Abstract

A monoclonal antibody specific for a common acute lymphoblastic leukemia (ALL) antigen has been generated and characterized. This antibody (J-5) is reactive with leukemic cells from most patients with non-T-cell ALL and some patients with chronic myelocytic leukemia in blast crisis. J-5 antibody is not reactive with leukemic cells from patients with either T-cell ALL, chronic lymphocytic leukemia, acute myeloblastic leukemia, or stable-phase chronic myelocytic leukemia. In addition to diagnostic applications, anti-common ALL antigen monoclonal antibody has been used to study the effect of specific serotherapy in the treatment of ALL. In the first patient with ALL treated with J-5 antibody, a 90% reduction in circulating lymphoblasts occurred within 1 hr of starting antibody infusion. Despite continued serotherapy, however, lymphoblasts persisted in peripheral blood and bone marrow. Analysis of cell surface markers during serotherapy suggested that resistance to antibody-mediated lysis in vivo may have been due to antigenic modulation of leukemic cells in response to J-5 monoclonal antibody.

Introduction

The method for generation of monoclonal antibodies by somatic cell hybridization has now been successfully used to produce antibodies specific for various cell surface proteins of normal human lymphocytes. These have included histocompatibility antigens (26), Ia-like antigens (17), peripheral T-cell antigens (16), and thymocyte differentiation antigens (15, 19). Malignant cells also often express these normal surface antigens, and monoclonal antibodies to these antigens can therefore serve as useful probes in determining the cellular derivation and state of differentiation of leukemic cells. As previous studies with conventional antisera have shown (18, 24, 27), these factors have restricted the clinical application of cytotoxic antisera with high titer and unique specificity. In addition to diagnostic applications, J-5 monoclonal antibody has been used to evaluate the use of serotherapy in the treatment of ALL. This report describes the characterization of this reagent and presents our preliminary experience with the first treatment of a patient with ALL with anti-CALLA monoclonal antibody.

Materials and Methods

Anti-CALLA Monoclonal Antibody. The generation and characterization of J-5, a monoclonal antibody specific for CALLA, has been developed in our laboratory (20). The specificity of J-5 monoclonal antibody was determined by indirect immunofluorescence assay on cell lines and cryopreserved leukemic cells which had previously been analyzed with rabbit anti-CALLA. In addition, immune precipitation experiments with J-5 antibody and rabbit anti-CALLA confirmed that both antisera identify a cell surface glycoprotein with molecular weight of 95,000 to 100,000 (14).

J-5 antibody-producing hybridoma cells were grown as ascites tumors in pristane-primed BALB/c mice. J-5 monoclonal antibody was purified from ascites by precipitation with ammonium sulfate, followed by reconstitution and dialysis against phosphate-buffered saline. Purified antibody was then heat inactivated at 56°C for 30 min, ultracentrifuged at 100,000 × g for 20 min, adjusted to a final concentration of 25 mg/ml, filtered (0.45 µMillex), and stored at −70°C. Purified J-5 antibody was also tested for presence of endotoxin using the Limulus amebocyte lysate test (Microbiological Associates), for hepatitis B surface antigen using radioimmunoassay, and for bacterial and fungal sterility. In addition, each lot of J-5 antibody was shown to be unreactive with a panel of HLA typing cells, which included cells from 34 different individuals.

1 Presented at the Conference on Cell Markers in Acute Leukemia, March 4 and 5, 1980, Bethesda, Md. This work was supported in part by NIH Grants CA 056518, CA 25389, AI 12069, and CA 19589.
2 To whom requests for reprints should be addressed, at Division of Tumor Immunology, Sidney Farber Cancer Institute, 44 Binney St., Boston, Mass. 02115.
3 Recipient of a Fellowship from the Cancer Research Institute.
4 The abbreviations used are: ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; CALLA, common acute lymphoblastic leukemia antigen; FACS, fluorescence-activated cell sorter.
Analysis of Peripheral Blood and Bone Marrow Samples. Samples of peripheral blood and bone marrow were drawn into heparin syringes, and mononuclear cells were separated by ficoll-hypaque density sedimentation. Three monoclonal antibodies (J-5, anti-la, and anti-T3) were used to phenotypically characterize mononuclear cells in each population. Each of these antibodies has been characterized and described previously. Anti-la (17) is specific for human la-like antigen, while anti-T3 (16) is reactive with all peripheral T-lymphocytes. Purified mononuclear cells were characterized with these specific antibodies using a standard indirect immunofluorescence assay and fluorescein-conjugated goat anti-mouse immunoglobulin (Meloy Laboratories, Springfield, Va.) as a developing reagent. Cells were then analyzed on the FACS (Becton, Dickinson, Mountain View, Calif.), which determined the intensity of fluorescence for 40,000 cells in each sample. Results are displayed as a histogram, plotting intensity of fluorescence versus cell number for each reagent. Background fluorescence was determined by incubating cells with nonreactive ascites (J-0) and fluorescein-conjugated goat anti-mouse immunoglobulin.

Results

Specificity of Anti-CALLA Monoclonal Antibody. The unique specificity of J-5 antibody was first demonstrated by examining the reactivity of J-5 ascites with cryopreserved leukemic cells which had previously been phenotyped with rabbit anti-CALLA (Table 1). Cells from 138 patients have been tested with both reagents and, except for 3 instances, J-5 reactivity correlated with that of rabbit anti-CALLA, demonstrating specificity for leukemic cells from most patients with non-T-cell ALL and some patients with CML blast crisis. In almost all cases, fluorescence with J-5 closely paralleled reactivity with rabbit anti-CALLA. There was no J-5 reactivity with leukemic cells from patients with T-cell ALL, AML, or stable-phase CML.

The reactivity of J-5 antibody with normal hematopoietic cells has also been evaluated by indirect immunofluorescence assay and quantitative analysis on the FACS. J-5 antibody has not been reactive with normal peripheral blood mononuclear cells, purified T-cells, B-cells, null cells, monocytes, granulocytes, platelets, thymocytes, spleen cells, or adult bone marrow. We have also not found significant reactivity with cells from 18-week fetal liver, bone marrow, or thymus or with regenerating bone marrow after either chemotherapy, autologous transplantation, or transplantation from HLA-matched donors. Although J-5 reactivity with normal hematopoietic cells has not been demonstrable by indirect immunofluorescence assay, immune precipitation experiments have identified a glycoprotein with a molecular weight of 95,000 to 100,000 in normal bone marrow. Further biochemical characterization of this antigen is currently in progress, and the relationship of this antigen to the CALLA identified on leukemic cells remains to be determined.

The specificity of J-5 antibody has also been tested in a microcytotoxicity assay with rabbit complement by measuring release of $^{51}$Cr from labeled target cells. These experiments demonstrated that J-5, which is a murine IgG2A antibody, readily initiates complement-mediated lysis of CALLA-positive cells but has no effect on CALLA-negative cells. As shown in Chart 1, greater than 50% lysis was observed with ascites dilutions of 1:10,000 on CALLA-positive cells, LAZ 221 (10), and NALM-1 (12), but no lysis was noted at any dilution on CALLA-negative cells, LAZ 388, and CEM. Both LAZ 221 and LAZ 388 are cell lines derived from a single patient with ALL. The difference in expression of CALLA is reflective of the derivation of LAZ 221 from leukemic cells, while LAZ 388 is derived from Epstein-Barr virus-transformed B-cell lymphoblasts.

Serotherapy of ALL with Anti-CALLA Monoclonal Antibody. To evaluate the clinical efficacy and toxicity of J-5 monoclonal antibody, an experimental protocol for specific serotherapy of ALL has been initiated at the Children's Hospital Medical Center and the Sidney Farber Cancer Institute. Only multiply relapsed patients with ALL who are not receiving chemotherapeutic agents are considered eligible for this study. The first patient treated under this protocol was a 7-year-old girl, and her case is presented in detail to illustrate both the potential efficacy and some of the problems which will have to be overcome if serotherapy is to be effectively utilized in the treatment of ALL.

Case History. In D. M., the diagnosis of ALL was initially established in February 1976 at the age of 3 years and 4 months, when she presented with malaise and pallor. Physical examination showed moderate enlargement of the liver and spleen without lymphadenopathy. WBC was 6900/cc mm with 51% lymphoblasts, and bone marrow aspirate contained 98% lymphoblasts. Histochemical stains demonstrated that these cells were periodic acid-Schiff positive and peroxidase negative. Treatment with vincristine and prednisone induced a complete remission, and subsequent systemic therapy on a previously described protocol (22) included 6-mercaptopurine, doxorubicin, L-asparaginase, and methotrexate. Chemotherapy

Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Rabbit anti-CALLA</th>
<th>Hybridoma J-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-T-cell ALL</td>
<td>51/74</td>
<td>50/74</td>
</tr>
<tr>
<td>T-cell ALL</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>2/13</td>
<td>0/13</td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>0/22</td>
<td>0/22</td>
</tr>
<tr>
<td>CML</td>
<td>5/8</td>
<td>5/8</td>
</tr>
<tr>
<td>Blast crisis</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Stable phase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Each fraction represents the number of positive populations over the total number tested. Leukemic cells were either positive or negative with both rabbit anti-CALLA and J-5 monoclonal antibody except as described in Footnote b.

† ALL cells from one patient and chronic lymphocytic leukemia cells from 2 patients were reactive with rabbit anti-CALLA but totally unreactive with J-5.
Use of Monoclonal Antibodies in ALL

was electively discontinued in August 1978 after 2.5 years of continuous complete remission. In December 1978, bone marrow relapse occurred and chemotherapy with vincristine, L-asparaginase, and prednisone was reinstituted. Additional chemotherapy during the next 8 months included actinomycin D, cyclophosphamide, 1-β-D-arabinofuranosylcytosine, and VM-26 (21), but a second remission was never achieved.

D. M. was admitted to our hospital on September 9, 1979, for serotherapy with anti-CALLA monoclonal antibody. Her last chemotherapy had been 4 weeks earlier with 1-β-D-arabinofuranosylcytosine and VM-26 (21). Her current medications were trimethoprim-sulfamethoxazole and phenytoin. In addition, allopurinol and i.v. fluids were started 18 hr before serotherapy. Examination of peripheral blood just prior to antibody infusion showed a WBC of 4800/cu mm with 51% lymphoblasts, 26% polymorphonuclear leukocytes, 21% lymphocytes, and 2% monocytes. Hematocrit was 32% and platelet count was 80,000/cu mm. Bone marrow aspirate was hypercellular and contained 80% lymphoblasts. Serum electrolytes, blood urea nitrogen, creatinine, bilirubin, and uric acid were normal. Serum glutamic-oxaloacetic transaminase was 48 microunits/ml, lactic dehydrogenase was 320 microunits/ml, and alkaline phosphatase was 143 microunits/ml. Arterial blood gases, chest X-ray, electrocardiogram, and urine analysis were normal.

Chart 2 depicts the numerical changes in peripheral blood mononuclear cells which occurred during serotherapy with anti-CALLA monoclonal antibody. On the first day of therapy, 85 mg J-5 antibody (5 mg/kg) were infused over a 4-hr period. The first blood count obtained during serotherapy was taken 1 hr after starting antibody infusion and showed a marked reduction in circulating blasts when compared to pretreatment values. This reduction in circulating blasts persisted during the 3 days of therapy and then gradually returned to pretreatment values. Fourteen days after starting serotherapy, the peripheral lymphoblast count was again over 2000 cells/cu mm. No further reduction in circulating blasts occurred on the second or third days of therapy when additional infusions of 170 mg J-5 antibody (10 mg/kg) were given. There were no consistent changes in granulocytes, monocytes, lymphocytes, or platelets which could be attributed to serotherapy. Total granulocyte counts dropped slightly during the 4-hr infusions on Days 1 and 2 but recovered after the infusions were completed. There was no change during the infusion on Day 3. Monocyte counts changed from 96/cu mm pretreatment to 138/cu mm on Day 5 (range, 19 to 203/cu mm). Normal lymphocyte counts changed from 1,008/cu mm pretreatment to 1,748/cu mm on Day 5 (range, 528 to 1,748/cu mm). Platelet counts changed from 80,000/cu mm pretreatment to 79,000/cu mm on Day 5 (range, 54,000 to 89,000/cu mm).

Serotherapy with J-5 antibody was clinically well tolerated. No adverse reactions were noted during antibody infusions, but fevers from 38.3°C–40°C developed between 12 and 18 hr after each infusion. The patient remained entirely asymptomatic during these febrile episodes. There were no changes in serum creatinine, electrolytes, blood urea nitrogen, urine analysis, or liver function tests during serotherapy.

Analysis of Leukemic Cell Phenotype. The cell surface phenotype of leukemic cells from this patient obtained prior to initiation of chemotherapy and at the time of initial relapse was typical of the majority of patients with ALL (23) and is shown in Chart 3. Lymphoblasts expressed both la and CALLA, as identified by anti-la and J-5 but were unreactive with T-cell specific antisera (anti-T3).

During serotherapy, cells were obtained from peripheral blood and bone marrow at various times and analyzed with these same monoclonal antibodies. The results of these studies are summarized in Table 2. Prior to serotherapy on Day 1, 63% of cells in peripheral blood expressed CALLA, but 90 min later only 1% cells remained reactive. Although there was an absolute decrease in circulating leukemic cells during this interval, cytocentrifuge smears of the cells obtained at 90 min still contained 44% lymphoblasts. This correlates well with the persistent reactivity with anti-la at 90 min (43% positive cells). The loss of J-5 reactivity persisted in every peripheral blood sample tested for 1 week after the first antibody infusion. On Day 8, however, peripheral blood lymphoblasts again reexpressed CALLA and 45% of cells were reactive with J-5 antibody.
FACS analysis of bone marrow cells (Table 2 ) also demonstrated a decrease in CALLA expression by leukemic cells during serotherapy. Prior to antibody infusion, 71% of bone marrow cells were reactive with J-5 antibody; but 48 hr after starting serotherapy, only 4% of cells remained CALLA positive. There was no change in expression of la antigen during this time. Two weeks after starting serotherapy, bone marrow lymphoblasts had reverted to their original phenotype and 94% of cells were CALLA positive. There was no significant change in bone marrow cellularity or differential during serotherapy.

### Table 2

<table>
<thead>
<tr>
<th>Cell surface phenotype</th>
<th>PB*</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of cells reactive on FACS analysis</td>
<td>J-0</td>
<td>J-5</td>
</tr>
<tr>
<td><strong>PB</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment, Day 1</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>90 min, Day 1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Day 8</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td><strong>BM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment, Day 1</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>Day 3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Day 14</td>
<td>7</td>
<td>94</td>
</tr>
</tbody>
</table>

* PB, peripheral blood; BM, bone marrow; NT, not tested.

In addition to use as diagnostic and prognostic indicators, monoclonal antibodies may also provide an additional therapeutic modality in the treatment of ALL. Monoclonal antibodies are well suited for use in serotherapy because their unique specificity and higher titer circumvent many of the difficulties associated with the use of conventional heteroantisera. Our initial studies, however, indicate that large quantities of monoclonal and cytotoxic antibody may not be sufficient to assure efficacy of passive serotherapy and that additional factors must be considered. In the first patient with ALL treated with J-5 antibody, leukemic cells rapidly became resistant to serotherapy. Analysis of cell surface markers during serotherapy indicated that resistance may have been due to antigenic modulation of leukemic cells. Antigenic modulation appeared to be a specific response to J-5 antibody which resulted in the loss of cell surface CALLA but did not alter the expression of an independent cell surface antigen such as la. This phenomenon has been previously described and well characterized as a response of murine thymus leukemia antigen to specific antibody (3, 25), but its significance as an escape mechanism of human tumor cells has not been established.

An alternative explanation for the change in expression of CALLA is that selection of preexisting CALLA-negative cells occurred during serotherapy. This appeared to be unlikely, because prior to serotherapy there was no evidence to suggest that such a population existed, and the percentage of lymphoblasts in peripheral blood and bone marrow using morphological criteria correlated closely with the percentage of CALLA-positive and la-positive cells by FACS analysis. In addition, the bone marrow aspirate taken just 48 hr after starting serotherapy contained predominantly CALLA-negative cells but showed no change in cellularity compared to the pretreatment aspirate.

Even though J-5 antibody infusion alone did not result in complete regression of leukemia in our patient, a partial response was noted, and serotherapy under more favorable conditions with a smaller tumor burden may be more effective. Although antigenic modulation may limit the efficacy of serotherapy in vivo, the use of rabbit complement to lyse leukemic cells in vitro may prove useful in the treatment of bone marrow prior to autologous transplantation. In addition, as further studies define the cellular mechanism of antigenic modulation, methods which will circumvent or inhibit this process may be developed. More significantly perhaps, J-5 antibody affected all leukemic cells in bone marrow as well as in peripheral blood, indicating that in the future J-5 monoclonal antibody may be more effectively utilized as a unique delivery system for other therapeutic agents.

### References

Use of Monoclonal Antibodies in ALL


Use of Monoclonal Antibodies as Diagnostic and Therapeutic Reagents in Acute Lymphoblastic Leukemia

Jerome Ritz, John M. Pesando, Jean Notis-McConarty, et al.


Updated version

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/11_Part_2/4771

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.