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

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Abstract

An accurate method of classification of the surface membrane characteristics of blast cells 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

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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 8382 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 Ia-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 (IgG-1K) or GPC-7 (IgG-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,
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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 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 be the most powerful in defining risk groups. It is clear that 12E7 reacts with cells possessing non-T as well as T-cell characteristics and with patients in both high- and standard-risk categories.

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...
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
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