Malnutrition and the Immune Response,
edited by R. M. Suskind.
Raven Press, New York © 1977.

Rosette-Forming Lymphocytes in Guatemalan Children with Protein-Calorie Malnutrition

*Gerald T. Keusch, **Juan J. Urrutia, **Oscar Guerrero, **Gustavo Casteneda, and †Steven D. Douglas

**Division of Infectious Diseases, Mt. Sinai School of Medicine, New York, New York 10029; **Division of Environmental Biology, Institute of Nutrition of Central America and Panama and Department of Pediatrics, Roosevelt Hospital, Guatemala City, Guatemala; and †Department of Medicine and Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455*

The ability of lymphocytes to bind sheep erythrocytes (SRBC) and to form rosettes (SRBC-R) is a marker for thymus-dependent lymphocytes (T-cells) (1). T-cells serve a major function in host defense mechanisms in cell-mediated immunity (CMI) and, for certain antigens, in antibody production by bursa equivalent lymphocytes (B-cells) as well (2). Previous reports of children in Africa and India have indicated that protein-calorie malnutrition (PCM) results in marked thymic atrophy, and diminished numbers and functional response of circulating T-cells (3–5). This study was undertaken to determine if malnutrition in Central America also affects the circulating T-cell pool.

PATIENTS AND METHODS

Children hospitalized at Roosevelt Hospital, Guatemala City, were selected for study. Since healthy, age- and sex-matched controls were not available, healthy North American and Guatemalan adults were used as a control population. Malnutrition was classified according to criteria of weight for age as a percentage of the 50th percentile of the INCAP standard. All five children in the 60–80% weight for age group were edematous (kwashiorkor) as were five of seven who were less than 60% weight for age (marasmic-kwashiorkor). Clinical diagnoses are shown in Table 1.

Heparinized venous blood was used to isolate mononuclear cells on a layer of Ficoll-Hypaque (6). Lymphocytes (10^6) suspended in medium 199 were added to 10^8 SRBC (total volume 1 ml). Heat-inactivated SRBC-adsorbed fetal calf serum (0.1 ml) was then added. The cells were gently mixed, incubated at 37°C for 5 min, sedimented at 200 *g* for 5 min, and then maintained at 4–5°C for 1 hour. The pellet was gently resuspended and

TABLE 1. *Number, classification, and diagnoses of study population*

Category	Number	Mean age (years)	Diagnoses[a]
>80% wt/age	3	3.7 ± 1.8[b]	pneumonia (2), piperazine intoxication
60–80% wt/age	5	3.9 ± 2.6	diarrhea (4), diarrhea + pneumonia
<60% wt/age	7	2.3 ± 1.3	diarrhea (4), pneumonia (3)

[a] Number of children with diagnosis in parentheses.
[b] ±1 Standard deviation.

transferred to a Neubauer counting chamber with a Pasteur pipette. A minimum of 200 lymphocytes were counted and both small (1–2 adherent SRBC) and large (3 or more adherent SRBC) rosettes were tallied.

Lymphocyte pellets were fixed in cold 1.5% glutaraldehyde in 0.1 M Na cacodylate containing sucrose and stored at 4°C. Cells were post-fixed in OsO_4, dehydrated and embedded in EPON, and *en bloc* stained with uranyl acetate. Samples were examined in a Siemens 102 electron microscope.

RESULTS

Hematological values for the children shown in Table 1 are given in Table 2. Children in all three nutritional categories were infected and anemic but did not show leukocytosis or a left shift. There were no significant differences between the three pediatric groups in the number of total circulating lymphocytes per cubic millimeter of blood.

The number of small, large, and total SRBC-R is shown in Table 3. In both the kwashiorkor (60–80% weight for age) and the marasmic and marasmic-kwashiorkor group (<60% weight for age), there was a marked reduction in large SRBC-R cells, and consequently a highly significant reduction in the total number of rosette-forming cells.

Electron microscopy showed frequent plasmacytoid cells (lymphocytes with abundant and dilated rough-surfaced endoplasmic reticulum) (Fig. 1).

TABLE 2. *Hematology values in study population*

	No.	Hemoglobin	Hematocrit	White bloodcount	% Lymphocytes
Control (adult)	5	14.6 ± 1.5[a]	46.0 ± 3.3	7423 ± 546	44.1 ± 5.1
Hospital patients					
>80% wt/age	3	10.6 ± 1.7	30.0 ± 6.2	9283 ± 929	51.3 ± 9.0
60–80% wt/age	5	8.8 ± 2.4	30.8 ± 2.6	7610 ± 2951	62.0 ± 9.7
<60% wt/age	7	8.7 ± 2.3	26.6 ± 5.4	8093 ± 1627	58.7 ± 7.6

[a] ± 1 Standard deviation.

TABLE 3. *Rosette-forming lymphocytes in adults and Guatemalan children with malnutrition*

Category	No.	SRBC-R		
		Small	Large	Total
Control (adult)	5	27.3 ± 6.7[a]	22.5 ± 3.5	49.8 ± 3.2
Hospital patients				
>80% wt/age	3	20.5 ± 5.8	17.8 ± 6.0	38.3 ± 3.6
60–80% wt/age	5	17.6 ± 4.5	3.1 ± 2.3	20.7 ± 5.9
<60% wt/age	7	17.7 ± 4.1	8.4 ± 7.2	26.1 ± 8.3

[a] ±1 Standard deviation.

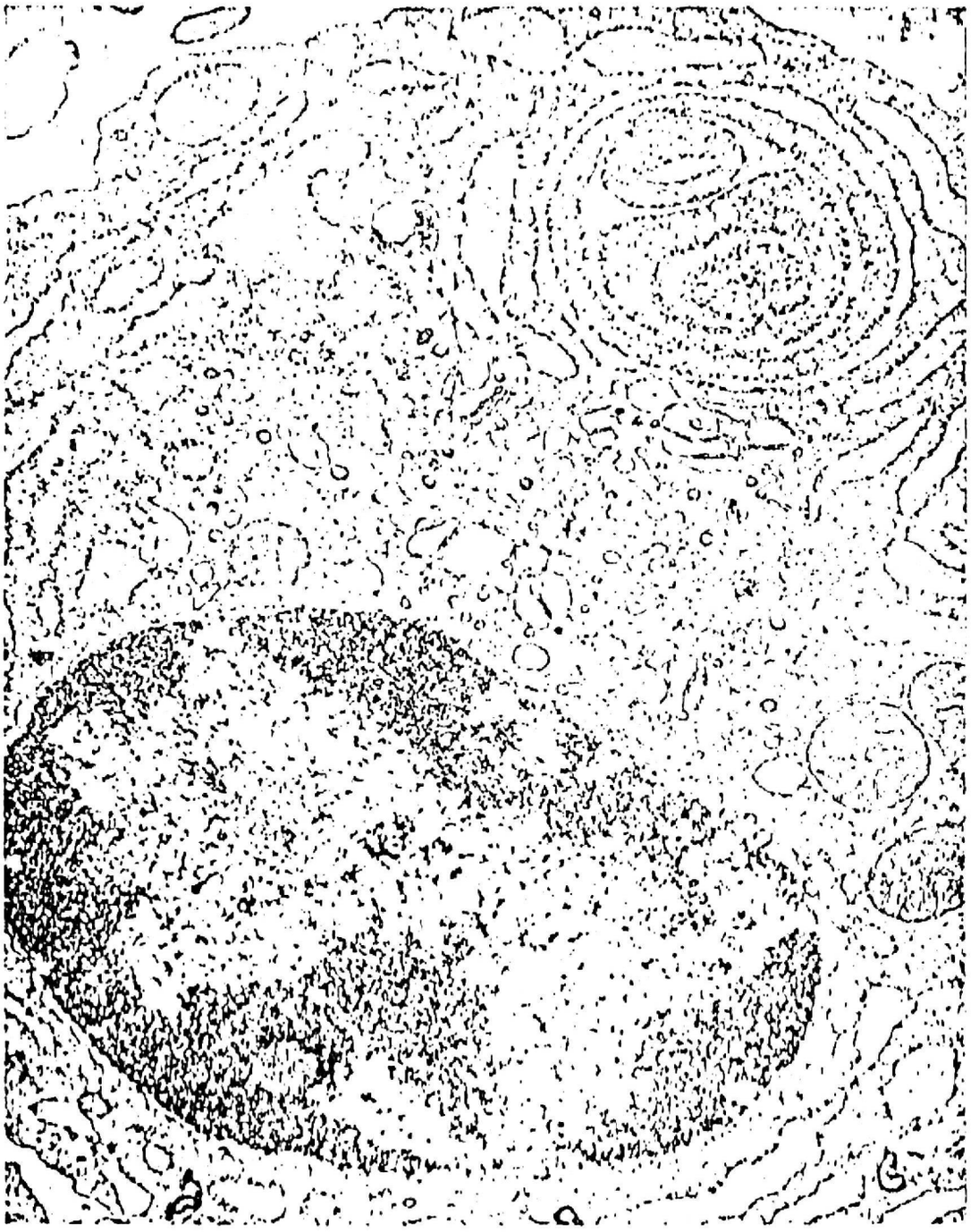

FIG. 1. Electron micrograph of a plasmacytoid cell obtained from the peripheral blood of a child with kwashiorkor. Note the very prominent Golgi zone. The cytoplasm contains numerous strands of rough-surfaced endoplasmic reticulum. Magnification ×20,000.

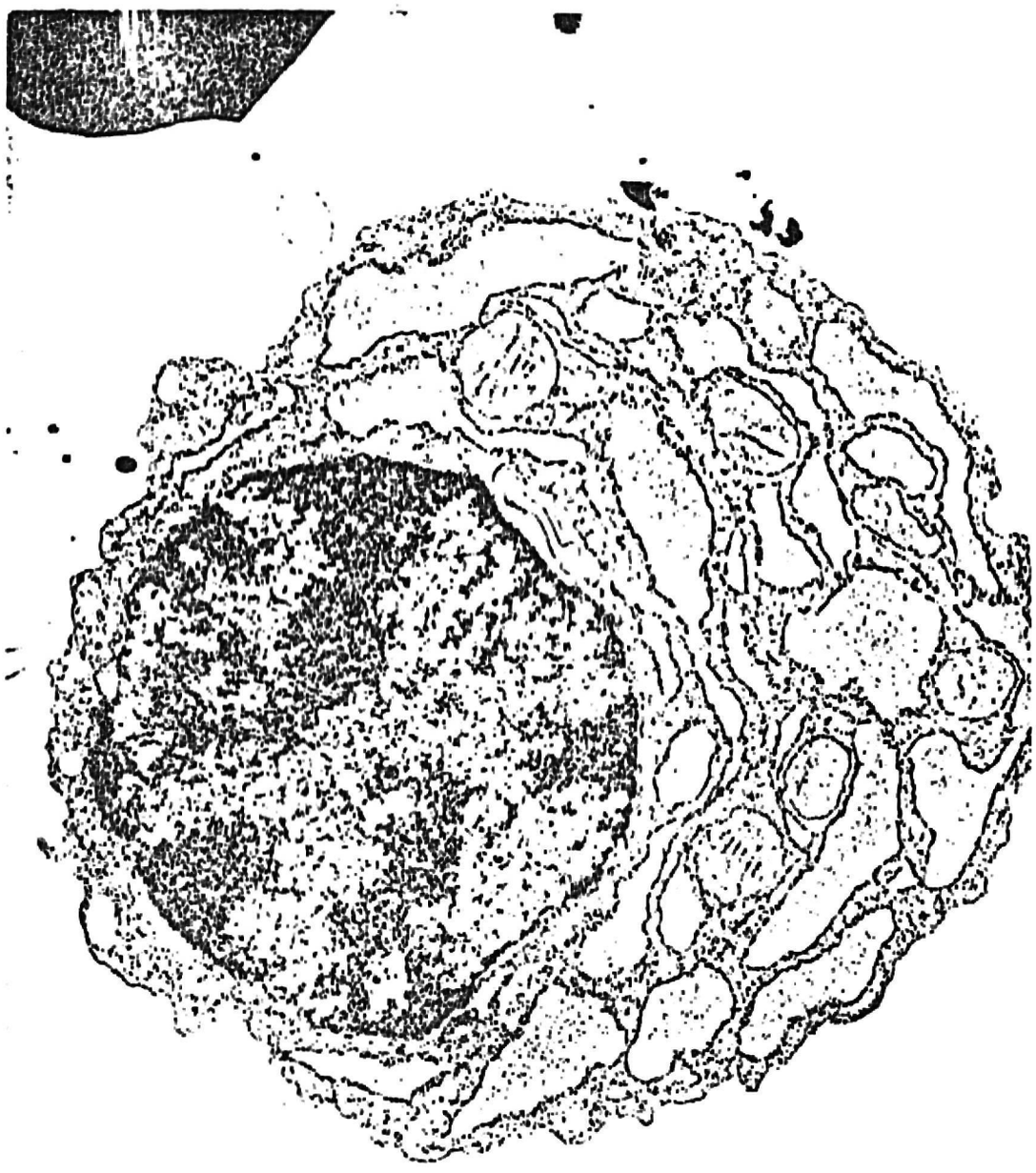

FIG. 2. Electron micrograph of a plasma cell obtained from the peripheral blood of a child with kwashiorkor. Note the very extensively dilated rough-surfaced endoplasmic reticulum. Magnification ×10,500.

In one kwashiorkor child, a circulating typical plasma cell (Fig. 2) was also observed. Quantitative studies have not yet been performed; however, such cells are extremely rare in normal populations.

DISCUSSION

This study documents a significant decrease in SRBC rosette-forming cells for large SRBC-R in Guatemalan children with malnutrition. Although the number of small SRBC-R was only slightly decreased, the total number of rosettes was markedly abnormal. Although the functional significance of small SRBC-R is not clear, diminished numbers of large SRBC-R generally will be reflected in impaired CMI responses *in vivo* and *in vitro* (1). There

was no difference in the total number or distribution of SRBC-R between the kwashiorkor or marasmic and marasmic-kwashiorkor groups. Three children with weight for age above 80% of the 50th percentile standard had near normal numbers of large SRBC-R cells, even though two had been admitted with etiologically undefined pneumonia.

Electron microscopic evidence of circulating, plasmacytoid cells (stimulated B-cells) is of considerable interest in view of the usual finding of normal or increased levels of serum immunoglobulin, particularly IgA and IgE (7,8), in children with malnutrition. This may be related to preservation of B-cell functions and the constant antigenic stimulation to which malnourished children are thought to be exposed, and hence might be considered as an exaggerated but otherwise normal response. This has been seen before in children ingesting *Phytolacca americana* (9) from which pokeweed mitogen is obtained. It is not clear whether finding circulating typical plasma cells is consistent with this concept. Continued observations will be required to determine how frequently this is seen in malnutrition and whether it has additional pathological significance.

These results are consistent with reports of diminished SRBC-R cells in children with malnutrition in other parts of the world (4,5). The lesion in CMI responsiveness that can be a consequence of deficient functioning T-cells is likely to be of major importance in explaining the adverse synergistic interaction between malnutrition and infection (7).

ACKNOWLEDGMENTS

The encouragement and advice of our teachers and colleagues, Drs. Nevin Scrimshaw, John E. Gordon, Moises Behar, and Leonardo J. Mata is gratefully acknowledged.

Supported in part by U.S. Public Health Service Grant AM-15970 and contract N01–DH–4–2859 from the National Institutes of Health.

Dr. Keusch is the recipient of a Career Development Award K04 AI 70847 from the National Institute of Allergy and Infectious Disease.

REFERENCES

1. Wybran, J., Carr, M. C., and Fudenberg, H. H. (1972): The human rosette-forming cell as a marker of a population of thymus derived cells. *J. Clin. Invest.*, 51:2537–2543.
2. Miller, J. F. A. P., Basten, A., Sprent, J., and Cheers, C. (1971): Interaction between lymphocytes in immune responses. *Cell Immunol.*, 2:469–495.
3. Smythe, P. M., Schonland, M., Brereton-Stiles, G. G., Coovadia, H. M., Grace, H. J., Loening, W. E. K., Maloyane, A., Parent, M. A., and Vos, G. H. (1971): Thymolymphatic deficiency and depression of cell mediated immunity in protein-calorie malnutrition. *Lancet*, 2:939–943.
4. Chandra, R. K. (1974): Rosette-forming T lymphocytes and cell-mediated immunity in malnutrition. *Br. Med. J.*, 3:608–609.
5. Ferguson, A. C., Lawlor, G. J., Jr., Neumann, C. G., Oh, W., and Stiehm, E. R.

(1974): Decreased rosette-forming lymphocytes in malnutrition and intrauterine growth retardation. *J. Ped.*, 85:717–723.
6. Böyum, A. (1968): Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.*, 21 (suppl. 97):77–89.
7. Faulk, W. P., Mata, L. J., and Edsall, G. (1975): Effects of malnutrition on the immune response in humans: A review. *Trop. Dis. Bull.*, 72:89–103.
8. Neumann, C. G., Lawlor, G. J., Jr., Stiehm, E. R., Swendseid, M. E., Newton, C., Herbert, J., Ammann, A. J., and Jacob, M. (1975): Immunologic responses in malnourished children. *Am. J. Clin. Nutr.*, 28:89–104.
9. Farnes, P., Barker, B. E., Brownhill, L. E., and Fanger, H. (1964): Mitogenic activity in *Phytolacca americana* (pokeweed). *Lancet*, 2:1100–1101.