Serotherapy of Acute Lymphoblastic Leukemia
With Monoclonal Antibody

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We tested the efficacy of passive serotherapy in the treatment of acute lymphoblastic leukemia in four patients who had relapsed while receiving standard chemotherapeutic agents. Each patient received multiple intravenous infusions of J-5 monoclonal antibody specific for common acute lymphoblastic leukemia antigen (CALLA). In the three patients with circulating leukemic cells, there was a rapid decrease in circulating blasts that began immediately after antibody infusion, but not all leukemic cells were cleared, and remaining cells appeared to be resistant to further serotherapy. Although J-5 antibody was also demonstrable on bone marrow lymphoblasts immediately after antibody infusion in one patient, there was no change in bone marrow cellularity or differential during serotherapy. Analysis of the cell surface phenotype of leukemic cells during serotherapy and in vitro studies with patient cells suggests that resistance to serotherapy was mediated in part by antigenic modulation of CALLA in response to J-5 antibody.

SEROThERAPY of human leukemia has, in the past, been limited by both the quantity and specificity of the antisera that have been available. The generation of homoantisera specific for human leukemia cells, but unreactive with normal cells, has required extensive absorption with normal tissues, and therefore, has been difficult to prepare in large quantities. Fully absorbed antisera also have relatively low titers of reactivity with tumor cells and often maintain some cross-reactivity with normal tissues. In addition, serotherapy with antisera produced in this fashion would necessitate the administration of large quantities of foreign proteins with the high risk of allergic reactions.

Many of these problems have been circumvented by the generation of hybridoma antibodies to human leukemia cells. We have recently produced a murine monoclonal antibody (J-5) specific for leukemic cells from 80% of patients with non-T-cell acute lymphoblastic leukemia (ALL) and 40% of patients with chronic myelocytic leukemia (CML) in blast crisis. The common ALL antigen (CALLA) identified by this monoclonal antibody has been shown to be a 100,000 mol wt glycoprotein, which had been previously identified by conventional rabbit antisera. Further studies have shown that CALLA is a member of a family of cell surface glycoproteins and that many normal hematopoietic as well as malignant cells express 100,000 mol wt glycoprotein antigens that are serologically related to CALLA. The common ALL antigen is unique, however, in that it is not expressed by normal hematopoietic cells from peripheral blood, spleen, thymus, or lymph node by indirect immunofluorescence assay. In addition, committed erythroid and myeloid stem cells do not express CALLA. Although normal bone marrow contains a small percentage of cells that are CALLA positive, the functional role of these cells has not been established.

This article details our experience with the clinical use of J-5 monoclonal antibody to test the efficacy of specific serotherapy in the treatment of acute lymphoblastic leukemia. Four patients with multiply relapsed ALL have been treated with intravenous infusions of purified J-5 antibody. Three patients had circulating lymphoblasts, and in each of these patients, dramatic reductions in numbers of leukemic cells in peripheral blood occurred in response to serotherapy. In each instance, however, leukemic cells also rapidly became resistant to antibody treatment. Phenotypic characterization of leukemic cells during the course of serotherapy and in vitro studies with patients' cells suggested that this resistance was mediated, at least in part, by antigenic modulation of leukemic cells.

SEROThERAPY PROTOCOL

All patients were treated under an experimental protocol for specific immunotherapy of acute lymphoblastic leukemia with monoclonal antibody. Patients were eligible for this study if the following conditions were met: (1) informed consent of patients and/or guardians; (2) multiple previous relapses and histologic confirmation of current relapse in bone marrow and/or peripheral
blood; and (3) demonstration of reactivity of leukemic cells with anti-CALLA monoclonal antibody. Patients were considered ineligible for serotherapy if any of the following were present: (1) intercurrent treatment with cytotoxic chemotherapeutic agents; (2) past history of hypersensitivity to animal serum or documented hypersensitivity to hybridoma antibody by intradermal skin test; and (3) impaired renal function with serum creatinine greater than 2 mg/dl.

All patients received allopurinol and intravenous fluids in addition to J-5 antibody, but no other medications were routinely given. Acetaminophen and diphenhydramine could be used if necessary to treat fevers or allergic responses to serotherapy. The duration of treatment was limited to 5 days and each patient went on to receive additional chemotherapy after serotherapy. The effect of serotherapy was monitored by enumerating leukemic cells in peripheral blood and bone marrow samples during the course of serotherapy and by analyzing these populations on the fluorescence activated cell sorter to determine their cell surface phenotype.

CASE REPORTS

Patient 1
D.M. initially presented at age 3 yr, 4 mo and treatment with vincristine and prednisone induced a complete remission. Subsequent therapy, which included 6-mercaptopurine, doxorubicin, t-asparaginase, and methotrexate, was electively discontinued after 30 mo of continuous complete remission. Four months later, bone marrow relapse occurred and despite intensive chemotherapy, which included actinomycin D, cyclophosphamide, cytosine arabinoside, and VM-26, a prolonged second remission was not obtained.

D.M. was admitted for serotherapy with anti-CALLA monoclonal antibody at age 7, 9 mo after relapse. Her last chemotherapy had been 4 wk earlier, and her current medications were trimethoprim-sulfamethoxazole and phenitoin. Laboratory studies showed a white blood cell count 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.

Patient 2
C.B. was referred for treatment of ALL at the age of 7. A complete remission was induced with vincristine and prednisone, and subsequent chemotherapy included doxorubicin, 6-mercaptopurine, and t-asparaginase. CNS relapse occurred 11 mo after diagnosis. She responded to intrathecal hydrocortisone and cytosine arabinoside but lymphoblasts reappeared in the cerebrospinal fluid 9 mo later. This second CNS relapse responded gradually to intrathecal methotrexate, but bone marrow relapse occurred 31 mo after initial diagnosis.

C.B. was admitted for serotherapy at age 9.5 yr. Her last course of chemotherapy had been 8 wk previously, and CNS radiotherapy had been administered 4 wk previously. Her current medications were digoxin and hydrochlorothiazide. Physical examination revealed large ecchymoses on all extremities. Laboratory studies showed a white blood cell count of 77,000/cu mm with 94% lymphoblasts, 4% polymorphonuclear leukocytes, 1% lymphocytes, and 1% monocytes. Hematocrit was 33% and platelet count was 60,000/cu mm.

Patient 3
J.P. initially presented at the age of 8 yr, 5 mo because of diffuse adenopathy. He was treated with methotrexate, vincristine, cyclophosphamide, 6-mercaptopurine, and prednisone, and therapy was electively stopped after 30 mo of continuous remission. Bone marrow relapse occurred after 1 yr off therapy, and a second remission was achieved with vincristine and prednisone. Subsequent chemotherapy included doxorubicin, 6-mercaptopurine, L-asparaginase, cytosine arabinoside, and intrathecal methotrexate.

Chemotherapy was electively stopped for the second time after another 30 mo of continuous remission, but bone marrow relapse occurred again 7 mo thereafter. A third remission was achieved, but bone marrow relapse occurred 9 mo later, while receiving chemotherapy.

J.P. was admitted for serotherapy at age 16 (7.5 yr after initial diagnosis). His last course of chemotherapy had been given 7 wk previously. He was taking no medication at the time of admission. On physical examination, spleen tip was palpable at the left costal margin, and temperature was 99.2°F. Laboratory studies showed a total white blood cell count of 800/cu mm with 42% lymphocytes, 1% monocytes, and 47% polymorphonuclear leukocytes. Hematocrit was 32% and platelet count was 80,000/cu mm. Bone marrow aspirate was hypercellular, containing 97% lymphoblasts.

MATERIALS AND METHODS

Anti-CALLA Monoclonal Antibody

The generation and characterization of J-5 monoclonal antibody has been described previously.4 J-5 antibody-producing hybridoma cells were grown as an ascites tumor in pristane-primed BALB/c mice. J-5 antibody (murine IgG,4) was purified from ascites by ammonium sulfate precipitation followed by reconstitution and dialysis against phosphate-buffered saline. Purified antibody was heat inactivated at 56°C for 30 min, ultracentrifuged at 100,000g for 20 min, adjusted to a final concentration of 25 mg/ml, filtered (0.45 µm Millex) to ensure sterility, and stored at −70°C prior to use. Each lot of purified J-5 antibody was tested for the presence of endotoxin using the Limulus Amebocyte Lysate test; for hepatitis B antigen using RIA; and for bacterial and fungal sterility prior to clinical use. In addition, each lot of J-5 antibody was shown to be totally unreactive with a panel of HLA typing cells, which included cells from 34 different individuals.

Analysis of Peripheral Blood and Bone Marrow Samples

Samples of peripheral blood and bone marrow taken before and during serotherapy were drawn into heparinized syringes, and mononuclear cells were separated by Ficoll-Hypaque density sedimentation. Because mature granulocytes as well as red cells are removed during this separation process, these cell preparations became relatively enriched for leukemic lymphoblasts. Cytocentrifugation of peripheral blood and bone marrow samples during the course of serotherapy and by analyzing these populations on the fluorescence activated cell sorter to determine their cell surface phenotype.
SERO THERAPY OF ALL

fluorescence of purified mononuclear cells were therefore used to determine the morphology of cells that were subsequently studied for the presence of specific cell surface antigens. Mononuclear cells that were not used for immediate analysis were cryopreserved in 5% DMSO using standard techniques.

In addition to J-5 antibody, other monoclonal antibodies were used to phenotype the mononuclear cells in each population. Each of these antibodies has been described previously. Anti-Ia

purified mononuclear cells were characterized with each of these reagents using a standard indirect immunofluorescence assay. One million cells were incubated with each hybridoma antibody (0.1 ml ascites at a 1:100 dilution) for 30 min and washed twice to remove excess antibody. Cells were then incubated with fluorescein-conjugated goat anti-mouse Ig (G/M FITC; Meloy Laboratories, Springfield, Va.) for 30 min and washed twice again prior to analysis. All incubations with antisera and washes were carried out at 4°C. Cells were analyzed on the fluorescence activated cell sorter (FACS-1; 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 pretreatment cells with nonreactive ascites (J-0) and G/M FITC. During the course of serotherapy, incubation of cells with G/M FITC alone was used to detect J-5 antibody bound to cells in vivo.

Serum J-5 Assay

J-5 monoclonal antibody was detected in patient serum by incubating heat-inactivated serum with known CALLA-positive cells, followed by fluorescence analysis of these cells for the presence of murine immunoglobulin. NALM-1 cells, grown in tissue culture, served as a source of cells with a stable expression of CALLA and were used to test all serum samples. One million NALM-1 cells were incubated with 0.1 ml patient serum for 30 min at 4°C followed by two washes and incubation with G/M FITC for another 30 min at 4°C. After two additional washes, cells were analyzed on the FACS as outlined above. Patient serum obtained prior to J-5 therapy was used as a control for background fluorescence.

In Vitro Culture and Modulation of Leukemic Cells

Mononuclear cells from peripheral blood or bone marrow from each patient were placed in tissue culture media and incubated at 37°C for either 24 or 48 hr prior to phenotypic analysis. Tissue culture media consisted of SMEM supplemented with 10% fetal calf serum (heat-inactivated), nonessential amino acids, pyruvate, glutamine, and penicillin-streptomycin (Microbiological Assoc., Bethesda, Md.). In these experiments, J-5 antibody was added to cell cultures at 37°C to determine whether antigenic modulation could be induced in vitro. To ensure antibody excess, 0.1 mg antibody/10⁶ cells was added. Nonreactive ascites (J-0) added to identical cultures served as a control for cultures to which J-5 had been added. Cells from J-0 and J-5 cultures were subsequently analyzed for cell surface reactivity with monoclonal antibodies as described above. The number of positive cells in each sample was determined by FACS analysis.

RESULTS

Patient 1

Preliminary results of serotherapy in this patient have been presented previously. The changes in peripheral blood lymphoblasts and granulocytes, which occurred during serotherapy with anti-CALLA monoclonal antibody, are presented in Fig. 1A. On the first day of serotherapy, 85 mg of J-5 antibody (5 mg/kg) was infused over a 4-hr period. The first blood count was obtained 1 hr after starting the infusion and showed a marked reduction in circulating blasts when compared to values obtained just prior to serotherapy (2450 blasts/cu mm versus 230 blasts/cu mm). On the second and third days of serotherapy, additional infusions of 170 mg J-5 (10 mg/kg) were given in a similar manner but no further reduction in circulating blasts occurred. The initial decrease in numbers of circulating blasts persisted for the duration of serotherapy and then blast counts gradually returned to pretreatment values. Total granulocyte counts dropped slightly during the 4-hr infusions on days 1 and 2 but recovered after the infusions were completed. No decrease in granulocyte counts occurred during the infusion on day 3. The numbers of circulating monocytes, normal lymphocytes, and platelets varied slightly during serotherapy, but there were no consistent changes that could be attributed to J-5 antibody infusion. A bone marrow aspirate obtained prior to antibody infusion on day 3 showed continued replacement by leukemic cells and no change in cellularity.

Coincident with the decrease in circulating blasts during the first J-5 antibody infusion, there was also a dramatic change in the cell surface phenotype of leukemic cells. Figure 2 demonstrates the patterns of reactivity of peripheral blood mononuclear cells with three monoclonal antibodies (J-5, anti-Ia, and anti-T3) as determined by indirect immunofluorescence and analysis on the FACS. Cells obtained before treatment (Fig. 2A) are compared with cells taken 90 min after starting serotherapy (Fig. 2B). The most striking difference is the loss of reactivity with J-5 antibody; whereas 63% of mononuclear cells in peripheral blood were J-5 positive before treatment, only 1% of cells were reactive 90 min later. Although some of this decrease was due to loss of leukemic cells from the circulation, cyt centrifuge smears of the cells analyzed in Fig. 2B still contained 44% lymphoblasts. This correlates well with the persistent fluorescence with anti-Ia (68% in Fig. 2A versus 43% in Fig. 2B), which is also reactive with the leukemic cells from this patient. In addition, as the percentage of leukemic cells decreased during serotherapy, there was a corresponding increase in circulating normal T-cells identified by anti-T3 (21% in Fig. 2A versus 37% in Fig. 2B).

Analysis of bone marrow cells taken prior to the third infusion of J-5 antibody also demonstrated a
Fig. 1. Changes in circulating lymphoblasts during sero-therapy with J-5 monoclonal antibody. The dose and duration of antibody infusions are indicated for each patient (○○, granulocytes; •••, lymphoblasts).
marked change in expression of CALLA by leukemic cells. Figure 3 compares the FACS analysis of bone marrow taken before treatment (Fig. 3A) with cells obtained 48 hr after the first infusion of J-5 antibody (Fig. 3B). Cytocentrifuge smears of these populations contained 76% and 81% lymphoblasts, respectively. Whereas leukemic cells were reactive with both J-5 and anti-Ia prior to therapy, only anti-Ia reactivity persisted at 48 hr. Thus, even though bone marrow cellularity was not affected by J-5 antibody infusion, the cell surface phenotype of bone marrow lymphoblasts had changed and these cells no longer expressed CALLA.

Immunofluorescence assay with G/M FITC alone was used to detect J-5 antibody bound to leukemic cells in vivo as a result of intravenous serotherapy. In both Figs. 2 and 3, fluorescence with G/M FITC alone was identical to fluorescence with nonreactive ascites (J-0) followed by G/M FITC, and did not appear to increase during serotherapy. Thus, although leukemic cells persisted in the peripheral blood and bone marrow during intravenous infusion of J-5 antibody, monoclonal antibody was not readily detectable on these leukemic cells. The loss of cell surface CALLA was therefore not due to blocking of antigenic sites by J-5 antibody administered in vivo.

The phenotypic pattern shown in Fig. 2B persisted in all peripheral blood samples analyzed during the 3-day course of serotherapy and for 4 days following the last antibody infusion. FACS analysis of cells obtained 8 days after starting serotherapy, however, was again similar to that found prior to serotherapy and shown in Fig. 2A. At this time, peripheral blood lymphoblasts were again reactive with J-5 antibody, demonstrating reexpression of CALLA by leukemic cells.

J-5 antibody in patient serum was detected by immunofluorescence assay using NALM-1 target cells as described in Materials and Methods. Using this assay, free J-5 antibody was present in all serum samples taken during the 3 days of serotherapy, including serum obtained just before the second and third infusions. Two days following the last infusion, J-5 antibody remained detectable in serum at a 1:10 dilution. Five days after completing serotherapy, however, J-5 antibody was no longer detectable in undiluted patient serum. This was also the first day that CALLA reexpression became evident on peripheral blood lymphoblasts.

Patient 2

Figure 1B summarizes the changes in circulating lymphoblasts and granulocytes that occurred during serotherapy in patient 2. A marked decrease in
lymphoblasts occurred in response to J-5 antibody infusions on the first, second, and fourth days of serotherapy. No infusion was given on the third day. Although there was an average decrease of 22,000 lymphoblasts/cu mm in response to each infusion of J-5, there was also a rapid recovery following each dose of antibody. Twenty-four hours after the last J-5 infusion, total lymphoblast count was 69,400/cu mm compared to 72,400/cu mm just prior to starting serotherapy. There was no consistent change in number of peripheral blood granulocytes, monocytes, or platelets during serotherapy.

The amount of J-5 antibody administered in each infusion was 3 mg (0.1 mg/kg) on days 1 and 2, and 7.5 mg (0.25 mg/kg) on day 4. This represents a 50-fold reduction in the dose of J-5 antibody compared to the amount given to patient 1. In addition, the duration of antibody infusion was shortened to 2 hr on days 1 and 2, and to 15 min on day 4. These changes were made to avoid the persistent antibody excess that had occurred in patient 1. As expected, with these lower doses, J-5 antibody was only detectable in serum obtained during the course of each infusion and for 1–2 hr after each infusion. J-5 antibody was not detectable in patient serum just prior to infusions on days 2 and 4.

Indirect immunofluorescence assay of peripheral blood mononuclear cells showed that, coincident with the decrease in circulating lymphoblasts and each J-5 antibody infusion, there occurred a loss of cells that expressed CALLA. On day 1, when the lymphoblast count fell from 72,400/cu mm to 47,700/cu mm 30 min after the J-5 infusion, the percentage of CALLA-positive cells on FACS analysis fell from 71% to 13%. Twenty hours later, however, the percentage of CALLA-positive cells of FACS analysis had returned to 66%. The number of circulating lymphoblasts had also increased by this time to 60,300/cu mm. This pattern was repeated with each of the following two J-5 antibody infusions.

Although both the numbers of circulating lymphoblasts and percentage of CALLA-positive cells decreased following J-5 antibody infusions, it appeared that the loss of CALLA reactivity was much greater than the decrease in circulating lymphoblasts. At the nadir of the lymphoblast count, following the first J-5 antibody infusion, cytocentrifuge smears showed that lymphoblasts still comprised 67% of the population, but only 13% of these cells were CALLA positive. Subsequently, as CALLA expression by lymphoblasts increased, there was only a slight increase in the percentage of lymphoblasts on cytocen-
trifuge smears by morphological criteria. As with patient 1, Ia expression by lymphoblasts could also be used operationally as an independent surface marker of leukemic cells. Indirect immunofluorescence with anti-Ia varied during serotherapy, as lymphoblasts were cleared from the peripheral blood, but greater than 70% of the remaining cells were consistently Ia positive.

The results of serotherapy in patient 2 were, therefore, similar to the effects noted in patient 1. Although in both patients J-5 infusion resulted in a rapid decrease in circulating lymphoblasts, not all leukemic cells were cleared, and the remaining cells no longer expressed CALLA. The decrease in CALLA expression represented a specific loss, since the expression of Ia antigen did not change. In addition, the loss of CALLA appeared to be temporally related to the presence of J-5 antibody, and reexpression of CALLA occurred when J-5 antibody was no longer detectable in the patient’s serum.

Patient 3

Prior to initiation of serotherapy, J.P. had marked leukopenia without circulating lymphoblasts, and the effects of J-5 infusion on leukemic cells could not be evaluated by examination of peripheral blood. Bone marrow aspirates were obtained before starting J-5 serotherapy and daily during the course of therapy. This patient received 5 mg antibody (0.1 mg/kg) on day 1, 10 mg antibody (0.2 mg/kg) on day 2, and 20 mg antibody (0.4 mg/kg) on day 4. No infusion was given on day 3, and in each instance, antibody was infused intravenously within 10–15 min.

During the course of serotherapy, there was no change in total circulating white blood cells (800/cu mm pretreatment to 800/cu mm posttreatment) or platelets (80,000/cu mm pretreatment to 86,000/cu mm posttreatment). At no time were lymphoblasts seen on peripheral blood smears, and the percentage of CALLA-positive cells in peripheral blood by FACS analysis varied from 0% to 2%. Bone marrow aspirates obtained each day of serotherapy showed replacement of normal bone marrow by leukemic cells and contained between 78% and 84% lymphoblasts. There was no significant change in either cellularity or differential during J-5 antibody therapy.

The cell surface phenotype of bone marrow mononuclear cells was assessed daily during antibody therapy by indirect immunofluorescence and FACS analysis. Bone marrow aspirates were obtained before J-5 infusion on days 1 and 2, and immediately after J-5 infusion was completed on day 4. FACS analysis on days 1 and 2 were identical and demonstrated that greater than 90% of cells were reactive with both anti-Ia and J-5. There was no reactivity with G/M FITC alone, indicating that J-5 antibody was not detectable on bone marrow lymphoblasts 24 hr after J-5 infusion. On day 4, however, when bone marrow cells were examined 15 min after intravenous antibody infusion, J-5 antibody was present on marrow lymphoblasts without detectable free CALLA. These results demonstrate that, although J-5 antibody was not present on lymphoblasts 24 hr after antibody infusion, rapid binding of intravenously administered J-5 antibody to bone marrow lymphoblasts did occur. The finding that bone marrow cellularity did not change despite binding of J-5 antibody to bone marrow lymphoblasts suggests that these cells were not susceptible to antibody-mediated lysis.

Patient 4

The numerical changes in peripheral blood lymphoblasts that occurred during serotherapy are shown in Fig. 1C. Each dose of antibody was given by i.v. infusion and varied from 8 mg (0.1 mg/kg) to 25 mg (0.3 mg/kg). On days 3 and 4, multiple infusions were administered. As shown in Fig. 1C, there was a consistent and rapid decrease in circulating lymphoblasts, which began immediately after each dose of J-5 antibody. The nadir of the lymphoblast count occurred 1–2 hr after antibody infusion and was followed by a rapid return of circulating lymphoblasts to pretreatment levels in 4-6 hr. Increasing the frequency of antibody infusions did not result in delaying the recovery of circulating blasts. The numbers of circulating myeloid cells, normal lymphocytes, and platelets did not change significantly during serotherapy.

The cell surface phenotype of circulating mononuclear cells was evaluated by immunofluorescence assay and FACS analysis during the course of serotherapy (Fig. 4). Each cell population was analyzed directly for the presence of hybridoma antibody with G/M FITC and for C3 with anti-human C3 FITC. In addition, the expression of CALLA and Ia antigens was determined by incubation with either J-5 or anti-Ia, followed by G/M FITC. As the dose of J-5 was gradually increased from 0.1 mg/kg on day 1 to 0.3 mg/kg on day 3, there was a progressive increase in the percentage of cells that had bound J-5 antibody in vivo. C3 was not detectable on circulating leukemic cells on day 1, but on all subsequent days, the percentage of cells coated with C3 closely paralleled the percentage of cells coated with J-5. J-5 and C3 coated cells, however, were only detectable immediately and 1 hr after antibody infusion. As shown in Fig. 4, the presence of murine antibody on leukemic cells preceded the decrease in the percentage of cells in the peripheral blood that expressed CALLA and Ia anti-
These changes in cell surface phenotype, which were coincident with the decrease in circulating blasts shown in Fig. 1C, indicate that J-5 antibody and C3-coated lymphoblasts were selectively and rapidly cleared from the peripheral blood after each antibody infusion. As increasing doses of antibody were administered (days 1–3), the change in phenotype became more evident. The first antibody infusion on day 4 consisted of 25 mg (0.3 mg/kg) and was the same as the first infusion of day 3. As shown in Fig. 4, this infusion resulted in a greater decrease in the percentage of CALLA and Ia-positive cells, but fewer cells were coated with antibody immediately after the 15-min infusion. These data suggest that by day 4, the rate of clearance of antibody-coated cells had increased, perhaps as a result of the more frequent doses of J-5 that were given.

Figure 4 also illustrates the recovery of cell surface phenotype that occurred after each J-5 antibody infusion. In this patient, the numbers of circulating blasts had almost returned to pretreatment levels 4 hr after each dose of antibody. J-5 antibody and C3-coated cells were no longer detectable at this time, and blasts that were present in the circulation expressed both CALLA and Ia antigen.

As with the previous three patients, J-5 hybridoma antibody was assayed in patients’ sera during the course of serotherapy. Free J-5 antibody was detected in undiluted serum 1–2 hr after antibody infusion but was not detectable 4 hr after infusion. As with patients 1 and 2, the presence of circulating J-5 antibody correlated closely with the phenotype of circulating lymphoblasts, and when free J-5 antibody was no longer detectable, there was a coincident reexpression of CALLA by circulating lymphoblasts.

**Toxicity of J-5 Antibody Infusions**

In all four patients, antibody infusions were well tolerated. All patients remained entirely asymptomatic during antibody infusions and no immediate adverse reactions were noted. Patients 1 and 2 developed fevers of 100–102°F between 6 and 18 hr after each antibody infusion. Patients remained asymptomatic during these febrile episodes, which responded to oral acetaminophen. Patient 3 had a low-grade fever (99.2°F) prior to serotherapy that subsequently resolved spontaneously. Patient 4 remained afebrile throughout his hospitalization. There was no other toxicity that could be attributed to serotherapy, including changes in urine analysis, serum creatinine, or hepatic enzymes. No patient developed skin rash, arthritis, or dyspnea.

**In Vitro Studies With ALL Cells**

Although the rapid changes in cell surface phenotype that occurred during serotherapy suggested that CALLA modulation had been induced by J-5 monoclonal antibody, it may also have been possible to account for these changes solely on the basis of in vivo depletion of CALLA-positive cells and emergence of preexisting CALLA-negative lymphoblasts. To study
this possibility, cryopreserved pretreatment lymphoblasts from each patient were placed in culture at 37°C with either J-5 antibody or negative control ascites (J-0). After either 24 or 48 hr, cells from these cultures were washed, counted, and assayed for expression of CALLA by indirect immunofluorescence assay with additional J-5 antibody and G/M FITC. Expression of Ia antigen was detected by incubation with anti-Ia followed by G/M FITC, and presence of hybridoma antibody on the cell surface was detected by incubation with G/M FITC alone. Cells from J-5 cultures and J-0 cultures were treated identically and analyzed on the FACS as previously described. The results of these experiments are shown in Table 1. In each patient, cells that had been incubated in the presence of J-5 antibody subsequently demonstrated a decreased expression of CALLA compared to cells that had been incubated with J-0. Decreased CALLA expression was not due to either loss or death of J-5 reactive cells in vitro, since numbers and viability of cells in J-0 and J-5 cultures remained comparable. In addition, there was no decrease in expression of Ia antigen, indicating that the loss of CALLA was a specific effect and not a general response of all membrane proteins to the presence of monoclonal antibody in vitro.

The ability of leukemic cells that had been modulated in vitro to reexpress CALLA was tested in patient 1. A bone marrow aspirate taken 2 wk after serotherapy demonstrated persistent replacement by leukemic cells, which again expressed CALLA. These cells were incubated with either J-5 antibody or nonreactive ascites (J-0) at 37°C for 72 hr and then analyzed on the FACS to determine their cell surface phenotype. As shown in Fig. 5A, greater than 90% of cells incubated with J-0 expressed CALLA. The phenotype of cells incubated with J-5 (Fig. 5B) was markedly different, and although murine antibody was detectable on 62% of the cells, there was no free CALLA and the total amount of CALLA present on these cells was markedly decreased. These in vitro “modulated” cells were then washed and placed in fresh media without J-5 antibody. After an additional 72 hr at 37°C, reexpression of CALLA was evident on FACS analysis (Fig. 5C). Cells that had been incubated with J-0 and then washed and placed in fresh media showed no change in phenotype, which remained identical to that shown in Fig. 5A. In addition, there was no change in expression of Ia by cells incubated with either J-0 or J-5 (data not shown), and cell number and viability at the end of the experiment were comparable in the J-0 and J-5 treated populations.

DISCUSSION

This article summarizes our experience with the use of a murine monoclonal antibody specific for a common ALL antigen (CALLA) for serotherapy of acute lymphoblastic leukemia. Four patients with relapsed ALL have been treated, and in the three patients with circulating lymphoblasts, there was a rapid clearance of blasts that began immediately upon intravenous infusion of J-5 antibody. In patient 1, who received a total dose of 25 mg/kg, the decrease in circulating blasts persisted during the 3 days of serotherapy and then gradually returned to pretreatment levels over a 2-wk period. Patient 2 received much smaller doses of J-5 (total dose, 0.45 mg/kg) and responded well to each antibody infusion, but the numbers of circulating blasts returned to pretreatment levels with 12–24 hr after each infusion. Patient 4 received a total dose of 1.9 mg/kg over 8 separate infusions, and although there was a rapid response to each dose of antibody, circulating blasts recovered within 4–6 hr after each infusion. There was no detectable change in bone marrow cellularity or differential during serotherapy in any patient.

Table 1. Modulation of Common ALL Antigen Induced by J-5 Antibody In Vitro

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<th>Patient</th>
<th>Incubation</th>
<th>Cell Number/ml</th>
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*Cells were obtained from bone marrow aspirates in patients 1 and 3 and from peripheral blood in patients 2 and 4.
†Cells from patients 1, 2, and 3 were incubated at 37°C for 24 hr, cells from patient 4 were incubated for 48 hr.
‡Viability was determined by Trypan blue dye exclusion.
§Percent positive cells was determined by analysis on the fluorescence activated cell sorter.
Analysis of peripheral blood mononuclear cells on the fluorescence activated cell sorter demonstrated that J-5 antibody was rapidly bound to leukemic cells during antibody infusion. This was best demonstrated in patient 4, where C3, in addition to J-5 antibody, was detected on leukemic cells, which then appeared to be rapidly and selectively cleared. In patient 3, J-5 antibody was detected on bone marrow lymphoblasts within 15 min after antibody infusion.

In addition to demonstrating that J-5 antibody bound to leukemic cells in vivo, we noted that a profound change in cell surface CALLA occurred in response to serotherapy. Thus, although there were marked reductions in numbers of circulating lymphoblasts during serotherapy, not all leukemic cells were cleared, and those cells that remained in the peripheral blood were no longer CALLA positive. In patient 1, the expression of CALLA by bone marrow lymphoblasts was also affected by serotherapy. Furthermore, the change in cell surface phenotype appeared to be related to the presence of free J-5 antibody in patient serum, and when antibody was no longer detectable, both bone marrow and circulating lymphoblasts reexpressed CALLA. These phenotypic changes appeared to be a selective and specific alteration in expression of CALLA without change in other cell surface proteins, such as Ia antigen.

Although the immediate response to serotherapy was dramatic, the recovery of circulating lymphoblasts was unfortunately just as dramatic. Several factors may have been responsible for this rapid development of resistance to serotherapy. Our first consideration was that selection of CALLA-negative lymphoblasts had occurred, but this seemed unlikely for several reasons: (1) prior to serotherapy, there was no evidence to suggest the existence of a distinct and large population of CALLA-negative lymphoblasts; (2) the changes in phenotype occurred so rapidly that they could not easily be explained by the loss of one population with subsequent replacement by another; and (3) the loss of cell surface CALLA appeared to be temporally related to the presence of free J-5 antibody and reexpression of CALLA by leukemic cells occurred as soon as J-5 antibody was no longer detectable in patient serum. In vitro studies were therefore undertaken to determine whether the change in surface phenotype during serotherapy could be the result of antigenic modulation of leukemic cells in response to specific antibody. These studies and others have demonstrated that modulation of CALLA by J-5 monoclonal antibody does occur and suggested that CALLA modulation resulted in resistance of leukemic cells to antibody-mediated lysis in vivo. Although antigenic modulation has been known to be a mechanism whereby murine leukemia cells can evade host immune defenses, this had not been previously demonstrated for human leukemia cells.

In another recent trial of serotherapy, Nadler et
al. demonstrated that tumor cell lysis was inhibited by free circulating tumor antigen that blocked the binding of monoclonal antibody to tumor cells. In our studies, which used smaller doses of antibody, we were unable to detect any serum blocking factors, and J-5 antibody was readily detected on circulating and bone marrow lymphoblasts immediately after antibody infusion on patients 2, 3, and 4. These three patients received much lower doses of antibody (approximately 1%-2%) than patient 1 to prevent prolonged antibody excess and to shorten the length of time that leukemic cells were maintained in a CALLA-modulated (and therefore J-5 resistant) phenotype. Unfortunately, decreasing the dose of antibody and increasing the frequency of administration did not result in improving the response to serotherapy.

Although CALLA modulation occurred in our patients, it may be possible that other factors also contributed to the resistance to serotherapy. Bernstein et al. have suggested that complement-mediated lysis may be an important effector system in the serotherapy of a transplantable murine leukemia with monoclonal antibody. Others have found that FC-receptor-bearing host cells are the primary effectors in serotherapy of murine lymphomas. We have noted that even though J-5 antibody rapidly lyzes CALLA-positive cells in the presence of rabbit complement, lysis with human complement in vitro is minimal. J-5 antibody does, however, activate human complement in vivo as evidenced by the rapid deposition of C3 on antibody-coated cells in patient 4. This suggests that the loss of leukemic cells during serotherapy was not the result of intravascular complement-mediated lysis, but rather may have been due to reticuloendothelial clearance of circulating cells that were coated with J-5 antibody and C3. This hypothesis might also explain the lack of effect of serotherapy on bone marrow lymphoblasts even though antibody was demonstrable on these cells.

Although passive serotherapy did not produce a persistent response in our patients, monoclonal antibodies may, in the future, become useful agents in the treatment of human leukemia. J-5 antibody infusion had a dramatic and specific effect on leukemic cells with minimal toxicity. The lack of persistent response was in part due to antigenic modulation of leukemic cells and perhaps also to the inability to natural effector systems to lyse antibody coated cells in vivo. Further studies of CALLA modulation in vitro may result in the development of methods to either inhibit or utilize antigenic modulation for therapeutic benefit. In this regard, recent studies have demonstrated that antigenic modulation is an unusual property of CALLA that is not shared by other cell surface proteins, such as Ia antigen or HLA. In addition, it may be possible to circumvent the lack of natural effectors by coupling J-5 antibody to either toxins or chemotherapeutic agents. J-5 antibody, which specifically binds to bone marrow as well as circulating leukemic cells, can then be used as a unique delivery system for other, more potent, cytotoxic agents.

ACKNOWLEDGMENT

We are indebted to Felice Coral, R.N., Joan McDowell, and John Daley for assistance in performing these studies and to Luci Grappi and Janice Powers for preparing this manuscript.

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Serotherapy of acute lymphoblastic leukemia with monoclonal antibody

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