Definition of the High-Risk Acute Lymphoblastic Leukemia Patient by Immunological Phenotyping with Monoclonal Antibodies

Theodore F. Zipf, Robert I. Fox, Jeanette Dilley, and Ronald Levy

Howard Hughes Medical Institute Laboratories [R. I. F., J. D., R. L.], Department of Medicine/Oncology [R. I. F., J. D., R. L.], and Department of Pediatrics [T. F. Z.], Stanford University Medical Center, Stanford, California 94305, and Division of Hematology/Oncology, Children’s Hospital at Stanford, Palo Alto, California 94304 [T. F. Z.]

Abstract

An accurate method of classification of the surface membrane characteristics of blasts from patients with acute lymphoblastic leukemia would allow a more definitive study of the nature of this disease. Monoclonal antibodies have been produced to the surface antigens of leukemic blasts from a patient with high-risk acute lymphoblastic leukemia. Two antibodies of interest were obtained from this immunization. These two, in combination with a monoclonal antibody with anti-la specificity, have been used to obtain surface phenotypes for patients with childhood acute lymphoblastic leukemia. Preliminary results indicate that the definition of a high-risk group, using these antibodies, is possible.

Introduction

Studies of immunological membrane markers on leukemic blasts from patients with ALL have already revealed considerable heterogeneity within this disease (2). The classification according to conventional T- and B-cell markers has led to the definition of subgroups with different prognoses. It is well known that those patients whose leukemic blasts have surface properties similar to those of either T- or B-cells have a markedly poorer overall response to therapy than do those with cells that have neither T- nor B-cell markers. Additional studies have demonstrated a correlation between clinical features at presentation and classification according to these markers (3). Several of the reliable prognostic factors derived from clinical characteristics at the time of presentation, i.e., level of blast cell count and sites of disease (13), appear to be a reflection of the degree of tumor dissemination which may, in turn, be determined by a unique immunological subtype. The latter may ultimately prove to be a more appropriate indicator of the optimum therapy for the patient. An accurate method of classification not only would allow a more definitive approach to treatment but also would be of great value in the further study of the properties of both the malignant cell and the disease-bearing host.

The homogeneity of monoclonal antibodies and their concomitant ability to detect single antigenic determinants makes them an attractive tool for phenotyping leukemic blast cells. The cell hybridization technique of Köhler and Milstein (5) makes possible the copious production of these antibodies and thereby the establishment of a battery of standardized reagents which could be universally available for diagnostic studies.

Two monoclonal antibodies have been produced, using leukemic cells from a patient (DOM) with features at presentation which are usually associated with a poor prognosis. One antibody, 12E7, detects an antigen with a molecular weight of 28,000, present in high density on cortical but not on medullary thymocytes (7). This antibody reacts weakly with normal peripheral lymphocytes and mitogen-stimulated lymphocytes. The second antibody, 17F12, reacts with an antigen with a molecular weight of 67,000, present on mature T-cells found predominantly in the medulla of the thymus, peripheral blood, and T-cell zones of lymphoid tissue (16). These 2 antibodies, in combination with monoclonal antibody against Ia-like molecules (6), were used to phenotype the leukemic blast cells of patients with ALL. In this report, we have been able to subdivide ALL into distinct groups, one of which appears to contain the ‘‘high-risk’’ patients.

Materials and Methods

The proportion of E-rosette-forming cells after 1 hr of incubation at 4 and 37°C was determined using methods described by Mills et al. (11). The assay of surface immunoglobulin was done using the F(ab′)2 fragment of fluorescein-conjugated rabbit anti-human immunoglobulin (Cappel).

The methods used for production and screening of the monoclonal antibodies have been described in detail previously (7). The immunizing and screening cells were lymphoblasts obtained from the peripheral blood of a 2.5-year-old boy with ALL (DOM). This patient presented with a large anterior mediastinal mass and hepatosplenomegaly. The WBC on admission was 460,000/cu mm, and 95% of these cells had lymphoblastoid morphology. The bone marrow aspirate contained 78% lymphoblasts. Greater than 90% of the lymphoblasts formed rosettes with sheep erythrocytes at 37°C. Blasts were present in the cerebrospinal fluid at the time of presentation.

The methods used in the labeling of the cell membranes, immunoprecipitation of the antigens and gel electrophoresis, have been described in detail elsewhere (4). Antigens on the cell surface were identified by the binding of monoclonal antisera, using indirect immunofluorescence. Determination of the relative amount of bound antibody was performed using the FACS as described by Loken and Herzenberg (8).

Leukemia cells in these studies were obtained as heparinized bone marrow or peripheral blood samples from patients at Stanford University and at the Children’s Hospital at Stanford. The patients included 16 children with ALL and 2 patients with the initial diagnosis of lymphoblastic lymphoma who subsequently developed leukemia transformation. Bone marrow specimens were studied for the children with ALL at the time of presentation and in the 2 cases of lymphoblastic lymphoma at the time of leukemic transformation. All patients with ALL were initially treated with the same standard antileukemic therapy (9).
Normal human thymocytes were obtained when thymus tissue was removed from children undergoing cardiac surgery.

Cell lines used in these studies include T-cell lines 8402, MOLT-4, and HSB2 and the B-cell lines 8392 and SB. Their origins and characteristics have been described previously (12).

**Results**

Three monoclonal antibodies were chosen for phenotyping the leukemic cells. Two of these, 12E7 and 17F12, were derived from the immunization with DOM cells (7, 16). The third, L243 (Ia), which binds to la-like molecules, was obtained from a separate immunization (6). Analysis with the FACS demonstrated that antibody 12E7 showed strong binding to E-rosette-positive ALL cells and weak binding to PBL. Weak staining was also observed when phytohemagglutinin-stimulated PBLs were stained. For the purposes of this study, the weak staining of PBL was set as the threshold above which leukemic cells were scored as positive or negative. When suspensions of thymocytes were stained with 12E7, the cells with the brightest fluorescence were those in the large-size category, presumably derived from thymic cortex. Antibody 17F12 showed binding to both PBL and E-rosette-positive ALL, but not to the B-cell lines. Thymocytes stained with this antibody showed highest fluorescence in the medium-sized cells, presumably derived from the thymic medulla. No cells in the thymocyte suspensions stained with Ia. Negative controls for staining included myeloma proteins MOPC-21 (lgG-1K) or GPC-7 (lgG-2aK), which were matched with the isotypes of 12E7 and 17F12, respectively. These controls gave assurance that the fluorescence patterns observed did not arise from nonspecific binding of antibody via Fc receptors. Cells from a series of patients were analyzed with antibodies 12E7, 17F12, and L243 using the FACS. Chart 1 shows an example of the results from 2 patients, one with T-cell ALL (left) and one with non-T, non-B ALL (right). In each case, a negative control incubated with a nonspecific myeloma protein is included. The leukemia cells showed either no staining with the monoclonal antibody or complete staining. For example, within the limit of this method, all of the leukemia cells in the patient shown on the left expressed the antigens detected by antibodies 12E7 and 17F12, and none expressed the Ia antigens detected by antibody L243 (phenotype ++ -). The cells from the patient on the right gave the converse staining pattern (phenotype - - -). Even though the leukemia cells from a given patient expressed a given antigen, they did so to a variable degree, i.e., showed a wide distribution of staining intensities. With 3 different antibodies, 8 phenotypes are possible. In a small series of ALL patients we have observed 5 different phenotypes (--- ---, ++ +++, and - + + have not been observed).

Table 1 shows the phenotype and presenting features of the 16 patients with ALL. No patients other than the donor of the immunizing cells presented with central nervous system disease. The clinical course and sites of relapse for those patients with treatment failure are given in Table 2. One patient, BAL, who expired while undergoing induction therapy as a result of hepatic failure of unknown etiology, was included in the treatment failures. Whenever possible, patients have been phenotyped at the time of relapse. To date, no change in phenotype has been observed out of 5 tested. The 2 patients with the diagnosis of lymphoblastic lymphoma were male, and in both cases the diagnosis was made from lymph node biopsy specimens. Both were reported to have less than 25% blasts in bone marrow aspirates or biopsies done at presentation. They were treated with contemporary regimens for childhood non-Hodgkin’s lymphoma and achieved periods without evidence of disease for 8 to 25 months prior to the development of marrow disease. Both then showed poor responses to antileukemic therapy and expired at 4 and 11 months after development of leukemia.

**Discussion**

The results presented here are of a preliminary nature with a small number of patients followed for a period of only about 18 months. Conclusions must, therefore, be of a tentative nature. Nevertheless, we think that we have obtained several results of significance which deserve further study. This effort to develop a more precise method of identifying the patient with a high probability for treatment failure was based on the premise that the antigenic content of the leukemic cell surface would vary, depending upon the disease subtype. Further, it was presumed that the expression of these antigens is intimately related to the biology of the diseased cell, and hence the response to any given mode of therapy. Thus,
monoclonal antibodies to the surface antigens of lymphoblasts with conventional T-cell markers were produced. The presenting clinical features of our index patient (DOM) were indicative of a high probability of treatment failure. Recent studies would indicate that statistically at least 2 of these risk factors were of independent predictive significance (13). The cells of this patient reacted with antibodies 12E7 and 17F12 but not with anti-La L243 (phenotype + + -).

Cells from 4 of the other 15 patients with ALL had the DOM phenotype. All of these were male and all had characteristics associated with T-like disease. However, only 2 of the 4 patients had marrow lymphoblasts which formed E-rosettes stable at 37°C. One of the patients, PER, whose blasts did not form rosettes at either 37 or 4°C, presented with a large anterior mediastinal mass. His leukocyte count was not elevated and no blasts were seen on the peripheral smear. The other, BAL, presented with a leukocyte count greater than 100,000/cu mm and hepatosplenomegaly greater than 10 cm. Previous reports have addressed the problem of detecting T-like disease with either a combination of antisera and E-rosettes or the use of antisera alone (10, 14). In view of the data presented in this report, we suggest that surface phenotyping with several monoclonal antibodies such as the ones used here may ultimately prove to be the more precise method. The clinical course of the patients with the DOM phenotype (Table 2) confirms that this phenotype carries a poor prognosis with present antileukemic therapy. DOM and the 3 other patients who achieved remission experienced only brief durations of initial remission, and all relapsed in bone marrow and simultaneously in at least one extramedullary site. The unresponsiveness to therapy of the 2 patients with lymphoblastic lymphoma with leukemia transformation (both of whom had the DOM phenotype) strengthens our conclusion that high-risk patients can be identified in this way. Of the 3 monoclonal antibodies used in this small study, 17F12 and L243 (anti-la) appear to react reciprocally, (+ -) and with patients in both high- and standard-risk categories. Power in defining risk groups. It is clear that 12E7 reacts more powerful in defining risk groups.

Whether reactivity with 12E7 will be an independent correlate with either a combination of antisera and E-rosettes or the use of antisera alone (10, 14). In view of the data presented in this report, we suggest that surface phenotyping with several monoclonal antibodies such as the ones used here may ultimately prove to be the more precise method. The clinical course of the patients with the DOM phenotype (Table 2) confirms that this phenotype carries a poor prognosis with present antileukemic therapy. DOM and the 3 other patients who achieved remission experienced only brief durations of initial remission, and all relapsed in bone marrow and simultaneously in at least one extramedullary site. The unresponsiveness to therapy of the 2 patients with lymphoblastic lymphoma with leukemia transformation (both of whom had the DOM phenotype) strengthens our conclusion that high-risk patients can be identified in this way. Of the 3 monoclonal antibodies used in this small study, 17F12 and L243 (anti-la) appear to react reciprocally, (+ -) and with patients in both high- and standard-risk categories. Whether reactivity with 12E7 will be an independent correlate of clinical outcome will need a larger study to decide. Antigens 17F12 and L243 appear to react reciprocally, (+ -) defining the high-risk group and ( - +) defining the standard-risk group.

Several investigators have hypothesized that the T and non-T leukemias represent 2 separate disease entities with different target cells and pathogenesis (1, 3, 15). According to this model, the T-like disease is of extramedullary origin, presumably lymphatic and similar to childhood lymphoblastic leukemia.
lymphoma. Thus, the leukemic presentation is a late development and represents the malignant extension of the disease to the bone marrow. In contrast, non-T, non-B ALL is considered to be a disease which is primarily of bone marrow origin. That the 2 patients with leukemic transformation of lymphoblastic lymphoma have the same phenotype as the patients with T-like ALL lends validity to this hypothesis.

The data at present are insufficient to allow meaningful speculation on the significance of the other phenotypes that we observed. Apparently, they represent different forms of the disease, and the collection of further data may indicate other high-risk groups. We would propose that large-scale prospective phenotyping be done in multiinstitutional studies with a suitable panel of monoclonal antibody reagents. In this way, we may be able to achieve a uniform typing system for this heterogeneous group of diseases.

References


Definition of the High-Risk Acute Lymphoblastic Leukemia Patient by Immunological Phenotyping with Monoclonal Antibodies

Theodore F. Zipf, Robert I. Fox, Jeanette Dilley, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/11_Part_2/4786

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.