Immune Responses in Patients with T-Cell Lymphoma Treated with an Anti-Idiotype Antibody Mimicking a Highly Restricted T-Cell Antigen


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ABSTRACT

We generated an IgG1 murine monoclonal anti-idiotype antibody (Ab2) to a highly restricted T-cell antigen designated glycoprotein (gp) 37 that is found on T-cell malignancies but not on normal cells. gp37 is identified by the murine monoclonal antibody SN2 (Ab1) against which the Ab2 was raised. Each of four patients with T-cell lymphoma predominantly confined to the skin received a minimum of four intracutaneous injections of aluminum hydroxide precipitated anti-idiotype murine monoclonal antibody (1 mg/injection) given every 2 weeks. For responding patients, injections were continued on a monthly basis. All tumors were measured along orthogonal major and minor axes, using a ruler and/or calipers, by the same observer. Tumor sizes were documented photographically. Three of the four patients developed specific idiotypic humoral immune responses, and two of the four patients also demonstrated idiotypic cell-mediated responses. Humoral responses included binding of the patients’ sera to the anti-idiotype antibody as well as specific inhibition of binding of the SN2 antibody (Ab1) to the anti-idiotype antibody (Ab2). Anti-anti-idiotypic (Ab3) from one patient’s serum bound specifically to the gp37-positive cell line MOLT-4 and also to semipurified gp37 antigen. Cell-mediated responses were demonstrated by specific proliferative response to the aluminum hydroxide precipitated anti-idiotype antibody by patients’ peripheral blood mononuclear cells. While three of the four patients had extensive disease and did not have clinical responses, one of the patients who had nine discrete skin tumors and peripheral blood involvement without other detectable disease had virtually complete disappearance of the tumors lasting over 11 months. Our results demonstrate that this particular anti-idiotype antibody can induce humoral and cellular immune responses, and at least in one patient led to a meaningful therapeutic response. Future trials should focus on immunocompetent patients with minimal disease.

INTRODUCTION

The immunotherapy of human cancer using tumor cells or tumor-derived vaccines has been disappointing for several reasons. It has been consistently difficult to obtain large quantities of purified tumor-associated antigens, which are often chemically ill-defined and difficult to purify. In addition, there remains the problem of the immunobiological response potential against tumor antigens, or in other words, the question whether a cancer patient can mount an effective immune response against his tumor. Tumor-associated antigens are often a part of ‘‘self’’ and usually evoke a very poor immune response in tumor-bearing hosts due to tolerance to the antigen. Immunobiologists have learned that a poor antigen can be turned into a strong antigen by changing the molecular environment of the haptenic structure. Such changes of the carrier have allowed for T-cell help to become active, making the overall immune response stronger. In addition, changing the carrier can also turn a tolerogenic antigen into an effective antigen. Furthermore, often the immunological status of the cancer patient is suppressed and only able to respond to certain T-dependent antigens and not to other antigen forms. From these considerations, it would be logical to introduce molecular changes into the tumor antigens before using them as vaccines. Unfortunately, this is impossible to accomplish for most tumor antigens because they are not well defined and are difficult to purify.

The network hypothesis of Lindemann (1) and Jerne (2) offers an elegant approach to transform epitope structures into idiotypic determinants expressed on the surface of Abs. They described the immune system as a network of interacting Abs and lymphocytes. According to this original network hypothesis, the idiotype-anti-idiotype interactions regulate the immune response of a host to a given antigen. Both idiotypes and anti-idiotypes have been used to manipulate cellular and humoral immunity. Idiotypes are distinguished by their topographical location on the immunoglobulin structures and are classified by their physical relation to the antigen binding site of the Ab (Ab1). If the Ab1 generates an Ab2 that binds to the antigen-

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Received 4/21/95; revised 6/21/95; accepted 6/29/95.

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3 The abbreviations used are: Ab, antibody; gp, glycoprotein; PHA, phytohemagglutinin P; TCR, T cell receptor; CPD, clonotype primer-directed; MAb, monoclonal antibody.
binding site (paratope) of Ab1, then this Ab2 is referred to as β type (3–5). Anti-idiotype Abs of the β type are thought to express the internal image of the antigen for Ab1. They can effectively mimic the three-dimensional structures of external antigens, and when injected into naïve animals or patients, the induced Ab3 has Ab1-like reactivity and is called Ab1’ to indicate that it might differ in its other idiotypes from Ab1 (6, 7).

We have generated an Ab2β anti-idiotype Ab (8, 9) to a murine mAb, designated SN2 (Ab1), that identifies a highly restricted T-cell antigen designated gp37 that is found on 70% of T-cell acute lymphoblastic leukemia and approximately 30% of T-cell lymphoma but not on normal or activated T-lymphocytes (10). The SN2 Ab does not have a cluster designation, and the function of the epitope is unknown. We have been unable to identify this antigen on any normal adult human tissues. The IgG1 anti-idiotype Ab generated to the SN2 Ab was shown to be an internal image by generating anti-anti-idiotypic (Ab3) responses which recognized gp37 in three animal models: mouse, rabbit (9), and nonhuman primates. This anti-idiotypic Ab was used to treat each of the patients reported in this clinical trial.

Lymphoma-specific T-cell clones were identified at the molecular level by virtue of their unique T-cell receptor β chain variable/diversity/joining region gene sequences using tandem primary and secondary PCR amplifications of skin-derived genomic DNA (11). Using this methodology, the lymphoma receptor β chain clonotype of the patient responsive to immunotherapy was tracked throughout the clinical course independent of the gp37 tumor marker corresponding to the anti-idiotypic Ab.

PATIENTS AND METHODS
Selection of Patients
All of the patients had T-cell lymphoma primarily confined to the skin. Three of the four patients had clinically and histologically classic mycosis fungoides, while one of the patients (patient 2) had a diffuse, large cell lymphoma. Patients were selected on the basis of SN2 (Ab1) Ab binding to their tumor. Baseline studies included complete physical examination, chest radiography, computer axial tomography examination of the abdomen, and routine blood counts and chemistries. Two of the patients (patients 3 and 4) were anergic to i.d skin testing with mumps, Candida, and trichophytin antigens. All of the patients had been off of prior therapy for at least 4 weeks, and staging was repeated at the conclusion of therapy. Informed consent was obtained under a protocol approved by the Institutional Review Board.

Treatment Schedule
The patients were treated with 1 mg aluminum hydroxide-precipitated anti-idiotype Ab intracutaneously every other week for 8 weeks, and then every 4 weeks if they demonstrated a clinical or immunological response. All of the patients received four total injections, except for patient 2 who received a total of 17 injections.

Disease Assessment
The patients’ cutaneous lesions were carefully mapped at the start of treatment and at each visit. Lesion size was specified by measurements of the major and minor axes of the infiltrates and volume by the product of these axes times the average thickness. The same observer (A. R. O.) performed the measurements.

Generation of Anti-Idiotype Ab for Clinical Trial
Murine mAb SN2 (IgG1-k) was used to immunize syngeneic BALB/c mice for the production of anti-idiotype Ab. Immunization of BALB/c mice, hybridoma fusion and cloning, selection of anti-idiotype (Ab2), and production of ascites in bulk quantities in mice were done as previously described (8, 9). The Ab2β anti-idiotype 4DC6 (IgG1-k) was purified from ascites by affinity chromatography on Protein A-CL Sepharose 4B columns. The purity of the isolated immunoglobulin (>95%) was determined by SDS-PAGE and high-pressure liquid chromatography techniques. Sterility, pyrogenicity, polynucleotides and Mycoplasma, and adventitious virus contaminations were tested in accordance with the United States Food and Drug Administration guidelines.

Preparation of Final Product
To 5-mg aliquots of purified monoclonal anti-idiotype (Ab2), 1 ml aluminum hydroxide (2% Alu-Gel S; Serva Fine Biochem, Inc., Garden City, Long Island, NY) was added (12). The volume was then adjusted to 10.0 ml with Dulbecco’s PBS, and the mixture was incubated on a vortex for 1 h at room temperature. The mixture was then centrifuged at 2000 rpm at 24°С for 10 min. The amount of Ab bound in the gel layer was determined by measuring spectrophotometrically the amount of unbound Ab in the supernatant. The aluminum hydroxide-precipitated Ab was stored at 4°С until use. These procedures were performed aseptically in a laminar flow hood, and the final product was sterile and clearly labeled as anti-idiotype 4DC6 Alu-Gel S and aliquoted into pyrogen-free, sterile glass vials.

The final product was tested for sterility, pyrogenicity, and general safety in guinea pigs before use. An Investigational New Drug Application was approved through the United States Food and Drug Administration (BB-IND 4515).

Assays for Sera Anti-Idiotypic Ab (Ab3)

Direct ELISA. Preimmune and hyperimmune patient sera were diluted serially and added to microtiter plate wells that had been coated with IgG1 or F(ab’2), fragments of immunizing anti-idiotype 4DC6 or an isotype- and allotype-matched control Ab2, 3H1 (13). Bound Ab was detected with alkaline phosphatase-labeled goat antihuman IgG (heavy and light chains)-specific Abs (Caltag Laboratories, South San Francisco, CA).

Inhibition RIA. Preimmune and hyperimmune patient sera samples were treated with murine immunoglobulins to remove anti-idiotypic and allotypic reactivities. Serial dilutions of sera were then tested for inhibition in the Ab1-Ab2 binding
assay. For the direct-binding inhibition assay between Ab1 and Ab2, purified Ab2 4DC6 was used to coat plates (500 ng/well), and the binding of radiolabeled SN2 (Ab1) to Ab2 was tested in the presence of different patients’ hyperimmune (Ab3) sera and Ab1.

To determine whether Ab3 sera compete with Ab1 for binding to gp37 antigen-positive MOLT-4 cells, the binding of radiiodinated Ab1 (SN2) was tested for inhibition in the presence of Ab3 sera. Approximately $2 \times 10^5$ MOLT-4 cells/tube were coincubated with different dilutions of the patients’ Ab3 sera (after preabsorption with normal mouse immunoglobulin) and a fixed amount of $^{125}$I-labeled Ab1 (SN2).

Detection of Anti-gp37 Antibodies in Patients Immunized with Ab2 4DC6

This assay was conducted to determine whether some of the Ab3 induced in patients by monoclonal murine Ab2 was of the Ab1 type and whether it would bind to gp37. Glycoproteins containing gp37 were isolated from cell membranes of MOLT-4 as described previously. Briefly, MOLT-4 cells were mechanically disrupted and subjected to differential centrifugation, which yielded a cell membrane preparation. The membrane proteins were solubilized from the membrane preparation by sodium deoxycholate treatment, and glycoproteins containing gp37 were isolated by affinity chromatography on a Lens culinaris lectin column. Semipurified preparation of gp37 ($2 \mu\text{g/ml}$) was coated onto 96-well microtiter plates and reacted with serial dilutions of the patients’ Ab3 sera. The antigen-Ab reaction was detected by using alkaline phosphatase-labeled antihuman immunoglobulin reagent. Preimmune sera and sera obtained from colon cancer patients treated with an unrelated Ab2 (3H1) were used as controls. Binding of purified Ab3 and Ab1 to gp37 antigen on the microtiter plate was also checked using the ELISA.

Purification of Anti-Idiotype Ab (Ab3) from Hyperimmunized Patients’ Sera

Fifty ml hyperimmune serum were passed over an immunoadsorbent column consisting of immobilizing anti-idiotype immunoglobulin (4DC6-IgG1) coupled to Sepharose 4B. Anti-idiotype Abs (Ab3) were eluted with 0.1 M glycine-hydrochloric acid buffer (pH 2.4). The eluted Ab was then passed over an immunoadsorbent column consisting of an unrelated isoa1toype-matched anti-idiotype immunoglobulin coupled to Sepharose 4B to remove anti-isotypic and antiallotypic reactivities. Ab that passed through was concentrated and used as purified Ab3. The isotype of Ab3 was determined with the ELISA using human anti-isotype-specific reagents (Tago).

Flow Cytometry Analysis with Purified Ab3

Antigen-positive MOLT-4 cells ($1 \times 10^5$/tube) were reacted with Ab1 (SN2) and Ab3 at 100 nM at 4°C for 60 min. After washing, the cells were incubated with either goat anti-mouse or goat antihuman F(ab’)$_2$ IgG-FITC-labeled Ab (Tago) for 30 min at 4°C. They were then washed twice, fixed in 2% paraformaldehyde, and analyzed by immune flow cytometry (FACS STAR, Becton Dickinson).

Immunoperoxidase Staining of Tumor Sections

The reactivities of monoclonal Ab1 and purified Ab3 at 10 $\mu\text{g/ml}$ solution were compared on surgical specimens of T-cell lymphomas by a very sensitive staining method (biotin-streptavidin reagents; Vector Laboratories, Burlingame, CA) as described in detail elsewhere (13). All sections were counterstained with Meyer’s hematoxylin. Pertinent specificity tests were performed, including a block of the endogenous peroxidase, omission of the first layer, or substitution of nonimmune homologous serum for the specific antiserum and P3–653 myeloma cell culture supernatant as the control.

To determine whether endogenous Ab3 Abs bound to relapsed tumor, the tumor sections were treated with PBS-BSA instead of Ab3 and then reacted with antihuman immunoglobulin reagents and stained as above.

Assay for Idiotype-specific Proliferative Response

Fresh peripheral blood mononuclear cells were isolated using standard Ficoll-Hypaque density gradient centrifugation methods, and $5 \times 10^5$ cells/well were incubated with different concentrations of 4DC6 Alu-Gel S and control 3H1 Alu-Gel S (10 ng-2 $\mu\text{g}$) in RPMI 1640 medium with 5% heat-inactivated FCS and penicillin and streptomycin. The nonspecific mitogen PHA was used as a positive control at 2 $\mu$g and 1 $\mu$g/well. After the cells were incubated for 5 days at 37°C in an atmosphere containing 5% carbon dioxide, they were pulsed with [3H]thymidine (1 $\mu$Ci/well) for 20 h. Data are expressed as mean counts (triplicate wells) per minute of [3H]thymidine incorporation. The SDs of the data were <10% for each determination.

Molecular Detection of Lymphoma-specific T-Cell Receptor Clonotypes

High molecular weight DNA was isolated from skin biopsies from patient 2 as previously described (15). Skin specimens were first minced extensively, and mononuclear cells were isolated from the cell suspension by density gradient centrifugation. Primary PCR amplifications of the TCRB-VDJ complex were achieved by using the following oligonucleotide primer set: The negative strand primer is derived from the TCR-J3 region repertoire. The positive strand primer is derived from each of the TCR-V3 family-specific genes spanning the 5’ V3 regions. Each reaction is defined by a TCR-J3 generic primer and a TCR-V3 family-specific primer. In this manner a set of 24 PCR amplifications were performed on each genomic DNA sample (11).

All PCRs were carried out against 1 $\mu$g DNA template in 100 $\mu$l adjusted to final concentrations of 10 $\mu$M Tris-HCl (pH 8.3), 50 $\mu$M KCl, 1.5 mM MgCl$_2$, 0.001% gelatin, 200 $\mu$M deoxynucleotide triphosphates, and 200 nM each primer, to which 2.5 units Taq polymerase (Perkin Elmer/Cetus, Norwalk, CT) were added. Temperature cycling was carried out in a DNA Thermal Cycler (Perkin Elmer/Cetus) as follows: an initial denaturation for 5 min at 94°C, then 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C for 30 cycles. Twenty-$\mu$l aliquots of all PCR amplifications within a set were electrophoresed through 1.2% agarose gels stained with ethidium bromide (1 $\mu$g/ml), visualized under UV light and
photographed, and scanned densitometrically. In this manner the predominant TCR-Vβ repertoire, corresponding to the T-cell clone of interest, was identified.

To ensure quality of DNA and integrity of the PCRs, internal control amplifications were run concurrently. Each genomic DNA sample was amplified using an intragen primer set that frames a 325-bp target sequence within the actin gene. In addition, reagent controls, consisting of the reaction mixture which scans the DNA template, were coamplified and tested for each of the 24 primer pairs.

The predominant TCR-VDJβ amplified product was directly ligated into PCR 1000 plasmid (Invitrogen, San Diego, CA), cloned, and sequenced using the dideoxy chain termination method (16). At least three separate clones were fully sequenced in both directions to obtain a consensus genetic sequence for the TCR-VDJβ segment.

From the TCR-VDJβ consensus sequence, a negative strand primer was constructed which spans a portion of the most 3' TCR-Vβ region, the highly diverse D region, and a portion of the flanking 5' TCR-Jβ region (Fig. 1). The clonotype VDJ primer was 36 nucleotides long and specific for the individual malignant T-cell clone. CPD-PCR was performed similar to the primary PCR, except that the primer set consisted of the predominant 5' positive strand TCR-Vβ family-specific primer and the clonotype-specific TCRβ-VDJ primer replaced the TCR-Jβ generic primer. Amplified products were electrophoresed in 1.2% agarose gel stained with ethidium bromide and visualized under UV light.

**RESULTS**

**Humoral Responses to Anti-Idiotype.** The development of humoral immunity induced by immunization with aluminum hydroxide-precipitated Ab2 4DC6 was assessed by testing sera obtained from patients before therapy and after each treatment with the vaccine. Hyperimmune sera (following the fourth injection of 4DC6) from three of four patients showed significant levels of total human antimouse Ab responses, including anti-iso/allo/ and anti-anti-idiotypic antibodies against immunizing Ab2 4DC6, as determined using the ELISA (Figs. 14, 3A, and 4A). Next, the sera from these immunized patients were treated with excess normal murine immunoglobulin (50 µg/ml) to block human Abs against isotypic and allotypic determinants and tested against F(ab')2 4DC6 anti-idiotype Ab in an ELISA. Absorbed sera from three of the four patients demonstrated strong reactivity with the F(ab')2 4DC6 (Figs. 2B, 3B, and 4B), whereas there was no reactivity with the F(ab'), fragment of an unrelated isotype- and allotype-matched Ab2 designated 3H1 (Figs. 2C, 3C, and 4C). Preimmune sera had no reactivity with the F(ab')2 4DC6. In addition, the responding patient’s serum (after the fourth injection) preabsorbed with normal mouse immunoglobulin demonstrated specific inhibition...
of binding of radiolabeled Ab1 Ab with Ab2 (Fig. 5), and there was no inhibition using preimmune sera. Although steric hindrance by Ab3 binding cannot be excluded in these assays, the results suggest the presence of true anti-anti-idiotypic Abs that share idiotypes with Ab1. Again, three of four patients were positive for Ab3 responses using this assay.

If Ab3 generated in patients has a similar binding site as Ab1, it should compete with Ab1 for binding to gp37 on MOLT-4 cells. A fixed amount of radiolabeled Ab1 was coincubated with different dilutions of patients’ Ab3 sera and preimmune sera absorbed with normal mouse immunoglobulin. As shown in Fig. 6, Ab3 sera from patient 2 inhibited the binding significantly (about 80% inhibition at 1:10 dilution), whereas Ab3 sera from patient 1 did not show any appreciable binding inhibition. No inhibition was obtained with Ab3 sera (after the fourth injection) from patient 3 or 4.

To determine whether Ab2 4DC6-immunized patients’ sera bound specifically to semipurified gp37 antigen, the binding of patients’ Ab3 sera to the gp37 antigen on the plate was tested using the ELISA. As shown in Fig. 7A, Ab3 sera from patient 2, obtained after the fourth immunization, at different dilutions, reacted significantly with gp37 antigen, whereas preimmune sera, Ab3 sera from patient 1, or Ab3 sera from one colon cancer patient treated with an unrelated Ab2 3H1 (17) showed marginal binding.

Next the Ab3 sera was affinity purified through Ab2-Sepharose 4B columns. Purified Ab3, which was predominately IgG, isolated from patient 2 was compared with purified Ab1 for binding to the gp37 antigen using the ELISA. As shown in Fig. 7B, there was almost identical binding to the gp37 antigen coated onto microtiter plates by Ab1 and Ab3, whereas purified Ab3 from a colon cancer patient treated with Ab2 3H1 did not show any binding. Purified Ab3 isolated from patient 2 did not show any binding to the control, unrelated purified carcinoembryonic antigen using the ELISA (data not shown). It demonstrated specific binding to the MOLT-4 cell line, which expresses cell surface gp37 antigen, without binding to the gp37 antigen-negative Raji cell line by immune flow cytometry (Fig. 8). The Ab3 from patients 1 and 3 showed only marginal reactivity to MOLT-4 cells or semipurified gp37 antigen.
Cellular Responses to Anti-Idiotype. Cellular immune responses were measured by the proliferation of peripheral blood mononuclear cells incubated with aluminum hydroxide-precipitated anti-idiotype Ab (4DC6) and its F(\(\text{ab}^{\prime}\)^2) fragment. Aluminum hydroxide-precipitated unrelated anti-idiotype Ab 3H1 and its F(\(\text{ab}^{\prime}\)^2) fragment were used as controls. Positive proliferative responses were seen in patients 2 and 3 (Figs. 9 and 10) that were somewhat comparable to the responses to the mitogen PHA. Preimmune cells had no proliferative response to the anti-idiotype Ab or its F(\(\text{ab}^{\prime}\)^2), whereas postimmune cells had a significant response. There was also a minor but significant response to the isotype-matched 3H1 aluminum hydroxide-precipitated anti-idiotype Ab that was significantly less than that of the 4DC6 response; this probably represents a response to the nonidiotypic components of the immunoglobulin molecule. Proliferative responses were first noted after the third injection and continued to increase with each ensuing injection. Flow cytometric analysis of the cultures demonstrated that >90% of the proliferating cells were CD4-positive T lymphocytes. The T-cell proliferative assay was not performed for patient 1, whereas patient 4 was anergic and did not even respond to PHA. We did not test T-cell proliferation in the presence of semipurified gp37 antigen due to a very limited supply, and thus could not establish...
the antigen specificity of the cellular immune responses induced in the lymphocytes.

Toxicity and Clinical Responses. Toxicity was minimal with only transient local reactions of mild erythema and mild to moderate induration at the injection site and mild fever and chills relieved by acetaminophen. The anti-idiotypic treatment did not have any deleterious effect on hematopoietic cells or on renal or hepatic function.

Patients were monitored for disease activity at each vaccination visit. Patients 1 and 4 had extensive patch and plaque stage infiltrates, and patient 3 had tumor stage mycosis fungoides. There were no SN2-positive circulating cells present in these patients. After four injections the patients did not have objective responses. Patient 1 had a significant anti-anti-idiotypic response (Figs. 2 and 5), but did not mount any significant anti-gp37 Ab response. This patient was lost to follow-up after the fourth injection. Patient 2 had discrete skin tumors and also had circulating lymphocytes that labeled with the SN2 Ab. This patient had no other measurable or detectable disease. After the second injection, there was marked induration at the injection site and a slight decrease in the size of the skin lesions. After the fourth injection, all nine skin lesions flattened to the surface of the skin. The decrease in the size of the tumors occurred as the titer of Ab3 in the sera rose during the first cycle of therapy. Numerous biopsies were obtained and only one of the skin lesions demonstrated residual tumor cells. These cells did not stain with either Ab1 (SN2) murine mAb or Ab3 hyperimmune sera using the immunoperoxidase assay. Photographs from one of the skin lesions are shown in Fig. 11 as well as a skin biopsy taken from the same tumor site at the completion of the fourth injection. Circulating SN2-positive cells also disappeared (data not shown). The patient was continued on monthly injections for...
an additional four injections (total of eight), and then the injections were discontinued. The tumors did not regrow, and there were no new tumors until 6 months after therapy was discontinued. At this time, there were no new tumors but some of the original sites became thicker; the most significant growth was in the lesion that demonstrated residual lymphoma in the earlier biopsy. The Ab3 titer had declined, and anti-idiotype vaccine therapy was reinitiated with an additional nine injections. The SD of the data was <10% for each determination. ■, 4DC6; □, 3H1; ●, 4DC6 F(ab')2; □, 3H1 F(ab')2; □, PHA.

**DISCUSSION**

The SN2 mAb identifies a highly restricted T-cell antigen, designated gp37, that is found on approximately 30% of patients with T-cell lymphoma and 70% of children with T-cell acute lymphoblastic leukemia, but is not found on normal T cells or other hematopoietic or nonhematopoietic tissues (10). We, therefore, felt this would be an ideal target antigen for an anti-idiotype vaccine. Tumors from all treated patients were gp37 antigen positive prior to the initial therapy. Three of four patients demonstrated an anti-anti-idiotype (Ab3) response to the anti-idiotype Ab, and two demonstrated specific idiotypic T-cell responses. Only patient 2 mounted antitumor cell Abs. Patient 2 had an excellent clinical response. We believe the clinical response was related to the fact that this patient did not have cutaneous anergy, and that this patient had the smallest tumor burden and the best immune response, including antitumor Ab response. We demonstrated binding of hyperimmune serum (Ab3), as well as purified Ab3 (predominately IgG), to the gp37 antigen and gp37 antigen-positive cell line MOLT-4. Unfortunately, we lost the pretherapy autologous tumor in a freezer thaw and, therefore, could not demonstrate Ab3 binding. After 11 months, the patients' tumors began to regrow at some of the original tumor sites. Despite an excellent active immune response to retreatment with anti-idiotype Ab, the tumors only partially regressed and then began to regrow. They were gp37 antigen negative at that time, suggesting that the lack of response was secondary to growth of gp37 antigen-negative variants. We ruled out the possibility that Ab3 blocked Ab1 binding by demonstrating the lack of binding of antihuman immunoglobulin or murine Ab2 to the tumor. Both reagents should have detected Ab3 if it was present on the tumor. Importantly, the clonotype primer-directed PCR demonstrated that despite the loss of the gp37 antigen, the T-cell receptor clonotype did not change (Fig. 12).

![Fig. 9](image_url) **T-cell proliferation assay with peripheral blood lymphocytes from patient 2 in the presence of 4DC6 Alu-Gel S, isoallotype-matched control 3H1 Alu-Gel S, 4DC6 F(ab')2, 3H1 F(ab')2, or PHA. Peripheral blood lymphocytes were isolated from fresh blood obtained after four immunizations and cultured with 100 ng 4DC6 Alu-Gel S or 3H1 Alu-Gel S and other stimulants, as described in “Patients and Methods.” [3H]Thymidine incorporation was measured in pre- and post-therapy samples. Data are expressed as mean cpm of triplicate wells. The SD of the data was <10% for each determination. ■, 4DC6; □, 3H1; ●, 4DC6 F(ab')2; □, 3H1 F(ab')2; □, PHA.**

![Fig. 10](image_url) **T-cell proliferation assay with peripheral blood lymphocytes from patient 3 in the presence of 4DC6 Alu-Gel S, isoallotype-matched control 3H1 Alu-Gel S, 4DC6 F(ab')2, 3H1 F(ab')2, or PHA. Peripheral blood lymphocytes were isolated from fresh blood obtained after four immunizations and cultured with 100 ng 4DC6 Alu-Gel S or 3H1 Alu-Gel S and other stimulants, as described in “Patients and Methods.” [3H]Thymidine incorporation was measured in pre- and post-therapy samples. Data are expressed as mean cpm of triplicate wells. The SD of the data was <10% for each determination. ■, 4DC6; □, 3H1; ●, 4DC6 F(ab')2; □, 3H1 F(ab')2; □, PHA.**
Fig. 12 The clonotype primer-directed PCR permitted the serial detection of the original T-cell lymphoma from skin-biopsied patient specimens (arrowheads). The pretreatment malignant T-cell clone generated a strong signal in the liquid hybridization/gel retardation electrophoretogram (Lane 1). The CPD-PCR was specific for the T-cell lymphoma of interest and did not generate a signal from normal, polyclonal peripheral blood mononuclear cell-derived DNA (Lane 2). The original lymphomatous T-cell clone was also detected in subsequent skin biopsies obtained during early immunotherapy (Lane 3), at the time of clinical partial remission (Lane 4), and, subsequently, during relapse (Lane 5) as evidenced by the signal bands generated on the ethidium bromide-stained agarose gels.

portion of the murine immunoglobulin served as a ‘‘carrier’’ to help promote the immune responses. It was also interesting that our anti-idiotype Ab was able to stimulate an in vitro helper T-cell proliferative response in treated patients. The relative roles of patient 2’s humoral and cellular immune responses in the clinical response are not known.

The purpose of this clinical study was to determine whether an anti-idiotype Ab that mimics the gp37 tumor-associated antigen could generate active immunity to this antigen in patients. Anti-idiotype Abs have been shown to generate active tumor immunity in animal models with both humoral immune responses and delayed-type hypersensitivity (19–27). Perhaps the first suggestion in humans that anti-idiotype responses might correlate with clinical responses was in patients treated with Ab1 Ab for colorectal cancer who developed anti-idiotypic antibodies and improved clinically (28). Subsequent clinical trials using polyclonal goat anti-idiotype (Ab2) vaccines for colorectal cancer (12) and monoclonal anti-idiotypes for malignant melanoma (29, 30) have demonstrated that anti-idiotype vaccine therapy leads to active immune responses. In a recent study in patients with B-cell lymphoma, idiotype-specific immune responses were induced against the surface immunoglobulin idiotype expressed by their tumors (31). In this study, two patients had clinical responses similar to our patients’ responses.

We believe our results demonstrate that anti-idiotype Ab can be effective in some patients. Because of the limited number of patients in our clinical trial, it is not possible to predict which patients will benefit from anti-idiotype vaccine therapy. Immu-
nototherapy will probably be most effective in patients without cutaneous anergy who have minimal tumor burden.

ACKNOWLEDGMENTS

We thank Dr. Maurice Barcos for assistance in the histology and immunohistochemistry studies and for the photomicrographs. Stuart Shauler assisted in the clinical care and assessments.

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