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

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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. However, the induction of human antibody to murine MoAb was observed in one patient who was treated over a 70-day period. Toxicities encountered were minimal and included fever (3 patients), back pain (1 patient), and arthralgias and myalgias (1 patient). This is the first reported clinical trial of (1) IgM MoAbs, (2) MoAb therapy in patients with AML, (3) combinations of MoAbs directed toward different myeloid differentiation antigens, and (4) MoAbs directed to glycolipids. The relative lack of toxicity and the positive effects of MoAb treatment in the reduction of leukemia cell counts permit the continued study of more innovative approaches to the treatment of AML with MoAbs.

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.[1-5] Patients with acute lymphocytic leukemia (ALL),[1] chronic lymphocytic leukemia (CLL),[2] T-cell lymphomas,[3] and nodular lymphocytic lymphoma[4,5] 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[1,6] and circulating blocking factor(s).[2-5] 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.[5]

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).[7-9] 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.[10] 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

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remission was attained with intermediate doses of Ara-C and L-asparaginase,[11] 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.[7,8] None of these four MoAbs are reactive with the colony-forming unit–granulocyte/monocyte (CFU-GM) or burst-forming unit–erythroid (BFU-E).[9] 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.[8,9] 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 3.5.[12] All antibody preparations were passed through a 0.2-μ filter and stored at 4°C. Cultures for aerobic and anaerobic bacteria and fungi were negative. Endotoxin assays, performed by the Limulus amoebacyte lysate assay (Associates of Cape Cod, Inc., Woods Hole, MA),[13] consistently revealed less than 2 ng endotoxin/mg MoAb.

Table 1. Monoclonal Antibodies Employed in This Study

MoAb	Isotype	Specificity: Normal Cells	AML Subclass	Reference
PM-81	IgM	Granulocyte/monocyte	M1-M5	8
AML-2-23	IgG2b	Monocyte/granulocyte	M4-M5	7,9
PMN 29	IgM	Granulocyte	M4	7,9
PMN 6	IgM	Granulocyte	M4	7,9

Cell Separations

Leukemia cells from patients treated in this study and cells from normal volunteers were harvested from the peripheral blood as previously described.[14] 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 (2×10^6) 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 16 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.

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*

Patient (FAB Class)	Monoclonal Antibody (% Cells Positive)			
	PMN 6	PMN 29	PM-81	AML-2-23
1(M2)	0 (0)†	0 (0)	86 (41)	0 (0)
2(M1)	11 (7)	59 (61)	75 (99)	22 (20)
3(M4)	54 (110)	70 (110)	92 (120)	94 (135)

*Binding was determined by indirect immunofluorescence and flow cytometry. MoAb-treated cells were considered positive when fluorescence exceeded the background level detected on cells treated with control MoAb.

†The number in parentheses is the mean fluorescence intensity (MFI) of MoAb-treated cells corrected 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 (2×10^6 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

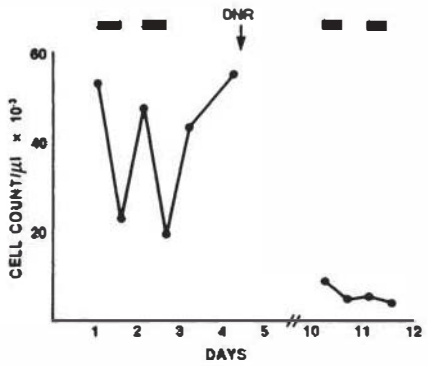

Fig. 1. Circulating blast count in patient 1 following MoAb PM-81 infusion. The peripheral blood blast cell count is plotted against time in relation to infusion of PM-81 MoAb (■). The amounts of PM-81 infused were 60, 60, 30, and 20 mg, respectively, during each of the indicated treatments. A dose of DNR (85 mg) was given on day 6. 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.

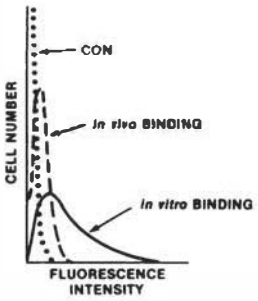

Fig. 2. Binding of MoAb PM-81 to blast cells from patient 1 obtained during PM-81 infusion. Blast cells were separated by Ficoll-Hypaque gradient centrifugation and incubated either with FITC-GAM alone (in vivo binding) or with saturating amounts of PM-81 followed by FITC-GAM (in vitro binding) and analyzed 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.

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.

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

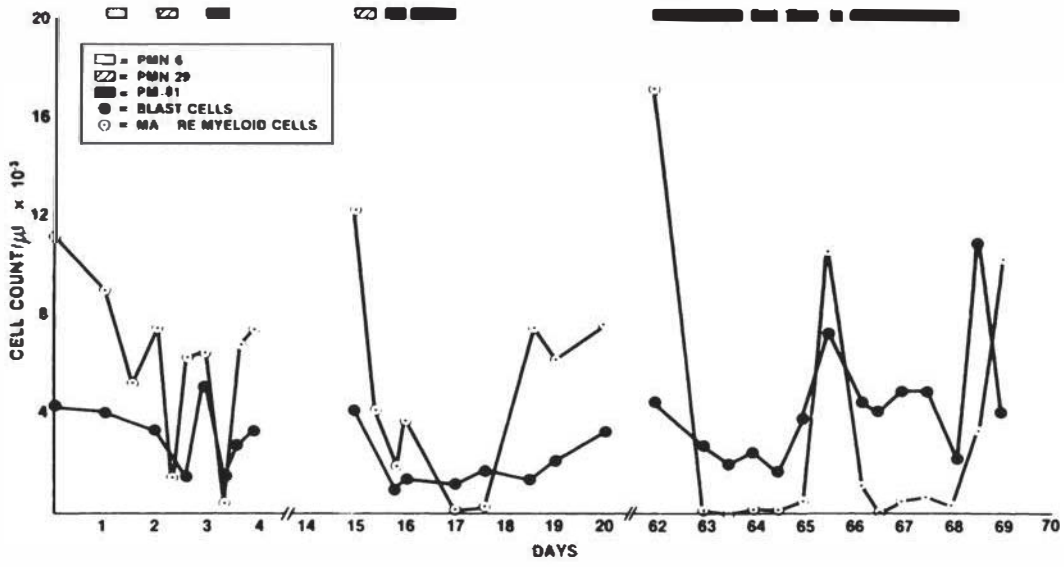

Fig. 3. Circulating blast and mature myeloid cell counts in patient 2 during MoAb treatment.

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 ^{125}I-labeled PM-81 (0.25 mg PM-81, 7×10^8 cpm/mg) was injected and serial measurements of plasma and cell-bound counts were performed. Rapid decrease in free plasma radioactivity occurred ($T_{1/2}$ = 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 ^{125}I-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

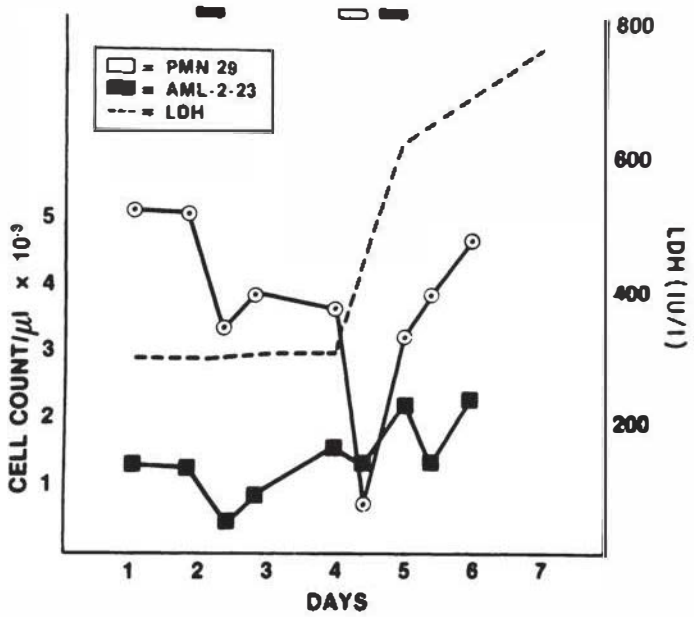

Fig. 4. Circulating blood cell counts and serum LDH levels in patient 3 during MoAb treatment. The effect of treatment on PMNs (○) and monocytes (■) is plotted in relationship to each MoAb infusion.

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