Monoclonal Antibodies to Myeloid Differentiation Antigens: In Vivo Studies of Three Patients With Acute Myelogenous Leukemia

By Edward D. Ball, George M. Bernier, Gibbons G. Cornell III, O. Ross McIntyre, Joseph F. O'Donnell, and Michael W. Fanger

Three patients with acute myelogenous leukemia (AML) in relapse were treated with intravenous infusions of one or more purified murine monoclonal antibodies (MoAbs) specific for differentiation antigens on normal and malignant myeloid cells. Three of the MoAbs used were IgM immunoglobulins that react with glycolipids, while the fourth, an IgG2b, reacts with a protein antigen. Peripheral blood leukemia cell counts decreased significantly, but transiently, during treatment. Evidence of in vivo binding of each MoAb to leukemia cells was obtained, although two of the four MoAbs could not be detected in the plasma following infusion, perhaps due to circulating blocking factors. Antigenic modulation was not encountered in these studies.

Several investigators have reported in vivo treatment of human leukemias and lymphomas with murine monoclonal antibodies (MoAbs) directed to a variety of tumor-associated antigens. Patients with acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), T-cell lymphomas, and nodular lymphocytic lymphoma have all been treated in this manner, with variable results. Several obstacles to such therapy have been identified, including temporary internalization (modulation) of the cell surface antigen targeted for therapy and circulating blocking factor(s). While most trials of MoAb therapy have produced only temporary decreases in tumor cell counts, toxicity has been minimal. Of interest is the report of the successful induction of complete remission in a patient with a B-cell nodular lymphocytic lymphoma using a monoclonal antibody directed to the idiotype expressed on the patient’s tumor cells.

We have recently prepared four MoAbs reactive with different normal myeloid differentiation antigens that are also expressed on leukemia cells from patients with acute myelogenous leukemia (AML). In this report, we describe the treatment of three patients with acute myelocytic leukemia (AML) with these MoAbs. These studies were conducted to determine (1) the safety of MoAb administration in patients with AML, (2) changes in concentrations of circulating blasts and polymorphonuclear cells (PMNs), (3) the presence of free and cell-bound MoAb after therapy, (4) rate of clearance of circulating MoAb, and (5) the presence of factor(s) in patient and normal plasma that block MoAb binding to cells. Three of the antibodies employed in this study are IgM and react with glycolipids. To our knowledge, this article describes the first trial of in vivo MoAb treatment with IgM MoAbs, the first report of human MoAb treatment targeted to glycolipids, and the first treatment of AML with MoAb directed to myeloid differentiation antigens. The lack of serious toxicity with these reagents permits numerous future applications of these and other MoAbs.

MATERIALS AND METHODS

Patient Selection

Patients with AML who were refractory to standard chemotherapy or who were poor risks for further chemotherapy were considered for MoAb treatment. Treatment of patients with MoAb was approved by the Committee for the Protection of Human Subjects, and all patients gave informed consent for all aspects of the therapeutic protocol.

Case Reports

Patient 1. R.B. was a 27-yr-old white female who developed AML in September 1981. At that time, her WBC was 8,600/µl with 2% blasts, and her bone marrow was hypercellular, with 15% blasts and 8% promyelocytes. Auer rods were present, and she was classified as FAB type M2. She achieved a complete remission (CR) with cytosine arabinoside (Ara-C), daunorubicin (DNR), and 6-thioguanine (6-TG), but relapsed in February 1982. A second
remission was attained with intermediate doses of Ara-C and \( L-\text{asparaginase} \), but the patient relapsed and MoAb therapy was started.

**Patient 2.** P.S. is a 65-yr-old white male who presented in February 1981 with fatigue, sore throat, and a platelet count of 12,000/µl. Bone marrow was diagnostic of AML (FAB M1), and his leukemic cells exhibited trisomy No. 8 on chromosomal analysis. He achieved a CR with Ara-C, DNR, and 6-TG, but relapsed in January 1982. Following reinduction with intermediate dose Ara-C, transient aplasia complicated by bacterial and fungal sepsis occurred, but leukemic cells reappeared. In May 1982, he was treated with cis-retinoic acid (provided by Hoffmann-LaRoche, Inc., Nutley, NJ) in the hope of inducing differentiation. He developed significant cutaneous toxicity, no improvement in his peripheral counts, and increasing transfusion dependency. He was begun on MoAb therapy in September 1982.

**Patient 3.** L.S. was a 65-yr-old white male who was well until September 1981, when he developed fever, adenopathy, and a rash. At that time, he was found to have a WBC of 46,000/µl, with 48% blasts and a bone marrow consistent with acute myelomonocytic (FAB M4) leukemia. A CR was attained with Ara-C and DNR, and he was maintained with Ara-C, DNR, 6-TG, and vincristine. In June 1982, he had a biopsy-proven recurrence of leukemia in the skin of his right buttock and posterior thigh. At that time, his peripheral blood and bone marrow were normal, and he responded to 1,500 rad of radiation therapy. In October 1982, he developed diffuse arthralgias and increasing cutaneous nodules of the face and neck, which biopsy showed to be leukemic. His bone marrow remained normal, and he was treated with MoAbs.

**Monoclonal Antibodies**

The MoAbs used in this study included PMN 6, PMN 29, and PM-81, all IgM class immunoglobulins, and AML-2-23, an IgG2b MoAb (Table 1). PMN 6 and PMN 29 react with mature granulocytes, while PM-81 and AML-2-23 react with both granulocytes and monocytes. Each of these MoAbs are cytotoxic in the presence of rabbit serum to normal and leukemic myeloid cells. None of these four MoAbs are reactive with the colony-forming unit–granulocyte/monocyte (CFU-GM) or burst-forming unit–erythroid (BFU-E).

PMN 6 and PMN 29 react with blast cells from patients with AML of the M4 subtype, PM-81 with all subtypes of AML, and AML-2-23 with the M4 and M5 subtypes. An IgM and an IgG2b MoAb of irrelevant specificity were used as controls for in vitro studies.

**Antibody Purification**

Hybridoma cells producing each of the above antibodies were grown in the peritoneal cavity of pristane-primed BALB/c mice. Ascitic fluid was collected aseptically and pooled. IgM MoAbs were purified by gel filtration through Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) using sterile pyrogen-free phosphate-buffered saline (PBS) (pH 7.4) as running buffer. AML-2-23 was purified on a protein-A-Sepharose column by elution at pH 7.4 buffered saline (PBS) (pH 7.4) as running buffer. AML-2-23 was purified by gel filtration through Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) using sterile pyrogen-free phosphate-buffered saline (PBS) (pH 7.4) as running buffer.

**Cell Separations**

Leukemia cells from patients treated in this study and cells from normal volunteers were harvested from the peripheral blood as previously described. Leukemia cells were separated by Ficoll-Hypaque gradient centrifugation. Normal polymorphonuclear leukocytes (PMNs) were harvested by sedimentation of Ficoll-Hypaque dense cells in 2% dextran.

**Antibody Binding Assays**

Binding of MoAbs to leukemic and normal cells was determined by indirect immunofluorescence and flow cytometry. Target cells (2 x 10⁶) were incubated with 50 µl of purified MoAb at 20 µg/ml for 30 min at 4°C, followed by the addition of a fluorescein isothiocyanate (FITC) coupled goat F(ab')₂ antibody directed to mouse immunoglobulin (FITC-GAM) (Boehringer-Mannheim, Indianapolis, IN) for another 30 min at 4°C. The cells were then analyzed for fluorescence on the Ortho (Westwood, MA) Cytofluorograph System 50H, with multichannel distribution analyzer 2103 and Ortho 2150 computer systems. Positive binding was defined as fluorescence on MoAb-reacted cells greater than on control MoAb-reacted cells.

**Blocking Studies**

Plasma from leukemic patients and normal controls was studied for the presence of blocking factors that might interfere with the binding of MoAb to cell surfaces. Plasma was incubated with equal volumes of varying concentrations of purified MoAb for 15 min at 4°C prior to addition of this mixture to target cells. Following incubation at 4°C for 30 min, FITC-GAM was added for an additional 30 min at 4°C. Cells treated in this manner were then analyzed by flow cytometry.

**Determination of Patient Antibody Production to Mouse Antibody by Enzyme-Linked Immunosorbent Assay (ELISA)**

MoAbs PM-81, PMN 29, and AML-2-23 were applied to individual wells of 96-well flat-bottom plates at 20 µg/ml in PBS and the plates incubated at room temperature (RT) overnight. The plates were washed with PBS and incubated with 5% bovine serum albumin (BSA) in PBS for 1 hr at 37°C. Control wells were treated with 5% BSA only. After washing, serially diluted normal and patient plasma (pre-, intra-, and posttreatment) were applied in duplicate to MoAb-treated and control wells. The plates were incubated for 2 hr at 37°C and washed with PBS. Alkaline phosphatase-labeled affinity-purified goat anti-human antibodies specific for either IgM or IgG (Kirkegaard & Perry Labs, Inc., Gaithersburg, MD) were added and the plates incubated at RT for 1 hr. The plates were again washed and p-nitrophenyl phosphate disodium (Sigma Chemical Corp., St. Louis, MO) was applied. The plates were observed for the appearance of yellow color, and the optical density (OD) at 405 nm of each well was determined on an ELISA reader after 15 min. The mean OD of duplicates of each serum dilution assayed against BSA alone (background) was subtracted from that of the same dilution assayed against MoAb.

**Monoclonal Antibody Administration**

Purified MoAb was administered by intravenous infusion. The dose to be administered was diluted into a final volume of 500 ml of...
normal saline containing 5% human albumin. Following preparation of the final solution to be infused into the patient, culture for bacteria and fungi was performed. Solutions of antibody were usually administered over 8 hr, while the patient was being monitored closely for changes in vital signs or symptoms of allergic reaction. The duration of infusion in patient 2 was 24 hr on one occasion and 7 days on another (see Fig. 3). In order to minimize potential allergic reactions, patients 2 and 3 were premedicated with 50 mg diphenhydramine hydrochloride and 100 mg hydrocortisone immediately before each MoAb infusion.

Rationale for MoAb Dose and Infusion Rate

The dosage of MoAb was initially calculated based on the amount of MoAb that saturated a given number of leukemia cells in vitro and an estimate of the patient’s leukemia cell burden. We infused each dose over 8 hr because other investigators had noted less toxicity with slow compared to rapid infusions.2 We modified this approach in patient 2 after the first series of infusions revealed favorable effects on the blast cell counts, with little toxicity. Therefore, we increased both the amount and the duration of the MoAb infusion in an effort to produce a more sustained depression in the blast count.

Study Parameters

Patients treated with MoAbs were observed for changes in the white blood cell and differential counts, hemoglobin, platelet count, prothrombin time, partial thromboplastin time, urinalysis, serum creatinine, alkaline phosphatase, glutamic oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), lactic dehydrogenase (LDH), and isoenzyme profile and 5’ nucleotidase.

Antibody Binding

Monoclonal Antibody Therapy of AML

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Free MoAb in the serum of patients during and after treatment was measured by incubating PMNs sequentially with plasma samples and FITC-GAM before, during, and after therapy, and studied by flow cytometry. Blast cells from patients treated with MoAb were harvested at various times during treatment and studied for the presence of cell surface mouse antibody by adding FITC-GAM. Aliquots of the same cell preparations were treated in parallel, with the addition of purified MoAb followed by the FITC-GAM and flow cytometric analysis to quantify free binding sites for MoAb.

A skin nodule biopsy was performed on patient 3 (who had leukemia cutis) during the infusion of the AML-2-23 antibody. Cells from the skin nodule were dissociated by forceps and passed through a steel screen to make a single-cell suspension. Aliquots of these cells were then incubated either with FITC-GAM or with purified AML-2-23 antibody followed by FITC-GAM and studied by flow cytometry.

RESULTS

Monoclonal Antibody Binding

The results of pretreatment cytofluorographic analysis of MoAb binding to the leukemia cells from the patients treated in this study are shown in Table 2. The leukemia cells of patient 1 reacted strongly with the PM-81 MoAb but not with PMN 6, PMN 29, or AML-2-23. The cells of patient 2 reacted strongly with PM-81 and, to a lesser extent, with PMN 29. At the time of this analysis, significant numbers of more differentiated cells (promyelocytes and myelocytes) were present, which probably accounted for the relatively strong reaction of PMN-29 with these cells.

Table 2. Binding of MoAb to Leukemia Cells From AML Patients

<table>
<thead>
<tr>
<th>Patient (FAB Class)</th>
<th>PMN 6</th>
<th>PMN 29</th>
<th>PMN-81</th>
<th>AML-2-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(M2)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>86(41)</td>
<td>0(0)</td>
</tr>
<tr>
<td>2(M1)</td>
<td>11(7)</td>
<td>59(61)</td>
<td>75(99)</td>
<td>22(20)</td>
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<tr>
<td>3(M4)</td>
<td>54(110)</td>
<td>70(110)</td>
<td>92(120)</td>
<td>94(135)</td>
</tr>
</tbody>
</table>

The number in parentheses is the mean fluorescence intensity (MFI) of MoAb-treated cells correct for the MFI of control MoAb-treated cells.

Blast cells from patient 3 obtained at his first relapse reacted with all four of the MoAbs. Binding studies of PM-81 to normal PMNs and blast cells revealed that both cell populations (2 × 10⁶ cells, respectively) showed saturable binding at approximately 3 µg/ml of the PM-81 MoAb.

Effects of Treatment

Patient 1

Following the first infusion (60 mg of PM-81 over 8 hr), the peripheral blood blast cell count fell from 54,000/µl to 22,000/µl (Fig. 1). Twelve hours later, however, the blast cell count returned to 48,000/µl. The next administration of PM-81 (60 mg) produced a depression in the blast count to 19,000/µl. Again, however, the blast count rose to 43,000/µl within 12 hr and to 55,000/µl 24 hr later. While more antibody was being prepared, the patient was treated with DNR (25 mg/sq m), which decreased the blast count to 5,000/µl before it began to rise. On day 10, a 30-mg dose of PM-81 was administered, and her blast count fell from

Fig. 1. Circulating blast count in patient 1 following MoAb PM-81 infusion. The peripheral blood blast cell count is plotted against time in relation to infusion of PM-81 MoAb (II). The amounts of PM-81 infused were 60, 80, 30, and 20 mg, respectively, during each of the indicated treatments. A dose of DNR (85 mg) was given on day 5. The blast cell count decreased to 9,000/µl by day 8 and remained at this level for the 3 days prior to the next MoAb infusion.
Fig. 2. Binding of MoAb PM-81 to blast cells from patient 1 obtained during PM-81 infusion. Blast cells were separated by Ficoll-Hypaque gradient centrifugation and incubated either with FITC-GAM alone (in vivo binding) or with saturating amounts of PM-81 followed by FITC-GAM (in vitro binding) and analyzed on the cytofluorograph. Cells (CON) obtained before therapy were incubated with control IgM MoAb and FITC-GAM and compared to cells reacted with PM-81 MoAb. Evidence of in vivo binding is seen from this analysis, although saturation of all PM-81 binding sites was not achieved. The degree of binding of control cells incubated with PM-81 (not shown) is identical to that of cells exposed in vivo to PM-81, and these in vitro binding curves are superimposable.

Blast cells harvested at 1 hr and 5 hr during the first MoAb infusion and 1 hr after completion of the infusion revealed detectable amounts of the PM-81 antibody bound to leukemia cells. However, addition of saturating amounts of PM-81 in vitro showed that only about 25% of available antigenic sites had been occupied in vivo (Fig. 2). Plasma obtained 1 hr after the infusion was completed revealed high levels of free PM-81 antibody, as determined by the ability of this patient’s plasma to bind to normal PMNs in vitro. These studies suggested that antigenic modulation did not occur during the period of infusion of the PM-81 MoAb, since the number of antigenic sites was unchanged on cells exposed to PM-81 in vivo compared to pretreatment cells. Similar results were obtained during the subsequent infusions, though the percentage of cells demonstrating in vivo antibody binding during the third and fourth infusions was lower than that observed in the first and second infusions.

Patient 2

The first treatment involved a series of infusions of PMN 6, PMN 29, and PM-81 MoAbs; the second treatment consisted of PMN 29 and PM-81; while the final treatment involved the use of PM-81 only (Fig. 3). At the onset of therapy, the patient had a white blood cell count of 18,000/μl, with 22% blasts and 50% myeloid cells at various levels of maturation. The treatment plan was to use two different MoAbs (PMN 6 and PMN 29) to remove more mature myeloid cells before using PM-81, which reacted more strongly with the patient’s blast cells. Infusion of PMN 6 resulted in a drop of the mature myeloid cell (PMNs, bands, myelocytes, and promyelocytes) count from 9,000/μl to 5,000/μl. Another dose of PM-81 (20 mg) produced a modest decrease in white count to 4,000/μl. Due to the patient’s grave clinical condition, which included probable sepsis, the patient and her physicians elected to withhold any further therapy, and she expired 1 wk later.

During the first infusion of the PM-81 antibody to patient 1, she developed an urticarial eruption on her legs, which responded promptly to a single dose of diphenhydramine hydrochloride (50 mg i.v.) and hydrocortisone (100 mg i.v.). Prior to the next three infusions, she was premedicated with these drugs and experienced no further skin reactions or other adverse effects, with the exception of mild febrile reactions.
to 5,000/μl. The following day, treatment with PMN 29 produced a fall in the mature myeloid count from 7,400/μl to 1,600/μl and a decrease in the blast count from 3,400/μl to 1,400/μl. On the third day, PM-81 produced a fall in the mature myeloid count from 6,600/μl to 400/μl and a fall in the blast count from 5,300/μl to 1,400/μl. A return to pretreatment levels occurred within 1 day. Cells from a bone marrow aspirate performed on day 3 revealed no measurable cell-bound MoAb. During this treatment course, toxicity was limited to complaints of back pain during infusion of the PMN 6 antibody, which diminished as the rate of infusion was decreased.

On day 15, infusion of PMN 29 (60 mg over 8 hr) decreased the mature myeloid count from 12,000/μl to 4,100/μl and the blast count from 4,300/μl to 2,200/μl. PM-81 was then administered over 8 hr (60 mg) and 24 hr (210 mg). The blast count remained in the range of 1–2,000/μl, while the mature myeloid count dropped to 0 and remained low for nearly 24 hr. No toxicity, other than mild fever, was observed during this treatment. In view of the prolonged effect on reduction of the blast cell count, a larger dose (600 mg) of PM-81 was given by continuous infusion (4 mg/hr) over 7 days, starting on day 62 (with interruptions for blood transfusions) (Fig. 3). A fresh bottle of MoAb was prepared daily for this longer infusion. The mature myeloid count fell from 17,300/μl to 180/μl and the blast count from 4,500/μl to 1,800/μl. On day 65, when the infusion had been stopped for blood transfusion, major increases in both mature myeloid and blast counts were noted, but these counts fell again upon resumption of the infusion. On day 68, after therapy was stopped, the blast count peaked at 11,400/μl before falling again to the pretreatment level. The serum LDH concentration tripled (200–600 IU/liter, isoenzymes 2 and 3) during each of these treatments. During the infusion of PM-81, MoAb could not be detected in the patient’s plasma or bound to cells from the peripheral blood or bone marrow. On day 64, a bolus of 125I-labeled PM-81 (0.25 mg PM-81, 7 × 10⁸ cpm/mg) was injected and serial measurements of plasma and cell-bound counts were performed. Rapid decrease in free plasma radioactivity occurred (T½ = 3.5 hr), with plasma counts still detectable at 24 hr but not at 48 hr. Peripheral blood cell-bound radioactivity was detectable only immediately after infusion of the 125I-labeled PM-81.

**Patient 3**

This patient was treated with MoAbs PMN 29 and AML-2-23. Since this patient had no circulating leukemia cells at the time of therapy and only a small percentage of blasts in the bone marrow, we monitored normal blood cell counts and investigated the ability of the AML-2-23 antibody to bind to leukemia cells in the patient’s leukemic skin nodules. Reductions in the peripheral blood neutrophil count and monocyte count occurred during infusion of MoAb AML-2-23 (60 mg over 12 hr) (Fig. 4). Infusion of PMN 29 (70 mg over 12 hr) produced a decline in the neutrophil count to 500/μl. Several hours later, infusion of AML-2-23 (60 mg over 12 hr) had no effect on the blood cell counts. Unbound PMN 29 was detectable in plasma following the PMN 29 infusion, but free AML-2-23 was not present during or after the infusion. Serum LDH
concentrations (isoenzymes 2 and 3) increased from 300 to 800 IU/liter after MoAb treatment (Fig. 4). Removal of one of the patient’s leukemic nodules during the last infusion and isolation of these cells in vitro revealed that detectable levels of leukemia cell-bound MoAb AML-2-23 were present. However, the binding sites were well below saturation (approximately 20% of saturation). During infusions of both antibodies, the patient experienced myalgias, arthralgias, and fever, none of which necessitated discontinuation of therapy. During treatment with AML-2-23, microscopic hematuria was noted. However, no change in the serum creatinine or BUN occurred.

Evidence of Plasma Inhibitors of MoAb Binding to Cells

Plasma samples from the patients treated in this study were found to have varying amounts of PM-81 inhibitors (Fig. 5). Binding studies of purified PM-81 in the presence and absence of pretreatment plasma from patient 2 indicated that 2–10 μg/ml of PM-81 was neutralized by a plasma substance, while plasma from patients 1 and 3 and normal donors had no blocking activity. Plasma samples obtained during the treatment of patient 2 with PM-81 did not block PM-81 binding in vitro and did not contain detectable amounts of free PM-81 (as determined by binding to PMNs in vitro). These findings provided presumptive evidence that soluble factors and cell-bound antigen were capable of complexing all of the infused PM-81 MoAb. In contrast, the plasma of patient 1, which did not contain inhibitors of PM-81, did have free MoAb following treatment with PM-81.

Analysis of plasma samples obtained from patient 3 before and after an infusion of AML-2-23 revealed that the quantity of AML-2-23 blocking factor increased after the infusion (Fig. 6). Free plasma AML-2-23 MoAb was not detectable immediately after a 60-mg infusion. These results also suggested that soluble and cell-bound antigens (on skin leukemia cells) were capable of binding all of the infused MoAb. Plasma from patient 2 and from normal donors did not contain significant levels of blocking factors for MoAbs PMN 29 and PMN 6 (data not shown)—findings that are consistent with our ability to detect free MoAb PMN 6 and PMN 29 in the circulation of this patient after infusion of these MoAbs.

Patient Antibody Production to Mouse Ig

Plasma from patient 2 was found to contain human antibody of the IgG class reactive with murine MoAb, first noted on day 62 of therapy. The titer (defined as
the dilution that gave 50% maximal binding) of this antibody increased from 1:100 on day 62 to 1:1,600 on days 73 and 77. This antibody reacted similarly with IgM MoAb PMN 29 and the control IgM MoAb, but did not react with IgG MoAb AML-2-23, suggesting that the patient’s antibody is directed mainly toward μ chain.

**DISCUSSION**

These studies represent the first reported clinical trials of (1) IgM class MoAbs, (2) MoAb therapy in patients with AML, (3) combinations of MoAbs directed toward different myeloid differentiation antigens, and (4) MoAbs directed to glycolipid antigens. No significant toxicity was observed in any of the patients. Perhaps more importantly, the reduction in circulating leukemia cell count and the serum rise of intracellular enzymes indicated that leukemia cell kill was achieved in these patients.

MoAb therapy in mice has demonstrated that IgG (but not IgM) antibodies lead to an enhanced ability of phagocytic cells to mediate leukemia cell lysis,16,17 probably through antibody-dependent cellular cytotoxicity (ADCC) or by opsonization of target cells. Although it is possible that, in the present study, MoAb coating led to transient sequestration of leukemic cells in extravascular sites, the depression of peripheral blood neutrophil and blast cell counts concomitant with a rise in leukocyte LDH isoenzymes indicate that myeloid cell lysis did occur in vivo. The mechanisms of this cytolysis are at present unclear. They probably do not include direct complement-mediated lysis, since the MoAbs employed in this study did not mediate significant cell lysis in vitro using human complement. Furthermore, there is no evidence for the existence of IgM-specific Fc receptors on human neutrophils or monocytes.18 On the other hand, IgM MoAb-mediated cytolysis may occur through fixation of C3b to complement receptors on phagocytic cells in the absence of activation of the subsequent complement components. Alternatively, or in addition, since these MoAbs bind to normal phagocytic cells as well as leukemia cells, bridging of target and effector cells might have occurred without the participation of complement or Fc receptors.

With the introduction of the concept that tumors may issue from a pool of proliferating and self-renewing cells representing only a fraction of the tumor,19 and the increasing evidence that various factors may influence the rate of proliferation and degree of differentiation in cells that have emerged from this pool, our findings gain greater significance. Thus, although it would be desirable to eliminate or greatly reduce the size of the tumor stem cell pool (as presumably occurs in that fraction of patients achieving long-term remissions or cures with chemotherapy), it is possible that treatments eliminating cells at stages beyond the stem cell level could reduce the disease morbidity or could induce kinetic events in the stem cell pool, thereby rendering it more sensitive to chemotherapy.

Following infusion of MoAbs, in vivo binding to leukemia cells, although subsaturating, was demonstrated in each patient. Moreover, in patient 1, the inability to saturate leukemia cells occurred despite the presence of 0.5–1 µg/ml of free PM-81, suggesting the existence of different subpopulations of leukemia or normal myeloid cells with diverse affinities for the antibody. Differences were demonstrated between patients in the quantity of free plasma MoAbs subsequent to administration. Free plasma MoAbs PM 6 and PMN 29 were demonstrable in patient 2, but PM-81 was not detectable during or after infusion in this patient. Free PMN 29, but not AML-2-23, was detectable in patient 3. Clearance of free MoAb could occur by binding to cells, binding to soluble factors, catabolism, or by immunologic elimination resulting from the development of human anti-mouse antibody. Binding to cells and to soluble factors is the most likely reason for the rapid clearance of MoAbs observed in these studies. Elimination due to anti-mouse antibody is likely to have occurred only during the later infusion given to patient 2 when anti-mouse antibody was detected.

Antigenic modulation, a temporary loss of cell surface antigen induced by antibody binding,4 was not noted in these trials. In vitro studies have revealed an insignificant level of modulation of the antigens defined by the MoAbs used in this study. Moreover, cells taken from patients treated with these MoAbs were found to have unchanged densities of antigen. A problem encountered by other investigators reporting in vivo MoAb therapy has been the presence of serum blocking factors, presumably antigen. In the course of these treatments, we have shown that the levels of blocking factors for PM-81 and AML-2-23 in the plasmas of patients 2 and 3 were in the range of 2–10 µg/ml, while blocking factors for PM-81 in patient 1 and PMN 29 in patients 2 and 3 were negligible. It is conceivable that our failure to achieve saturation of leukemic cells was due, in part, to the presence of blocking factors, since the rate of MoAb infusion was 4–10 µg/ml (plasma)/hr. We assume that the soluble blocking factors are antigens similar or identical to those on the cell surface of myeloid cells. In the case of AML-2-23, material from the plasma of patient 3, which specifically bound to an AML-2-23 MoAb affinity column, could block the in vitro binding of AML-2-23 to cells, thus indicating the likelihood that soluble antigen was inhibiting in vivo binding.
In these studies we have demonstrated that IgM MoAbs can be safely administered to patients. Since there are numerous potential in vivo applications of MoAbs, including imaging studies and delivery of cytotoxic agents to tumor cells, this finding provides a rationale for continued investigation with these and similar antibodies. Secondly, apparently because of plasma blocking substances, it was difficult to saturate myeloid leukemia cells with these MoAbs, even when large amounts were used. The occurrence of such blocking factors may be important in explaining the inability of the host’s normal immune system to destroy leukemia cells in vivo. Last, it may be difficult to lyse all leukemia cells using MoAbs alone. An alternative approach to augmenting MoAb efficacy might be to enhance the ability of the immune system to phagocytize antibody-coated cells by increasing the number of monocyte/macrophage Fc receptors. Recent studies have shown that gamma interferon is capable of increasing monocyte/macrophage Fc receptors 10-fold. Approaches to antibody-mediated therapy might involve the coadministration of biologic response modifiers, such as gamma interferon, in conjunction with MoAb. Alternatively, it may be possible to couple MoAbs to cytotoxic drugs, radiopharmaceuticals or toxins, or to use combinations of MoAb therapy and chemotherapy as new approaches to the treatment of the myeloid leukemias.

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