The Nature of Childhood Leukemia and Lymphoma

J. H. Kersey, MD, K. J. Gajl-Peczalska, MD, PhD, P. F. Coccia, MD, and M. E. Nesbit, MD

One hundred consecutive newly diagnosed cases of leukemia and lymphoma in children from 0 to 16 years of age presenting at the University of Minnesota from 1973 to 1977 were studied. Clinical features were correlated with phenotypic features of blast cells, including surface markers and cytomorphology. Four groups with distinct clinical and pathologic features emerged from the study: a) The acute leukemias of the "null" or "undifferentiated" group were those in which the malignant cells carried distinctive null leukemia surface antigen and lacked features of either T cells (E-rosette positivity) or B cells (surface immunoglobulin positivity). These cases occurred most frequently in the series (56% of total cases), peaked in incidence at 6 years, were associated with extensive bone marrow involvement, lacked distinguishing cytomorphologic features, and had the best response to therapy of all groups. b) The acute myelogenous leukemias, including those with myeloid, monocytoid, or erythroid features or a combination of the above, had extensive bone marrow involvement and the characteristic morphology. This group was seen with intermediate frequency and showed an intermediate response to therapy. c) Leukemia–lymphomas of the T-cell group were frequently associated with mediastinal masses and other masses, a cytomorphology which was different from the B-cell group but similar to the null group, and high white cell counts. These cases occurred with intermediate frequency (14%) and had a worse prognosis than the null group. d) Leukemia–lymphomas of the B-cell group had monoclonal surface immunoglobulin with μ-heavy and either κ or λ light chain. These patients were least frequent in the series, frequently presented with abdominal masses, and had a characteristic Burkitt cell morphology. Prognosis was the worst of all patients in our series. These data suggest that the major phenotypic groups of childhood leukemia and lymphoma have differing prognoses and should receive differing forms of therapy. Clinical and pathologic features of each group are sufficiently distinctive to suggest that they may have different causes. (Am J Pathol 90:487–496, 1978)

HUMAN HEMATOPOIETIC NEOPLASMS have been the subject of intensive study in recent years. However, the nature of the cells involved, combined with methodologic limitations, has resulted in conclusions that were frequently nonreproducible or without strong scientific basis. Several problems are inherent in such studies. For example, the lymphocyte is perhaps unique in human biology and pathology because of the remarkable alterations in appearance and metabolic activity that result when lymphocyte activation occurs following physiologic stimulation with antigen.[1] The activated lymphocyte has many of the metabolic and morpho-

From the Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota Medical School—Minneapolis, Minneapolis, Minnesota.

Supported in part by Grants HL-07145, CA-15548, CA-07306, CP-16228, CA-18083, and CA-17034 from the US Public Health Service and Grant 340 from the American Cancer Society.

Accepted for publication October 6, 1977.

Address reprint requests to Dr. J. H. Kersey, Box 609 Mayo, University of Minnesota Medical School—Minneapolis, Minneapolis, MN 55455.

logic features generally observed in malignant cells. Because of the dramatic changes in activated lymphocytes, it is not surprising that a number of traps exist for individuals studying lymphoproliferative diseases. An example of problems inherent in the purely morphologic approach is derived from the studies of Barr et al,[2] which suggest that multipotential stem cells have morphologic features identical to small lymphocytes. A new approach to the analysis of lymphoid malignancies was suggested by observations indicating that subsets of normal lymphocytes bear specific membrane surface markers.[3,4] In recent years a significant body of evidence has been collected indicating that the surface phenotypes of normal lymphocytes are frequently retained in malignancies of lymphoid cells.[5] This fact, combined with the observation that most human leukemias and lymphomas are monoclonal, ie, derived from a single lymphoid clone,[6] has resulted in the ability to readily determine the membrane phenotype of most lymphomas and acute leukemias. Childhood hematopoietic malignancies as studied in our institution by combined clinical, immunologic, and morphologic analyses is the subject of this report.

Materials and Methods

Analysis of a Series of Childhood Leukemia-Lymphomas Using Multiple Parameters

The material presented represents the experience in immunopathologic characterization of childhood leukemias at the University of Minnesota from 1973 to July 1977. For the past 3 years, it has been routine for our laboratory to perform surface marker evaluations on all new patients between the ages of 0 and 16 years with leukemia and lymphoma at the time of hospital admission. Studies reported here include in almost all instances patients who were studied at diagnosis and prior to therapy. Bone marrow was examined in patients with leukemia, and lymph nodes were usually examined in patients with lymphoma. Excluded were tissues that were found to have less than 50% of cells that were morphologically malignant. In the Minnesota series there were 100 evaluable children, including 86 with acute leukemia and 14 with lymphoma. The distinction between leukemia and lymphoma was based on the percentage of blasts in the marrow: greater than 25% indicates leukemia. In all instances, tumor cell suspensions were made for study. In many cases, results of T- and B-cell marker analysis of suspensions were confirmed using frozen section material. In all cases the tumor material was evaluated for a) the presence of receptors for sheep red cells (E) using rosettes according to a previously described method[7] and b) surface immunoglobulin (SIg) using fluorescein-conjugated polyvalent and monospecific antiserums as previously described.[8] Monospecific antiserums were specific for μ, γ, and α heavy chains and κ and λ light chains. In cases in which it appeared possible that membrane immunoglobulin was absorbed, the material was cultured overnight and restudied the following day. Frequently, complement receptors were studied using chicken red cells (cE) as a morphologic marker, rabbit antibody (A), and mouse serum as a complement (C) source. In many cases, mixed rosette studies were utilized to determine cells carrying sheep cells and complement receptors utilizing our previous method.[9] Fc receptors were studied using cEA rosettes and also by using aggregated immunoglobulin. Human T-lymphocyte antigen (HTLA) was studied using antithymocyte serum which was absorbed extensively with red cells and cells from patients with chronic lymphatic leuke-

mia of B-cell origin.[7] "Null" antiserums were produced by immunization of goats with non-T, non-B leukemic cells followed by extensive absorption with cells from patients with acute myelogenous leukemia, T-cell leukemia, B-cell chronic lymphocytic leukemia (CLL), and B-lymphoblastic cell lines. The absorbed antiserum was nonreactive with the absorbing cells and with peripheral blood lymphocytes in a complement-mediated cytotoxic assay.[10]

To be considered as positive in rosette assays, greater than 50% of morphologically malignant cells had to be positive; confirmation of positivity of malignant cells was obtained using stained cytocentrifuge preparations. Results in the SIg assay were considered positive if more than 70% of B lymphocytes (by polyvalent antiserums) carried one heavy and one light chain.

Results

Data from 100 cases of childhood lymphoid leukemias and lymphomas in Minnesota from 1973 to 1977 are presented in Table 1. Four distinct groups emerged in the analysis: a) the null, undifferentiated, non-T, non-B group; b) the acute myelogenous group (including erythroid and monocytoid leukemias); c) the T-cell or thymocyte-type group; and d) the B-cell group. Each of these groups had distinctive clinical and morphologic features.

The first group represents patients with malignancies that are E-rosette negative and SIg negative and thus termed null or undifferentiated. This group contained the greatest number of patients in our group, 56 (56% of the total), and also responded to therapy the best of all four groups. These cases were almost always associated with early and extensive bone marrow involvement, a median age of 6 years, no sex predominance, and a tumor cell morphology which, in our experience, was indistinguishable from the E-rosette–positive group.[11] There was generally, but not always, an absence of mediastinal masses. Three patients with Ph^1-positive leukemia, including one with chronic myelogenous leukemia in blast crisis, were observed in this group. Fifteen of these patients were studied using the null antiserums, and all but 2 were positive in the cytotoxicity assay.

The second group contained 21 patients with acute myelogenous, erythroid, or monocytic leukemia. This group tended to be associated with extensive bone marrow involvement, no sex predominance, a median age of 9, the absence of cell surface markers, and a characteristic morphology and cytochemistry. Chromosomal abnormalities were frequently seen in this group, and response to therapy was intermediate in comparison with the other groups.

The third group, that in which the malignant cells were E-rosette–positive and hence of T-cell or thymocyte type, contained 14 patients (14% of the total). This group was associated with a predominance of males, and the median age was 12 years. Nodal and mediastinal masses were frequently seen. The morphology of these malignant cells was

Table 1—Major Phenotypic Groups of Childhood Leukemias and Lymphomas (Minnesota Series, N = 100)

	Null, undifferentiated, non-T, non-B	Acute myelogenous (including erythroid and monocytoid)	T cell or thymocyte type	B cell
Frequency	56/100 (most frequent)	21/100 (intermediate)	14/100 (intermediate)	9/100 (least frequent)
Suspected origin	Bone marrow	Bone marrow	Thymus or lymph node	Germinal centers of lymph nodes & Peyer's patches
Involvement				
Bone marrow	++++	++++	+	+
Blood	+	++	++++	++
Lymphatics (presence of masses)	+	+	++++	++++
Age	Younger (median = 6)	Intermediate (median = 9)	Older (median = 12)	Older (median = 11)
Male-Female Ratio	Equal	Equal	Male predominance	Male predominance (7/9)
Cytology				
Morphology	Not distinctive, lympho-blastic	Myeloid, erythroid	Not distinctive	Frequently Burkitt cell
Chemistry	Not distinctive	Peroxidase-positive	Frequently acid phosphatase+	Lipid stains positive
Surface markers	Null antigen-positive	None	E-rosette-positive, human thymus antigen (HTLA)-positive	SIg-positive (monoclonal IgM)
Chromosomal abnormality	Some (Ph^1)	Frequent	None described	Some ($14q^+$ $8q^-$)
Response to therapy	Best	Intermediate	Intermediate	Worst

different from the SIg-positive (Burkitt's) group but was similar to the E-rosette–negative group.[11] E-rosette–positive disease have been variously called Sternberg's sarcoma, poorly differentiated lymphoblastic lymphoma, and acute lymphoblastic leukemia. Similar clinical features of this group have been described by others.[12,13]

The fourth group contained 9 patients in whom malignant cells were SIg-positive and therefore of the B-cell phenotype. In all cases the surface immunoglobulin appeared to be monoclonal, always with μ heavy and either κ or λ light chain. This group was associated with male predominance and a median age of 11, ie, older than the null, undifferentiated group. These patients presented with abdominal primaries and a morphologically characteristic Burkitt cell, ie, a cell with large nucleus, prominent nucleoli, basophilic cytoplasm, and large lipid-containing cytoplasmic vacuoles. Similar clinical characteristics of this group of B-cell or Burkitt leukemia–lymphoma patients were also described by other groups.[14,15]

In the 100 patients of this study we saw no instances in which the malignancy did not belong in one of the four groups and no instances in which the malignancy had features of more than one group. For example, we saw no instance of E-rosette–positive and SIg-positive disease or null antigen-positive and E-positive or SIg-positive disease.

Childhood lymphoid malignancies have sometimes been divided into lymphomas (ie, patients with masses and little or no marrow involvement) and leukemias (ie, those with extensive marrow involvement). With one exception, all (55/56) of the null group, ie, the E-rosette–negative, SIg-negative patients had the characteristics of leukemia. The B-cell-Burkitt–SIg–positive group presented with masses in all instances, although extensive bone marrow involvement was sometimes seen. The E-rosette–positive group presented with varying combinations of peripheral blood, nodal, mediastinal, and bone marrow involvement. White counts were generally higher and the degree of marrow involvement was generally less than those of the null group.

In the Minnesota series, an extensive analysis of survival of children with malignant lymphoma and leukemia was performed, and results were published.[11] In general, the children with the lymphomas and leukemias were treated similarly, using multiagent chemotherapy with vincristine, prednisone, L-asparaginase, and methotrexate according to Childrens' Cancer Study Group protocols. In the T-cell group and the acute myelogenous leukemia group, survival was intermediate between that of the B-cell-Burkitt–SIg–positive group and the null group. Survival in the null subgroup was generally good, with 85% survival at 30 months. B-cell-

Burkitt lymphoma patients had very poor survival, with all patients dead at 6 months. Patients with E-rosette–positive (T-cell) leukemia–lymphoma had an intermediate prognosis. Similar results indicating best survival for the null group, worst survival for the Burkitt's group, and intermediate survival for the T-cell group have been reported by others.[16-18]

We next examined the T-cell malignancies in further detail to evaluate the significance of variables which might affect prognosis. Patients with E-rosette–positive leukemia tended to have high white cell counts, although the count in 3 of 10 patients was less than 50,000/cu mm. Patients with E-rosette–positive malignancies also tended to have mediastinal masses, although 4 of 10 did not. There is a suggestion that survival might correlate somewhat with white cell count, although 1 patient with a count of 31,000/cu mm is dead at 13 months, and, conversely, 1 patient with 475,000/cu mm is alive at 14 months. Thus, larger numbers of patients are necessary to determine whether white cell count and rosette positivity are useful as independent variables in determining prognosis. Of the 4 patients with E-rosette–positive lymphomas, ie, those with masses but no bone marrow involvement, 2 had primaries in the skin, 1 had an apparent primary in the mediastinum and 1 had no evident primary site.

Studies Using Xenoantiserums

It is likely that development of antiserums that are specific for each of the major phenotypic subgroups of leukemia and lymphoma will be useful for a variety of purposes, including diagnosis, monitoring, and etiologic and therapeutic studies. This is especially true for the null leukemias in which no other surface markers are positive and for which the cellular origin is not known. As noted earlier, we have produced antinull leukemia serums that are used in complement-dependent cytotoxicity assays. The Minnesota null antiserums have not reacted with any acute myelogenous leukemias, acute T leukemias, acute B leukemias, lymphoblastoid cell lines or PHA-stimulated lymphoblasts. The antiserums gave positive results in 13 of 15 cases of null leukemia in the cytotoxicity assay; the nature of the 2 nonreactive cases remains to be defined. Null antiserums with apparently the same specificity have been described by others.[19] Other laboratories have developed xenoantiserums with apparent specificity for null leukemia using immunofluorescent binding assays.[16,20] Currently, the nature of the null antigen(s) detected by these various antiserums remains unclear; they are probably either differentiation antigens or leukemia-specific antigens. A normal cellular subset bearing these null antigens has not been found.

Ia-Like Antigens

We and others have found that acute leukemias and lymphomas frequently bear antigens also found in normal B lymphocytes, granulocytes, and monocytes but not in T lymphocytes.[21-24] These antigens are termed B cell or Ia (immune associated) because of their occurrence on B cells and their similarity to murine antigens located in the major histocompatibility complex and are felt to be related to genes associated with immune function. The presence of these Ia-like antigens has been detected using both xeno-antiserums and alloantiserums. Because of the widespread distribution of these Ia-like antigens on normal cells, their presence on leukemia cells does not provide a clear picture of the cellular origin other than to suggest a non-T origin in those cases that are positive.

Conclusion

Our studies suggest that childhood lymphoid malignancies can be divided into four major groups, each with distinct clinical and prognostic features. In the first group, malignant cells lack features of B or T lymphocytes but bear a characteristic null antigen; this comprises the largest proportion of cases of acute leukemia of childhood and has the best prognosis. The cellular origin of this group of leukemias is now known. The second group includes myeloid malignancies, including acute myelogenous, erythroid, and monocytoid leukemias. This comprises approximately one fifth of all the childhood leukemias, has typical cytomorphologic features, and has an intermediate prognosis. The third group is that in which the malignant cells bear the T phenotype. This group has characteristic clinical features and an intermediate prognosis. The last group, that in which the malignant cells are of the B-cell phenotype are surface-immunoglobulin (μ heavy chain)–positive. This is the least frequent in our series, is identical to Burkitt lymphoma, and has the worst prognosis.

In conclusion, surface marker analysis has provided clues that are useful for understanding human lymphoid malignancies. We hope that further evaluation of childhood leukemias and lymphomas using immunologic methods will provide useful data that will assist in the prevention and treatment of these frequent malignancies.

References

1. Greaves MF, Owen JJT, Raff MC: T and B lymphocytes: Origins, Properties, and Roles in Immune Responses. New York, American Elsevier Publishing Co., Inc., 1973
2. Barr RD, Whang-Peng J, Perry S: Hemopoietic stem cells in human peripheral blood. Science 190:284–285, 1975

3. Möller G (ed): T and B lymphocytes in humans. Transplant Rev 16:1–217, 1973
4. Kersey JH, Gajl-Peczalska KJ: T and B lymphocytes in humans: A review. Am J Pathol 81:446–458, 1975
5. Thierfelder S, Rodt H, Thiel E (editors): Haematology and blood transfusion. Immunological Diagnosis of Leukemias and Lymphomas, Vol 20. New York, Springer-Verlag Inc., 1977
6. Fialkow PJ: Glucose-6-phosphate dehydrogenase (G-6-PD) markers in Burkitt lymphoma and other malignancies.[5] pp 297–305
7. Kersey JH, Nesbit ME, Hallgren H, Sabad A, Yunis EJ, Gajl-Peczalska KJ: Evidence for origin of certain childhood acute lymphoblastic leukemias and lymphomas and thymus-derived lymphocytes. Cancer 36:1348–1352, 1975
8. McKenna RW, Parkin J, Kersey JH, Gajl-Peczalska KJ, Peterson LC, Brunning RD: Chronic lymphoproliferative disorder with unusual clinical, morphologic, ultrastructural and membrane surface marker characteristics. Am J Med 62:588–596, 1977
9. Gajl-Peczalska KJ, Chartrand S, Bloomfield CD, Corte J, Coccia PF, Nesbit ME, Kersey JH: Lymphocytes bearing receptors for both sheep erythrocytes and complement in patients with neoplastic and non-neoplastic diseases. Clin Immunol Immunopathol 8:292–299, 1977
10. Corte J, Kersey JH: In preparation
11. Coccia, PF, Kersey JH, Gajl-Peczalska KJ, Krivit W, Nesbit ME: Prognostic significance of surface marker analysis in childhood non-Hodgkin's lymphoproliferative malignancies. Am J Hematol 1:405–417, 1976
12. Sen L, Borella L: Clinical importance of lymphoblasts with T markers in childhood acute leukemia. N Engl J Med 292:828–832, 1975
13. Tsukimoto I, Wong KY, Lampkin BC: Surface markers and prognostic factors in acute lymphoblastic leukemia. N Engl J Med 294:245–248, 1976
14. 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
15. Mann RB, Jaffe ES, Braylan RC, Nanba K, Frank MM, Ziegler JL, Berard CW: Non-endemic Burkitt's lymphoma: A B cell tumor related to germinal centers. N Engl J Med 295:685–691, 1976
16. Greaves MF, Janossy G, Roberts M, Rapson, NT, Ellis RB, Chessels J, Lister TA, Catovsky D: Membrane phenotyping: Diagnosis, monitoring and classification of acute 'lymphoid' leukemias.[5] pp 61–76
17. Borella L, Sen L, Dow LW, Casper JT: Cell differentiation antigens versus tumor-related antigens in childhood acute lymphoblastic leukemia (ALL). Clinical significance of leukemia markers.[5] pp 77–86
18. Belpomme D, Lelarge N, Mathé G, Davies AJS: Etiological, clinical and prognostic significance of the T-B immunological classification of primary acute lymphoid leukemias and non-Hodgkin's lymphomas.[5] pp 33–46
19. Rodt H, Netzel B, Thiel E, Jäger G, Huhn D, Haas R, Götze D, Thierfelder S: Classification of leukemic cells with T- and O-ALL-specific antisera.[5] pp 87–96
20. Minowada J, Tsubota T, Nakazawa S, Srivastava BIS, Huang CC, Oshimura M, Sonta S, Han T, Sinks LF, Sandberg AA: Establishment and characterization of leukemic T-cell lines, B-cell lines, and null-cell line: A progress report on surface antigen study of fresh lymphatic leukemias in man.[5] pp 241–252
21. Billing D, Rafizadeh B, Drew I, Hartman G, Gale R, Terasaki P: Human B-lymphocyte antigens expressed by lymphocytic and myelocytic leukemia cells. I. Detection by rabbit antisera. Br J Haematol 144–167–178, 1976
22. Fu SM, Winchester RJ, Kunkel HG: The occurrence of the HL-B alloantigens on

the cells of unclassified acute lymphoblastic leukemias. J Exp Med 142:1334–1338, 1975

23. Kaplan J, Ravindranath Y, Peterson WD Jr: T and B lymphocyte antigen-positive null cell leukemias. Blood 49:371–378, 1977
24. Kersey JH, Coccia PF, Bloomfield CD, Nesbit ME, McKenna RW, Brunning RD, Hallgren H, Gajl-Peczalska KJ: Surface markers define human lymphoid malignancies with differing prognoses.[5] pp 17–24

[*End of Article*]