Monoclonal Anti-Idiotype Antibody Therapy of B-Cell Lymphoma: The Addition of a Short Course of Chemotherapy Does Not Interfere With the Antitumor Effect Nor Prevent the Emergence of Idiotype-Negative Variant Cells

By David G. Maloney, Sherri Brown, Debra K. Czerwinski, Tina Marie Liles, Sarah M. Hart, Richard A. Miller, and Ronald Levy

The Ig idiotype of B-cell lymphoma can be used as a tumor-specific target. Prior trials with monoclonal anti-idiotype antibodies alone and combined with r-interferon have shown significant antitumor activity. In some patients, idiotype-negative tumors emerged after treatment. In this trial, patients with relapsed non-Hodgkin's lymphoma were treated with two identical courses of monoclonal anti-idiotype antibody therapy. Concurrent with the second course, at a time when idiotype-negative cells were suspected to be proliferating, a pulse dose of chlorambucil was administered. Tumor biopsies obtained before the first and second courses of treatment and at relapse were analyzed for idiotype expression and proliferation. Thirteen patients received 24 courses of antibody with minimal toxicity. Eleven had tumor regression, with 1 complete remission, 8 partial remissions, and 2 minor remissions, with freedom from progression lasting a median of 7 months in responding patients. Idiotype-negative tumor cells appeared in some relapse specimens despite the use of chlorambucil. In retrospect, this was not surprising because there was no increase in the proliferative rate of these tumors at the time the drug was used. Anti-idiotype antibodies continue to demonstrate antitumor activity against B-cell lymphoma with minimal toxicity. The mechanism of the effect is presumed to involve both direct antiproliferative effects of the antibody on the tumor cells as well as indirect, more long-lasting effects on the host. The addition of a mild chemotherapeutic agent in the dose and schedule used here to the second cycle of antibody therapy did not interfere with the antitumor effect, nor did it decrease the emergence of idiotype-negative cells.

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HUMAN B-CELL non-Hodgkin's lymphomas (NHLs) provide, through clonal expression of cell surface Ig, a target for monoclonal antibody (MoAb) therapy. The combination of antibody heavy and light chain variable region segments creates unique antigenic determinants (collectively termed the idiotype) that can be used for the production of monoclonal anti-idiotype antibodies. These antibodies have been shown to identify cells bearing the tumor idiotype both in peripheral blood samples and in tissue sections, as well as detecting low levels of secreted idiotype bearing Ig in the serum.

We, and others, have shown that monoclonal anti-idiotype antibodies can be prepared against a patient's tumor-specific idiotype determinants. These antibodies have significant antitumor activity in patients with B-cell lymphoma. We have previously reported trials of anti-idiotype antibody alone and in combination with r-interferon (r-IFN). Additional studies with radiolabeled or toxin-conjugated antibodies directed against the idiotype or other cell surface molecules also have shown antitumor activity.

An important problem limiting the effectiveness of this therapy has been the presence in most patients of small numbers of tumor cells with mutant forms of the surface Ig that can escape the effects of the MoAb. In previous trials, comparison of tumor biopsies pretreatment and posttreatment has shown the emergence of tumor cells unreactive with the treatment antibody in approximately half of the patients treated either with anti-idiotype antibody alone or with anti-idiotype antibody combined with r-IFN. In all but one case, the cells retained their surface Ig. Alteration in the idiotype has been shown to result from somatic mutation in the Ig variable region genes, which is a property of normal B cells as well as certain types of B-cell tumors. The change in tumor composition toward idiotype-negative cells is evidence for a strong selective pressure from the anti-idiotype antibody. It can occur rapidly, and has been documented as soon as 1 month after treatment.

In the current trial, we attempted to decrease the emergence of idiotype-negative variants by combining a short course of a cell cycle-active chemotherapy with the MoAb therapy at a time when idiotype-negative cells were suspected to be proliferating. Whenever possible, samples of tumor cells were obtained before each course of therapy and at the time of tumor relapse. The tumor cells were analyzed for idiotype expression and for the percentage of cells in various phases of the cell cycle. This study confirmed the antitumor effect of monoclonal anti-idiotype antibodies that were unimpeded by the chemotherapy. However, the single pulse of chemotherapy used at the time chosen was not sufficient to prevent the outgrowth of idiotype-negative variant tumor cells.

MATERIALS AND METHODS

Patient selection. All patients included in this trial gave informed consent in accordance with the guidelines established by the Human Investigation Committee at Stanford University Medical Center. Eligibility criteria for entrance into the study to begin preparation of a custom anti-idiotype antibody included diagnosis of recurrent NHL, a peripheral lymph node accessible for biopsy of
greater than 2 cm in diameter, a projected survival of longer than 1 year, and the absence of concurrent malignancy or other major medical problems. Immunophenotyping of the biopsy was performed to confirm the presence of clonal surface Ig. After take biopsy, patients were treated in the customary fashion with chemotherapy and/or radiation therapy, as indicated by their clinical situation. Later, when custom monoclonal anti-idiotypic antibodies were available for therapy (see below), the patient was reevaluated. Eligibility criteria for the patient to embark on treatment included measurable disease, a serum idiotypic protein level of less than 100 μg/mL, and a repeat lymph node biopsy or fine needle aspiration confirming continued reactivity of the anti-idiotypic antibodies with the tumor cells. The anti-idiotypic antibody or antibodies were required to react with greater than 90% of the tumor cell population as determined by flow cytometry. In some cases, more than one MoAb was needed to achieve this goal. Patients had not received therapy for their tumor for at least 4 weeks and had stable or progressive disease before beginning the experimental treatment. Thirteen patients fulfilled these criteria and were entered onto this trial.

Monoclonal anti-idiotypic antibodies. Custom made, patient-specific, murine monoclonal anti-idiotypic antibodies were produced by IDEC Pharmaceuticals Corp (Mountain View, CA) by methods previously described in detail. Antibodies used in treatment were greater than 90% pure IgG and were prepared in accordance with an Investigational New Drug Exemption (IND).

Trial design. Each patient received two courses of monoclonal anti-idiotypic antibody therapy separated by a 4-week interval. Each course used exactly one-half of the available antibody. A 5-day course of chemotherapy with chlorambucil (16 mg/m²/d) was initiated with the beginning of the second course of antibody.

Patients received therapy in a hospital setting. Acetaminophen and diphenhydramine were administered before antibody infusion and every 4 hours as needed. Anti-idiotypic antibody was administered over 4 to 8 hours by intravenous (IV) infusion using a 0.22-μm in-line filter at a maximum rate of 150 mg/h. Treatment was administered every other day until one-half of the total available antibody was used. The initial dose of antibody was 225 mg (rounded to the nearest whole vial), with subsequent doses escalated in each patient based on sequential monitoring of serum murine antibody levels. Prior experience with responding patients indicated that building trough levels and sustained antibody excess was desirable. Accordingly, doses were increased by 100 to 150 mg to achieve 48-hour (trough) levels of murine antibody of greater than 25 μg/mL and immediate postinfusion peak levels of greater than 200 μg/mL. Because the pretreatment serum idiotypic level and the tumor bulk of each patient varied, the antibody doses were individualized in each patient. The number of antibody infusions ranged from 6 to 10 in each course and depended on both the doses used and the availability of antibody. The second course of antibody therapy was administered using the identical doses and schedule as the first course, irrespective of the serum levels achieved.

The serum level of idiotypic and murine anti-idiotypic antibody were monitored immediately before, immediately after and 24 hours after each infusion by methods previously described. Complete blood count (CBC), general chemistry panel, and platelet count were obtained before each antibody infusion. Staging with physical exam, chest x-ray, lymphangiogram, and computed tomography (CT) scans of disease sites (as appropriate) were performed before each cycle and monthly until disease progression was noted. Bone marrow biopsies were performed at study entry and were repeated if all other measurable disease sites responded completely. Repeat lymph node biopsies or fine needle aspirations were performed before the second course of treatment and at the first evidence of tumor relapse or progression in all patients with accessible tumor sites. Cells from these matched tissue specimens were analyzed for surface idiotypic expression and proliferative fraction as described below.

Clinical responses were scored according to objective measurement of disease. A complete response (CR) was defined as the disappearance of all detectable sites of disease. A partial response (PR) was defined as greater than 50% reduction in the sum of the product of the perpendicular diameters of all the measured lesions. A minor response (MR) was defined as a reduction of less than 50% but greater than 25%. Responses were required to last at least 1 month with the observation of response noted on two separate occasions. Freedom from progression was scored from the time treatment was initiated until the time disease progression occurred. Disease progression was defined as the increase in size of any lesion of greater than 25% of the product of the perpendicular diameters or the appearance of any new lesion.

Immunophenotyping. Immunofluorescence staining of single-cell suspensions of tumor tissue samples was performed using a variety of MoAbs and F(ab')2 fragments of goat antihuman Ig chains and analyzed as previously described by flow cytometry. An estimate of the number of T cells, B cells, and idiotypic-positive and idiotypic-negative tumor cells was made by staining with anti-T-cell reagents, anti-light chain reagents, and anti-idiotypic antibodies. Antigen expression in frozen tissue specimens was also analyzed by the immunoperoxidase staining method, as previously described.

Cell cycle analysis. Matched tissue samples from pretreatment, from pre-course II, and from the time of tumor progression were obtained from six patients. Two-color immunofluorescence staining was used to analyze both surface antigen expression and DNA content simultaneously. Mononuclear cells were isolated by centrifugation over Ficol-Hyphaque, washed, and resuspended in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) with 0.05% sodium azide. Cell surface antigens (surface Ig, idiotype, CD3, CD4, CD8) were identified either directly, by using fluorescein-conjugated antibodies, or indirectly, by unlabeled antibodies followed by fluoresceine-conjugated goat F(ab')2 antimmouse Ig (Caltag, South San Francisco, CA). After washing, the cells were fixed in ice-cold 100% methanol for 30 minutes. For DNA staining, the cells were then washed and resuspended in PBS with 1% BSA, sodium azide, and 5 μg/mL propidium iodide (Sigma, St Louis, MO). Cells were incubated for at least 30 minutes before analysis and a minimum of 50,000 cells were analyzed using a FACS 440 (Becton Dickinson, San Jose, CA). The percentages of cells identified with greater than 1N DNA was then determined for the idiotypic-positive and idiotypic-negative tumor cell populations. An excitation wave length of 488 nm was used. Green fluorescence was detected using a 530/30 band pass dichroic filter and red fluorescence was detected using a 645/35 filter.

RESULTS

Therapeutic trial of anti-idiotypic antibody and chlorambucil. A total of 13 patients were treated and are evaluable. Clinical characteristics of these patients are shown in Table 1. All but one patient had failed multiple prior treatment regimens. Two patients had received anti-idiotypic antibody under earlier trials. Twelve of 13 patients had an initial diagnosis of low-grade follicular small cleaved cell (number 8) or follicular mixed lymphoma (number 4). Nine of 13 patients were biopsied at the beginning of therapy, three to follicular mixed, four to diffuse large cell, one to follicular large cell, and one to diffuse mixed large
<table>
<thead>
<tr>
<th>Patient No./ Sex/Age</th>
<th>Tissue Diagnosis</th>
<th>Prior Therapy</th>
<th>Disease Sites</th>
<th>Antibody Isotype</th>
<th>Pre-Rx Id Level (μg/mL)</th>
<th>Antibody Used Each Course (mg)</th>
<th>Doses Achieving Target Serum Levels</th>
<th>Target Serum Levels</th>
<th>Tumor Response</th>
<th>Freedom From Progression (mo)</th>
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<tr>
<td>C01/F/38</td>
<td>FSC</td>
<td></td>
<td>Lymph nodes, Bone marrow</td>
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<td>4.2/1.5</td>
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<td></td>
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<td>FSC, FLC, F&amp;DLC</td>
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<td>Lymph nodes, Skin, Kidneys</td>
<td>IgG1</td>
<td>4.8/5.0</td>
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<td>MR, PR</td>
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<td>&lt;0.1/&lt;0.1</td>
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<td>SD, PR</td>
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**Abbreviations:** FSC, follicular small cleaved cell; FM, follicular mixed large and small cell; FLC, follicular large cell; DLC, diffuse large cell; N & Di, nodular and diffuse intermediate cell; DM, diffuse mixed large and small cell; Chl, chlorambucil; CVP, cyclophosphamide, vincristine, prednisone; BCEPP, bleomycin, cyclophosphamide, etoposide, procarbazine, prednisone; XRT, radiation therapy; Mab, monoclonal anti-idiotype antibody; MACOP-B, methotrexate, Adriamycin, cyclophosphamide, vinblastine, prednisone; DHAP, decadron, Ara-C, cisplatin; CMOPP, cyclophosphamide, vincristine, procarbazine, prednisone; SD, stable disease; P, progression.

*Target serum level is the 48-hour postinfusion serum level of > 25 μg/mL.

†Clinical response to course I of therapy evaluated before course II. Clinical response to course II evaluated after course II.

§Rat monoclonal anti-idiotype antibody.

Pools of multiple anti-idiotype clones.
and small cleaved cell lymphoma. Nine of 13 patients had evidence of bone marrow involvement and 10 of 13 patients had tumor masses greater than 5 cm in diameter.

**Pharmacokinetics.** The intention was to administer two identical courses of antibody therapy. Detailed antibody pharmacokinetic data are shown for a typical patient (C-02) in Fig 1 and are used to illustrate how the data in Table 1 were derived. In this case, the initial two doses of 240 mg did not achieve antibody excess in the serum and the dose was escalated as shown. After escalation to 540 mg, persistent antibody excess was obtained. In the second course of treatment, the same dose and schedule achieved target serum levels by dose 2, and higher serum levels overall. In the first course, four of seven treatments achieved target serum levels and in the second course, six of seven treatments achieved target serum levels. As shown in Table 1, the pre-course I idiotype level was 3.2 µg/mL, whereas the pre-course II idiotype level was 1.7 µg/mL.

The details of therapy for all patients are shown in Table 1. Pre-therapy serum idiotype levels ranged from less than 1 to 36 µg/mL. Eight patients were treated with a single monoclonal anti-idiotype antibody, while five patients were treated with mixtures of two or more anti-idiotype antibodies. The majority of the MoAbs were of the murine IgG1 isotype. The number of doses, the cumulative dose administered, and the number of doses that achieved target trough levels (>25 µg/mL at 48 hours posttreatment) are also shown in Table 1. In most cases, the second course of treatment resulted in higher serum levels of antibody, and a greater number of infusions that exceeded the target serum values. This could be attributed to the antitumor effects of the first course of treatment leading to a lower tumor burden and a lower pre-course II serum idiotype level.

**Tumor responses.** The clinical responses to therapy are shown in Table 1. All 13 patients received course I of the trial. Two patients did not receive course II of the trial as scheduled. One of these patients (C-11) had course II aborted due to the inability to achieve adequate serum levels despite administration of a cumulative dose of more than 5 g of antibody in course I, and an apparently increasing pre-course II serum idiotype level without change in tumor bulk. A second patient (C-06) had course II aborted due to continued disease progression. Neither of these patients received the pulse dose of chlorambucil chemotherapy. In an additional two patients (C-09, C-10), the planned dose of chlorambucil was reduced by 50% (8 mg/m² for 5 days) because of prior sensitivity to alkylator therapy and neutropenia. Of the 13 treated patients, 11 had objective evidence of tumor regression. Nine patients achieved a complete or partial response (1 CR and 8 PR), with freedom from progression ranging from 3 to 31 months (average of 9.4 months; median of 7 months). An additional two patients had minor responses, with freedom from progression lasting 6 and 7 months, respectively. Overall, freedom from progression in all 13 patients averaged 7.8 months, again with a median of 7 months. Examples of partial tumor regressions in two patients are presented in Fig 2. Patient C-02 had an enlargement of pelvic lymph nodes that underwent partial regression after therapy, as shown by comparison of the radiographs from pre-therapy and 4 months post-therapy. Patient C-10 also had an antitumor response of greater than 50% (illustrated in the lower portion of Fig 2). This is an example of a response after the first course of MoAb therapy, before the combined course of antibody and chlorambucil.

**Toxicity.** A total of 195 antibody infusions were administered over two courses to 13 patients with minimal adverse reactions. No patient produced a detectable human antimurine antibody response (HAMA). The most common reactions associated with antibody infusion included fever and/or rigors (>38.5°C in 10 infusions) and mild symptoms of allergy (urticaria or skin rash, increased respiratory secretions, or mucosal edema) in a total of 23 infusions. These reactions occurred most often during the first few infusions of each course, and in patients who had elevated pretreatment idiotype levels. These reactions were controlled as needed with acetaminophen, diphenhydramine, or meperidine. In one patient (C-08) with a high number of tumor cells circulating in blood, a more serious reaction, characterized by bronchospasm, fever, and flank pain, developed on the second dose of antibody therapy. These symptoms rapidly resolved with medication, and the patient continued on therapy with minimal further reactions. During course I (antibody alone), platelet counts transiently decreased in 3 of 13 patients (to 40,000 to 50,000/mm³), were unchanged in 7 of 13 patients, and increased significantly in 2 of 13 patients. Five of 13 patients had significant increases in platelet counts at the end of course I, while 8 of 13 had no change. The granulocyte count was transiently decreased to less than 1,500/mm³ in 3 of 13 patients after initial antibody infusions. Three additional patients had granulocyte counts decrease transiently during the first
course of therapy to less than 500/mm³; however, two of three of these patients started therapy with a granulocyte count of less than 1,000 cells/mm³. The granulocyte count rapidly returned to baseline or higher levels after the completion of antibody treatments. Transient mild elevations in liver function tests (SGOT) occurred in three patients. No significant changes from baseline occurred in serum creatinine or blood urea nitrogen (BUN). After the pulse dose of chlorambucil administered with course II, leucopenia and decreases in platelet count occurred as expected.

Analysis of proliferating cell subsets. Matched tissue specimens were obtained in 6 of the 13 treated patients. Tumor samples from biopsies taken pre-course I, pre-course II, and at the time of relapse were analyzed for surface expression of Ig, idiotype, and DNA content. An example of the FACS data for the pre-course I and pre-course II biopsies for patient C-10 is shown in Fig 3. The majority of the cells in the pre-course I biopsy were of the IgM(k) phenotype and reacted with the treatment anti-idiotype antibody (anti-LV-1). This is displayed on the y axis. After the first course of antibody therapy, a partial regression of the tumor occurred. Rebiopsy before the combined treatment with antibody and chlorambucil again showed that the tumor cells had surface Ig [IgM(k)], but they did not react with the treatment antibody, although they retained partial
Fig 3. Analysis of tumor cells from pre-course I and pre-course II lymph node biopsies for patient C-10. Tumor cells were stained for surface expression of Ig by two different monoclonal anti-idiotype antibodies, and for DNA content by propidium iodide (PI). The number of cells in the right upper and right lower quadrants of each panel indicate cells with greater than diploid DNA. Pre-course I cells stained with anti-LV-1 and anti-Al-1 anti-idiotype antibodies. Tumor cells from the pre-course II biopsy only stained with the nontreatment anti-Al-1 anti-idiotype antibody. This demonstrates the powerful selective pressure of the anti-idiotype antibody. The percent of proliferating cells was roughly the same in the two biopsies. There was no increase in the percent of proliferation in the LV-1-negative tumor population.

Table 2. Percent of Cells Proliferating in Tumor Biopsies

<table>
<thead>
<tr>
<th>Patient</th>
<th>Percent of Cells in G2/M and S Phase of the Cell Cycle*</th>
<th>Clinical Response</th>
<th>Tumor Progression</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Course I</td>
<td>Pre-Course II</td>
<td>At Tumor Progression</td>
</tr>
<tr>
<td>C-10</td>
<td>1.7</td>
<td>2.3</td>
<td>7.9† (7)</td>
</tr>
<tr>
<td>C-04</td>
<td>5.2</td>
<td>8.4</td>
<td>7.1 (4)</td>
</tr>
<tr>
<td>C-13</td>
<td>3.9</td>
<td>2.4</td>
<td>3.3 (13)</td>
</tr>
<tr>
<td>C-12</td>
<td>4.6</td>
<td>6.6</td>
<td>15.6 (13)</td>
</tr>
<tr>
<td>C-02</td>
<td>9.0</td>
<td>11.3†</td>
<td>16.6 (14)</td>
</tr>
<tr>
<td>C-03</td>
<td>3.3</td>
<td>2.9</td>
<td>3.8 (6)</td>
</tr>
</tbody>
</table>

Time of biopsy for tumor progression in months from initiation of course I is in parentheses.

*Percent of cells in G2/M and S phase of cell cycle determined by FACS analysis of propidium iodide-stained cells.
†Idiotype selection noted “yes” if greater than 40% of tumor cells on analysis of tumor biopsy are nonreactive with the anti-idiotype antibody(s) used for therapy.
‡Tumor biopsies performed by fine needle aspiration.
second cycle of antibody therapy was administered with the pulse dose of chlorambucil chemotherapy.

The percentage of cells in G2/M and S phase of the cell cycle from the serial biopsies in all six patients with matched biopsies are presented in Table 2. These tumor populations displayed a range of baseline proliferation from 1.7% to 9.0%. For comparison, between 60% and 75% of the cells of an in vitro proliferating cell line from a patient with large cell lymphoma were in G2/M and S phase using this method (data not shown). Comparison of the pre-course I tissue with the pre-course II tissue showed no significant change in any of the six patients. Therefore, we did not demonstrate a compartment of cells with an increased proliferative rate after exposure to anti-idiotype antibody despite the fact that at least two patients (C-10 and C-13) had evidence of significant idiotype selection before the second cycle of anti-idiotype antibody therapy.

Analysis of the relapsing tumors showed an increased percentage of cells in G2/M and S phase of the cell cycle in three of six cases (patients C-10, C-12, and C-02, biopsied at 7, 13, and 14 months post-MoAb treatment, respectively). In two of these three patients, significant idiotype selection was noted; However, there was no difference in proliferation between idiotype-positive and idiotype-negative cell populations. A third patient (C-03) also showed idiotype selection at the time of tumor progression, but never demonstrated an increased rate of cell proliferation. Lastly, it is interesting that in one patient with idiotype selection noted in the pre-course II biopsy (C-13), idiotype reexpression was noted in the tumor progression biopsy obtained 13 months after treatment. These observations suggest that idiotype selection occurs without requiring an increased rate of proliferation in the idiotype-negative cells. However, the interpretations of idiotype selection and reexpression are limited by the inability to sample the same tumor tissue at different points in time. Thus, site to site variations in the tumor composition may also complicate this analysis.

Overall, the data indicate that a strong antitumor effect was induced by the anti-idiotype antibodies, resulting in the elimination of the idiotype-positive cells in many patients, but no recruitment of idiotype-negative tumor cells into the cell cycle.

**DISCUSSION**

This trial further demonstrates that MoAb therapy directed against the tumor-specific idiotypes expressed on the cell surface Ig of B-cell lymphomas has antitumor activity in patients who have failed multiple attempts at conventional chemotherapy. The overall response rate (1 CR and 8 PR in 13 patients) is similar to our previously published data using antibody alone or in combination with α-IFN. Freedom from disease progression averaged 7.8 months for all patients, and 9.4 months for responding patients (median of 7 months). In our prior trials with anti-idiotype antibody alone, 2 of 14 patients had complete tumor remissions lasting more than 5 years. In the antibody plus IFN trial, 3 of 11 patients had complete tumor regressions, all of which persist with no further therapy greater than 5 years. In the current trial, the one completely responding patient has relapsed at 31 months posttreatment. Overall, patients in this trial had more advanced disease and had received a greater number of prior treatment regimens (chemotherapy and radiation therapy) than patients reported in the prior trials.

In this trial, 195 infusions of MoAb were administered to 13 patients in the form of two identical treatment courses that were individualized for each patient. No patient made an immune response against mouse Ig. Minimal toxicity was associated with the treatment and, when seen, occurred during the initial infusions of each course.

Due to the production and purification characteristics of each antibody, the amount of anti-idiotype available for treatment varied for each patient. In the current trial, our goal was to make comparisons within individual patients of two identical courses of antibody with and without the drug chlorambucil. Therefore, in each case the available antibody was divided into two equal portions and administered in two identical treatment schedules. During the first course, the MoAb was administered in escalating doses and adjusted in an effort to achieve free circulating serum antibody levels. During the second course, the identical dose and schedule were used. In each patient, the serum level of antibody attained varied and was dependent on a variety of factors. The serum idiotype level, rate of production of idiotype protein by the tumor cells, and tumor burden are all factors that increase antibody consumption. Because of these factors, a wide range of doses and amounts of antibody were used.

In general, patients who had greater tumor bulk and higher pretreatment idiotype levels required higher doses of antibody to reach target serum levels. In two patients (C-12 and C-13), partial remissions occurred even though sustained serum levels of antibody were not achieved, despite large cumulative doses. Overall, higher serum levels were obtained in the second cycle of therapy, likely reflecting a response to the first cycle and a lower tumor burden and lower serum idiotype levels.

The novel aspect of this trial was an attempt to decrease the emergence of idiotype-negative variants by a cycle of chemotherapy administered at a time when the idiotype-negative cells were presumed to have been proliferating. The chemotherapy chosen, pulse chlorambucil, is a standard treatment for low-grade lymphoma. However, all of these patients had previously been treated with at least one and often multiple courses of therapy with alkylating agents, and had relapsed. The presumption was that tumors in these patients would be resistant to alkylators on the basis of kinetic rather than genetic mechanisms. Our analysis of the pre-course II tumor biopsies did not show an increased rate of cell proliferation compared with the pre-course I biopsies. Moreover, significant idiotype selection was observed already to have occurred before course II in several patients. As we have observed in the past, the idiotype-negative cells retained cell surface Ig, although in one case decreased intensity of surface Ig staining was noted (C-12). We found that idiotype-negative tumor cells...
did not have an increased rate of proliferation. Thus, in retrospect, it was unlikely that they would be more susceptible to killing by a cell cycle active chemotherapy drug than the idiotype-positive cells.

The addition of chlorambucil to the second cycle of antibody therapy complicates the assessment of clinical response to the anti-idiotype antibody. However, the majority of these patients had been heavily pretreated with chemotherapy and radiotherapy, often with histologic conversion from a low-grade to an intermediate-grade histology and would be unlikely to receive significant clinical benefit from a single pulse dose of chlorambucil. In addition, in 10 of 13 patients some clinical improvement was seen after the first cycle of anti-idiotype antibody alone, before the second course with chlorambucil. These results compare favorably with the outcome of other MoAb trials and with conventional cytotoxic therapies for similar patients.17

The mechanism of tumor regression induced by anti-idiotype antibodies is not known. In murine models and in transplanted human tumor xenografts, accessory cells that can kill antibody-coated cells appear necessary.18 Increased host T-cell “infiltrates” were found in the tumor biopsies of some patients who later responded to anti-idiotype antibody therapy, suggesting a possible role in tumor regression.19 In the current trial, the addition of chlorambucil did not decrease the emergence of idiotype variant cells, indicating that the antitumor effect of the antibodies was not impeded. It was theoretically possible that the chlorambucil would inhibit host effector cells that might be necessary for anti-idiotype activity. Because the response rate and duration were similar to that in our previous trials, no such negative effect was apparent.

In our prior studies, late clinical remissions have been observed to occur. It is interesting to speculate that the treatment with the anti-idiotype antibody induced a host antitumor response, or invoked a “network” reaction that resulted in the control of tumor growth.20 These cases are being investigated for the presence of a humoral or cellular endogenous host antitumor activity.

This study, and our previous trials, used monoclonal anti-idiotype antibodies that were custom-produced for each patient. This process currently requires the acquisition of viable tumor cells and is time-consuming. It involves several steps, including the isolation of the tumor idiotype and the production and screening of hybridoma clones to identify the tumor-specific anti-idiotype antibodies. Identification of clinically suitable patients is complicated by the time required to produce a custom antibody in suitable quantities required for treatment. Because of these issues, this approach is not feasible for the majority of patients with B-cell lymphoma. However, it is possible that with newly developed technologies of molecular biology that this process will be simplified and shortened.21 Recently, a panel of monoclonal anti-idiotype antibodies reactive with the tumor cells from multiple patients with B-cell lymphoma has been identified.22 Trials with these antibodies are underway, and antitumor activity has been observed. In the future, therapy earlier in the course of a patient’s disease, with a single antibody or possibly a combination of antibodies against shared idiotopes may address the problem of idiotype variant tumor cells. Despite the logistical difficulties of this approach, the clinical activity shown in heavily pretreated patients and the lack of serious toxicities make it an example of how therapy directed against receptors on tumor cells that are specific and involved in signal transduction can be worthwhile.

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Monoclonal anti-idiotype antibody therapy of B-cell lymphoma: the addition of a short course of chemotherapy does not interfere with the antitumor effect nor prevent the emergence of idiotype-negative variant cells

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