

Insulin Binding of Acute Lymphocytic Leukemia Cells

By Elaine C. Esber, Donald N. Buell, and Sanford L. Leikin

Because of differences in insulin binding of cultured lymphoid cell lines, T- and B-cell surface receptor and 125 I-insulin binding studies were performed on the bone marrow and peripheral blood leukocytes of 13 children with active acute lymphocytic leukemia. Based on surface receptors, nine patients had null-cell disease and four had T-cell disease. The mean per cent insulin binding of the bone marrow cells from the null-cell patients was $10.0\% \pm 8.1$ and from the T-cell patients was $0.18\% \pm$

0.13 . The mean insulin binding of the cell suspensions of the peripheral blood from the null-cell patients was $7.3\% \pm 7.5$ and $0.07\% \pm 0.06$ from the T-cell patients. Displacement studies with nonradioactive insulin indicated that null leukemic cells bore specific binding sites. These results indicated that there may be metabolic as well as surface membrane heterogeneity among the acute lymphocytic leukemias of childhood.

LYMPHOCYTE surface receptor studies indicate that childhood acute lymphocytic leukemia (ALL) is a heterogeneous disorder.¹⁻³ The majority of leukemic cells in approximately 25% of ALL patients bear surface receptors of the T (thymus-dependent) lymphoid system. In a small percentage of patients, the abnormal cells have surface immunoglobulins and bind IgG aggregates^{4,5} indicating a B (thymus-independent) cell origin. However, the leukemic cells of the majority of patients with ALL lack T- and B-cell receptors. These individuals have been designated as having "null-cell" leukemia. A number of established lymphoid cell lines derived from subjects with a variety of hematopoietic disorders have been examined for insulin binding. These investigations have shown differences in the hormone-binding capacity⁶ of various lymphoid cell lines, and have led us to study the relationship of lymphocyte surface receptors and insulin binding of leukocytes from children with ALL.

MATERIALS AND METHODS

Peripheral blood and/or bone marrow specimens were obtained from 13 ALL patients with active disease. Nine of these were newly diagnosed and previously untreated. Four were in relapse having been treated previously with prednisone, vincristine, L-asparaginase, mercaptopurine, and methotrexate. The per cent pathologic cells (lymphoblasts and other leukemic cells) in their bone marrow specimens ranged from 59% to 99% (mean = 90.2%). The per cent pathologic cells in their peripheral blood ranged from 41% to 95% (mean = 76.2%). In addition, specimens were obtained from four patients with ALL who were undergoing treatment and were in hematologic remission. Their bone marrows contained less than 5% pathologic cells.

The presence of various cell surface receptors was detected on mononuclear cells obtained by Ficoll-Hypaque gradients⁷ as follows.

From the Children's Hospital National Medical Center, Washington, D.C., and the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Submitted December 1, 1975; accepted March 12, 1976.

Supported by Public Health Service Grant Ca-03888 and a grant from the Leukemia Society of America.

Address for reprint requests: Sanford L. Leikin, M.D., Children's Hospital National Medical Center, 2125 13th St. N.W., Washington, D.C. 20009.

© 1976 by Grune & Stratton, Inc.

T-Lymphocyte Markers

*Rosette-forming lymphocytes (RFL).*⁸ Lymphocyte suspensions (1.2×10^6 in 0.4 ml) were incubated with an equal volume of a 0.5% suspension of neuraminidase-treated sheep red blood cells (SRBC) for 15 min at 37°C, centrifuged at 130 g for 10 min, and then incubated for 1 hr at 4°C. The suspensions were then gently agitated. The per cent RFL was obtained by counting at least 200 lymphocytes in a wet preparation and determining those having three or more attached SRBC. Cyto-centrifuge preparations were prepared and stained to identify the rosette-forming cell type.

*Human T-lymphocytic antigen (HTLA).*⁹ The assay was performed by determining the per cent cytotoxicity as detected by the trypan blue dye exclusion method after the mononuclear cell suspension had been exposed to heterologous anti-human thymocyte antiserum in the presence of rabbit complement. This antiserum had been absorbed with cultured human B cells and was demonstrated to be cytotoxic for human T lymphocytes.

B-Lymphocyte Markers

*Surface immunoglobulins (SIg).*¹⁰ Suspensions of 10^6 mononuclear cells were incubated with polyvalent (IgG, IgM, and IgA) fluorescein-conjugated goat anti-immunoglobulin for 30 min at room temperature. The percentage of fluorescent cells was determined by counting at least 200 cells per slide with a Leitz fluorescent microscope.

*Aggregated IgG (F_c) receptors.*¹¹ Cells bearing aggregated human gamma globulin (F_c) receptors were determined by incubating suspensions of mononuclear cells in 2% BSA buffer (10^6 cells in 0.05 μ l) with 0.05- μ l volumes of varying dilutions of fluoresceinated heat-aggregated gamma globulin for 30 min at room temperature. The cells were then washed three times with buffer, and the percentage of fluorescent cells was determined in a manner similar to that used for surface immunoglobulin-bearing cells.

In all of the above studies monocytes were identified by phagocytosis of latex 1.0- μ beads in autologous plasma and excluded from counting.

Insulin Binding

The insulin-binding studies were performed using leukocytes from sedimented or Ficoll-Hypaque separated mononuclear cell suspensions that contained a minimum of 85% lymphoid cells. The binding assay was performed by incubating 2.5×10^7 cells/ml from the buffy coat or Ficoll-Hypaque suspension for 90 min at 15°C in a HEPES buffer with ¹²⁵I-monoiodinated insulin (kindly provided by M. Lesniak and P. DeMeys, Diabetes Branch, NIAMDD, NIH).⁶ At the end of the incubation period, 0.2 ml of the cell suspension was layered over an equal volume of fetal calf serum and centrifuged for 1 min in a Beckman Microfuge (Model 152). The supernatant unbound insulin was aspirated, and the radioactivity in the cell pellet was determined in a Nuclear-Chicago gamma counter. Displacement curve data were generated by adding increasing amounts of unlabeled insulin to replicate tubes of cells plus ¹²⁵I-insulin prior to incubation. The percentage of ¹²⁵I-insulin specifically bound was calculated by subtracting the percentage of ¹²⁵I-insulin bound in the presence of unlabeled insulin (nonspecific) from the percentage bound in the absence of unlabeled insulin (total). Displacement curves using the established cell line IM-9 were generated as a standard with each experiment. These lymphoid cells had B cell characteristics.¹² Statistical analysis was performed by the Student's t test.

RESULTS

The ALL patients with active disease had blood and marrow suspensions that contained greater than 85% (mean 89.6%) and 89% (mean 95.0%) pathologic cells, respectively. The results of the lymphoid surface receptors and insulin-binding studies of the patients with acute lymphocytic leukemia with active disease are shown in Tables 1 and 2. Nine of these 13 patients were considered to have null-cell disease based on the finding of low percentages of RFL's and HTLA, SIg- and F_c -positive cells in the bone marrow suspensions, and the ab-

Table 1. Lymphocyte Surface Receptors and Insulin Binding of Bone Marrow Cells From Acute Lymphocytic Leukemia Patients

	RFL (%)	HTLA (%)	Slg (%)	F _c (%)	Specific Binding (%)
Null cell					
P.K.	1.0	0.0	0.0	—	10.3
C.S.	3.0	0.0	1.0	—	3.3
K.D.	0.5	0.0	0.5	0.5	29.0
H.B.	3.0	0.0	1.0	—	12.3
M.P.	5.0	5.0	1.5	1.0	12.2
L.T.	4.1	0.0	0.5	0.5	5.9
K.B.	0.0	0.0	1.0	0.0	1.4
K.A.	1.0	0.0	1.0	—	5.9
C.L.	1.0	0.0	1.0	—	9.8
Mean					10.0 ± 8.1
T cell					
G.S.	56.0	97.8	0.5	0.5	0.2
N.J.	91.5	98.1	0.0	—	0.2
K.M.	18.0	71.4	0.4	—	0.3
J.A.	78.0	94.7	0.0	—	0.0
Mean					0.18 ± 0.13

sence of pathologic cells with rosette formation in cytocentrifuged preparations of their bone marrows. The peripheral blood suspensions of three of the nine patients with null-cell leukemia contained more than 10% RFL-positive cells, and one had 7% Slg-positive cells. These probably represented residual normal T and B peripheral blood lymphocytes, respectively.⁵ Four patients were categorized as having T-cell leukemia based on the finding of increased percentages of RFL-positive and HTLA-positive bone marrow cells and the identification in cytocentrifuge preparations of spontaneous rosette-forming pathologic cells in their bone marrow cell suspensions.

The mean percentage specific insulin binding of the IM-9 line on 18 different determinations was 19.0% ± 8.54%. The specific binding of the peripheral blood cells from six normal subjects was 0.55% ± 0.30%. These results were

Table 2. Lymphocyte Surface Receptors and Insulin Binding of Peripheral Blood From Acute Lymphocytic Leukemia Patients

	RFL (%)	HTLA (%)	Slg (%)	F _c (%)	Specific Binding (%)
Null cell					
C.S.	1.5	7.5	1.5	—	2.6
K.D.	0.5	0.0	0.5	0.0	19.5
H.B.	20.0	5.7	2.0	—	9.4
L.T.	10.3	0.0	3.0	0.5	3.7
K.B.	23.5	0.0	7.0	4.0	1.4
Mean					7.32 ± 7.5
T cell					
N.J.	47.5	90.2	11.5	—	0.1
K.M.	10.5	72.1	1.5	—	0.0
J.A.	85.0	97.3	0.0	—	0.1
Mean					0.07 ± 0.06

lower than those reported by another group of investigators,¹³ probably due to the different cell concentration we employed.

The mean per cent insulin binding of the cell suspensions from the bone marrow of nine patients with null-cell disease was $10.0\% \pm 8.1\%$. In contrast, the mean per cent insulin binding of the bone marrow of four patients with T-cell disease was $0.18\% \pm 0.13\%$. The bone marrow suspension from one patient with T-cell disease demonstrated no binding. The mean specific binding of insulin to cells from the null-cell and T-cell types of ALL patients were significantly different ($p < 0.005$). The mean per cent binding found in the cell suspensions from the bone marrow of four patients with null-cell disease who were in hematologic remission was $2.1\% \pm 0.21\%$. This mean was significantly less than that of patients with florid null-cell leukemia ($p < 0.025$).

The mean per cent insulin binding of the cell suspensions from the peripheral blood of the null-cell patients was $7.32\% \pm 7.5\%$. In contrast, the mean per cent insulin binding of the cell suspensions from the blood of three patients with T-cell disease was $0.07\% \pm 0.06\%$. The peripheral blood suspensions from one of the T-cell patients demonstrated no binding. The difference between the means of the null-cell and T-cell ALL patients was significant ($p < 0.05$).

Figure 1 shows representative displacement curves that were generated when increasing concentrations of unlabeled insulin were added to replicate suspensions of bone marrow cells from the children with null-cell and T-cell ALL. Increasing concentrations of unlabeled insulin reversed the binding in the suspensions of the null-cell patients but not of the T-cell patients.

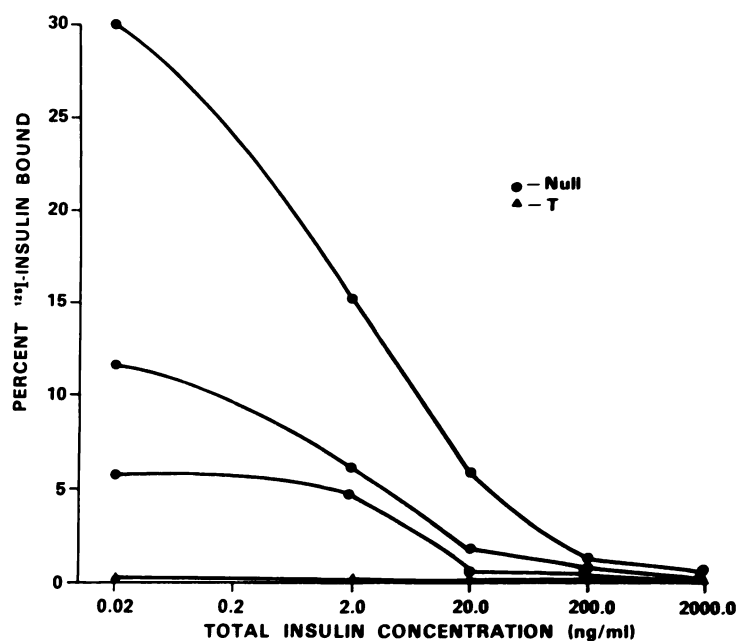


Fig. 1. Representative displacement curves generated in replicate bone marrow suspensions from null-cell and T-cell ALL patients.

DISCUSSION

Analyses of lymphocyte surface receptors on ALL cells have indicated that the leukemic cells in this disease can be divided into several groups.¹⁻⁵ Our results confirmed these reports. The pathologic cells from the bone marrow of a majority of our patients lacked T- and B-lymphocyte receptors. The pathologic cells in bone marrow from a minority of the patients bore receptors characteristic of T-cell leukemia. Some divergence was present between the results of RFL and HTLA studies in the peripheral blood suspension of N.J. and the peripheral blood and bone marrow suspensions of K.M. (see Tables 1 and 2). Lack of correlation between these two markers has been observed by other investigators.¹ It is possible that the antiserum is detecting malignant cells derived from T lymphocytes very early in differentiation, i.e., cells that may not yet have developed sheep erythrocyte receptors. It is also conceivable that malignant cells retain HTLA but lose sheep erythrocyte receptors. It is not currently possible to distinguish between these two alternatives.

In addition, our results indicate that leukemic cells from patients with active null-cell disease but not those from patients with T-cell leukemia bind significant amounts of radioactive insulin. The reversibility of the binding when unlabeled radioactive insulin is added is strong evidence that these null cells bear specific binding sites on their surfaces. That it is specifically the leukemic cells which bear these receptors is supported by the finding of lower mean per cent binding in bone marrow suspensions from patients in remission than from those with active disease. The possibility that the differences in insulin binding between the different types of abnormal cells are due to binding by monocytes¹³ (see below) appears unlikely because none of the patients' bone marrow suspensions contained more than 1% monocytes and in only two of their peripheral blood suspensions have more than 1% monocytes been found. The lack of insulin binding in the cells of patients with T-cell leukemia is consistent with results obtained using several human cultured T-cell lines.¹⁴ Although the most likely explanation for the decreased binding in these cells is an absence of the specific receptor, it is also possible that these receptors are "buried" in the membrane and therefore inaccessible, or that they are shed rapidly and lost in the medium. There is a wide range of per cent specific binding in the bone marrows from the patients with null-cell leukemia. Similar observations have been made between different cultured lymphoid cell lines.¹⁴ This variability could be due to technical factors, since day to day differences in per cent binding were also noted in the IM-9 line used as a positive control. However, it is also possible that heterogeneity also exists between the leukemic cells from these individuals.

Insulin receptors are found in liver and adipose tissue as well as in peripheral blood leukocytes and lymphoid cell lines. Their exact role in cellular metabolism has not yet been determined. It has been suggested that these binding sites are important in the regulation of intracellular carbohydrate and lipid metabolism in adipose tissue. The finding in patients with insulin-resistant diabetes of serum antibodies that reduce insulin binding to specific receptors on their circulating monocytes¹⁵ does, however, suggest that these sites play a role in hormonal-cellular interactions.

The pathophysiologic significance of insulin binding sites on leukemia cells is unknown. Insulin stimulates glucose¹⁶ and alpha-aminoisobutyric acid uptake in normal rat thymocytes¹⁷ and has also been reported to cause increased membrane ATPase and glucose uptake in the human lymphoid line, 1788.¹⁸ It is possible, therefore, that the clinical differences^{19,20} seen between these various forms of ALL may be related to these observed metabolic differences. It is notable, therefore, that of the ten patients in this series who have been followed for more than 1 yr, only one of six null-cell patients, but three of four T-cell patients have relapsed. Additional observations and follow-up will be required, however, to determine the prognostic implications of these findings.

The report that monocytes rather than lymphocytes are the insulin-binding cells in normal human peripheral blood mononuclear leukocyte preparations¹⁰ raises the question of whether the leukemic null cells are of monocytic rather than lymphocytic origin. However, the lack of Fc receptors, as determined by fluorescent-labeled aggregated gamma globulin, and preliminary studies of the lack of null-cell content of nonspecific esterase, myeloperoxidase, and particle phagocytosis, characteristic of monocytes, do not support this hypothesis.

The results of this study provide additional evidence of the heterogeneity of childhood ALL. Binding of ¹²⁵I-insulin provides an additional marker with which to characterize this heterogeneity. Further investigations of the surface membrane and metabolic characteristics of leukocytes from such patients may provide a better understanding of the nature of this disease.

REFERENCES

1. Kersey JH, Gajl-Peczalska KJ, Nesbit ME: The lymphoid system: Abnormalities in immunodeficiency and malignancy. *J Pediatr* 84:789-796, 1974
2. Borella L, Sen L: T and B lymphocytes and lymphoblasts in untreated acute lymphocytic leukemia. *Cancer* 34:646-651, 1974
3. Esber E, DiNicola W, Movassaghi N, Leikin S: Lymphocyte surface receptors in acute lymphocytic leukemia. *Pediatrics* 56:788-792, 1975
4. Flandrin G, Brouet JC, Daniel MT, Preud'homme JL: Acute leukemia with Burkitt's tumor cells: A study of six cases with special reference to lymphocyte surface markers. *Blood* 45:183-188, 1975
5. Davey FR, Gottlieb AJ: Lymphocyte surface markers in acute lymphocytic leukemia. *Am J Clin Pathol* 62:818-822, 1974
6. Gavin JR III, Gordon P, Roth J, Archer J, Buell D: Characteristics of the human lymphocyte insulin receptor. *J Biol Chem* 248:2202-2207, 1973
7. Boyum A: Separation of leukocytes from blood and bone marrow. Introduction. *Scand J Clin Lab Invest* 21 (Suppl 97):7, 1968
8. Weiner MS, Bianco C, Nussenzweig V: Enhanced binding of neuraminidase-treated sheep erythrocytes to human T lymphocytes. *Blood* 42:939-946, 1973
9. Smith R, Terry W, Buell D, Sell K: An antigenic marker for human thymic lymphocytes. *J Immunol* 110:884, 1973
10. Pernis B, Ferrarini M, Forni L, Amante L: Immunoglobulins on lymphocyte membranes, in Amos B (ed): *Progress in Immunology*. New York, Academic, 1971, p 95
11. Dickler HB, Kunkel HG: Interaction of aggregated gamma globulin with B lymphocytes. *J Exp Med* 136:191-196, 1972
12. Fahey JL, Buell DN, Sox HC: Proliferation and differentiation of lymphoid cells: Studies with human lymphoid cell lines and immunoglobulin synthesis. *Ann NY Acad Sci* 190:221-234, 1971
13. Schwartz RA, Bianco AR, Handwerker BS, Kahn CR: Demonstration that monocytes rather than lymphocytes are the insulin binding cells in preparations of human peripheral blood mononuclear leukocytes: Implications for studies of insulin-resistant states in man. *Proc Natl Acad Sci USA* 72:474-478, 1975
14. Gavin J, Buell D: Insulin and growth hormone receptors on human lymphoid cell lines. (Manuscript in preparation)
15. Flier J, Kahn R, Roth J, Bar R: Anti-

bodies that impair insulin receptor binding in an unusual diabetic syndrome with severe insulin resistance. *Science* 190:63-65, 1975

16. Boyett JD, Hofert JF: Stimulatory effect of insulin on glucose metabolism of thymus lymphocytes. *Horm Metab Res* 4:163-167, 1972

17. Goldfine ID, Gardner JD, Neville DM Jr: Insulin action on isolated rat thymocytes. I. Binding of 125 I-insulin and stimulation of aminoisobutyric acid transport. *J Biol Chem* 247:6919-6926, 1972

18. Hadden JW, Hadden EM, Wilson EE,

Good RA, Caffey RG: Direct action of insulin on plasma membrane ATPase activity in human lymphocytes. *Nature (New Biol)* 235:174-177, 1972

19. Ravindranath Y, Kaplan J, Zuelzer WW: Significance of mediastinal mass in acute lymphoblastic leukemia. *Pediatrics* 55:889-893, 1975

20. Sen L, Borella L: Clinical importance of lymphoblasts with T markers in childhood acute leukemia. *N Engl J Med* 292:828-832, 1975