

INDUCTION OF IMMUNE RESPONSES IN PATIENTS WITH B-CELL LYMPHOMA AGAINST THE SURFACE-IMMUNOGLOBULIN IDIOTYPE EXPRESSED BY THEIR TUMORS

LARRY W. KWAK, M.D., PH.D., MICHAEL J. CAMPBELL, PH.D., DEBRA K. CZERWINSKI, B.S., SARAH HART, B.S., RICHARD A. MILLER, M.D., AND RONALD LEVY, M.D.

Abstract Background. The idiotypic determinants of the surface immunoglobulin of a B-cell lymphoma can serve as a clonal tumor-specific marker, which may have implications for immunotherapy. We sought to determine whether idiotype-specific immune responses against this autologous antigen could be induced in patients with B-cell lymphoma.

Methods. Nine patients were selected who had minimal residual disease or a complete remission after chemotherapy. Each received a series of subcutaneous injections of the immunoglobulin derived from his or her tumor cells (immunoglobulin-idiotype protein), which had been conjugated to a protein carrier and mixed with an immunologic adjuvant.

Results. In seven of the nine patients the injections induced sustained idiotype-specific immunologic responses of the humoral type (two patients), the cell-mediated type (four patients), or both (one patient). The

use of an adjuvant was essential for these immune responses. The induced antibodies bound specifically to autologous immunoglobulin idiotype, inhibited the binding of murine monoclonal antiidiotype antibodies, and bound autologous tumor cells. Cell-mediated responses were demonstrated by the specific proliferation of immune peripheral-blood mononuclear cells to the soluble immunoglobulin-idiotype protein in vitro. The tumors of both of the patients with measurable disease regressed completely. Toxicity associated with the vaccine was minimal and consisted only of mild reactions at the site of intramuscular injection.

Conclusions. These results demonstrate that autologous immunoglobulin idiotype can be formulated into an immunogenic, tumor-specific antigen in humans with B-cell lymphoma, and they provide the background for large-scale trials of active specific immunotherapy of this disease. (N Engl J Med 1992;327:1209-15.)

IMMUNOGLOBULIN molecules are composed of heavy and light chains, which possess highly specific variable regions at their amino termini. The variable regions of heavy and light chains combine to form the unique antigen-recognition site of the immunoglobulin protein. These variable regions contain determinants (molecular shapes) that can themselves be recognized as antigens or idiotypes. B-cell tumors are composed of clonal proliferations of cells synthesizing a single antibody molecule with unique variable regions in the heavy and light chains. B-cell lymphomas are neoplasms of mature resting and reactive lymphocytes, which generally express synthesized immunoglobulin on the cell surface. The idiotypic determinants of the surface immunoglobulin of a B-cell lymphoma can thus serve as a tumor-specific marker for the malignant clone. Studies in animals as well as in humans have demonstrated the usefulness of the immunoglobulin idiotype as a tumor-specific antigen for the study of the biology of B-cell lymphoma in vitro and as a target for passive immunotherapy in vivo.¹⁻⁵ Furthermore, active immunization against idiotypic determinants on malignant B cells has produced resistance to tumor growth in several models of syngeneic tumors,⁶⁻¹⁶ as well as specific antitumor therapy against established tumors.^{17,18} Moreover, preclinical studies in subhuman primates

have demonstrated that optimal immunization with immunoglobulin derived from human lymphomas requires conjugation of the protein to a strongly immunogenic carrier protein (e.g., keyhole-limpet hemocyanin [KLH]) and emulsification in an adjuvant.¹⁹ These results provided the rationale for testing autologous tumor-derived idiotypic surface immunoglobulin as a therapeutic "vaccine" against human B-cell lymphoma.

We immunized nine patients with B-cell lymphoma with the immunoglobulin-idiotype protein derived from their own tumors after they had been treated with chemotherapy and their tumors were in remission. The primary objective of this study was to determine whether immune responses against this autologous antigen could be induced in such patients.

METHODS

Selection of Patients

All patients had a histopathological diagnosis of non-Hodgkin's lymphoma. Immunophenotypic studies of the tumors showed that they were of B-cell origin, with the surface expression of immunoglobulin molecules composed of both heavy and light chains in a monotypic pattern (Table 1). Patients were selected who had minimal residual disease or who were in complete remission after chemotherapy. Seven patients had had only a single regimen of chemotherapy, and two had had multiple regimens (Table 1). The base-line studies used to evaluate clinical disease in all patients before immunization with idiotypic immunoglobulin included complete physical examination, chest radiography, routine blood counts and chemistry tests, and bipedal lymphangiography, with or without abdominal and pelvic CT scanning. None of the patients had received any chemotherapy for at least six months, and none received antitumor therapy during the study. All underwent restaging during immunization and at the completion of the series of immunizations, according to the objective procedures used at base line. In addition, three patients with rapidly progressive recurrent lymphoma were enrolled in a separate study to assess safety; all three

From the Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, Calif. Address reprint requests to Dr. Kwak at the Biological Response Modifiers Program, National Cancer Institute, Frederick, MD 21702-1201.

Supported by a grant (CA-33399) from the National Cancer Institute. Dr. Kwak is the recipient of a Young Investigator Award from the American Society of Clinical Oncology. Dr. Levy is an American Cancer Society Clinical Research Professor.

required reinstitution of chemotherapy shortly after enrollment, did not complete the series of immunizations, and were not studied further.

Isolation and Conjugation of Autologous Immunoglobulin Idiotype

Lymphoma cells from a biopsy specimen were fused with the hypoxanthine-aminopterin-thymidine (HAT)-sensitive heterohybridoma K6H6/B5.²⁰ Hybrid cells selected from HAT medium that secreted immunoglobulin with the type of heavy and light chains corresponding to the known immunophenotype of the tumor specimen were expanded into larger volumes (1 liter), and the supernatants were collected and pooled.

The tumor immunoglobulin-idiotype protein was purified from bulk supernatant by affinity chromatography (antihuman immunoglobulin-Sepharose). After the protein was adsorbed to the immunoadsorbent matrix, the immunoglobulin idiotype was eluted with 0.1 M glycine-hydrochloric acid buffer (pH 2.4). The purity of the protein (>90 percent) was determined by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis. Before conjugation, hemocyanin from the keyhole limpet (*Megathura crenulata*) (Calbiochem, San Diego, Calif.) was passed over a QAE Zeta Prep 15 disk (LKB, Broma, Sweden) to reduce the level of endotoxin to less than 1000 EU (endotoxin units) per milliliter. Equivalent volumes of immunoglobulin idiotype and KLH (both at a concentration of 1 mg per milliliter and sterilized by filter) were mixed together, and sterile glutaraldehyde (Sigma, St. Louis) was added, for a final concentration of 0.1 percent. The immunoglobulin-KLH conjugate was then dialyzed extensively against physiologic saline. The final product was tested for sterility and general safety before it was used.

Immunologic Adjuvant

The immunologic adjuvant used in this study was the vehicle component of SAF-1 (Syntex adjuvant formulation 1) described by Allison and Byars.²¹ It is composed of 5 percent (wt/vol) squalane (BASF, Parsippany, N.J.), 2.5 percent Pluronic L121 polymer (Aldrich Chemical, Milwaukee), and 0.2 percent polysorbate (Tween 80, Sigma) in phosphate-buffered saline.

Assays for Serum Antiidiotypic Antibody

Direct Enzyme-Linked Immunosorbent Assay

For the direct enzyme-linked immunosorbent assay (ELISA), preimmune and hyperimmune serum samples from each patient were diluted over wells of a microtiter plate that had been coated with either autologous immunoglobulin idiotype or a panel of four isotype-matched human tumor immunoglobulins of unrelated idiotype. Bound antibody was detected with horseradish peroxidase-goat antihuman light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype (Caltag Laboratories, South San Francisco).

Inhibition ELISA

Preimmune and hyperimmune serum samples from each patient were incubated on microtiter plates that had been coated with autologous immunoglobulin idiotype. Each plate was washed, and then a murine monoclonal antiidiotype antibody was added to the wells. Bound murine monoclonal antibody was detected with horseradish peroxidase-conjugated goat antimouse IgG antibodies (Caltag).

Inhibition Cell-Binding Assay

Tumor cells were obtained from a cryopreserved lymph-node-biopsy specimen obtained before treatment; the specimen was shown by flow cytometry to have 21 percent CD3-positive T cells (antihuman Leu-4 fluorescein isothiocyanate, Becton Dickinson, San Jose, Calif.), 4 percent lambda-light-chain-bearing normal B cells (fluorescein isothiocyanate-goat F(ab')₂ antihuman lambda, Tago, Burlingame, Calif.), and 75 percent kappa-light-chain-

Table 1. Characteristics of Patients with B-Cell Lymphoma.

PATIENT No. (SEX/AGE)	HISTOLOGIC SUBTYPE*	SURFACE IMMUNO- GLOBULIN	PRIOR THERAPY† (No. OF CYCLES)	MONTHS SINCE LAST THERAPY	SITE OF MEASURABLE DISEASE
Group 1					
1 (F/41)	FM	IgM lambda	CVP (8)	9	Submandibular lymph node
2 (M/56)	FM	IgA lambda	CVP (10)	8	None
3 (M/47)	FSC	IgM kappa	Chl/P (12)	6	None
4 (F/43)	FSC→DM	IgM kappa	mAb, CEPP (4), ABMT	7	Cutaneous mass
5 (M/59)	FSC	IgM kappa	CVP (9)	8	None
Group 2					
6 (M/45)	FSC	IgM kappa	CVP (10)	6	None
7 (M/50)	FM	IgM kappa	CVP (8)	18	None
8 (F/25)	FSC	IgM kappa	CVP (9)	20	None
9 (M/56)	FLC	IgG kappa	[¹³¹ I]anti-CD20, ABMT	12	None

*FM denotes follicular mixed large cell and small cleaved cell; FSC follicular small cleaved cell; FSC→DM follicular small cleaved cell, with transformation to diffuse mixed large cell and small cleaved cell; and FLC follicular large cell.

†CVP denotes cyclophosphamide, vincristine, and prednisone; Chl/P pulsed chlorambucil and prednisone; mAb antiidiotype monoclonal antibody; CEPP cyclophosphamide, etoposide, prednisone, and procarbazine; ABMT autologous bone marrow transplantation; and [¹³¹I]anti-CD20 radiolabeled monoclonal antibody.

bearing tumor cells (fluorescein isothiocyanate-goat F(ab')₂ antihuman kappa, Tago) (data not shown). Indirect staining with a murine antiidiotype monoclonal antibody revealed binding of 61 percent of the lymph-node cells.

Cryopreserved cells (1×10⁶) prepared by Ficoll-Hypaque centrifugation were incubated first with 50 μl of hyperimmune or preimmune serum or 1 μg of unlabeled antiidiotype monoclonal antibody in a total volume of 100 μl at 4°C for 30 minutes. After washing, the cells were incubated with a saturating concentration of biotinylated antiidiotype monoclonal antibody at 4°C for 30 minutes, washed twice again, and then incubated with streptavidin-phycoerythrin (Becton Dickinson) at 4°C for 30 minutes. They were then washed twice, fixed in 2 percent paraformaldehyde, and analyzed with a cell sorter (FACS 440, Becton Dickinson).

Purification of Human Antiidiotypic Antibody from Hyperimmune Serum

Twenty milliliters of hyperimmune serum was passed over an immunoadsorbent column consisting of autologous immunoglobulin idiotype coupled to Sepharose 4B, and antiidiotypic antibodies were eluted with 0.1 M glycine-hydrochloric acid buffer (pH 2.4). The presence or absence of antibodies of the IgG class in the purified preparation of antiidiotypic antibodies was determined with a detector consisting of horseradish peroxidase-goat antihuman IgG antibodies (Tago) specific for the gamma heavy chain. The presence of mu and gamma heavy chains and both kappa and lambda light chains was established by sodium dodecyl sulfate-polyacrylamide-gel electrophoresis.

Assay for Idiotype-Specific Proliferative Response

Proliferation assays were adapted from the techniques of Chain et al.²² Fresh peripheral-blood mononuclear cells (PBMCs), prepared by Ficoll-Hypaque gradient centrifugation, were washed and plated at a concentration of 4×10⁵ cells per well in Iscove's modified Dulbecco's medium (IMDM) with 1 percent human AB serum (IMDM-1 percent AB). KLH or autologous immunoglobulin idiotype at concentrations of 0 to 100 μg per milliliter in IMDM-1 percent AB preparation was added in quadruplicate. After the cells were incubated for three days at 37°C in an atmosphere containing 5 percent carbon dioxide, they were transferred to a preparation of IMDM and 5 percent fetal-calf serum containing recombinant interleukin-2 (30 U per milliliter, a gift from Cetus, Emeryville, Calif.). The plate was then incubated for two days and pulsed for 16

to 20 hours with ^3H -labeled thymidine (1 μCi per well). Data are expressed as mean ($\pm\text{SEM}$) counts per minute of [^3H]thymidine incorporation.

Expansion of Immune PBMCs

Initial five-day cultures of PBMCs established as described above were expanded in the IMDM-5 percent fetal-calf serum containing interleukin-2 (30 U per milliliter) for three weeks. Harvested cells were replated in IMDM-1 percent AB containing autologous immunoglobulin idiotype and fresh irradiated (5000 R) autologous PBMCs (4×10^5 cells per well) as antigen-presenting cells for five days, before pulsing with [^3H]thymidine.

RESULTS

Immunization Schedule

All patients received subcutaneous injections of 0.5 mg of immunoglobulin-idiotype protein conjugated to KLH at time 0 and at 2, 6, 10, and 14 weeks, followed by booster injections at 24 and 28 weeks. Patients in the first trial (group 1) received immunoglobulin idiotype-KLH alone for the first three immunizations, then immunoglobulin idiotype-KLH emulsified in the Pluronic polymer-based adjuvant formulation for all subsequent immunizations. Although marked responses to KLH were observed after a single immunization, no idiotype-specific immune responses were evident before the adjuvant was added to the program for this first group of patients. Therefore, patients in the second trial (group 2) received the entire series of immunizations with this adjuvant. In all nine patients, the production of idiotype-specific antibody and the proliferative responses of PBMCs *in vitro* were determined immediately before each immunization and one to two months after the last immunization. The KLH carrier served as a convenient internal control for the immunocompetence of each patient. All patients had both humoral-antibody and PBMC proliferation to the KLH protein except Patient 4, who had only the latter response.

Humoral Responses to Idiotype

Antibody responses were screened by analysis of preimmune and immune serum samples collected at various times in the direct ELISA (see the Methods section). Responses indicated by a significant increase in the level of antibody binding to autologous immunoglobulin idiotype in hyperimmune serum as compared with preimmune serum were then confirmed by a competition ELISA (see the Methods section). Three patients were classified as having an antibody response on the basis of this two-step analysis (Fig. 1 and 2, showing both types of assays). It is clear that immunization with autologous immunoglobulin-idiotype protein induced titers of antiidiotypic antibody that either directly bound (Fig. 1) or inhibited the binding (Fig. 2) of a murine antiidiotype monoclonal antibody to immunoglobulin idiotype on the plate. The specificity of the humoral response for the idiotype of the immunoglobulin was demonstrated by a lack of binding of hyperimmune se-

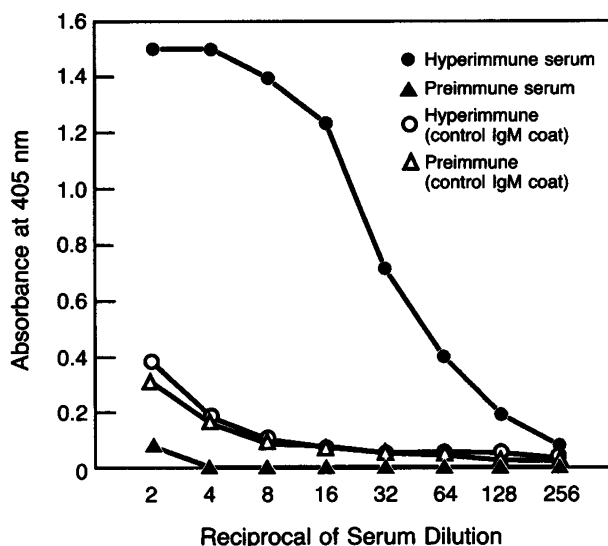


Figure 1. Serum Antiidiotypic-Antibody Titers Induced after Repeated Immunizations with Autologous Immunoglobulin Idiotype in Patient 1.

Preimmune and hyperimmune serum samples from the patient were diluted over wells of a microtiter plate that had been coated with either autologous immunoglobulin idiotype or a panel of four isotype-matched human immunoglobulins of unrelated idiotype. Bound antibody was detected with horseradish peroxidase-conjugated goat antihuman light-chain antibodies directed against the light chain not present in the immunoglobulin idiotype. For clarity, only one representative immunoglobulin coat of irrelevant idiotype is shown, in which there is a lack of marked binding activity of these serum samples.

rum to a panel of isotype-matched human immunoglobulins of unrelated idiotype (Fig. 1) or by a lack of inhibition of a panel of heterologous idiotype-antiidiotype systems (Fig. 2). Furthermore, the inhibition of each antiidiotype-monoclonal-antibody reagent was specific; that is, each monoclonal antibody could be blocked from binding to its corresponding immunoglobulin idiotype only by hyperimmune serum obtained from the same patient (data not shown).

The kinetics of the antibody responses elicited are shown in the screening ELISAs represented in Figure 3. Increases in the serum titer of antibody binding autologous immunoglobulin idiotype were not detectable in Patient 1 (group 1) until after the fourth immunization in the series (Fig. 3). In contrast, both responding patients from group 2 had increases in antibody titers at earlier points, with increases first observed after a single immunization (Fig. 3; Patient 7 is representative of both these patients). In each patient, these increases in antibody titer peaked after the fifth immunization. The *in vitro* responses of these three patients have persisted at peak levels for at least 9 months (range, 9 to 15).

The antiidiotypic antibodies produced in Patient 1 were purified by affinity chromatography and were shown to contain heterogeneous light chains as well as

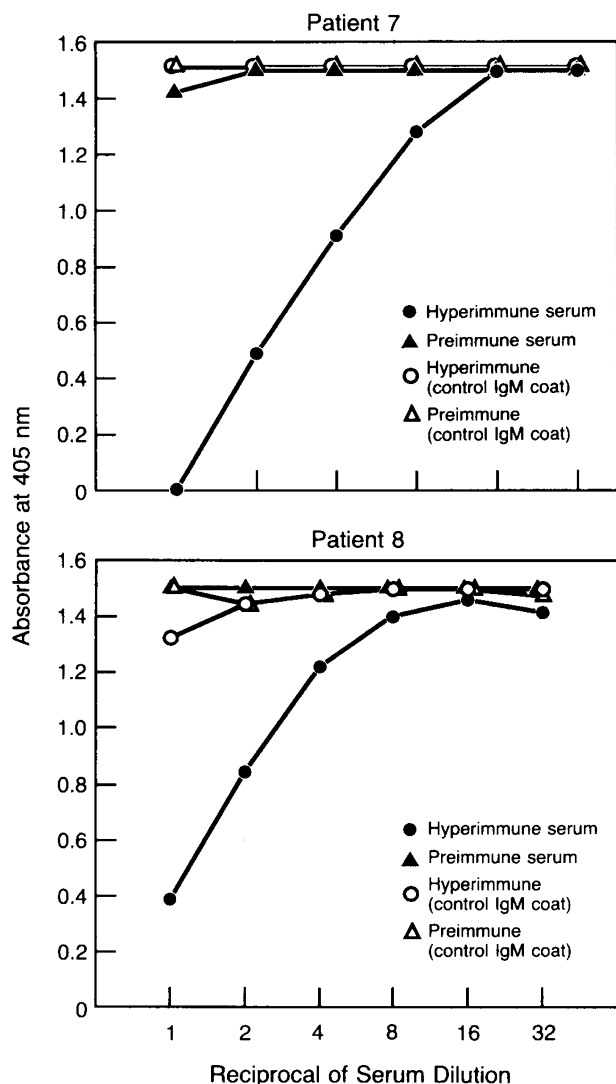


Figure 2. Inhibition of Binding of a Murine Antiidiotypic Monoclonal Antibody to Autologous Immunoglobulin Idiotype by Hyperimmune Serum in Patients 7 and 8.

Preimmune and hyperimmune serum samples were diluted over microtiter plates that had been coated with autologous immunoglobulin idiotype. The plates were washed, and culture supernatant containing a corresponding murine antiidiotypic monoclonal antibody was added to the wells. Bound murine monoclonal antibody was detected with horseradish peroxidase-conjugated goat antimouse IgG antibodies. The specificity of the hyperimmune samples was tested by coating plates with a panel of isotype-matched immunoglobulins of unrelated idiotype and substituting for the antiidiotypic monoclonal antibody an antiidiotypic monoclonal antibody corresponding to the respective control IgM coat. Each panel shows a lack of inhibition of one representative heterologous immunoglobulin idiotype-antiidiotypic system by each of the serum samples.

unrelated idiotype under the same experimental conditions (data not shown). Affinity-purified antiidiotypic antibodies from the hyperimmune serum of the two other patients who had idiotype-specific humoral responses were also demonstrated to bind autologous tumor cells (data not shown).

Cellular Responses to Idiotype

Cellular immune responses were measured by the proliferation of PBMCs in response to KLH and to autologous immunoglobulin idiotype at concentrations ranging from 1 to 100 μ g of soluble protein per milliliter in five-day in vitro cultures. Table 2 shows the results of assays of peak hyperimmune PBMCs from all patients. None of the preimmune PBMCs demonstrated any preexisting proliferation in response to autologous immunoglobulin idiotype that was greater than the proliferation in culture medium alone (data not shown). Hyperimmune PBMCs from all patients showed strong proliferative responses to the KLH carrier. The finding of primary interest was the hyperimmune proliferative responses to immunoglobulin idiotype that were detected in five patients. Although their responses were of a lower magnitude than parallel responses to KLH, Patients 3, 4, 6, 8, and 9 were classified as responders on the basis of reproducible increases, sustained over multiple time points, in the levels of activity in wells containing immunoglobulin idiotype, as compared with levels in wells containing medium alone. Patients who had occasional increases in activity in wells containing immunoglobulin idiotype as compared with medium alone were classified as nonresponders (Patients 1 and 5).

Increases in proliferation in response to autologous immunoglobulin idiotype in the two responding patients in group 1 were not detectable until after the fourth immunization of the series. The proliferative responses in the three responding patients in group 2 required a minimum of two immunizations. However, in two of these patients, peak responses were observed after additional immunizations had been administered (Table 2, Patients 8 and 9). These proliferative re-

IgG heavy chains. The presence of antibodies of the IgG class was confirmed by a specific ELISA (see the Methods section). This patient's antibody titer was successfully boosted with a single administration of immunoglobulin idiotype-KLH in adjuvant when the humoral response declined after a period of 15 months (data not shown).

Antibody Binding to Autologous Tumor

We also tested the ability of the idiotype-specific humoral response to bind autologous tumor cells in vitro. As shown in Figure 4, the binding of a labeled murine antiidiotypic monoclonal antibody to tumor cells from a lymph-node specimen obtained from Patient 8 before treatment was inhibited by her hyperimmune serum but not by her preimmune serum. The specificity of the blocking by the hyperimmune serum from this patient was confirmed by the lack of inhibition of a heterologous antiidiotypic monoclonal antibody binding to tumor cells from another patient expressing an isotype-matched immunoglobulin of

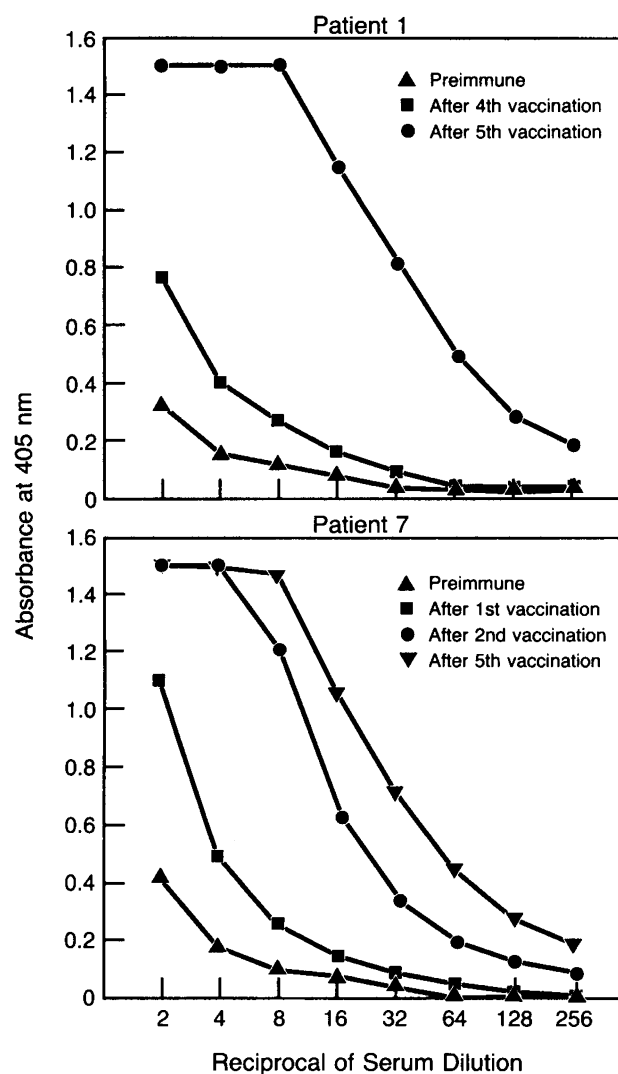


Figure 3. Kinetics of the Humoral Antiidiotypic Responses Elicited by Immunization with Immunoglobulin Idiotype in Patients 1 (Group 1) and 7 (Group 2).

The serial serum antibody titers in each panel were measured in a single screening ELISA as described in the legend to Figure 1.

sponses to immunoglobulin idiotype in both groups of patients have persisted during 9 to 14 months of follow-up.

Flow-cytometric analysis of cultures demonstrating proliferation in response to immunoglobulin idiotype revealed a predominance of cells staining positively for CD4 (>95 percent), suggesting the phenotype of the responding cell subpopulation (data not shown). These cultures could be successfully expanded for approximately four weeks by stimulation alternately with interleukin-2 and immunoglobulin idiotype-pulsed autologous irradiated PBMCs as antigen-presenting cells (Fig. 5). The specificity of the responses for immunoglobulin idiotype was confirmed by a lack of proliferation in response to an isotype-matched human immunoglobulin

of unrelated idiotype, as compared with medium alone (data not shown).

Toxicity and Disease Activity

Toxicity was minimal in all 12 patients (including the 3 with rapidly progressive disease). All patients had transient local reactions characterized by mild erythema, induration, and discomfort, without skin breakdown at the injection sites. Administering the components of the vaccine (immunoglobulin idiotype-KLH and adjuvant) separately in one patient with a moderate local reaction and in another with a moderate systemic reaction characterized by fever, rigors, and diffuse arthralgias revealed that the adjuvant was the component associated with these reactions. Both of these moderate reactions resolved completely after 24 to 48 hours. The only laboratory abnormality associated with immunization was a mild elevation (less than twice the normal value) in the serum creatine kinase concentration 24 hours after immunization in an occasional patient.

All patients were also closely monitored for disease activity by means of repeated physical examinations and routine laboratory and radiographic studies. Of the two patients with measurable tumor at the initiation of immunization, one (Patient 1) had complete regression of a single 2.5-cm left submandibular lymph node, documented by CT, and the other (Patient 4) had complete regression of a 4.5-cm cutaneous

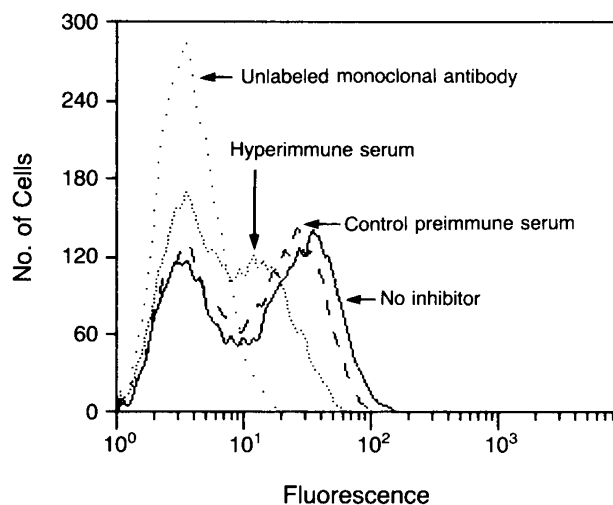


Figure 4. Inhibition of Binding of a Murine Antiidiotype Monoclonal Antibody to Tumor Cells from Patient 8 by Her Hyperimmune Serum.

Single-color fluorescence histograms from a single experiment represent staining of lymphoma cells with a biotinylated anti-idiotype monoclonal antibody after incubation of the cells with hyperimmune serum containing antiidiotypic antibody, control preimmune serum, or unlabeled monoclonal antibody or without inhibitor. The specificity of the hyperimmune serum from this patient was confirmed by the lack of inhibition of a heterologous antiidiotype-monoclonal-antibody reagent binding to tumor cells from another patient expressing immunoglobulin of the same isotype under the same experimental conditions (data not shown).

Table 2. In Vitro Proliferative Responses of PBMCs to KLH and Immunoglobulin Idiotype.

PATIENT No.	NO. OF IMMUNIZATIONS	PROLIFERATION IN MEDIUM	RESPONSE TO KLH			RESPONSE TO IMMUNOGLOBULIN IDIOTYPE		
			10 μ g/ml	50 μ g/ml	100 μ g/ml	10 μ g/ml	50 μ g/ml	100 μ g/ml
			<i>mean ($\pm$SEM) counts per minute for [^{3}H]thymidine incorporation by PBMCs*</i>					
Group 1								
1	5	1,721 $\pm$ 612	5,459 $\pm$ 1305	6,938 $\pm$ 402	ND	747 $\pm$ 234	3,117 $\pm$ 689	ND
2	6	137 $\pm$ 55	2,153 $\pm$ 560	2,697 $\pm$ 731	ND	188 $\pm$ 33	355 $\pm$ 40	ND
3	5	14,003 $\pm$ 1827	34,799 $\pm$ 4906	58,190 $\pm$ 1816	83,646 $\pm$ 3768	14,394 $\pm$ 6177	22,764 $\pm$ 5264	40,102 $\pm$ 4036
4	4	4,477 $\pm$ 584	6,767 $\pm$ 531	16,020 $\pm$ 1517	10,541 $\pm$ 560	9,629 $\pm$ 596	7,380 $\pm$ 382	8,182 $\pm$ 1138
5	7	4,380 $\pm$ 394	24,577 $\pm$ 898	ND	31,058 $\pm$ 781	5,362 $\pm$ 463	7,366 $\pm$ 372	ND
Group 2								
6	2	12,887 $\pm$ 1435	39,996 $\pm$ 2826	91,237 $\pm$ 1965	113,508 $\pm$ 5139	32,922 $\pm$ 3282	29,584 $\pm$ 5167	ND
7	5	6,702 $\pm$ 544	54,957 $\pm$ 2753	ND	67,571 $\pm$ 7341	7,162 $\pm$ 1179	ND	5,779 $\pm$ 481
8	5	7,645 $\pm$ 731	71,152 $\pm$ 3172	ND	81,842 $\pm$ 3162	10,769 $\pm$ 341	ND	17,977 $\pm$ 1334
9	6	8,953 $\pm$ 767	68,118 $\pm$ 3858	ND	67,657 $\pm$ 1013	6,419 $\pm$ 997	ND	20,332 $\pm$ 1379

*Each mean (quadruplicate wells) was calculated from assays of peak hyperimmune autologous PBMCs. ND denotes not done.

lymphomatous mass on the right arm. These patients remain in remission 24 and 10 months, respectively, after completing immunization. Of the patients who were in remission and completed the series of immunizations, one has had a recurrence (Patient 5); all the other patients remain in remission after a median follow-up of 10 months.

DISCUSSION

The idiotype of the immunoglobulin expressed on the surface of a B-cell lymphoma cell can function as a tumor-specific antigen. A major obstacle limiting the success of passive immunotherapy of B-cell lymphomas with a murine antiidiotype monoclonal antibody has been the emergence of tumor cells with mutated forms of their surface immunoglobulin.²³ Active immunization with purified immunoglobulin, by virtue of induction of a polyclonal immune response directed against multiple idiotypic determinants on this mole-

cule, may overcome this obstacle. The results presented here show that immunoglobulin idiotype can serve as an immunogen (antigen) eliciting both humoral and cell-mediated immunologic responses. Seven of nine patients with B-cell lymphoma had sustained idiotype-specific immune responses of a humoral type (two patients), cell-mediated type (four patients), or both types (one patient). The responses specific for this autologous protein were significantly increased over base-line levels, although they were not equal in magnitude to those generated against the KLH carrier. This was an expected result, given the strongly immunogenic nature of the exogenous antigen as compared with an autologous protein. The humoral antiidiotypic response elicited was clearly capable of binding autologous tumor in vitro. We presume that this induced antibody bound to tumor cells in vivo; however, this could not be proved because at the time of their immune responses the patients

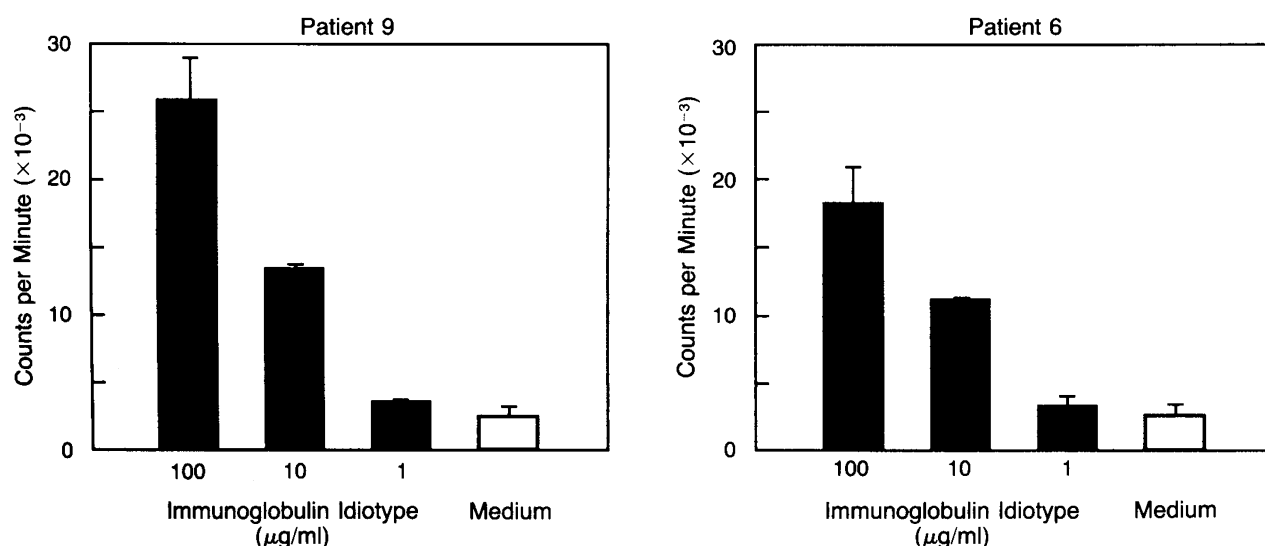


Figure 5. Expansion and Restimulation of Immune PBMCs in Patients 9 and 6.

PBMCs proliferated in vitro in response to autologous immunoglobulin idiotype after expansion of the primary five-day cultures (described in the Methods section) with interleukin-2 for three weeks and restimulation with idiotypic-immunoglobulin protein at various concentrations. Values are means $\pm$ SEM.

were all in complete clinical remission. The present study also establishes the requirement for an immunologic adjuvant, such as the Pluronic polymer-based vehicle used.

The concept of a therapeutic tumor vaccine might involve two different strategies. The goal of one approach is to induce immune resistance in the host against the regrowth of tumor cells that remain after primary therapy. Such an approach is exemplified by clinical trials of whole-tumor-cell or cell-lysate vaccines administered to patients with colon cancer²⁴ and to patients with melanoma²⁵ who are at high risk for relapse after standard therapy. The alternative approach is to administer the vaccine as therapy for clinically apparent disease. Several trials in which melanoma-cell-lysate vaccines have been administered without prior cytoreductive therapy have reported objective antitumor responses. In these studies, the induction of tumor-specific immunologic responses appears to be a requisite for the clinical responses observed, and the inability to elicit immune responses in the presence of gross tumor is a factor limiting the success of this approach.²⁶⁻²⁸

In this study we adapted a vaccine approach to B-cell lymphomas. We reasoned that the group in which we would have the best chance to demonstrate that patients could mount an immune response to components of their own tumor would be patients whose tumors were in remission but whose previous therapy had been limited. It was possible to induce a low level of specific humoral and cellular immunity in such patients. There may be considerable latitude for optimizing the immunogenicity of the vaccine by modifying its "foreignness" by molecular engineering and by using better immunologic adjuvants. If more-potent vaccines can be formulated, then the approach can be extended to immunization of patients with greater tumor burdens. The successful induction of immune responses to the KLH carrier suggests that chemotherapy-induced immunosuppression is not an obstacle to active immunotherapy administered as an adjunct to cytoreductive drug therapy.

A potential limitation of this approach is that the use of autologous immunoglobulin-idiotype protein as the immunogen requires that a vaccine be produced individually for each patient and that accessible tumor tissue be available as starting material. Methods to refine and streamline the production of the purified immunoglobulin-idiotype product would thus be desirable.

We are indebted to Roxena McElderry for skilled nursing assistance, to Dan Levi and Marlo Lenox for excellent technical assistance, and to Nancy Edwards and Phyllis Bussey for assistance in the preparation of the manuscript.

REFERENCES

1. Stevenson GT, Stevenson FK. Antibody to molecularly-defined antigen confined to a tumor cell surface. *Nature* 1975;254:714-6.
2. Stevenson GT, Elliott EV, Stevenson FK. Idiotype determinants on the surface immunoglobulin of neoplastic lymphocytes: a therapeutic target. *Fed Proc* 1977;36:2268-71.
3. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med* 1982;306:517-22.
4. Meeker TC, Lowder J, Maloney DG, et al. A clinical trial of anti-idiotype therapy for B cell malignancy. *Blood* 1985;65:1349-63.
5. Brown SL, Miller TA, Horning SJ, et al. Treatment of B-cell lymphomas with anti-idiotype antibodies alone and in combination with alpha interferon. *Blood* 1989;73:651-61.
6. Sirisinha S, Eisen HN. Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. *Proc Natl Acad Sci U S A* 1971;68:3130-5.
7. Jørgensen T, Gaudernack G, Hannestad K. Immunization with the light chain and the V_L domain of the isologous myeloma protein 315 inhibits growth of mouse plasmacytoma MOPC315. *Scand J Immunol* 1980;11:29-35.
8. Daley MJ, Gebel HM, Lynch RG. Idiotype-specific transplantation resistance to MOPC-315: abrogation by post-immunization thymectomy. *J Immunol* 1978;120:1620-4.
9. Bridges SH. Participation of the humoral immune system in the myeloma-specific transplantation resistance. *J Immunol* 1978;121:479-83.
10. Freedman PM, Autry JR, Tokuda S, Williams RC Jr. Tumor immunity induced by preimmunization with BALB/c mouse myeloma protein. *J Natl Cancer Inst* 1976;56:735-40.
11. Sugai S, Palmer DW, Talal N, Witz IP. Protective and cellular immune responses to idiotype determinants on cells from a spontaneous lymphoma of NZB-NZW F1 mice. *J Exp Med* 1974;140:1547-58.
12. Stevenson FK, Gordon J. Immunization with idiotype immunoglobulin protects against development of B lymphocytic leukemia, but emerging tumor cells can evade antibody attack by modulation. *J Immunol* 1983;130:970-3.
13. George AJT, Tutt AL, Stevenson FK. Anti-idiotypic mechanisms involved in the suppression of a mouse B cell lymphoma, BCL₁. *J Immunol* 1987;138:628-34.
14. Kaminski MS, Kitamura K, Maloney DG, Levy R. Idiotype vaccination against murine B cell lymphoma: inhibition of tumor immunity by free idiotype protein. *J Immunol* 1987;138:1289-96.
15. Campbell MJ, Carroll W, Kon S, et al. Idiotype vaccination against murine B cell lymphoma: humoral and cellular responses elicited by tumor-derived immunoglobulin M and its molecular subunits. *J Immunol* 1987;139:2825-33.
16. Campbell MJ, Esserman L, Byars NE, Allison AC, Levy R. Idiotype vaccination against murine B cell lymphoma: humoral and cellular requirements for the full expression of antitumor immunity. *J Immunol* 1990;145:1029-36.
17. George AJT, Folkard SG, Hamblin TJ, Stevenson FK. Idiotype vaccination as a treatment for a B cell lymphoma. *J Immunol* 1988;141:2168-74.
18. Campbell MJ, Esserman L, Levy R. Immunotherapy of established murine B cell lymphoma: combination of idiotype immunization and cyclophosphamide. *J Immunol* 1988;141:3227-33.
19. Campbell MJ, Esserman L, Byars NE, Allison AC, Levy R. Development of a new therapeutic approach to B cell malignancy: the induction of immunity by the host against cell surface receptor on the tumor. *Int Rev Immunol* 1989;4:251-70.
20. Carroll WL, Thielemans K, Dille J, Levy R. Mouse x human heterohybridomas as fusion partners with human B cell tumors. *J Immunol Methods* 1986;89:61-72.
21. Allison AC, Byars NE. An adjuvant formulation that selectively elicits the formation of antibodies of protective isotypes and of cell-mediated immunity. *J Immunol Methods* 1986;95:157-68.
22. Chain B, McCafferty I, Wallace G, Askenase PW. Improvement of the in vitro T cell proliferation assay by a modified method that separates the antigen recognition and IL-2-dependent steps. *J Immunol Methods* 1987;99:221-8.
23. Meeker T, Lowder J, Cleary ML, et al. Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. *N Engl J Med* 1985;312:1658-65.
24. Hoover HC Jr, Surdyke MG, Dangel RB, Peters LC, Hanna MG Jr. Prospectively randomized trial of adjuvant active-specific immunotherapy for human colorectal cancer. *Cancer* 1985;55:1236-43.
25. Bystryn JC, Oratz R, Harris MN, Roses DF, Golomb FM, Speyer JL. Immunogenicity of a polyvalent melanoma antigen vaccine in humans. *Cancer* 1988;61:1065-70.
26. Berd D, Maguire HC Jr, McCue P, Mastrangelo MJ. Treatment of metastatic melanoma with an autologous tumor-cell vaccine: clinical and immunologic results in 64 patients. *J Clin Oncol* 1990;8:1858-67.
27. Mitchell MS, Harel W, Kempf RA, et al. Active-specific immunotherapy for melanoma. *J Clin Oncol* 1990;8:856-69.
28. Hoon DS, Foshag LJ, Nizze AS, Bohman R, Morton DL. Suppressor cell activity in a randomized trial of patients receiving active specific immunotherapy with melanoma cell vaccine and low dosages of cyclophosphamide. *Cancer Res* 1990;50:5358-64.