

with red cell stroma and human serum and did not react with either human Ig or human red cells. The majority of antisera to HL-A were supplied by the Istituto di Genetica Medica, Torino. For HL-A terminology see "Joint Report" in *Histocompatibility Testing 1972* (Munksgaard, Copenhagen, 1973), p. 619. For the third SD (AJ) locus see (4). Rabbit antisera to human Ig and goat antiserum to rabbit Ig, with and without fluorescent label, were prepared by Dr. G. Trinchieri, according to the technique described by L. Amante and M. Giuriani [*Boll. Ist Sieroterapico Milanese* 48, 407 (1969)]. Horse antiserum to human lymphocytes (ALS) was a gift of Schweiz. Serum & Impfstitut, Bern. Nutrient medium was RPMI, supplemented with 10 percent normal rabbit serum, repeatedly absorbed with human red cells and buffy coat. Lymphocytes from defibrinated peripheral blood were purified by centrifugation on Urografin and adherent and phagocytic cells were eliminated according to technique of Mayr *et al.* (4). Lymphocytotoxicity was carried out according to the NIH microtechnique [D. L.

- Brand *et al.*, *Histocompatibility Testing 1970* (Munksgaard, Copenhagen, 1970), p. 357].
15. B. Pernis, L. Forni, L. Amante, *J. Exp. Med.* 132, 1001 (1970).
  16. P. Cresswell, M. J. Turner, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* 70, 1603 (1973).
  17. N. Nakamuro, N. Tanigaki, D. Pressman, *ibid.*, p. 2863.
  18. P. A. Peterson, L. Rask, J. B. Lindblom, *ibid.*, in press.
  19. O. Smithies and M. D. Poulik, *Science* 175, 187 (1972).
  20. P. A. Peterson, B. A. Cunningham, I. Berggård, G. M. Edelman, *Proc. Natl. Acad. Sci. U.S.A.* 69, 1697 (1972).
  21. R. Ceppellini, in *Progress in Immunology* (Academic Press, New York, 1971), p. 973.
  22. M. L. Bach, S.-W. Huang, R. Hong, M. D. Poulik, *Science* 182, 1350 (1973).
  23. The work done in Michigan was supported in part by NIAID grant AI-11335 and in part by the Children's Leukemia Foundation of Michigan.

12 November 1973

The separated mononuclear cells were washed three times and preincubated with latex particles so that contaminating monocytes, which phagocytosed the particles, could be distinguished from lymphocytes and lymphoblasts. These mononuclear cells were then resuspended in phosphate-buffered saline, mixed with washed SRBC's, and incubated at 4°C for 1 hour according to the method of Jondal *et al.* (3). Cells were considered to be positive when three or more SRBC's were bound to the surface of latex-negative cells. Confirmatory SRBC rosette data were obtained by using cytocentrifuge-prepared, stained slides.

Antiserum to human thymocytes was produced by multiple immunizations of a goat with viable human thymocytes. The antiserum was subsequently absorbed with human erythrocytes and lymphocytes from a patient with CLL of B lymphocyte origin, in which more than 90 percent of cells were bearing surface immunoglobulins. After absorption, the antiserum was used at a 1 : 100 dilution in a cytotoxicity assay. Equal volumes of antiserum, undiluted rabbit complement, and lymphocytes were incubated at 37°C for 45 minutes, after which the cells positive for trypan blue were determined. Complement controls were subtracted to give the reported figures.

Lymphocyte surface immunoglobulins were detected by using fluorescein conjugated monovalent antisera against  $\mu$ ,  $\gamma$ , and  $\alpha$  heavy chains and  $\kappa$  and  $\lambda$  light chains in a previously described method (11). Reported results are totaled from the heavy or light chain determinations.

In peripheral blood of normal individuals, 60 determinations yielded 50 to 78 percent lymphocytes forming rosettes with SRBC's (Table 1). In three persons with CLL, 1 to 6 percent of peripheral lymphocytes bound SRBC's, indicating that the majority of the lymphoid cells were not of T cell

## Acute Lymphoblastic Leukemic Cells with T (Thymus-Derived) Lymphocyte Markers

**Abstract.** Five of nine children with acute lymphoblastic leukemia had lymphoblasts that bound sheep erythrocytes or reacted with antiserum to thymocytes, suggesting involvement of T (thymus-derived) cells. When lymphoblasts from all patients were examined by immunofluorescence they were found to lack a marker for B (bone marrow or bursa-equivalent) cells, that is, the presence of surface immunoglobulins.

A heterogeneous population of lymphocytes are present in the peripheral blood. Bursa-equivalent or bone marrow-derived lymphocytes (B cells) are characterized by surface immunoglobulins and receptors for a complement component (C3) and aggregated immunoglobulin (1-3). Human T (thymus-derived) lymphocytes lack the above receptors but bind washed sheep red blood cells (SRBC's) in a rosette distribution (3, 4). Using these markers, other investigators have studied the lymphocytes of human leukemia. Chronic lymphatic leukemia (CLL) represents, in most cases, a monoclonal proliferation of B lymphocytes (5, 6). Burkitt's lymphoma of both African (7) and non-African (8) types often arises in B lymphocytes. Similarly, some leukemias and lymphomas could be expected to be of T cell origin. The cells of a patient with malignant lymphoma that appeared to originate in the thymus were of T origin, as shown by the SRBC rosette technique (9). Additionally, a cell line comprised of T lymphoid cells was established in continuous culture from a patient with acute lymphatic leukemia (10).

In order to study the possibility that acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, involves T cells, we studied lymphoblasts directly after removal from

peripheral blood. We now report that lymphoblasts from some of these children form rosettes with SRBC's and react with antiserum to thymocytes and thus carry membrane markers for T cells.

Patients with ALL or CLL confirmed by biopsies of bone marrow, and normal persons as controls, were included in the study. X-ray evaluation of the mediastinum was performed on all patients with ALL. Chronic lymphatic leukemia patients were adults with significant lymphocytosis (more than 30,000 lymphocytes per cubic millimeter). At the time the children with ALL were studied, they were not on treatment and had a significant percentage of lymphoblasts (46 to 99 percent) in the peripheral blood. Peripheral blood was separated on a Ficoll-Hypaque gradient.

Table 1. T and B surface markers from peripheral blood lymphoid cells from normal controls and from patients with leukemia. Numbers in parentheses indicate the number of individuals tested.

Patients	T cells		B cells
	Rosette-forming cells	Thymocyte antigen (% cytotoxicity)	Surface immunoglobulins* (%)
Normal controls	50-78% (60) (1100-2800/mm <sup>3</sup> )	50-70 (13)	14-33 (10)
Chronic lymphatic leukemia	1-6% (3) (1200-2700/mm <sup>3</sup> )	0-5 (6)	0-99 (12)
Acute lymphoblastic leukemia	1-68% (9) (660-151,500/mm <sup>3</sup> )	3-69 (4)	1-24 (7)

\* Determined by immunofluorescence.

Table 2. Clinical data and lymphoid surface markers from patients with acute lymphoblastic leukemia; N.T., not tested.

Patient	Sex and age	Thymic enlargement (by x-ray)	Peripheral blood				
			Lymphoblasts	Lymphocytes	Rosette-forming cells (%)	Thymocyte antigen (%)	Surface immunoglobulins (%)
1. P.F.	Male, 4 years	No	79% (189,600/mm <sup>3</sup> )	14% (33,600/mm <sup>3</sup> )	68	69	8
2. J.J.	Male, 5 months	No	99% (216,800/mm <sup>3</sup> )	1% (2,200/mm <sup>3</sup> )	34	N.T.	N.T.
3. E.B.	Male, 16 years	Yes	85% (134,300/mm <sup>3</sup> )	14% (20,600/mm <sup>3</sup> )	50	42	4
4. C.T.	Male, 14 years	No	46% (1,900/mm <sup>3</sup> )	24% (1,000/mm <sup>3</sup> )	28	N.T.	N.T.
5. D.P.	Male, 12 years	No	90% (29,600/mm <sup>3</sup> )	8% (2,600/mm <sup>3</sup> )	3	64	4
6. C.L.	Male, 17 years	No	94% (261,300/mm <sup>3</sup> )	1% (2,800/mm <sup>3</sup> )	1	N.T.	1
7. S.O.	Female, 14 years	No	62% (4,300/mm <sup>3</sup> )	33% (2,300/mm <sup>3</sup> )	30	N.T.	24
8. C.B.	Female, 16 years	No	52% (1,600/mm <sup>3</sup> )	48% (1,400/mm <sup>3</sup> )	37	N.T.	12
9. R.S.	Female, 5 years	No	90% (4,680/mm <sup>3</sup> )	5% (260/mm <sup>3</sup> )	6	3	17

origin. In children with ALL, 1 to 68 percent of peripheral lymphoid cells bound SRBC's. Also, significant numbers of lymphoblasts formed rosettes with SRBC's in four patients with ALL; in these four children (patients 1 to 4 in Table 2) morphologic evidence of binding of SRBC's to lymphoblasts was found. Further evidence of binding of SRBC's to lymphoblasts is indicated in these four patients in whom the total number of cells binding SRBC's was greater than the number of mature lymphocytes contaminating the preparations (Table 2)—for example, patient 1 had 14 percent lymphocytes and 68 percent cells binding SRBC's. The remaining five patients (patients 5 to 9) had fewer cells binding SRBC's than mature lymphocytes; also in these individuals no morphological evidence of SRBC's binding to lymphoblasts could be detected.

Results of cytotoxicity testing, obtained with the use of antiserum to thymocytes, gave 50 to 70 percent cells positive for thymocyte antigen in peripheral blood of 13 normal individuals and 0 to 5 percent positive cells in patients with CLL (Table 1). These results are similar to those of the assay for cells binding SRBC's. Peripheral blood lymphocytes from four individuals with ALL were tested with antiserum to thymocytes. In three, results were similar to those obtained with the rosette assay, showing significant lymphoblast cytotoxicity in patients 1 and 3 and absence of cytotoxicity in patient 9. The fourth individual, patient 5, although not demonstrating rosette-forming lymphoblasts, was found to have 64 percent cells positive for thymocyte antigen. These data suggest that lymphoblasts may carry one or both of the surface markers characteristic of T cells.

Cells bearing surface immunoglobulins (B cells) of ten normal individu-

als gave a mean of 21 percent (range, 14 to 33 percent) (Table 1). In patients with CLL the range was from 0 to 99 percent; most patients had more than 60 percent. Seven patients with ALL gave values ranging from 1 to 24 percent (Table 2). Values of 8, 4, and 4 percent were found in individuals 1, 3, and 5, respectively, all of which appeared to involve T cells by the previous tests. The higher values for cells bearing surface immunoglobulins, 24, 12, and 17 percent for individuals 7, 8, and 9, respectively, were found in individuals with higher percentages of contaminating lymphocytes in peripheral blood. Thus, as reported by others, we were unable to detect significant surface immunoglobulins in lymphoblasts of patients with ALL (12).

The data presented here indicate that some cases of ALL of childhood involve proliferation of cells with surface characteristics of T cells. The tests employed in our study do not permit us to distinguish whether the ALL's, involving cells with T markers, originate in thymus, bone marrow, or elsewhere. Origin within the thymus is suggested in the one patient who had thymic enlargement. Absence of thymic enlargement in remaining cases suggests origin in T lymphocytes of bone marrow or extramedullary origin; thymic origin with early peripheralization is an alternative explanation which cannot be ruled out, however.

In four cases of ALL we were unable to detect lymphoblasts with T or B cell markers. These data suggest that some ALL's originate in undifferentiated cells or that dedifferentiation may frequently be associated with malignant transformation of lymphoid cells, or both. The latter possibility is suggested by the observation that even in leukemias with the presence of T cell markers, some lym-

phoblasts carried T markers while others did not.

Of interest concerning possible T and B cell origin of human leukemias are studies which indicate that, in mice, virus- or radiation-induced leukemias often involve T cells while chemically induced leukemias often involve B cells (13, 14).

*Note added in proof:* Since submission of this report additional cases of ALL with T cell markers have been reported (15).

JOHN H. KERSEY

ANDREJ SABAD

KAZIMIERA GAJL-PECZALSKA

HELEN M. HALLGREN

EDMOND J. YUNIS, MARK E. NESBIT

*Departments of Laboratory Medicine and Pathology, and*

*Pediatrics, University of Minnesota, Minneapolis 55455*

#### References and Notes

1. C. Bianco, R. Patrick, V. Nussenzweig, *J. Exp. Med.* **132**, 702 (1970).
2. H. B. Dickler and H. Kunkel, *ibid.* **136**, 191 (1972).
3. M. Jondal, G. Holm, H. Wigzell, *ibid.*, p. 207.
4. M. Papamichail, E. J. Holborow, H. I. Keith, H. L. F. Currey, *Lancet* **1972-II**, 64 (1972).
5. H. M. Grey, E. Rabellino, B. Pirofsky, *J. Clin. Invest.* **50**, 2368 (1971).
6. A. C. Aisenberg and K. J. Bloch, *N. Engl. J. Med.* **287**, 272 (1972).
7. E. Klein, G. Klein, J. S. Nadkarni, H. Wigzell, P. Clifford, *Cancer Res.* **28**, 1300 (1968).
8. K. J. Gajl-Peczalska, J. Kersey, M. Nesbit, R. A. Good, in preparation.
9. J. L. Smith, C. R. Barker, G. P. Clein, R. D. Collins, *Lancet* **1973-I**, 74 (1973).
10. J. Minowada, T. Ohnuma, G. E. Moore, *J. Natl. Cancer Inst.* **46**, 891 (1972).
11. K. J. Gajl-Peczalska, B. H. Park, W. D. Biggar, R. A. Good, *J. Clin. Invest.* **52**, 919 (1973).
12. J. D. Wilson and G. J. V. Nossal, *Lancet* **1971-II**, 788 (1971).
13. H. S. Kaplan, *Cancer Res.* **27**, 1325 (1967).
14. N. Haran-Ghera and A. Peled, *Nature (Lond.)* **241**, 396 (1973).
15. C. Borella and L. Sen, *J. Immunol.* **111**, 1257 (1973); A. Chin, J. Saiki, J. Trujillo, R. Williams, *Clin. Immunol. Immunopathol.* **1**, 499 (1973).
16. We thank Drs. R. A. Good and W. Krivit for continued encouragement and assistance. Supported in part by a contract from the Special Virus Cancer Program (71-2261).

20 August 1973