Treatment of Two Patients With B Cell Lymphoma With Monoclonal Anti-Idiotype Antibodies

By E.M. Rankin, A. Hekman, R. Somers, and W. ten Bokkel Huink

Mouse monoclonal anti-idiotype antibodies have been used to treat two patients with progressive advanced B cell non-Hodgkin's lymphoma. Transient falls in the level of circulating malignant cells and idiotype immunoglobulin were produced, and free unbound monoclonal antibody was identified in the serum. Homing of the antibodies to tumor cells in the blood, bone marrow, ascites, and lymph nodes was demonstrated in both patients. Although large amounts of anti-idiotype antibody were given (3.8 g and 5.8 g), no toxic effects were seen, and no antibodies to the foreign mouse protein were made. There was no modulation of the antigen from the tumor cells and no indication of immunoselection. There was evidence of large-scale tumor cell destruction, but only a modest reduction in tumor size. The killing of the tumor cells was mediated by the reticuloendothelial system and not by complement.

HUMAN malignant B cell tumors arise from a proliferation of a single clone of cells. The immunoglobulin (Ig) that is expressed and in some cases secreted by the tumor cells is limited to the expression of one single $V_H$ and $V_L$ region, and to a single light chain. The unique variable region of the Ig, the idiotype, may be considered a model tumor marker, since it is found only on the malignant cells. Antibodies directed against this target, anti-idiotype antibodies, would have the potential to destroy malignant tissue while leaving the residual normal lymphoid tissues intact.

Monoclonal anti-idiotype antibodies have been used by Levy and colleagues for the treatment of eight patients with non-Hodgkin's lymphoma; a durable complete remission was obtained in one patient in whom treatment was free of side effects; partial remissions were induced in five patients. In several patients, problems with anti-mouse antibodies, antigen modulation, or tumor cell heterogeneity were encountered. Polyclonal anti-idiotype antibodies raised in sheep have been given to four patients with B cell leukemia and to three patients with lymphoma. There were transient reductions in the level of circulating lymphocytes, but no long-lasting antitumor effect was seen. After each infusion, there was evidence of antigen modulation with alterations in the density and distribution of the surface antigen, and resistance to complement-mediated lysis.

We have developed a fast and reliable method for the manufacture of mouse monoclonal anti-idiotype antibodies. Studies of the effects in vivo of these antibodies are being performed, and the first results are presented here. The aims of the study are threefold: (1) to determine the immunological effects of treatment and the method by which a response, if any, is produced; (2) to determine the toxic effects of the administration of mouse immunoglobulin; (3) to establish the best schedule for treatment. Such trials are of crucial importance, since even if the monoclonal anti-bodies are ineffective as antitumor agents when used alone, they may still have a role as carriers of toxins, radioisotopes, or chemotherapeutic agents.

MATERIALS AND METHODS

Manufacture of Monoclonal Anti-Idiotype Antibodies

The method by which the monoclonal anti-idiotype antibodies were raised has been described previously. Immunization with spleen cells of patient TOP yielded the antibody T2; after immunization with patient KOS' lymph node cells, two anti-idiotype antibodies, K1 and K2, were obtained. The specificity of the antibodies was proven by the lack of reaction with a variety of normal cells, 14 cell lines representing different stages of lymphoid and myeloid cell differentiation and a wide range of different non-Hodgkin's lymphomas. Each antibody precipitated immunoglobulin heavy and light chains from surface-labeled homologous tumor cell lysates, and their target antigen was capped by anti-light chain antibodies.

From the two antibodies against KOS idiotype, K1 was chosen for treatment, since it had a higher affinity for the tumor cells than K2, as judged by immunofluorescence. Also, unlike K2, K1 did not modulate the antigen as tested by visualizing bound antibody with fluorescein-labeled anti-mouse Ig after various times of incubation. Generally, monoclonal antibodies against idiotype or light chain were found to be less capable of induction of capping and modulation of surface Ig than polyclonal antisera, possibly because of a lower degree of crosslinking of the antigens. T2 and K1 were IgG2a immunoglobulins.

Antibody Purification

Monoclonal antibodies were purified from ascitic fluid of pristane-primed Balb/C mice injected with 2 to $5 \times 10^6$ hybridoma cells. Ascitic fluid was collected aseptically. T2 antibodies were isolated

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by ion exchange chromatography on DEAE Sephadex A50 (Pharmacia, Uppsala, Sweden) in 50 mmol/L Tris/HCl buffer, pH 7.5, with 0.1 mol/L NaCl. Under these conditions, the antibodies did not bind to the column. K1 antibodies were prepared by precipitation with ammonium sulfate at 40% saturation. Both antibody preparations were dialyzed against 0.14 mol/L NaCl, centrifuged for 45 minutes at 100,000 g to remove aggregates, passed through a 0.22-μm filter and stored at 4 °C. The antibodies were 80% to 90% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Cultures for aerobic and anaerobic microorganisms were negative. Absence of endotoxins was tested by the Limulus amoebocyte lysate assay (Malinkroft, Bethesda, Md) and by pyrogenicity tests in vivo in rabbits. Before administration, the antibodies were again passed through a 0.22-μm filter, then diluted to the desired concentration with 0.14 mol/L NaCl with human serum albumin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) as a protein carrier.

**Patients**

**Patient TOP.** In July 1980, a 71-year-old woman was found to have stage IV poorly differentiated diffuse non-Hodgkin's lymphoma with involvement of the lymph nodes, spleen, bone marrow, and a peripheral lymphocytosis (7 x 10^9/L). A partial remission of disease was obtained after several different chemotherapy regimens had been given; systemic treatment was stopped in September 1981. Two courses of radiotherapy were given to the spleen, and one course was given to enlarged nodes in the neck. The spleen was removed in December 1982.

When anti-idiotype therapy was begun in October 1983, there was massive abdominal disease with ascites and generalized lymphadenopathy. The patient complained of fatigue; her Karnofsky performance status was 70. The hemoglobin was 7.7 mmol/L, the platelet count was 230 x 10^9/L, and the white cell count was 19 x 10^9/L with 66% lymphocytes. All surface Ig-positive cells in blood, bone marrow, and lymph node also stained with the anti-idiotype antibody T2. The serum biochemistry, liver function, and renal function were normal except for a creatinine clearance of 40 mL/min. There was no paraprotein band in the serum and no excess of monoclonal light chains in the urine.

**Patient KOS.** A 62-year-old man was seen in October 1979 and a stage IV non-Hodgkin's lymphoma was diagnosed. Cervical node biopsy showed a nodular well-differentiated pattern, and the bone marrow showed a diffuse poorly differentiated lymphoma. A partial remission of disease was obtained with a regimen that combined chemotherapy with radiotherapy to the site of presenting bulk disease.

In March 1983, a node biopsy, performed because of increasing lymphadenopathy, showed a change to a higher-grade malignancy with a diffuse poorly differentiated pattern. A variety of investigational chemotherapeutic agents were tried without success.

When treatment with anti-idiotype antibody was begun in December 1983, the patient had rapidly advancing disease with diffuse lymphadenopathy and with massive involvement of the para-aortic and mesenteric groups, obstruction of the inferior vena cava, and considerable ascites. The patient's Karnofsky performance status was 40. The blood count at the time treatment was begun was: Hb 5.9 mmol/L, platelet count 255 x 10^9/L, white cell count 6.5 x 10^9/L, of which 37% were lymphocytes; of these 16% had x-IgM surface immunoglobulin, and 12% stained with the anti-idiotype antibody K1. K1 reacted with 31% of bone marrow cells. Of the cells isolated from the ascites fluid before treatment, 63% were idiotype positive tumor cells. Serum biochemistry, liver function, and renal function tests were normal; the creatinine clearance was 70 mL/min, and the serum protein immunoelectrophoresis was normal. There was no serum paraprotein band and no excess of monoclonal light chains in the urine.

**Study Parameters**

Blood samples were taken at regular intervals during and after therapy and the following measurements were made: full differential blood count, serum fibrinogen and fibrin degradation products, prothrombin and partial prothrombin times, complement (C1q globulin, C3 globulin, C4 globulin, C3d, CH50 titer, C1q binding test), serum electrolytes, renal function and liver function tests, and serum protein immunoelectrophoresis. Free idiotype Ig, unbound mouse anti-idiotype and human anti-mouse Ig antibody were measured as described below. Radiographs of the thorax and ultrasound scans of other sites of evaluable disease were taken at intervals. Computerized axial tomography (CAT scan) was performed before treatment began and when indicated thereafter. Creatinine clearance was measured regularly, and the urine was examined daily for the presence of blood, proteins, and casts.

The study protocol was approved by the Ethical Committee of The Netherlands Cancer Institute. Both patients gave written informed consent to the trial.

**Antibody Administration**

Thirty minutes before treatment was begun, the patient was given 500 mg aspirin and 25 mg benadryl orally; allopurinol was given to the patients throughout the treatment period in case of massive cell lysis. Before each antibody infusion, a skin test for immediate hypersensitivity to mouse protein was performed with a 0.1-mL solution containing 0.002 mg of mouse antibody. The patients were carefully monitored for any evidence of anaphylaxis or complications of cell destruction such as hypotension, fever, disseminated intravascular coagulation, etc.

**Assays for Idiotypic Ig and Anti-Idiotype Antibody**

The level of circulating free idiotype in the serum or ascites fluid was determined by a solid phase sandwich enzyme immunoassay with biotinyl conjugates of the anti-idiotype antibody. Idiotypic IgM was isolated from KOS serum by affinity chromatography on Sepharose-linked K1, using elution with 0.1 mol/L glycine/HCl, pH 2.5. This was used as reference preparation to quantify the assay of the idiotype in the serum.

The presence of circulating mouse anti-idiotype antibody in serum or ascites was detected by enzyme immunoassay on lymphoma cells fixed in Terasaki microtiter plates. Titration of the antibody preparations used for treatment showed that the limit of detection was 0.2 g/mL. Both methods have been described previously.

**Immunofluorescence**

Mononuclear cells were isolated from peripheral blood, bone marrow, ascites fluid, and cell suspensions of lymph node by centrifugation over Ficoll/Hypaque (Nyegaard, Oslo). The following monoclonal antibodies and polyclonal antisera were used for direct and indirect immunofluorescence, which was performed as described previously: fluorescein-conjugated rabbit anti-human IgG, IgM (Dako, Copenhagen), fluorescein-conjugated goat F(ab), anti-mouse IgG (Tago, Burlingame, Calif), fluorescein-conjugated goat anti-mouse Ig (Nordic Diagnostics, Tilburg, The Netherlands), fluorescein-conjugated rabbit anti-human complement (C3c + C3d) (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, OKT3, OKT4, OKT8 (Ortho-diagnostics N.V., Beerse, Belgium) and fluorescein-labeled anti-T3 antibody (Dr H. Spits, Netherlands Cancer Institute, Amsterdam). The degree of cell saturation in vivo by the anti-idiotype after
treatment was determined by comparing the staining obtained by fluoresceinated anti-mouse antibody with and without a prior exposure in vitro to the anti-idiotypic antibody.

**Punch Biopsy of Lymph Nodes**

Fine needle aspirations of involved lymph nodes were made using a 23-gauge needle attached to a 20-mL disposable syringe held in a pistol grip (Cameco, Sweden). A portion of the material was smeared directly, air dried and stained routinely with Giemsa; the rest was suspended in Dulbecco’s modified Eagle’s medium/fetal calf serum (DMEM/FCS) with heparin (1,000 U heparin per milliliter of medium).

**Cell Kinetic Studies**

Suspensions of lymphocytes from blood, bone marrow, lymph node, and ascites fluid were prepared, and the percentage of S-phase cells was determined by DNA staining with ethidium bromide and flow cytometric analysis by Dr L. Smets, Department of Experimental Cytology.

**Monocyte Functional Assays**

Monocytes were isolated from whole patient blood using the elutriation technique, and were tested for their capacity to mediate antibody-dependent cellular cytotoxicity with anti-D antibody-coated human red blood cells as the target. Spontaneous cellular cytotoxicity using K562 cells as a target was measured in a 5'Cr release assay. Activity of monocytes was measured by chemiluminescence. Five million monocytes were suspended in 250 µL RPMI supplemented with 10% FCS and incubated in ethidium bromide and flow cytometric analysis by Dr L. Smets, Department of Experimental Cytology.

**Indium Labeling of Lymphocytes**

The procedure used for 111In-labeling of the lymphocytes has been described elsewhere. After injection of labeled lymphocytes, blood samples were taken at regular intervals for lymphocyte count and radioactivity measurements, and γ-camera images were obtained.

**Antibodies Against Mouse Immunoglobulin**

Passive hemagglutination was used to test the patients’ serum for the presence of antibodies against mouse immunoglobulins. Human blood group O erythrocytes were fixed with 0.06% glutaraldehyde and coated with mouse immunoglobulin (of the same isotype as that used for treatment) by exposure to 1.2 mmol/L CrCl3. Twenty microliter- aliquots of the red cell suspension were incubated with 20 µL of test serum dilutions for 90 minutes at room temperature in V-bottomed microtiter plates (Greiner, Nurtlingen, GFR). The plates were then centrifuged for two minutes at 225 g, rested at an angle of 60° for ten minutes and then read. In each test, normal human serum, rabbit anti-mouse immunoglobulin (Nordic), normal rabbit serum, and uncoated erythrocytes were used as controls. Rabbit sera were absorbed with uncoated human erythrocytes before the test. A positive hemagglutination reaction was indicated by the persistent formation of a clump of erythrocytes, and a negative reaction by a diffuse band of erythrocytes in the bottom of the well.

**RESULTS**

**Patient TOP**

The effect of different schedules of administration on the circulating lymphocytes and demonstration of homing of the antibody to all tumor sites. Immediately before treatment, immunofluorescence studies showed that 80% of the lymphocytes were B cells with κ IgM IgD IgG surface immunoglobulin. All B cells stained with the anti-idiotypic antibody T2, although as with anti-κ, there was a considerable variation between cells in the strength of the fluorescent reaction. Circulating free antigen was barely detectable in the serum when measured either by the sandwich enzyme immunoassay or by the ability of the serum to inhibit the binding of T2 to TOP cells in immunofluorescence experiments. The effect of different schedules of antibody administration was determined by monitoring the level of circulating lymphocytes in the blood (Fig 1).

The effects of three different schedules of administration were explored. In the first, the antibody was given as an infusion over six hours, the dose being doubled daily. Transient falls in the level of circulating lymphocytes were seen but this was not dose-dependent since 20 mg produced the same proportional fall as did 160 mg. In the second schedule (on day 20) the dose was rapidly escalated from 10 mg/h to 150 mg/h. The lymphocytes fell continuously through the period of the infusion but rose again to pretreatment level when therapy was stopped. In an attempt to prevent this rise at the end of the infusion, a third regimen explored the effects of a rapid bolus dose of 150 or 300 mg followed by a low-dose continuous infusion of 20 mg/h (Fig 1, treatments a, b, and c). Again the fall in the number of lymphocytes, although substantial (on

![Fig 1.](image-url)
Ten milligrams of the antibody was sufficient to coat the blood lymphocytes in vivo as detected by reaction with fluorescein-labeled goat anti-mouse Ig; the tumor cells in the lymph nodes and bone marrow were saturated with T2 during the third regimen. Cryostat sections of a lymph node removed at the end of the infusion on day 63 were examined for immunofluorescence and immunoperoxidase staining with goat anti-mouse Ig antibodies. Only the malignant tumor cells had been coated in vivo with the anti-idiotype antibody. After 1,300 mg of antibody, the cells in the ascites were coated with T2 and they remained so even three days after the end of the infusion. Free T2 antibody was detectable in the serum after the second regimen, declining over 24 hours from a maximum of 12 μg/mL at the end of the infusion. After the third regimen, free T2 was detectable for three days in the serum, but never attained measurable levels in the ascites.

With the aid of fluorescence activated cell sorter (FACS) analysis, fluctuations in the amount and degree of antibody coating of the tumor cells were seen at different times during and after treatment, the reaction frequently being stronger 24 hours after the end of the infusion than immediately at the end of the infusion. The proportional number of T cells in the circulation rose and fell in an inverse relationship with the B cells. There was no change in the fluorescence intensity of the cells stained with OKT3 during or after treatment.

The third schedule was applied three times with total doses of 700, 950, and 1,300 mg. As there was no clinically detectable response, and the peripheral lymphocyte count was rising inexorably, further treatment with anti-idiotype antibody was stopped. Unexpectedly, the lymph nodes afterwards decreased in size, and two weeks later were 10% smaller than they were before treatment was begun. However, the patient's condition had deteriorated, and she was started on prednisolone. Because steroids themselves may exert an antitumor effect, all the data presented here refer to events before the introduction of steroids. The patient died three months after the end of treatment; permission for postmortem was not given.

**Lack of Toxicity of Anti-Idiotype Antibody Therapy**

The patient was closely monitored throughout the treatment period. Apart from one febrile episode of short duration, no symptoms developed that were attributable to the treatment, in particular there were no chills, bronchospasm, or hypotension. There was no hematological toxicity and no disturbance of liver or renal function; creatinine clearance remained at the pretreatment level of 40 mL/min. There was no evidence of activation of the complement pathway or of disseminated intravascular coagulation.

**Absence of Host Response to Mouse Protein**

A total of 3,800 mg of T2, a mouse immunoglobulin of the IgG2a subclass, were given to patient TOP. No antibodies to the mouse protein were detectable even one month after the last treatment.

**Anti-Idiotype Antibody Did Not Modulate the Antigen In Vivo**

Before treatment, the peripheral blood lymphocytes showed considerable variation in the strength of the fluorescent reaction with anti-κ and anti-IgM antisera, and with the T2 antibody. No antigenic modulation was observed in vitro. At the end of the infusions, only cells showing a moderate staining were detectable, but no antigen-negative tumor cells were observed. Twenty-four hours later brightly staining cells were again detectable. To test whether these changes in antigen density were caused by changes in cell populations or by partial modulation, the weaker staining cells found at the end of therapy, when there was excess circulating anti-idiotype, were washed and put into short-term culture in medium. Their reaction with anti-κ or with T2 was tested at various intervals up to 24 hours, but no increase in the strength of fluorescent reaction was seen.

**Observations on the Changes of Tumor Cell Populations**

It was important to establish whether the circulation was repopulated as a result of cell division or by migration of cells from an extravascular reservoir into the blood. Blood lymphocytes showed no change in DNA synthesis by tritium-labeled thymidine incorporation or autoradiography at the time of maximal rebound (data not shown). The percentage of S-phase cells in the blood before and after treatment was unaltered at 2%. In the lymph node, the percentage varied, 7.5% of the cells were in S-phase before treatment, 2% at the end of treatment, and 14% of the cells were in S-phase, most of them in early S-phase, four hours after the end of the infusion (Fig 2). Because at this time a substantial part of the repopulation of the blood had already occurred, it was unlikely that this was caused by the increased proliferation of the lymph node cells.

It is possible that the transient fall in peripheral lymphocytes seen during treatment was the result of...
Fig 2. Flow cytometer histogram showing the number of S-phase cells in the lymph node at different times during the treatment period. (A) Before treatment: 7.5% of the cells are in S-phase; (B) after the infusion of 1,300 mg T2 antibody: 2% of the cells are in S-phase; (C) four hours after the end of an infusion of 950 mg of T2 antibody: 14% of the cells are in S-phase. After the G1 peak, the scale of the ordinate is increased tenfold.

Fig 3. Comparison between the level of circulating In-labeled lymphocytes and the total number of lymphocytes in the blood during treatment with T2 anti-idiotype antibody; A: In-labeled lymphocytes, counted with a γ-counter; : total number of lymphocytes in the blood, counted with Coulter counter.

Antibody-induced alterations by the antibody of the circulation pathways of the tumor cells. This was investigated using peripheral lymphocytes labeled with Indium-111 oxine, as is described in detail elsewhere. Labeled cells (about 80% idiotype positive tumor cells) were injected, blood samples were taken at regular intervals and the total number of lymphocytes (determined by the Coulter counter) and the radioactivity of the labeled cells were compared. This was done at a time when the patient was not receiving treatment and once during treatment with T2 antibodies. Without administration of antibodies, the labeled cells disappeared slowly from the circulation. In contrast, during treatment the labeled lymphocytes showed rapid fluxes into and out of the circulation in the first hours and then disappeared altogether, while the unlabeled cells followed the well-established pattern in this patient of a transient fall and then a return to baseline levels (Fig 3). Serial γ-camera scans showed that during treatment the labeled cells rapidly accumulated in the liver (N.B.: the patient had had a splenectomy).

A remarkable effect of the antibody was seen within the lymph node. Serial aspiration biopsies (kindly performed by Dr P. van Heerde), showed a clear increase in the percentage of cells showing lysis during the period of anti-idiotype therapy. The results are shown in Table I and Fig 4.

Treatment With Anti-Idiotype Antibody Improved Monocyte Function

At intervals before, during, and after the treatment period, a monocyte fraction of the peripheral blood was prepared. The functional properties of these monocytes were measured in two ways, by antibody-dependent cellular cytotoxicity (ADCC) using lysis of rhesus-positive red cells in the presence of an anti-rhesus antiserum as the test system; and by chemiluminescence induced by phagocytosis. As can be seen from Fig 5, there was a dramatic improvement in the efficiency with which the monocytes killed the cells in the ADCC. After therapy, the monocyte function measured in this system was normal, whereas before treatment it was negligible. The phagocytic function of the monocytes also improved during treatment, but had not reached normal levels by the time therapy was stopped (data not shown).
Table 1. The Relation Between Anti-Idiotypic Therapy, Cell Lysis, and Recruitment Into S-Phase in Tumor Cells From Lymph Nodes

<table>
<thead>
<tr>
<th>Day of Treatment</th>
<th>Amount of Antibody Infused (mg)</th>
<th>No. of Lytic Cells* (%)</th>
<th>No. of Cells in S-Phase* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>1</td>
<td>7.5</td>
</tr>
<tr>
<td>20</td>
<td>300 mg</td>
<td>1</td>
<td>Not tested</td>
</tr>
<tr>
<td>23</td>
<td>720 mg</td>
<td>1</td>
<td>Not tested</td>
</tr>
<tr>
<td>40</td>
<td>None</td>
<td>5–10</td>
<td>2</td>
</tr>
<tr>
<td>50†</td>
<td>950 mg</td>
<td>20–25</td>
<td>14</td>
</tr>
</tbody>
</table>

*These two indices were measured on samples taken at the same time from the same lymph node.
†Biopsy was done four hours after the end of the infusion, in the other cases, biopsy was done immediately after therapy was finished.

Patient KOS

When therapy was begun, there was massive abdominal disease causing considerable mechanical problems with ascites and pitting edema to the level of the umbilicus. The patient's poor condition precluded extensive investigation for research purposes. We were concerned that he should have the opportunity to benefit from any therapeutic effect resulting from antibody administration. Our previous experience with patient TOP had been encouraging, since anti-idiotypic therapy in that case had been free from side effects.

Before treatment, the patient's serum contained 300 μg idiotypic immunoglobulin per milliliter. This was used to monitor the effects of treatment, since the number of circulating malignant cells was too low (12% of 2.4 × 10⁹/L lymphocytes) for this purpose. Our plan was to establish as rapidly as possible the amount of antibody required to remove all the free idiotypic, to produce saturation of the lymph node cells and an excess of mouse antibody in the circulation.

No untoward effects were seen with a four-hour trial infusion of 5 mg K1 antibody. The first dose of 50 mg was given over one hour. All subsequent treatments were given at a rate of between 100 to 200 mg antibody per hour. It can be seen from Fig 6 that removal of free idiotypic from the circulation correlated with the detection of free K1 antibody in the blood. K1 was detectable in the circulation at a level of 20 μg/mL at the end of a total dose of 1,200 mg. This free K1 had nearly disappeared by the following morning when a very small amount of free idiotypic was again identified. At the end of the 1,200-mg dose, cells from a lymph node were saturated with K1 antibody in vivo. However, tumor cells from the ascites were not coated with K1, and no free K1 was detectable in the ascites.

During a break from treatment over the Christmas period, the level of free idiotypic returned almost to the pretreatment level. Three more treatments of 800 mg, 600 mg, and 1,000 mg of K1 were given. Transient falls in the level of free idiotypic were seen. Free idiotypic returned to much higher levels over 24 hours than in the first treatment period. Free K1 antibody

Fig 4. Cytological smears from punch biopsies of the lymph node of patient TOP. (A) Before treatment began. Three tumor cells can be seen with large nuclei, dense nucleoli, and small amounts of cytoplasm. (B) At the end of the treatment period. Bizarre cells in various stages of lysis can be seen (→). One cell is undergoing mitosis.

Fig 5. Cytotoxic activity of monocytes isolated from the blood of patient TOP at different times during treatment: 0, before treatment; A, day 12; A, day 26; O, day 52.
was found in the circulation after therapy, reaching a maximum of 5 μg/mL, but it had always disappeared within 24 hours of the end of treatment. The lymph node cells were coated but not saturated with K1 on day 14, but after the 1,100 mg dose on day 15, no anti-idiotype could be detected on the lymph node cells. This time the ascites cells were weakly stained.

During the period of treatment, there was no evidence of antigen modulation by the antibody. Before and after treatment, all B cells reacted with K1; thus, there was no immunoselection. At no time during or following treatment was there any detectable antibody response to the large amount of mouse protein administered. Serial biopsies showed that there was no significant increase in the number of necrotic cells in the lymph nodes after antibody treatment.

No change in the intra-abdominal tumor was seen on tomography, but the femoral and iliac nodes were about 10% smaller after 5.8 g of K1 antibody. Treatment with anti-idiotype antibody was stopped, since increasingly high doses of antibody were required for the same effect. Six weeks later, the patient was started on steroids. He died four months after the anti-idiotype treatment.

**DISCUSSION**

In this study, we report the results of a trial of treatment with monoclonal anti-idiotype antibodies in two patients with advanced lymphoma in an aggressive phase. We have defined the effect of various schedules of administration on the malignant cells, demonstrating homing of the antibody to the different tumor sites. Transient falls in the level of circulating malignant cells and free antigen were produced, and unbound monoclonal antibody was identified in the serum of both patients. In both cases, a minimal tumor response was seen.

Other investigators have observed that the effect of anti-idiotype antibodies has been limited by antigenic modulation, heterogeneity of the tumor or the development of antibodies against the mouse protein. These problems were not encountered in our patients.

Apart from fever of short duration on one occasion, no toxicity was seen in either patient. This contrasts with the experience of others with the use of monoclonal antibodies in vivo, which has been associated sometimes with temporary alterations in renal function, and fever, chills, hypotension, or respiratory distress.

We have been unable to explain why the treatments given to patient KOS on days 14, 15, and 16 were less effective than those given earlier. The level of free idiotype had not substantially altered, there was no increase in the level of circulating tumor cells, and there was no anti-mouse antibody in the serum. Antibody titer and affinity were not different.

The phenomenon of transient falls in the circulating tumor cells resulting from antibody treatment is well documented. In some patients, the level of circulating lymphocytes had risen above the pretreatment baseline by the end of the treatment. The rapid repopulation of the blood by tumor lymphocytes, a phenomenon also seen after leukopheresis is consistent with the presence of an extravascular pool of accessible tumor cells in the tissues that can exchange with the blood.

The low number of S-phase cells in the blood and the low tritium-labeled thymidine incorporation suggests that mature, nonproliferating cells migrated into the blood. The percentage of S-phase cells was higher in the lymph node several hours after the end of treatment. But since the number of malignant cells in the blood had already increased when the lymph node cells were still in early S-phase, it seems unlikely that the repopulation was caused by this enlarged dividing fraction. Rather, the increase in the number of S-phase cells in the lymph node after treatment can be explained by the fact that reduction of malignant cell density increases the proportion of cells in the growth fraction.

The mechanisms by which anti-idiotype antibodies exert their antitumor effects are not established. It is known that macrophages mediate the killing of tumor cells in vitro, in the presence of murine IgG2a monoclonal antibody to the tumor. Both antibodies T2 and K1 were of the IgG2a subclass. The indium-labeling experiments suggest that the reticuloendothelial system was responsible for the removal of circulating tumor cells, a finding in accord with that of oth-
ers. This would explain the persistence of antibody-coated cells in the ascitic fluid. It may be that the massive tumor cell destruction seen during therapy resulted in a shortage of macrophages, thereby producing an “effector cell shortage” limiting the benefits of the antibody infusion. However, time was allowed between the different treatments to permit recovery of the reticuloendothelial system. Unexpectedly, tests of macrophage and monocyte function showed improvement in patient TOP during the period of treatment, with monocyte function returning to normal (Fig 5). In the case reported by Miller et al, a lymph node biopsy before treatment showed infiltration by reactive T cells, more than two thirds of which were “helper” cells. A biopsy of the regressing lymphoma lesions after therapy showed that there were large numbers of macrophages and activated T cells present. In our patients, no change was seen in the low numbers of T cells and macrophages in the nodes.

Nevertheless, in Fig 4 the lysis of tumor cells in a lymph node induced by treatment is clearly seen and is suggestive of a direct lethal effect of the antibody on the malignant cell population. It is unlikely that this effect was mediated by complement, since in vitro the antibodies were not cytotoxic with human complement. Studies in vivo showed there was no alteration in the complement status of either patient, and human complement (C3c + C3d) was not detectable on the surface of antibody-coated tumor cells during treatment.

An essential question requiring further investigation is raised by the studies of Kubagawa et al. They have shown that clonal involvement in several different B cell malignancies could be traced back to the pre-B cell stage, before the expression of membrane Ig. If this is the case, malignant precursor cells will escape from the effect of anti-idiotype antibodies. It is of obvious importance for future therapeutic applications of anti-idiotype antibodies to establish the nature of the tumor stem cell in B cell lymphoma.

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REFERENCES

Treatment of two patients with B cell lymphoma with monoclonal anti-idiotype antibodies

EM Rankin, A Hekman, R Somers and W ten Bokkel Huinink