A Clinical Trial of Anti-Idiotype Therapy for B Cell Malignancy

By Timothy C. Meeker, James Lowder, David G. Maloney, Richard A. Miller, Kristiaan Thielemans, Roger Warnke, and Ronald Levy

Eleven patients with B lymphocytic malignancy were treated with mouse monoclonal anti-idiotype antibodies. All but one of the patients in this study had received extensive prior treatment with conventional lymphoma therapy. All antibodies were prepared against, and uniquely reactive with, the patient’s own tumor. Ten patients were treated with a single antibody, but one patient received three antibodies concurrently. The treatment protocol initially used an escalating dose schedule that was intended to evaluate toxicity, pharmacokinetics and, eventually, to achieve appreciable levels of free mouse antibody in the circulation. The last two patients received substantial initial doses. Tumor sampling was performed before and during therapy to evaluate tissue penetration by antibody. None of the patients had serum paraproteins by routine clinical testing, but six had idiotype protein detectable by a sensitive immunoassay at levels greater than 1 \( \mu g/mL \), two of which were greater than 200 \( \mu g/mL \).

Most human B lymphocytic malignancies are thought to be derived from a single original transformed B cell.\(^1\) This implies that the cells of the tumor uniformly express an immunoglobulin molecule that is unique for each tumor. Antibodies can be made that are directed toward these unique (idiotypic) structures in the variable region of the immunoglobulin molecule.\(^5\) Such antibodies are essentially tumor-specific and are extremely useful for the study of the biology of these malignancies.\(^6\)\(^7\)

Recently, several groups have used mouse monoclonal antibodies in therapeutic trials of human cancer.\(^8\)\(^9\)\(^10\) Anti-idiotype antibodies should have a distinct advantage for this purpose as compared with antibodies directed at other normal differentiation antigens on malignant tissue.\(^11\)\(^12\)\(^13\) Because of their high degree of tumor specificity anti-idiotype antibodies provide an estimate of the ultimate potential of monoclonal antibodies in therapy. We have treated and reported a patient with B cell lymphoma who had a dramatic clinical response induced by an anti-idiotype antibody.\(^21\) In this article, we update his response and describe the results of clinical trials in an additional ten patients.

MATERIALS AND METHODS

Patient Selection

The patients in this study were selected according to two distinct sets of criteria. Patients were first selected for the production of an anti-idiotype antibody if they had: a surface immunoglobulin-positive lymphocytic neoplasm, no serum paraprotein, tumor tissue of \( \approx \) 2-cm diameter easily accessible for biopsy, a projected longevity of at least one year, and absence of other serious medical problems. Once anti-idiotype antibodies became available for these patients, they were reevaluated. They were then required to have disease that was objectively evaluable and a Karnofsky status of \( \geq 20\% \). After three patients were treated, an additional criterion included a serum idiotype protein level of less than 200 \( \mu g/mL \). The standard pretherapy evaluation included physical examination by several investigators, hemoglobin, hematocrit, white blood cell count and differential, platelet count, serum protein electrophoresis (SPEP), serum immunoelectrophoresis (SIEP), quantitative immunoglobulins, a general chemistry survey, urinalysis, creatinine clearance, chest x-ray, biper-
mouse immunoglobulin. To assay serum idiotype, 96-well polystyrene plates (Dynatech, Alexandria, Va) were coated for at least two hours with 50 μl per well of specific mouse monoclonal anti-idiotype antibody at 10 μg/mL in phosphate-buffered saline (PBS). The plates were washed at this point and after all subsequent incubations with 0.9% sodium chloride plus 0.05% NP-40. Idiotype protein at a known concentration was serially diluted in the plate in parallel with patient serum samples and incubated for 60 minutes. Fifty microliters of specific mouse monoclonal anti-idiotype antibody conjugated to biotin was then added to each well at an appropriate dilution and incubated for 20 minutes. Avidin conjugated to horseradish peroxidase (HRP) (Vector, Burlingame, Calif) was then added to each well for 20 minutes. Last, 100 μL of substrate solution (150 μg/mL of 2,2' azinodi-3-ethylbenzylthiazoline sulfonic acid, and 0.001% hydrogen peroxide in 50 μmol/L citric acid buffer pH 4.0) was added per well and incubated in the dark. The optical density of each well was read at 407 nm on a Dynatech MR580 microelisa autoreader interfaced to a Hewlett-Packard 85 microcomputer. Curves generated from each sample were analyzed by a "least-squares fit" program and compared to the standard value.

To assay serum mouse immunoglobulin, microtiter plates were coated with goat anti-mouse immunoglobulin (Tago, Burlingame, Calif) diluted 1:200 in PBS. Patient serum samples and standards were serially diluted in PBS with 1% bovine serum albumin (BSA) in the plate and incubated for 30 to 60 minutes. Goat anti-mouse immunoglobulin HRP (Tago) diluted 1:1,500 was added for 30 minutes, and the plate was then developed with substrate solution and analyzed as above.

To assay human anti-mouse immunoglobulin, a plate was coated with a mouse antibody of the same isotype as the treatment antibody. The treatment antibody could not be used because we wanted to exclude the concurrent measurement of serum idiotype. Patient serum and a standard were serially diluted and incubated for one hour. The next stage was goat anti-human kappa chain conjugated to HRP mixed with goat anti-human lambda chain conjugated to HRP in a 1:3,000 dilution of each (Tago). Development with substrate solution and analysis followed. Values were quantitated by comparison to a standard of 10 μg/mL of affinity purified human anti-mouse antibody taken from a patient from another clinical trial who had made an anti-mouse immunoglobulin immune response.12

**Immunophenotyping of Tumor Cells**

Tumor samples from lymph nodes, spleen, peripheral blood, or bone marrow were obtained at various times before and during monoclonal antibody therapy. Each specimen was divided into portions for routine pathologic analysis, immunoperoxidase staining of frozen sections, and immunofluorescence analysis of single cell suspensions by techniques that have been previously described.14-16

**Plasmapheresis and Leukopheresis**

Plasmapheresis was performed on the first three patients to lower the serum level of idiotype protein. Three to five liters of plasma were exchanged per procedure using either a Haemonetics or IBM machine and replaced with 50% fresh-frozen plasma, 25% normal saline, and 25% albumin. In addition, leukopheresis was performed on patient FS to decrease the number of circulating idiotype-positive cells. Approximately 1011 cells were removed per leukopheresis procedure.

**Therapy**

The patients were routinely premedicated with 650 mg of acetaminophen and 50 mg of diphenhydramine. Antibody doses of less than 10 mg were diluted in 250 mL of 5% albumin. Larger doses of antibody were diluted in normal saline. Antibody infusions were scheduled to be completed in four hours, but some were extended to minimize rate-dependent side effects. The longest infusion took 23 hours.

A thorough physical examination, hematology panel, chemistry panel, urinalysis, and creatinine clearance were done prior to each

### Table 1. Pretherapy Clinical Features

<table>
<thead>
<tr>
<th>Pt/Age/Sex</th>
<th>Diagnosis</th>
<th>Prior Therapy</th>
<th>Extent of Disease</th>
<th>B Symptoms</th>
<th>Associated Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK/67/M</td>
<td>NLPD</td>
<td>Interferon, CVP, bleomycin</td>
<td>Nodes, liver, spleen, scalp nodules</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DLPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS/45/M</td>
<td>PLL</td>
<td>CHOP, splenectomy, leukopheresis</td>
<td>Blood, bone marrow</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BL/36/F</td>
<td>NLPD</td>
<td>Chlorambucil, CVP, BVP, doxorubicin, vinblastine</td>
<td>Nodes, blood, bone marrow, spleen</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>DLPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD/44/M</td>
<td>LCL</td>
<td>Radiation, MTX with rescue</td>
<td>Supraclavicular mass, right lung mass, mid-abdominal mass</td>
<td>—</td>
<td>Mid-abdominal radiotherapy 1.400 rad over seven days completed five days prior</td>
</tr>
<tr>
<td>BJ/40/F</td>
<td>NML</td>
<td>CHOP, BVP, cyclophosphamide, CCNU</td>
<td>Nodes, liver, spleen, blood, bone marrow</td>
<td>+</td>
<td>Splenectomy</td>
</tr>
<tr>
<td>CJ/39/M</td>
<td>NLPD</td>
<td>CVP, C-MOPP, CHOP, chlorambucil</td>
<td>Nodes, blood, liver, spleen</td>
<td>+</td>
<td>Splenectomy</td>
</tr>
<tr>
<td>CP/42/M</td>
<td>NLPD</td>
<td>Interferon</td>
<td>Nodes, bone marrow</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CG/53/F</td>
<td>NML</td>
<td>Interferon, CVP, splenectomy</td>
<td>Nodes, liver, bone marrow</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>TG/36/M</td>
<td>NLPD</td>
<td>Splenectomy, CVP</td>
<td>Nodes, bone marrow, blood</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KL/59/M</td>
<td>LCL</td>
<td>Radiation, CHOP, etoposide, bleomycin</td>
<td>Nodes, nasopharynx, hypopharynx</td>
<td>+</td>
<td>Hypopharyngeal radiotherapy completed five days prior</td>
</tr>
<tr>
<td>PE/38/M</td>
<td>NLPD</td>
<td>CVP, ChlVP</td>
<td>Abdominal mass, bone marrow</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

NLPD, nodular lymphocytic, poorly differentiated lymphoma; DLPD, diffuse lymphocytic, poorly differentiated lymphoma; PLL, polyclonal lymphocytic leukemia; LCL, large cell lymphoma; NML, nodular mixed lymphocytic lymphoma; CVP, cyclophosphamide, vincristine, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; BVP, bleomycin, vincristine, prednisone; MTX, methotrexate; C-MOPP, cyclophosphamide, mustard, vincristine, prednisone, procarbazine; ChlVP, chlorambucil, vincristine, prednisone.
Table 2. Pretherapy Laboratory Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Immuno-phenotype</th>
<th>Immunophenotype</th>
<th>Pretherapy Serum Immunoglobulin (mg/dL)</th>
<th>Mouse Isotype</th>
<th>Highest Single Dose (mg)</th>
<th>Peak Serum Mouse Level (μg/mL)</th>
<th>Total Dose (mg)</th>
<th>Duration of Therapy (d)</th>
<th>Anti-mouse* Binding to Tumor</th>
<th>Response</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>μ, λ</td>
<td>5</td>
<td>IgG2b</td>
<td>120</td>
<td>50</td>
<td>1,530</td>
<td>27</td>
<td>None</td>
<td>LN +</td>
<td>CR</td>
<td>None</td>
</tr>
<tr>
<td>FS</td>
<td>μ, κ</td>
<td>400</td>
<td>IgG1</td>
<td>560</td>
<td>50</td>
<td>2,101</td>
<td>17</td>
<td>(20)</td>
<td>PBL +</td>
<td>PR</td>
<td>Fever, chills, dyspnea, rash</td>
</tr>
<tr>
<td>BL</td>
<td>μ, κ</td>
<td>243</td>
<td>IgG1</td>
<td>900</td>
<td>50</td>
<td>2,101</td>
<td>18</td>
<td>None</td>
<td>PBL +</td>
<td>LN −</td>
<td>Fever, chills, dyspnea, thrombocytopenia</td>
</tr>
<tr>
<td>RD</td>
<td>μ, λ</td>
<td>0.10</td>
<td>IgG1</td>
<td>600</td>
<td>330</td>
<td>1,993</td>
<td>57</td>
<td>None</td>
<td>ND</td>
<td>PR</td>
<td>None</td>
</tr>
<tr>
<td>BJ</td>
<td>μ, κ</td>
<td>0.02</td>
<td>IgG2b</td>
<td>800</td>
<td>107</td>
<td>2,492</td>
<td>29</td>
<td>(17)</td>
<td>LN +</td>
<td>MR</td>
<td>Fever, chills, thrombocytopenia</td>
</tr>
<tr>
<td>CJ</td>
<td>μ, κ</td>
<td>2.20</td>
<td>IgG1</td>
<td>700</td>
<td>136</td>
<td>3,079</td>
<td>40</td>
<td>(24)</td>
<td>PBL +</td>
<td>PR</td>
<td>Fever, rigor, thrombocytopenia</td>
</tr>
<tr>
<td>CP</td>
<td>μ, λ</td>
<td>0.01</td>
<td>IgG1</td>
<td>730</td>
<td>269</td>
<td>3,080</td>
<td>21</td>
<td>(13)</td>
<td>LN +</td>
<td>NR</td>
<td>Fever, rigor, rash, neutropenia</td>
</tr>
<tr>
<td>CG</td>
<td>μ, κ</td>
<td>0.01</td>
<td>IgG1</td>
<td>600</td>
<td>242</td>
<td>3,173</td>
<td>38</td>
<td>None</td>
<td>LN +</td>
<td>PR−MR</td>
<td>Mild chills</td>
</tr>
<tr>
<td>TG</td>
<td>μ, λ</td>
<td>3.26</td>
<td>IgG2a</td>
<td>800</td>
<td>270</td>
<td>1,775</td>
<td>21</td>
<td>(10)</td>
<td>PBL +</td>
<td>BM +</td>
<td>Fever, chills, hypertension, azotemia, facial palsy</td>
</tr>
<tr>
<td>KL</td>
<td>μ, λ</td>
<td>0.01</td>
<td>IgG1</td>
<td>600 t</td>
<td>230</td>
<td>600</td>
<td>7</td>
<td>None</td>
<td>ND</td>
<td>NE</td>
<td>None</td>
</tr>
<tr>
<td>PE</td>
<td>μ, κ</td>
<td>14.50</td>
<td>IgG2a</td>
<td>783</td>
<td>240</td>
<td>3,183</td>
<td>42</td>
<td>None</td>
<td>ND CR−PR</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

*Normal value 45 to 250 mg/dL.
†Normal value 500 to 1,800 mg/dL.
‡Normal value 90 to 450 mg/dL.

antibody dose. Serum samples were collected immediately before, immediately after, one hour, and four hours after each dose. Chest and abdominal x-rays were repeated at least weekly. Computerized tomographic studies were repeated as indicated. Accessible tumor (blood, bone marrow, lymph nodes, or pleural effusion) were sampled when warranted. Clinical responses were measured by objective studies. A partial response was defined as a 50% reduction in all measurable disease, and a complete remission was the absence of measurable disease.

RESULTS

Patient Characteristics

A summary of the clinical features of the treated patients is shown in Tables 1 and 2. Eight patients had nodular lymphoma, one had diffuse poorly differentiated lymphocytic and large cell lymphoma, one had diffuse large cell lymphoma, and one had prolymphocytic leukemia. Three women and eight men, ages 36 to 67 years (mean, 46 years) were treated. Except for the initial patient, PK, the biopsies from which the anti-idiotype antibodies were prepared were obtained between April 1982 and June 1983. These ten patients were treated with antibody from January 1983 to August 1984. The mean time from obtaining tissue to therapy was approximately one year. Ten of 11 patients had been previously treated with multiple courses of chemotherapy and/or radiotherapy. Seven patient’s tumors were progressing despite conventional therapy before they began anti-idiotype therapy. Among the other four patients, CG, who had received no therapy for 18 months, had biopsy-proven progressive liver disease associated with an elevated serum alkaline phosphatase and evidence of slow lymph node enlargement. Patient FS required weekly leukopheresis to maintain control of his disease. Patient TG had relapsed in lymph nodes and peripheral blood (WBC...
100,000) after primary chemotherapy. A single cycle of chemotherapy was administered to decrease serum idiotype and circulating cells but regrowth of disease was documented prior to antibody therapy. Patient CP had received prior therapy only with interferon, to which he had a partial response. Three of the other patients had been treated with interferon, and one of them (PK) had responded on two separate occasions. Five patients had B symptoms characterized by fevers, night sweats, and fatigue.

At the time of therapy, three patients had granulocyte counts of less than 2,000/µL, one patient had a hemoglobin of less than 10 g and five had platelet counts of less than 150,000/µL. Eight patients had hypogammaglobulinemia. Quantitation of IgM, IgG, and IgA showed no consistent pattern of depression. No paraprotein was detected by either SPEP or SIEP. However, the serum of each patient was studied for idiotype with an immunoassay sensitive to approximately 10 ng/mL. This technique revealed small amounts of idiotype protein in most of the patients (Table 3).

Four patients had rapidly progressive disease requiring local palliation proximate to the time of therapy. Patient KL had bulky hypopharyngeal and paratracheal disease compromising his airway. He required endotracheal intubation and 2,400 rad of emergent radiotherapy completed five days prior to antibody infusion. He subsequently died of aspiration pneumonia seven days after a single dose of antibody. Patient RD required 1,400 rad of radiotherapy over seven days to an abdominal mass for pain relief. This therapy was completed five days prior to the initiation of antibody therapy. Patients BJ and CJ underwent splenectomies during antibody therapy for relief of pain and cytopenias. Evaluation of clinical response in these patients relied on anatomic areas independent of these therapeutic interventions. No steroids or cytotoxic agents were administered to any patient from one month before therapy until therapy and subsequent clinical follow-up were complete.

**Plasmapheresis**

Plasmapheresis was used to lower serum idiotype levels in patients PK, FS, and BL. Patient PK, with an initial idiotype level of 2.5 µg/mL, showed transient falls in serum idiotype as a result of plasmapheresis, but the serum idiotype was found to rebound to approximately prepheresis values by the next day. In this patient, three successive pheresis procedures resulted in no sustained effects (Fig 1A). In patient FS, a trial course of plasmapheresis was performed three months prior to antibody therapy to determine whether idiotype could be lowered and to confirm that manipulation of serum idiotype would have no independent effect on his tumor. The results showed that the serum idiotype (IgM) could be reduced approximately 50% by each whole volume plasma exchange and, after three successive daily procedures, a level of 10% of the initial value could be obtained (Fig 1B). However, after discontinuing the procedure, the level rose over seven days to the initial value. Similar results were noted in a repeat pheresis just prior to antibody therapy (Fig 2). Pheresis procedures and results for patient BL were comparable to those for patient FS (Fig 3).

The special circumstances of patient FS allowed a calculation of the rate of production of his idiotype. The tumor and IgM idiotype were confined to the intravascular space allowing the use of a single-compartment model. Assuming constant rates of production and degradation, the half-life of the serum idiotype can be calculated. After pheresis, seven days were required to reach a new steady state (Fig 1B). This is equivalent to four half lives. Using a standard pharmacokinetic equation, his tumor was calculated to secrete 18 mg of idiotype per hour.27

We conclude that some patients can be treated from a more favorable point if plasmapheresis is used. Some patients, however, (such as PK) apparently have a large extravascular pool of idiotype (perhaps those with predominantly extravascular tumor) and cannot
have their serum idiotypic substantially reduced by this approach.

Clinical Trial of Anti-Idiotype Therapy

Rationale. In this study, it was assumed that the anti-idiotype antibody needed to bind to the tumor cells to have an effect. An attempt was therefore made to infuse enough antibody to overcome serum idiotypic and permit penetration of solid tumor tissue. The initial patients on this protocol were treated with an escalating dose schedule. These patients were evaluated for side effects at lower doses before receiving large doses. Serum mouse immunoglobulin levels were followed and used to guide therapy, and antibody doses

Fig 2. The treatment course of patient FS is shown. Both (A) and (B) show the leukopheresis procedures (L), plasmapheresis procedures (P), and doses of antibody. (A) Serum idiotypic and serum anti-idiotype levels. Initially, five days of plasmapheresis reduced the serum idiotypic to 40 µg/mL. Once a plateau of serum idiotypic was reached, anti-idiotype therapy was begun. Small doses of antibody resulted in small decreases in serum idiotypic; 280 mg of anti-idiotype initially drove the idiotypic to zero, but it rebounded until plasmapheresis and anti-idiotype were reinitiated. The maximum level of anti-idiotype (50 µg/mL) was achieved transiently after 800 mg of antibody in two doses. After antibody therapy was completed, the serum idiotypic rose to values above the initial value for approximately one week and then returned to pretreatment values. (B) Change in absolute lymphocytes/µL in the peripheral blood and the anti-idiotype level. Leukopheresis can be seen to effect the lymphocyte count minimally. The first dramatic fall in the lymphocytes occurred with a 7-mg dose of antibody despite persistence of free serum idiotypic. A sudden and dramatic change in circulating tumor was seen when significant anti-idiotype levels were achieved for 48 hours. After antibody infusions were terminated, a very gradual increase in lymphocytes heralded a slow tumor regrowth over the subsequent six months.

Fig 3. Clinical course of patient BL. Plasmapheresis (P), serum idiotypic, and anti-idiotype levels are plotted. Initially, plasmapheresis reduced serum idiotypic to 20% of its initial value. Antibody therapy was begun, and increasing reductions in serum idiotypic occurred with larger doses of antibody. However, idiotypic reductions were transient; therefore, adjunctive plasmapheresis was used repeatedly. After a 680-mg dose, a lymph node biopsy was done and indicated no antibody penetration. A final larger dose was administered, but serum levels rose only to 50 µg/mL. No clinical response was observed in this case.
were administered at two- to three-day intervals to permit modulated cell surface idiotype to be reexpressed. Once high levels of serum mouse immunoglobulin were achieved, further treatment was delayed until the level fell (Fig 4). Accessible tumor in lymph nodes, bone marrow, and peripheral blood was sampled before and during therapy to document that antiidiotype still reacted with the tumor in vitro, to determine if mouse anti-idiotype had penetrated the relevant tumor tissue, and to be sure that antigenic modulation was not defeating the therapeutic goal.

Because the antibody used for each patient was different, each treatment represented a unique pharmacokinetic experiment. Six patients were treated with a mouse IgG1 antibody, two with an IgG2a antibody, and two with an IgG2b. Patient KL received three different antibodies, one of each isotype (Table 3). Presumably, all antibodies differed in affinity for their respective idiotype determinants. The patients also differed in the bulk and major sites of tumor, number of idiotype-positive circulating cells, body surface area, amount of serum idiotype and the kinetics of its production, the status of the reticuloendothelial system, including prior splenectomy, and the kinetics and degree of antigenic modulation. These variables all influenced the disposition of infused antibody.

Basic pharmacokinetics. The correlation between infused antibody dose and serum level achieved is diagrammed in Fig 4 for patient RD. This patient illustrates the relationship in its most simple form because there was no significant amount of idiotype protein in the serum, and the tumor was relatively inaccessible (ie, no bone marrow, blood, or spleen involvement). Small doses of antibody resulted in low, but measurable, serum levels which increased in relation to the given dose. After the second dose of 600 mg, a serum anti-idiotype level of 333 µg/mL was achieved. In this patient, mouse antibody levels declined with a half-life of 4.2 days, a result of metabolic degradation and penetration to extravascular tumor. Two additional patients (CG and CP) had no significant serum idiotype and no circulating idiotype-positive cells. The pharmacokinetics observed in these patients were similar to those in RD. Peak serum concentrations up to 300 µg/mL could be achieved with repeated doses of 400 to 700 mg and the serum half-life of mouse immunoglobulin in these two patients ranged from two to four days.

Anti-idiotype interaction with serum idiotype. None of the patients had a paraprotein level detectable by standard clinical testing. Four patients (PK, CJ, PE, and TG) had 2 to 15 µg/mL of serum idiotype. In these cases, small doses of anti-idiotype antibody produced transient decreases in the serum idiotype followed by rapid clearance of circulating murine immunoglobulin coincident with a rising serum idiotype level. Single doses of 50 to 400 mg eliminated idiotype from the serum. All the tumor responses observed in these patients occurred after the elimination of circulating idiotype and the establishment of free antibody levels (see below).

Two patients (FS and BL) had high idiotype levels of 400 and 300 µg/mL, respectively. This was of concern because of the possibility of toxicity resulting from immune complexes and because of the prohibitive amount of murine antibody that would have been necessary to achieve an excess in the serum. Even after plasmapheresis, the serum of patient FS contained 40 µg/mL of idiotype protein. Because of this, peak serum anti-idiotype levels were attenuated and the half-life of the infused anti-idiotype was short (Figs 2 and 3). Despite large single (BL, 900 mg; FS, 560 mg) and cumulative (BL, 2,101 mg; FS, 1,530 mg) doses, serum idiotype rapidly returned toward pretherapy levels, necessitating continued plasmapheresis during therapy.

Immune response against mouse immunoglobulin. Five of 11 patients made antibody against mouse immunoglobulin between days 10 and 24 of therapy. An immune response was most dramatic in patient CP (Fig 5), the only patient who had not had prior immunosuppressive therapy and one of the two who was not hypogammaglobulinemic. Among the other patients, the development of an anti-mouse response was not predicted by the degree of hypogammaglobulinemia, the absolute number of peripheral mononuclear cells, the percent T lymphocytes, or the blood helper/suppressor T cell ratio. A clinical syndrome often heralded the presence of anti-mouse Ig antibody prior to its detection by enzyme-linked immunosorbent assay (ELISA) (see below). When anti-idiotype was infused in the face of anti-mouse, serum levels of mouse immunoglobulin were lower and the anti-idiotype half-life was shorter. No patient had a clinical response occur after anti-mouse became measurable. Patients who did not make anti-mouse could receive prolonged courses of therapy (Fig 4).

Tissue penetration. Three patients had enough tumor cells in the blood to analyze. Infused anti-idiotype was demonstrated to bind to these cells and cause antigenic modulation. Surprisingly, anti-idiotype could cause cell clearance without first clearing the serum idiotype protein. For instance, in patient FS, a 7-mg dose cleared cells even though there was still a large excess of idiotype protein in the serum (Fig 2). Eight patients had at least one biopsy of a tissue tumor site (six lymph node and two bone marrow)
within 24 hours after a large antibody dose (100 to 680 mg). In six of these cases, mouse antibody could be detected binding to tumor cells in the sample (Fig 6). Modulation could also be demonstrated in several of the samples. Patient BL was the only patient who had no evidence of tissue penetration by antibody.

Penetration into other body compartments was investigated in patient RD. This patient had a chylous pleural effusion associated with a pleural-based tumor mass. As shown in Fig 4, pleural fluid antibody levels were about one tenth of those in the serum. Antibody levels in the pleural effusion continued to rise 24 hours after a large dose, demonstrating delayed penetration into this tissue space. The cerebrospinal fluid contained no detectable antibody 24 hours after a 200-mg dose.

We anticipated that the presence of easily accessible tumor would dramatically alter the serum kinetics of mouse antibody. Certainly, patients FS and BL, who had tumor cells in blood and bone marrow, achieved only low serum levels of anti-idiotypic; however, these patients also had high levels of idioype protein. Patients BJ and CJ both had massive hepatosplenomegaly and similarly achieved only low serum levels. Both of these patients subsequently had their spleens removed, but no major change in peak serum levels and serum half-life occurred, implying that the major site of antibody consumption was not the massive spleen.

Toxicity

The clinical toxicity observed in this study is outlined in Tables 3 and 4. These toxicities occurred in patients who had circulating tumor cells, serum idiotype of a level >1 μg/mL, or an anti-mouse antibody response. Patients who did not have target protein or cells in the blood at the time of infusion could tolerate antibody infused at rates as high as 200 mg/h with side effects. Similarly, patients with residual excess mouse antibody from a prior dose had no toxicity. Most patients experienced side effects at some point, which included chills, fever, transient dyspnea, headache, nausea, emesis, diarrhea, or myalgias. When any of these occurred, antibody infusions were slowed or stopped and the patient was treated with diphenhydramine, acetaminophen, prochlorperazine, meperidine, or oxygen as needed. In almost all cases the infusion could be resumed, initially at a slower rate, but eventually at the same or an even higher rate, without the recurrence of symptoms.

During episodes of clinical toxicity, three patients (CP, TG, FS) had low serum complement levels (C3, C4, CH50). We observed minor reactions in several patients 24 to 36 hours after an infusion when their anti-idiotypic level was falling because of consumption by anti-mouse or idioype. This implied that an equivalence point between antibody and antigen was reached.
Fig 6. Penetration by mouse antibody and antigenic modulation of the tumor cells in patient CG is documented by indirect immunofluorescence and immunoperoxidase techniques. (A through C) The horizontal axis displays fluorescence on a logarithmic scale with increasing signal to the right; the vertical axis displays cell number. The solid lines represent fluorescence of spleen cells pretherapy. The dotted lines represent fluorescence of lymph node cells removed shortly after a 400-mg dose of antibody. Panel A shows an irrelevant control antibody (6GB) followed by fluoresceinated goat anti-mouse immunoglobulin documenting that mouse antibody had reached the patient's tumor cells in vivo. Panel B shows indirect staining with the anti-idiotype antibody (1E11), indicating a decreased maximal signal after therapy consistent with antigen modulation. Both anti-μ and anti-κ were similarly decreased (data not shown). Panel C shows that both tissue specimens contain B lymphocytes (positive with the B1 antibody) that were morphologically malignant. (D through F) Frozen sections of lymph node were stained with the indirect immunoperoxidase method and methylene blue counterstain (original magnification ×200; current magnification ×180). (D through F) Pre-therapy lymph node. No staining is evident with an irrelevant control antibody (D). Intense staining of the entire morphologically malignant population is seen with anti-idiotype antibody (E) and pan-B lymphocyte antibody TD15 (F). (G through I) Post-therapy lymph node. Staining with irrelevant control antibody reveals a positive population of cells in the periphery of the nodule documenting penetration of the lymph node by anti-idiotype antibody (G). The central portion of the nodule stains with anti-idiotype (H), and the majority of cells stain with T015 (I).
and manifested as clinical toxicity at a time remote from an antibody infusion.

Three of the six patients who had serum levels of idiotype protein greater than 1 μg/mL had characteristic side effects with each antibody dose until the idiotype protein had been eliminated. In this group, reactions usually occurred during the first hour of an infusion. Fever and rigor were prominent features of this syndrome and transient thrombocytopenia also occurred.

Four patients had reactions to antibody after they had developed an immune response against mouse immunoglobulin. The initial manifestation of this syndrome often occurred one or two hours after an antibody infusion and one or two days prior to the detectability of anti-mouse antibody in the serum. One patient (CP) received 1,840 mg of antibody over four days after an immune response had begun (Fig 5). The infusions resulted in a sustained neutropenia of less than 500 cells/μL, a diffuse erythematous rash, and a mild transaminase elevation. These symptoms subsided over several days after cessation of antibody therapy. A second patient (TG) was treated after he had development of anti-mouse antibody. He experienced fever, rigor, and hypotension followed by a facial nerve palsy and mild renal impairment (creatinine up to 2.0, BUN up to 43). The hypotension and renal failure resolved after several days, but the facial nerve palsy persisted.

**Clinical Response**

The most dramatic tumor response was seen in the first patient, PK, who remains in an unmaintained complete remission more than three years posttherapy. The treatment course of patient PK has been previously described. Recent assays for idiotype-positive cells in blood and bone marrow and assays for serum idiotype protein are completely negative. Five other patients have had clinically significant responses; these consisted of four partial remissions and one with a 40% reduction of tumor mass. All responses began between eight and 16 days after the initiation of therapy. Except in patient FS, responses were gradual over a period of weeks. The partial responses were of short duration in four patients. Within one to six months, the tumors had returned to their pretherapy states. Patient PE has maintained a 90% reduction of tumor mass for three months and may have a sustained response. Five patients derived minimal or no benefit from the antibody therapy. BJ, TG, and CP developed anti-mouse antibody at about the time tumor response would have been anticipated. BJ had an initial decrease in spleen...
Fig 7. Bone marrow sections and aspirate films from a patient (FS) with prolymphocytic leukemia. Prior to (A through C) and after (D through G) monoclonal antibody treatment. Note the heavy mononuclear cell infiltrate in the marrow biopsy in (A) as compared with (D). Hematoxylin and eosin, ×350. The lymphocytic infiltrate is readily apparent in (B) and inapparent in (E). Wright-Giemsa, ×800. Staining of bone marrow biopsy frozen sections with anti-idiotype antibody highlights numerous tumor cells prior to therapy (C) and infrequent cells after therapy (F) compared with the post-treatment goat anti-mouse control (G). Immunoperoxidase with methylene blue counterstain, ×350.
size that was not sustained. Patient KL died of aspiration pneumonitis seven days after a single dose of antibody, prior to the time a clinical response would have been expected.

The clinical course of patient FS is detailed in Fig 2. His disease had initially involved the spleen, which was removed. Subsequently, a leukemic pattern developed with infiltration of the bone marrow and circulating abnormal lymphoid cells as high as 400,000/µL. His disease proved completely insensitive to chemotherapy. Prior to antibody therapy, he required weekly leukopheresis to maintain his white count at 60,000/µL. More intensive leukopheresis was unsuccessful in further lowering his white cell count despite the removal of 2 x 10^11 cells per day for five consecutive days. By comparison, his response to antibody therapy was dramatic, consisting of a sustained reduction of circulating idiotype-positive cells in his peripheral blood and clearing of the bone marrow. This response did not occur until idiotype was eliminated from the serum and substantial serum anti-idiotype levels were achieved (Fig 2). He achieved a normal total white count, which persisted for more than ten weeks. A bone marrow aspirate and biopsy showed a marked decrease in tumor cells by standard morphology and immunologic staining (Fig 7). However, occasional idiotype positive cells could still be detected. Eventually tumor cells reappeared and the white cell count rose gradually over a period of six months to the pretherapy level. Interestingly, in the week after antibody therapy was discontinued, the serum idiotype protein level rebounded and exceeded pre-treatment levels despite the sustained reduction in tumor cells. No extravascular sites of tumor could be identified. The few remaining tumor cells secreted increased amounts of idiotype protein in vitro (data not shown) but in addition, the metabolism of the idiotype protein may have changed.

Patient RD had explosive disease including a painful abdominal mass which required local therapy prior to antibody therapy. Other sites of measurable disease outside the radiated area included a mass behind his diaphragmatic crura, a pleural-based mass, a chylous pleural effusion and a large fixed left supraclavicular node. His symptoms (abdominal pain, anorexia, and fatigue) abated after the first week of antibody therapy (Fig 4). Ten days into therapy, after the third dose of 50 mg, the supraclavicular mass was palpably smaller. Over the next few weeks, this mass disappeared, and the other sites of disease were gradually reduced. The disease relapsed one month after the last dose of antibody with growth of intraabdominal and pleural tumor, but the supraclavicular node did not recur. Retreatment with monoclonal antibody stabilized the rapidly growing disease, but a second remission was not achieved.

Patient CJ had progressed despite multiple attempts at chemotherapy with massive hepatosplenomegaly, retroperitoneal adenopathy, peripheral adenopathy, circulating idiotype-positive cells and bone marrow involvement. After five doses of antibody, no clinical response was evident, and a splenectomy was performed to reduce his tumor bulk, relieve his pain, and improve his thrombocytopenia. After several higher doses of antibody, a response was documented by lymphangiogram (Fig 8). As the tumor volume decreased, his night sweats and fatigue disappeared. By day 24, an antibody response against mouse immunoglobulin occurred, and his disease began to worsen.

The treatment course of patient CG is shown in Fig 9. This patient had failed chemotherapy and had developed fatigue and right upper quadrant fullness. She had enlarged paraortic and iliac nodes evident on lymphangiogram and progressive hepatic disease that was documented by percutaneous liver biopsy. Her serum alkaline phosphatase level was two to three times normal. Eighteen days into therapy, after five doses of anti-idiotype antibody (up to 400 mg per dose), her serum alkaline phosphatase had fallen from 276 to 98, her LAG had improved, and her symptoms abated. However, within six weeks of completing antibody treatment, lymph nodes regrew and serum alkaline phosphatase rose. A second antibody treatment course resulted in the return of the serum alkaline phosphatase to normal, but there was no further shrinkage of lymph nodes.

Patient PE initially had a massive mesenteric and retroperitoneal mass, subcutaneous nodules, and lymphadenopathy, which completely regressed after CVP chemotherapy. He relapsed intraabdominally and his disease slowly progressed despite further chemotherapy. He was treated with 400 mg of anti-idiotype antibody twice weekly for three weeks and another dose of 784 mg was infused two weeks later. His tumor, as measured by CT scan, shrank dramatically (Fig 10). Bone marrow biopsy, focally positive prior to therapy, was negative four weeks after the first dose of antibody. His serum idiotype level, 14.5 µg/mL pretherapy, has remained undetectable since the initial dose of antibody. His response has persisted for three months after anti-idiotype antibody therapy.

DISCUSSION
This study is an extension of our earlier experience with the use of mouse monoclonal antibodies for cancer therapy.12,13 Anti-idiotype therapy differs in two important aspects from earlier attempts at tumor therapy with other antibodies. First, anti-idiotypes are essentially tumor-specific, whereas most antibody trials in human cancer have been directed at differentiation antigens that are present on both normal and
malignant cells. Anti-idiotype therapy is an important model system to evaluate the added benefit that might be derived when the target is tumor-specific. The price of specificity in this system, however, is that each patient requires a different, "tailor-made" antibody. In a survey of 15 different anti-idiotypes screened on 100 different unrelated lymphomas, we have not detected a cross-reaction. In addition, each patient has biological and clinical features that make him unique. Another potentially important point is that the antibodies used in this trial differed with regard to class, affinity, and epitope recognized on the immunoglobulin molecule of the patient's tumor cells.

Second, anti-idiotype antibodies are potentially capable of exerting functionally significant effects on tumor cells. It has been hypothesized that anti-idiotype antibodies have a critical role in the regulation of B cell growth and differentiation. This hypothesis has been supported by work in many systems for the study of normal and malignant B cell growth. In addition, monoclonal anti-idiotype antibodies can exert a direct antiproliferative effect on tumor cell growth in vitro. It is possible that anti-idiotypes are therefore capable of regulating the growth of human tumor cells, either directly or indirectly via a network of host immune responses.

We have shown that the infusion of high doses of mouse antibody can be performed safely, but it must be done in a careful fashion. Acute side effects generally are correlated with the presence of serum idiotype protein, an anti-mouse immune response or circulating...
idiotypic-positive tumor cells. Serum idiotypic and antimouse immunoglobulin response can both complex mouse anti-idiotypic and cause an acute immune complex syndrome that involves the consumption of complement. The timing of these reactions suggests that a specific ratio of antibody and antigen is needed to cause toxicity. Once this ratio is exceeded and anti-idiotypic excess is achieved, further antibody can be infused without additional toxicity. Clinical manifestations of the interaction of idiotypic and anti-idiotypic consisted of fever, rigor with dyspnea, arthralgias, and headache, with thrombocytopenia occurring less commonly. This syndrome usually occurred toward the start of an antibody infusion. By contrast, the initial manifestations of the interaction of anti-idiotypic with anti-mouse immunoglobulin often followed an antibody infusion and preceded the detection of anti-mouse antibody by several days. The infusion of mouse immunoglobulin in the presence of an established anti-mouse response led to significant clinical problems and resulted in a facial palsy, hypotension, and transient acute tubular necrosis in one patient. A third toxicity syndrome consisting predominantly of dyspnea has been reported by other investigators and is probably caused by the agglutination of antigen-positive cells by antibody.15

We have explored the pharmacokinetics of the infused antibodies. In a patient with no serum idiotypic or accessible tumor in blood or bone marrow, peak serum antibody levels could be predicted by the dose given and the serum volume. The half-life of IgG1 mouse antibodies in such cases was approximately four days. Infused antibody could be consumed by serum idiotypic protein or by circulating tumor cells. In the presence of either, peak serum levels were lower and half-life was shorter. In this trial, no tumor responded significantly until serum idiotypic was eliminated and substantial levels of free antibody were reached.

Plasmapheresis was helpful in removing serum idiotypic prior to antibody treatment for two of three patients in this series. We found that three pheresis procedures were optimal and the serum idiotypic in one patient with primarily intravascular tumor could be reduced by 90%. Future improvements in pheresis or immunoabsorbent technology may extend the usefulness of this maneuver.

We consider serum idiotypic the primary cause of failure of therapy for patient BL. In spite of plasmapheresis and large antibody doses, residual idiotypic protein prevented mouse antibody from reaching lymph nodes (Fig 3). Tumor cells are ultimately the source of the idiotypic protein. Reduction in their number should result in lower protein levels.32 Leukopheresis, as performed in patient FS, was not helpful in decreasing the number of idiotypic positive tumor cells in the blood. However, more intensive leukopheresis...
attempts or the use of other modalities (including cytotoxic chemotherapy) for cytoreduction prior to the initiation of antibody therapy need to be explored further.

The behavior of the serum idiootype in patient FS was somewhat puzzling. He had a dramatic reduction in tumor cell burden (>90%) as judged by blood and bone marrow studies. No other site of disease could be found. In spite of this response, his serum idiootype continued to rebound during therapy, and one week after therapy it rose transiently to levels higher than pretherapy. It is tempting to speculate that exposure to the anti-idiotype antibody caused the few remaining tumor cells to differentiate and to secrete high amounts of protein. Other mechanisms such as selective escape of high secreting cells or altered metabolic turnover of idiotype protein are also possible.

Another obstacle to therapy with murine antibody is an immune response against mouse immunoglobulin. This was seen in five of 11 patients. No patient initiated or continued a tumor response after antimouse antibody appeared. Because of this and because of the toxicity we observed, we now consider antimouse antibody to be a reason to terminate therapy.

Our clinical results to date remain encouraging. Six of 11 patients with a variety of different types of B cell tumor achieved significant responses despite the fact that all of the patients in the study had tumors which were far advanced and most were refractory to standard therapies. The responses were documented by objective studies and correlated with symptomatic improvement. However, the duration of these responses has been short in four of the patients treated. Two responding patients have been treated a second time with definite stabilization of disease, but in neither was a second remission achieved.

At this point, with a limited experience, we can only speculate about the variables that determine success or failure of this therapy. Clearly, the negative impact of serum idiotype protein, tumor bulk, and anti-mouse immune response have been documented here. In addition, we have recently observed that a significant fraction of patients with follicular lymphoma have more than one clone of tumor cells, each expressing a different idiotype. Furthermore, it is apparent that within a given B cell clone, there can be idiomorphic heterogeneity as a result of somatic mutations in the V region. Preliminary results indicate that this latter phenomenon played a significant role in the escape of patients CJ and CG tumors from therapy in the current study.

The full impact of idiomorphic heterogeneity, resulting either from multiple clones or from V region mutations, needs further study in the current series of patients and in the future. To circumvent this problem, it may become necessary to use more than one anti-idiotype antibody for each patient.

A better understanding of the mechanisms of antitumor effect of anti-idiotype antibodies is also necessary if the clinical results are to be improved. In many patients with B cell lymphoma, particularly follicular lymphoma, there is evidence of ongoing control of the tumor by the host. For instance, it has been pointed out that the B cell tumors are heavily infiltrated with T cells and other reactive cells. In addition, spontaneous remissions of follicular lymphoma are well documented. Whatever the mechanism of anti-idiotype effect, it may be augmenting a preexistent antitumor process in the host. The different intrinsic biological properties of the tumors as well as the different types of host tumor interaction are important in determining outcome of antibody therapy. For instance, it is unlikely that the complete and durable response in patient PK is caused solely by the direct killing of tumor cells by antibody in conjunction with complement or host effector cells. Rather, a prolonged active process of tumor control must have been triggered. The kinetics of the response in patient FS, on the other hand, is consistent with a direct effect of antibody on the tumor cells. Efforts are currently in progress to define the variables that predict clinical outcome so that the full potential of this modality may eventually be realized.

ACKNOWLEDGMENT

We would like to thank Carol Doss, Mary Kroos, Jane Bindl, Julie Gralow, and Tryg Stratte for technical assistance, and the nursing staff of the Stanford Clinical Cancer Research Center for enthusiastic support.

REFERENCES

ANTI-IDIOTYPE THERAPY


A clinical trial of anti-idiotype therapy for B cell malignancy
TC Meeker, J Lowder, DG Maloney, RA Miller, K Thielemans, R Warnke and R Levy