes.

DNCB sensitization and challenge were administered according to instructions on sample kits which were made available by courtesy of the Pittway Corporation, Baltimore, MD. Challenge doses were administered 2 wk after sensitization and were read after 72 hr.

Results. T and B cell-rosetting values for individual children with degrees of kwashiorkor, marasmus, and nutritional edema are shown in Table I. Children with clinically apparent concomitant fever are designated by the letter "c". There seems to be a gradation from low to higher T cell values in each group which correlates to some extent with the degree of clinical severity of PCM, and the three children with florid (+++) kwashiorkor show the lowest values. Averaged values for the combined groups and for the controls, and the ranges of rosetting percentages in the PCM and control groups, are shown in Table II.

The high B values in the four children with nutritional edema may reflect the fact that each of the four had clinically apparent fever

TABLE I. PERCENTAGE OF ROSETTE-FORMING LYMPHOCYTES IN INDIVIDUAL CHILDREN.

Age (mo)	PCM type[a, b]	Degree	% B	% T
9	K[a]	+++	30[c]	23
48	K[a]	+++	—[d]	43
60	K	+++	13	36
17	K[a]	++	24	48
24	K[a]	++	39[c]	33
9	K	+	—[d]	49
22	K	+	30	—
11	K	+	28	37
4	K	+	26	47
18	M	+++	38[c]	50
33	M[a]	+++	32	29
24	M	+++	30	58
8	M	+++	—[d]	60
20	NE	+++	56[c]	60
42	NE	+++	42[c]	62
26	NE[a]	+++	—[d]	63
25	NE	+++	58[c]	47
13	NE	++	49[c]	35

[a] Designates children who died within a few days of admission.

[b] K = kwashiorkor, M = marasmus, NE = nutritional edema.

[c] Designates concurrent infection.

[d] Designates no data.

associated with pneumonia, dysentery, severe diarrhea, or pneumonitis. It is recognized that children with a known history of concurrent or recent infection have higher concentrations of serum immunoglobulins than children without this history (9). For this reason the B values of the PCM children were divided into those of children with and without concurrent infection. As Table III shows, the averaged B values of those with concurrent infections were significantly higher than those without apparent infection.

Figure 1 is a summary chart of the percentages of T and B lymphocyte rosetting in individual children and in adult controls. It can be seen that all kwashiorkor T values are below 50% while all control T values are above 50%. The chart also emphasizes the wider rosetting ranges in PCM children than in controls.

DNCB tests. In a separate group of protein-calorie malnourished children, five hospitalized children from 1–4 yr old were given sensitizing doses of 2,4-dinitrochlorobenzene

TABLE II. TOTALED AVERAGED ROSETTING VALUES AND RANGES FOR ADULT CONTROLS AND PCM CHILDREN.

PCM type	Degree	Average % B	Average % T
K	+++	(2)[a] 21	(3) 34
K	++		
	+	(5) 29	(5) 43
M	+++		
	++	(3) 33	(4) 44
NE	+++		
	++	(4) 51	(5) 53
Average PCM		(14) 36	(17) 46
Range		13–39	23–63
Average adult control		(22) 31	(22) 63
Range		22–38	54–72

[a] Figures in parentheses indicate the number of tests.

TABLE III. AVERAGED B ROSETTING VALUES OF PCM CHILDREN WITH AND WITHOUT CLINICAL INFECTION AT TIME OF ADMISSION.

	% B	
	(14) 36% total av.	
av. uninfected	26% (7)	(7) 43% av. infected
range	13–30%	30–39%

(DNCB) within 1 or 2 days after admission. None of these children responded to the first challenge dose administered 2 wk later; four failed to respond to a second challenge. As Fig. 2A shows, only one of four responded to the first challenge even after a second sensitizing dose, and one of the children with kwashiorkor failed to respond to challenge after a third sensitizing dose. Figure 2B shows that of six children who were not sensitized until they had clinically recovered after refeeding therapy, five responded vigorously to the first challenge, including three who had had kwashiorkor.

Discussion. Tests on this limited number of children with moderate to severe malnutrition indicate that those who had kwashiorkor had generally lower T lymphocyte rosetting values than those with other types of PCM.

Children with kwashiorkor seem to have particular disabilities: low thymus weights (10), low serum transferrin and albumin levels (11), subnormal lymphocyte transformation values (2), thymic atrophy (12), and high susceptibility to bacterial infections (8). Also, globulin synthesis was found to be relatively unaffected by kwashiorkor unless a child acquired an infection, when it increased 3-fold (13). Finally, Stitaya and his colleagues (14) reported markedly lower serum levels of all complement proteins, ex-

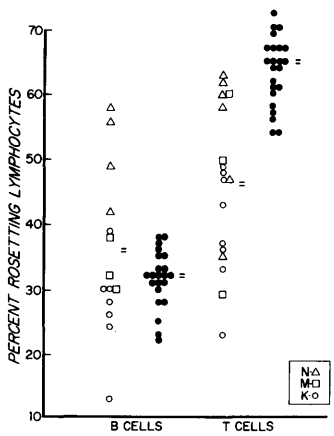

FIG. 1. Overnight rosetting values in individual PCM children (open symbols) and in adult controls (closed black circles). K = kwashiorkor, M = marasmus, N = nutritional edema. All PCM values are before refeeding. Double hash marks indicate group averages. All kwashiorkor T values are below 50%, all control values above 50%. All B values above the group average are in children with concomitant infections.

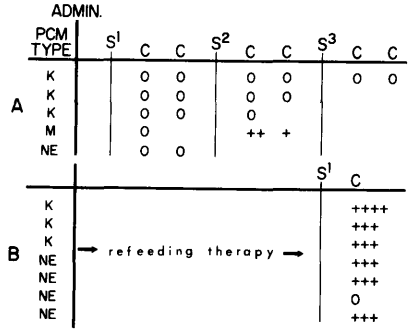

FIG. 2. Responses to challenge at 2-wk intervals after sensitization with DNCB. 2A represents responses when the initial sensitization was administered before refeeding. 2B represents responses when the initial sensitization was given after refeeding. S = sensitization, C = challenge, 0 = no response, + to ++++ = degree of response.

cept C4, in PCM than in normal children, and lower in kwashiorkor than in marasmus.

The DNCB results were similar to those reported by Edelman and his coworkers with DNFB (dinitrofluorobenzene) in Thailand (6). Whether the tolerance-like state apparent in the nonresponders might be maintained in some of these children during months or years could not, of course, be determined, but the results raise the question whether kwashiorkor might impose degrees of immunosuppression on children who are constantly exposed to high doses of new infections.

All of the children in the study were significantly subnormal in all of the criteria of growth for age published by the Indian Council of Medical Research (15). According to histories given by parents, only three of the children were brought to the hospital because of acute failure to thrive. The others were brought in because of fever, persistent productive cough, pneumonia, dysentery, or prolonged or recurrent diarrhea. Several were heavily parasitized by giardia or ascaris; one had a recent, one a past history of measles. The wide ranges in rosetting values may reflect the interactions between PCM and these many types of past and present infections.

Summary. T and B lymphocyte rosetting values were obtained for 18 children with kwashiorkor, marasmus, or nutritional edema. T cell values were subnormal in all malnutrition classes, but were lowest in children with kwashiorkor. Four of five malnourished children who were sensitized with 2,4-dinitrochlorobenzene (DNCB) before refeeding failed to respond to repeated subsequent challenges; five of six children who were sensitized after refeeding responded strongly to the first challenge.

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