Cellular and Humoral Immune Response to N-Glycolyl-GM3 Elicited by Prolonged Immunotherapy With an Anti-Idiotypic Vaccine in High-Risk and Metastatic Breast Cancer Patients

Marcelo D. Guthmann, Mónica A. Castro,* Gabriela Cinat,† Cecilia Venier,* Leonardo Koliren,‡ Roberto J. Bitton,* Ana María Vázquez,§ and Leonardo Fainboim**

Summary: In this study, the immunogenicity and toxicity profile of 1E10, an anti-idiotypic vaccine mimicking the N-glycolyl-GM3 ganglioside, was investigated with an extended vaccination protocol. The year-long vaccination scheme consisted of 6 biweekly intradermal injections (induction phase), followed by 10 monthly boosters (maintenance). Nineteen patients with high-risk (stage III) or metastatic breast cancer were vaccinated with different dose levels of 1E10 (0.5, 1, and 2 mg). The humoral and cellular responses to 1E10 and the targeted ganglioside were assessed at baseline and throughout the treatment. Local skin reactions represented the most common adverse event (National Cancer Institute Toxicity Criteria (NCIC) grades I and II), followed by mild flu-like symptoms lasting for 1 to 2 days. Two patients were removed from the study because of vaccine-related hypersensitivity reactions. A third patient was removed from the study after a transient loss of consciousness with uncertain relation to the vaccine. All patients showed a strong antibody response to the targeted ganglioside. In addition, ganglioside-specific T-cell responses were recorded in 5 of 13 evaluable patients. Vaccination with 1E10 was immunogenic and relatively well tolerated. Because similar results were observed with the 3 tested dose levels, the 0.5-mg dose level was selected for future trials.

Key Words: gangliosides, N-glycolylneuraminic acid, anti-idiotypic antibodies, cancer vaccines, breast cancer

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Because gangliosides have been identified as tumor-associated antigens capable of inducing an antibody response,[1,2] they have received attention as possible targets for cancer therapy. Gangliosides are a family of sialylated glycolipids that are normal components of the cell membrane.[3] They have been found to be important actors in multiple aspects of cellular interaction with the environment and with transmembrane signaling. As such, they are involved in cancer progression[4] and have become the focus of several immunotherapeutic approaches.[5,6] Not all gangliosides are equally immunogenic. N-acetyl-GM3, the most abundant ganglioside in normal serum[7] and one of the main gangliosides on the cell surface,[8] is one of the most immunologically tolerated members of the family. In contrast, N-glycolyl-GM3 is not expressed in normal human tissues because of a species-specific genetic mutation that abrogates the biosynthesis of N-glycolylneuraminic acid (Neu5Gc).[9] Neu5Gc has been reported, however, in human tumors, and its presence might be derived from dietary sources or an as yet unknown alternate synthesis pathway.[9] N-glycolyl-GM3 is expressed in melanoma[10] and breast cancer (BC) cells[11] and is highly immunogenic. As a result, it has been considered a target of choice for immunotherapy.

Different strategies to target N-glycolyl-GM3 have been tested. N-glycolyl-GM3 was combined with the outer membrane protein complex of *Neisseria meningitides*[12] to form very small-sized proteoliposomes (VSSPs). Unusually high antiganglioside antibody titers have been induced in BC patients treated with this vaccine.[13]

A second cancer vaccine targets N-glycolyl-GM3 by means of an anti-idiotypic monoclonal antibody (mAb) called 1E10. This Ab2 mAb was generated from a BALB/c mouse immunized with an Ab1 (P3) mAb shown to be specific for N-glycosylated sialic acid on mono- and disialogangliosides.[14]

Anti-idiotypic antibodies have been broadly used as cancer vaccines. Advantage has been achieved from the fact that they are easier to purify and scale up than the antigens they mimic. In addition, because carbohydrate antigens are less immunogenic than peptide antigens, it was hypothesized that an increased immune response would be obtained through immunization with the

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From the *Hospital de Clínicas José de San Martín, University of Buenos Aires, Buenos Aires, Argentina; †Instituto de Oncología Ángel Roffo, University of Buenos Aires, Buenos Aires, Argentina; ‡Hospital Dr. Carlos G. Durand, Buenos Aires, Argentina; and §Center of Molecular Immunology, Havana, Cuba.
Reprints: Marcelo D. Guthmann, Hospital de Clínicas José de San Martín, University of Buenos Aires, Córdoba 2351, Piso 3 Sala 4, 1120 Buenos Aires, Argentina (e-mail: ruculeufu@arnet.com.ar).

peptidic internal image of a carbohydrate antigen. Anti-idiotypic vaccines targeting GD3[15] and GD2[16] have been tested for their ability to induce ganglioside-specific antibodies with diverse results.

The immunogenicity of 1E10 was previously examined in melanoma[17] and BC patients[18] using a 6-dose vaccination protocol. Assessment of the humoral response indicated that high-titer anti-N-glycolyl-GM3 antibodies could be induced in almost all vaccinated patients. The induction of N-glycolyl-GM3–specific cellular immune responses has not been investigated to date, however. The purpose of this phase 1 clinical trial was to assess in BC patients the optimal 1E10 dose level and its immunologic and toxicity profiles using an extended 16-dose vaccination scheme.

PATIENTS AND METHODS

Study Population

Twenty patients were enrolled in this phase 1 study. The following criteria were used for inclusion: histologically confirmed diagnosis of BC; patients with American Joint Committee on Cancer (AJCC) stage III disease (high-risk BC) treated with primary surgery and adjuvant chemotherapy and/or radiotherapy (patients could be included in the study up to 2 years and not less than 28 days after finishing adjuvant treatment), patients with metastatic BC (slowly progressive or complete response disease), Eastern Cooperative Oncology Group performance status of 0 to 2, age of at least 18 years, life expectancy more than 6 months, adequate vital organ function (white blood cell count [WBC] ≥ 3500 cells/μL, neutrophil count ≥ 2000 cells/μL, platelet count $\geq 100{,}000$ cells/μL, serum creatinine < 1.5 mg/dL, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] < 3 times the upper limit of the institution's normal range, and total bilirubin < 2.0 mg/dL), and signed informed consent. Hormone treatment was allowed during the study.

Patients with the presence of any of the following criteria were not eligible for the study: brain metastatic disease, active acute or chronic infections, uncontrolled nonmalignant systemic disease, previous or concurrent malignancy (with the exception of correctly treated in situ cervix carcinoma and nonmelanoma skin cancer), known HIV infection, history of autoimmune disease, history of demyelinating or inflammatory central or peripheral nervous system disease, history of severe allergic reactions, concurrent use of corticosteroids (a washout period of 28 days was required), and pregnancy or lactation.

The trial protocol was approved by the institutional review boards and ethics committees of the involved investigational centers and was authorized by the Argentinean Authority for Drug, Food, and Medical Technology (ANMAT).

Vaccination Schedule

1E10 mAb (IgG1, κ) was purified for the clinical trial at the Center of Molecular Immunology, Havana, Cuba, as previously described.[18] Briefly, sterile purified 1E10 mAb was precipitated with aluminum hydroxide (Superfos Biosector, Fredrikssund, Denmark) to a final concentration of 2 mg/mL. The product was tested for sterility and pyrogenicity according to the US Pharmacopeia.

Patients were sequentially enrolled in the 0.5-, 1-, and 2-mg dose groups. Doses were partitioned in 250-μL intradermal injections located 5 cm apart in the arm, abdomen, or thigh. Six doses were administered biweekly (induction phase), followed by 10 monthly doses (maintenance phase).

Treatment Assessment and Safety

A complete history and physical examination, performance status, and hematologic and chemistry profiles were performed at baseline and before each treatment dose. A bone scan; computed tomography (CT) scan of the chest, abdomen, or pelvis; and mammography study were obtained at baseline and every 3 months during treatment.

Patients with metastatic disease were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines. Toxicity data were recorded using the National Cancer Institute Common Toxicity Criteria, version 2.0. Vaccination was discontinued in case of any evidence of disease relapse, progression, decline in performance status, or severe toxicity. Patients who received 1 or more vaccine doses were evaluable for toxicity and clinical results. Patients who received at least 4 and 6 vaccine doses were evaluable for humoral[17] and cellular responses, respectively.

Antibody Response

Anti-1E10 idiotype antibodies were determined by enzyme-linked immunosorbent assay (ELISA). Costar 3590 microtiter plates (Costar, Cambridge, MA) were sensitized with 0.5 μg per well 1E10-derived F(ab′)2 (Center of Molecular Immunology). Isotype control F(ab′)2 was used as a control. Serum samples were used diluted 1:5000. Bound IgG antibodies were detected with an alkaline phosphatase–conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Absorbency in control wells was subtracted to yield the specific anti-1E10 idiotype reactivity.

Anti-N-glycolyl-GM3 IgM and IgG antibodies were determined by ELISA using N-glycolyl-GM3–coated (20 ng per well) Falcon 3915 microtiter plates (Becton Dickinson, San Diego, CA). Control wells were treated with the carrier solvent alone (methanol high-performance liquid chromatography [HPLC] grade). After solvent evaporation for 2 hours in a vacuum, plates were blocked with 4% human serum albumin (Grifols, Buenos Aires, Argentina) in phosphate-buffered saline (PBS). Serum samples were used at a 1:200 dilution (for kinetics analysis) or in a 2-fold dilution series starting at a 1:200 dilution (for titration). Bound antibodies were detected with alkaline phosphatase–conjugated goat anti-human IgM or IgG (Jackson Immuno Research Laboratories). Absorbency in control wells was

subtracted to yield the specific anti-N-glycolyl-GM3 reactivity. Titers were defined as the inverse of the dilution yielding an absorbency of 0.1 and were obtained by interpolation in absorbency versus 1/dilution plots.

P3X63 Ag8 653 murine myeloma cells and B16 murine melanoma cells were incubated (2×10^5 per tube) with 20 μL sample sera. After washing, bound antibodies were detected with fluorescein isothiocyanate (FITC)–conjugated goat anti-human Ig (Sigma Chemical Company) and analyzed with a FACScalibur cytometer (Becton-Dickinson). Patients were considered responsive when 2 postvaccination samples had a 2-fold increase in fluorescence intensity with respect to baseline.

Measurement of IFNγ Secretion

Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed and resuspended in Dulbecco modified Eagle medium (Sigma Chemical Company) supplemented with 10% fetal calf serum (Natocor, Cordoba, Argentina). Cells were placed in round-bottom 96-well microtiter plates at 10^5 cells per well, and 1E10-derived (or the isotypic control) $F(ab')_2$ fragments were added at 5 μg/mL in a final volume of 200 μL per well. After 72 hours, culture supernatants were collected for interferon-γ (IFNγ) quantitation by ELISA. Cytokine release was expressed as the mean cytokine concentration obtained from triplicate stimulated supernatants minus cytokine released from control cultures.

Frequency of N-glycolyl-GM3–reactive cells was calculated by IFNγ ELISPOT assays as described elsewhere.[19] Autologous dendritic cells (DCs) were obtained from adherent monocytes cultured with 800 U/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) and 500 U/mL interleukin (IL)-4. Cryopreserved PBMCs (3×10^5 per well) and autologous DCs (3×10^4 per well) were placed in conical-bottom 96-well microtiter plates. N-glycolyl-GM3–containing liposomes (Center of Molecular Immunology[20]) were added to a final concentration of 10 μg gangliosides per milliliter. Equivalent amounts of unloaded liposomes were added to control wells. After a 24-hour culture, cells in each well were resuspended and transferred to triplicate wells (10^5 PBMCs per well) in an ELISPOT plate (Millipore, Bedford, MA) previously coated with an anti-IFNγ antibody (Pierce, Rockford, IL) and incubated for an additional 24 hours. Subsequent steps for detection of IFNγ-secreting cells followed standard ELISPOT procedures.[19] Patients with no response at baseline and with a posttreatment 2-fold increase in the number of spots in N-glycolyl-GM3–stimulated wells (vs. unstimulated wells) were considered to be responsive. Plates were scanned with an AID EliSpot Reader System and analyzed with its companion version 3.2 software (AID, Strassburg, Germany).

Statistical Analysis

The GraphPad Instat software package was used for statistical evaluation of the data. In the comparison of means of stimulated and unstimulated replicas, the Student *t* test was used. Null hypotheses were rejected, and significant statistical differences were assumed if $P < 0.05$.

RESULTS

Demographics

Twenty patients were accrued. Seven patients were assigned to the 0.5-mg dose level (1 did not start the treatment), 5 to the 1-mg dose level, and 7 to the 2-mg dose level. Clinical data of the 19 treated patients are summarized in Table 1: 14 were high-risk patients and 5 were metastatic BC patients. Two of the latter had locoregional unresectable relapse and soft tissue metastatic disease and were in complete remission after first-line chemotherapy. Three had slowly progressive metastatic disease compromising bone (2 patients) and soft tissue (1 patient). The median time from primary diagnosis was 12 months and 50 months for high-risk and metastatic BC patients, respectively. Thirteen patients had estrogen receptor–positive tumors, and 1 received concomitant hormonotherapy during treatment.

Toxicity

All the adverse events reported for the 19 treated patients are shown in Table 2. Local skin reactions such as erythema, pruritus, local pain, swelling, and small

TABLE 1. Patient Characteristics (n = 19)

Variables	Median (Range)
Age (y)	49 (22–72)
Median time from primary diagnosis, months	
Hig risk BC	12 (7–26)
Metastatic BC	50 (24–185)
	No. Patients (%)
High risk BC	
4–9 positive lymph nodes	9 (64)
> 9 positive lymph nodes	5 (36)
Metastatic BC	
Bone	2 (40)
Soft tissue (in CR)	1 (20)
Locoregional (in CR)	1 (20)
Soft tissue and locoregional	1 (20)
Primary therapy in high risk BC	
Surgery + CT + RT + HT	8 (58)
Surgery + CT + RT	3 (21)
Surgery + CT + HT	3 (21)
Previous treatment in metastatic BC	
Surgery + HT (1st line)	1 (20)
Surgery + CT (2nd line)	1 (20)
CT (1st line in CR)	2 (40)
CT (3rd line) + HT (3rd line)	1 (20)
Estrogen receptor status	
Positive	15 (80)
Negative	3 (15)
Unknown	1 (5)
Concurrent hormone therapy	
High risk BC	10 (70)
Metastatic BC	3 (23)

CR indicates complete response; CT, chemotherapy; HT, hormonotherapy; RT, radiotherapy.

TABLE 2. Adverse Events

	Dose Level Groups					
	0.5 mg*		1 mg†		2 mg‡	
	Adverse Event Grade					
	1/2	3/4	1/2	3/4	1/2	3/4
Injection site reactions						
Pain	8/–		5/–		5/–	
Erythema	3/1	1/–	4/1		15/1	
Pruritus	2/1		1/–		5/2	
Swelling	2/1	1/–				
Induration	4/–		12/–		2/1	
Constitutional symptoms						
Fever			1/4			
Fatigue	1/–		3/1		2/1	
Headache	–/1					
Musculoskeletal						
Arthralgia			2/2			
Myalgia			2/2			
Cramps	1/–					
Neurologic						
Tinnitus	–/9					
Syncope						1/–
Mood alteration					–/3	
Allergic						
Vasculitis				1/–		
Rhinitis	6/–				1/–	
Other						
Alkaline phosphatase	2/–					
Lactate dehydrogenase					2/–	
Anemia			3/–			
Hypertension					1/–	
Weight gain			1/–		6/–	

*64 vaccine doses were administrated in the 0.5-mg dose level.
†58 vaccine doses were administrated in the 1-mg dose level.
‡67 vaccine doses were administrated in the 2-mg dose level.
– denotes that no adverse events were recorded for the indicated adverse event grade.

hyperpigmented areas of induration (less than 0.5 cm) were the most frequently observed signs of toxicity. These injection site reactions disappeared after 4 to 7 days. Small hyperpigmented induration areas could persist for several months in some cases, however. Ten patients experienced mild to moderate flu-like symptoms consisting of myalgia, fever, headache, and fatigue. This syndrome usually started between 3 and 24 hours after the injection and persisted for 2 to 48 hours. These symptoms were self-limiting or relieved by nonsteroidal anti-inflammatory agents. There was no evidence of increased toxicity caused by repeated booster administration during the 10-month maintenance phase.

Three patients were withdrawn from the study because of the occurrence of adverse events. Patient 5 developed an extensive area of erythema (measuring approximately 15 cm) at the injection site after the fourth dose of 1E10 vaccine (0.5-mg dose). Patient 12 (1-mg dose) presented with induration and blisters at the injection site after the fifth dose. The pathologic findings of the lesion included signs of vasculitis. Clinical and serologic follow-up of this patient showed no positive markers for autoimmune disease. Patient 14 (2-mg dose) experienced a non–life-threatening episode of transient syncope 10 days after receiving dose 9 (maintenance phase), with an uncertain relation to the vaccine. Neurologic examination and brain magnetic resonance imaging (MRI) showed no sign of abnormality.

Patient 2 (0.5-mg dose, with skull metastasis at diagnosis) presented with episodes of tinnitus with an uncertain relation to the vaccine. A brain CT scan and inner ear MRI as well as audiometry and otorhinolaryngologist examinations showed no evidence of abnormalities. This patient was later withdrawn from the study because of disease progression.

Abnormalities observed in the laboratory tests were all grade 1 and were considered by the treating physicians as unlikely to be associated with vaccination.

Humoral and Cellular Response to 1E10

All patients were responsive to 1E10 vaccination and produced increasing antibody levels with specificity for the 1E10 idiotype throughout the induction phase (Fig. 1). Antibody levels were maintained after week 18 (not shown).

The secretion of IFNγ by PBMCs stimulated in vitro with 1E10-derived (or an isotype-matched negative control) $F(ab')_2$ was analyzed by ELISA. Fourteen patients were evaluable. All but 2 patients (from the lower dose group) showed an 1E10-specific increase in IFNγ secretion during treatment. Figure 2 depicts the baseline and maximal 1E10-specific IFNγ secretion for each patient. Maximal IFNγ secretion was attained at different time points (range: weeks 8–34). These results show that vaccination with 1E10 was immunogenic and yielded a strong idiotype-specific response involving

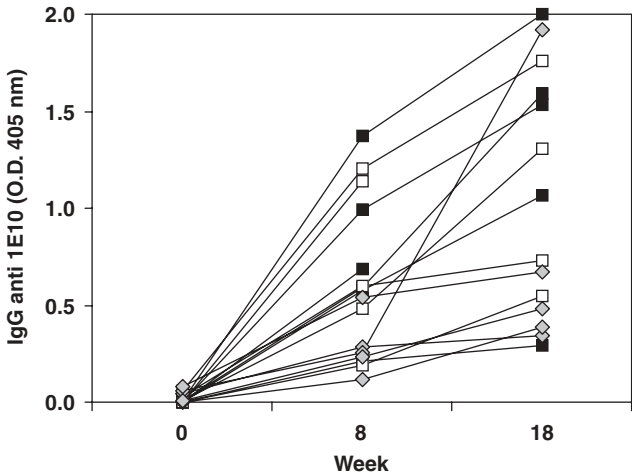

FIGURE 1. Serum antibodies specific for the 1E10 idiotope. The reactivity of serum antibodies was determined by ELISA against $F(ab')_2$ derived from 1E10. The absorbency from an isotype-matched negative control has been subtracted. Serum samples from 15 evaluable patients were tested at baseline, week 8, and week 18. Black squares indicate 0.5-mg dose; white squares, 1-mg dose; gray lozenges, 2-mg dose.

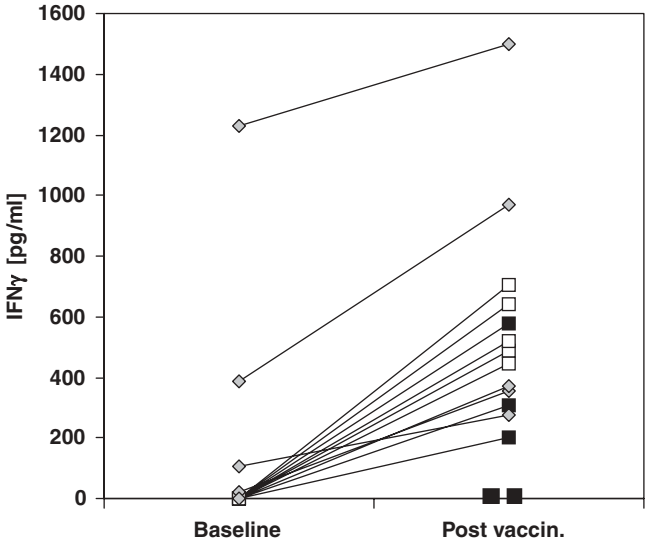

FIGURE 2. IFNγ secretion by PBMCs stimulated with 1E10. PBMCs were cultured in the presence of 1E10-derived (or a negative control) $F(ab')_2$. IFNγ concentration in the supernatant was then determined by ELISA. The baseline and maximal postvaccination 1E10-specific IFNγ secretion ($IFN\gamma_{1E10} - IFN\gamma_{isotype\ control}$) is indicated. Black squares indicate 0.5-mg dose; white squares, 1-mg dose; gray lozenges, 2-mg dose.

the humoral and cellular compartments of the immune system.

Anti-N-Glycolyl-GM3 Antibody Response

We investigated the time course of the production of N-glycolyl-GM3–specific antibodies in the 16 evaluable patients. All patients had undetectable IgM or IgG antibodies at baseline (titer < 200). After the first 4 doses (week 8), all patients produced IgM antibodies. A strong increase (> 10-fold) in anti-N-glycolyl-GM3 IgM antibodies was then observed over treatment time (maximal titer range: 900–12,800). There was no statistical difference in the mean of maximal titers between dose level groups (Fig. 3A). Thirteen patients also showed a strong IgG response (maximal titer range: 900–15,000). The means of maximal titers were not statistically different across the dose escalation (unpaired *t* test; see Fig. 3B).

IgM and IgG antiganglioside reactivity increased toward the end of the treatment (Fig. 4). Some patients showed a steady increase over time, whereas others had a sharp and early rise in antibody reactivity, followed by a temporary drop, which showed recovery by weeks 42 to 50. In some patients, IgG antibodies became detectable at a late point in treatment: patient 10 had detectable IgG beginning at week 42 (see Fig. 4B). Overall, the high frequency of responders and the increase in the intensity of the response during the treatment suggest that the extended vaccination schedule was effective in inducing an N-glycolyl-GM3–specific antibody response.

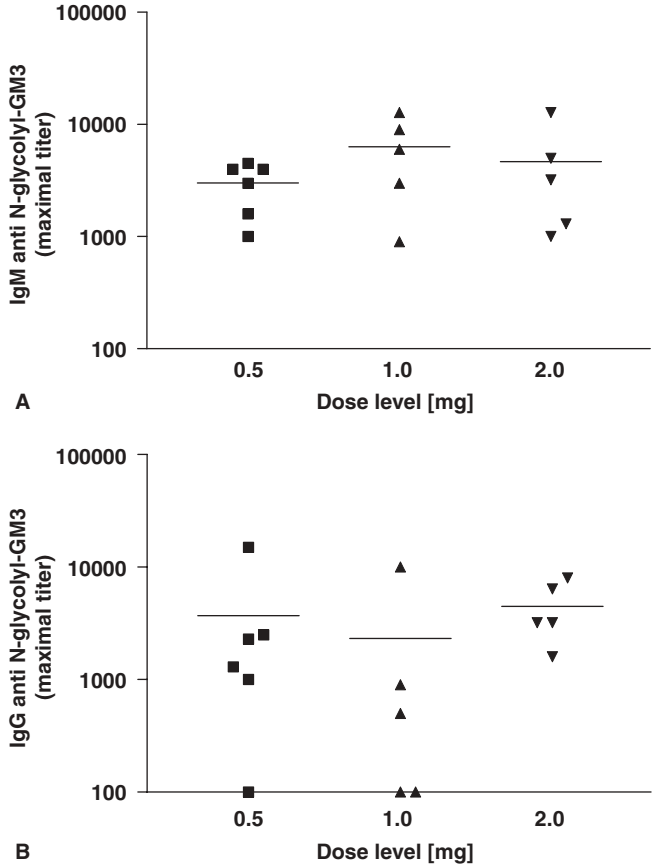

FIGURE 3. Serum antibodies specific for N-glycolyl-GM3. Specific IgM (panel A) and IgG (panel B) were titrated as described in the Patients and Methods section. Each patient's maximal titers are indicated, and the mean is shown for each dose level. Means were not statistically different (unpaired *t* test). Baseline antibodies were undetectable (<200 titer).

To study the specificity of the antibody response further, we assessed the binding of serum samples to a tumor cell line expressing N-glycolyl-GM3. Because no human N-glycolyl-GM3$^+$ cell line was available to us, we used P3X63 Ag8 653 (P3X63), a murine myeloma.[21] Anti-P3X63 antibodies had previously been elicited in BC patients vaccinated with N-glycolyl-GM3/VSSP.[13] 1E10 vaccination induced anti-P3X63 antibodies in 50% of the evaluated patients: 2 of 6 patients in the 0.5-mg dose group and 3 of 5 patients in the 1-mg and 2-mg dose groups (Fig. 5). It is noteworthy that all 5 patients with anti-N-glycolyl-GM3 antibody titers $\geq$ 8000 (patients 4, 9, 12, 15, and 20) produced P3X63-binding antibodies. In line with the latter observation, patient 11, with the weakest antiganglioside response, failed to produce tumor cell–reactive antibodies. Only 3 of the 7 remaining patients (with an intermediate antiganglioside response) produced P3X63-binding antibodies. Thus, the ability of serum antibodies to bind P3X63 seemed to be related to the intensity of the anti-N-glycolyl-GM3 response.

The B16 murine melanoma cell line expresses high levels of N-acetyl-GM3 but no N-glycolyl-GM3. We have

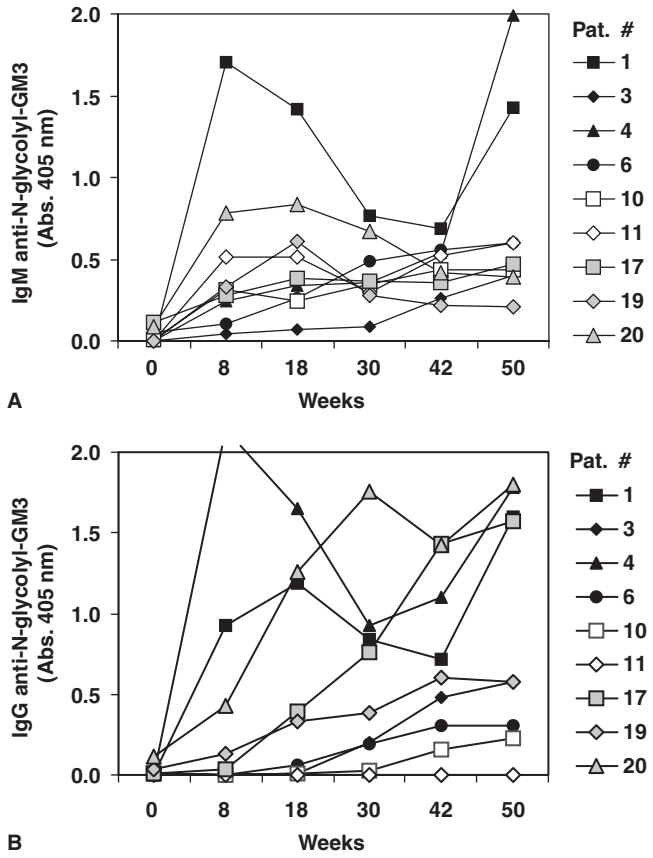

FIGURE 4. Time course of anti-N-glycolyl-GM3 antibody response. The results are shown for the 9 patients who completed the vaccination schedule. IgM antibodies (panel A) and IgG antibodies (panel B) are shown. Patient numbers are indicated. Black symbol indicates 0.5-mg dose; white symbol, 1-mg dose; gray symbol, 2-mg dose.

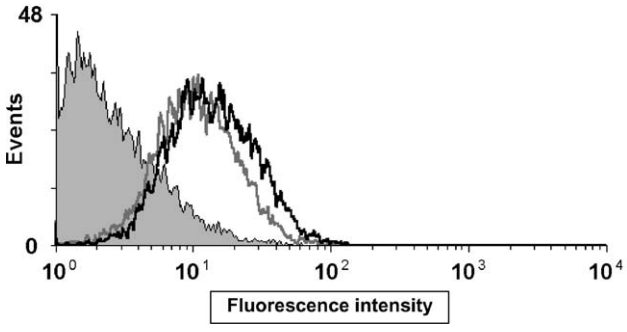

FIGURE 5. Serum antibody binding to P3X63 Ag8 653 mouse myeloma cell line. Cells were incubated with baseline and postvaccination serum samples, and binding of serum antibodies was monitored with FITC-labeled detection antibody. Staining with serum samples from patient 4 is shown and is representative of 8 responsive patients. The filled histogram shows the baseline sample. Gray line indicates week 8; black line, week 50. The increase in mean fluorescence intensity was 4.4-fold and 5.7-fold for week 8 and week 50, respectively.

recently reported the binding to B16 of serum antibodies derived from melanoma patients after treatment with an N-acetyl-GM3–based vaccine.[19] No detectable binding to B16 has now been observed for any of the 16 evaluable patients (data not shown). The tumor cell antibody binding thus seems to be dependent on N-glycolyl-GM3 expression.

N-Glycolyl-GM3–Specific Cellular Immune Response

We implemented ELISPOT assays to assess T-cell response to N-glycolyl-GM3. PBMCs were cultured in the presence of autologous DCs and N-glycolyl-GM3—loaded liposomes; negative control wells received unloaded liposomes. Specificity control wells received N-acetyl-GM3–loaded liposomes. The frequency of IFNγ-secreting cells was determined for baseline and postvaccination samples. Thirteen of 14 patients evaluable for cellular response were analyzed (not enough cells were recovered from patient 2's cryopreserved PBMC samples). Eight patients had no increase over the background frequency of IFNγ-secreting cells in any of the examined postvaccination samples. In contrast, 5 patients had a ganglioside-specific IFNγ response (Fig. 6). Additional specificity control wells run for patients 6 and 10 with N-acetyl-GM3–loaded liposomes indicated that the IFNγ response was specific for N-glycolyl-GM3 (not shown). The 5 patients with a cellular response were in both the lower and the upper dose level groups, with no dose-response correlation.

All IFNγ responses to N-glycolyl-GM3 were detected later than week 14. IFNγ secretion is thus an event detectable later than the antiganglioside humoral response. Positive responses were observed through week 42, confirming the convenience of an

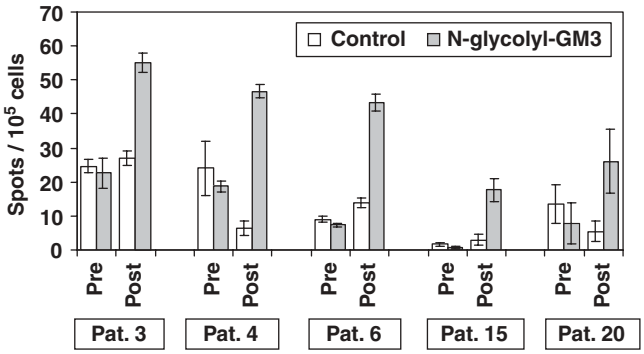

FIGURE 6. IFNγ secretion by PBMCs stimulated with N-glycolyl-GM3. Baseline and postvaccination PBMC samples were stimulated with autologous DCs and N-glycolyl-GM3–loaded (or control, unloaded) liposomes and cultured on ELISPOT plates. The mean spots from quadruplicate wells loaded with 10^5 PBMCs are shown for baseline and postvaccination samples from 5 responsive patients (bars indicate standard deviation). Stimulated and control means are statistically different for all shown posttreatment samples ($P < 0.05$, Student t test).

extended vaccination schedule to elicit an anti-N-glycolyl-GM3 response.

The small number of patients involved in the study prevented us from establishing a significant correlation between the immunologic and clinical outcomes.

Clinical Results

Fourteen patients completed the induction phase, and 9 patients finished the entire 16-dose treatment. The clinical outcome was similar at all dose levels (Table 3). Seven patients were removed from the study because of disease progression (3 progressed during the induction phase). High-risk BC patients relapsed with the following patterns: locoregional and soft tissue (1 patient), soft tissue (1 patient) and bone (2 patients). Three metastatic BC patients demonstrated disease progression during the study (1 patient in bone and 2 patients in soft tissue).

With a median follow-up from time of accrual of 31 months (range: 13–35 months), 11 patients with high-risk BC remain free of relapse. The median relapse free survival (RFS) and overall survival (OS) have not yet been reached. During this period, 1 patient with bone disease progressed and 1 patient with unresectable locoregional relapse in complete response (patient 14, who was withdrawn from the study for a toxicity event) remained disease-free.

DISCUSSION

Several murine anti-idiotypic antibodies have been evaluated over the past years as cancer vaccines, and their safety and immunogenicity have been established after prolonged monthly immunizations.[22] In addition, a significant survival advantage was observed in immunologic responders.[23]

We now show that treatment of BC patients with 1E10 in a 16-dose vaccination scheme was safe and well tolerated in most patients. Most adverse events related to the vaccine were local skin reactions. Local hypersensitivity reactions developed in 2 patients and could be more likely ascribed to the patients' predisposition to alum-mediated toxicity than to the extent of the vaccination protocol. Such reactions are rare but can be expected from aluminum-based salts.[24] It has been suggested that compounds such as aluminum hydroxide may trigger autoimmune reactions through bystander effects that favor the activation of autoreactive T cells in predisposed individuals.[25] The molecular mimicry approach (often the favored mechanism to trigger autoimmunity[26]) is not likely to induce autoimmunity when the antigen of interest is not expressed on normal tissues, however.[27] It is noteworthy that no severe treatment-related toxicity events were recorded during the extended maintenance phase.

In the present study, 1E10 vaccination elicited a strong and sustained immune response. 1E10-specific antibodies were detected in all evaluable patients. Similar results have been obtained previously with patients harboring metastatic disease.[17,18] Additionally, we assessed the involvement of T cells in the anti-idiotypic response and observed the induction of IFNγ secretion in most patients. These results are in line with the description of an in vivo idiotypic cascade involving anti-idiotypic and anti-anti-idiotypic B and T cells in mice immunized with the P3 mAb (Ab1).[28] The immunodominance of the variable (idiotype-containing) regions of P3 and 1E10 have been demonstrated recently.[29]

The extent of the efficacy of treatments involving murine antibodies has been shown to be related to the balance of anti-idiotypic and anti-isotypic responses.[30] The described ability of 1E10 to maintain its immunogenicity during an extended immunization protocol might be related to such a balance and is an expected consequence of the immunodominance of its variable regions.[29]

The rationale for using anti-idiotypic vaccines is their capacity to perform functional antigen mimicry. Thus, a response directed to the target antigen is expected. Accordingly, we detected a high-titer, dose-independent, anti-N-glycolyl-GM3 IgM response in all evaluable patients. Most patients (13 of 16 patients) also elicited an IgG class response.

This and previous studies with 1E10 showed that the humoral response could be efficiently induced with 6 biweekly injections (induction phase). In addition, we have now shown that the maintenance phase can induce an IgG response in patients who had an IgM-restricted response during the induction phase and can further increase early IgM and IgG responses.

High anti-N-glycolyl-GM3 IgM and IgG titers have also been obtained by immunizing BC patients with N-glycolyl-GM3/VSSP.[13] Most interestingly, as shown previously for the N-glycolyl-GM3/VSSP vaccine,[13] we have now shown that the 1E10 anti-idiotypic antibody is also able to induce antibodies reactive to a N-glycolyl-GM3$^+$ tumor cell line.

The frequency of responsive patients reported here is among the highest reported for vaccination protocols targeting ganglioside tumor antigens. Namely, fucosyl-GM1[31] and GM2,[32] when conjugated to keyhole limpet hemocyanin (KLH) and administered with the adjuvant QS-21, have been shown to be immunogenic in all (or almost all) treated cancer patients. A similarly high frequency of GD2-specific responses was obtained with an anti-idiotypic antibody[16] and with GD2 lactone-KLH/QS21.[33] Anti-GD3[34] and anti-N-acetyl-GM3[19] responses have been much less frequent.

TABLE 3. Clinical Outcome

	Treatment Dose				
	0.5 mg		1 mg		2 mg
	Disease Status at Time of Accrual				
	HRBC	MBC	HRBC	MBC	HRBC
Complete treatment	3	1	2		3
Uncompleted, progression		1	1	2	3
Uncompleted, toxicity		1	1		1

HRBC indicates high-risk breast cancer; MBC, metastatic breast cancer.

In studies in which the patient population showed a heterogeneous immune response to ganglioside immunization, the induction of specific antibodies was found to correlate with survival.[35,36] In other studies, however, in which most patients had a strong antiganglioside antibody response, this fact did not seem to have a clinical impact.[16] It is expected that successful immunotherapeutic strategies coordinately involve antibody and T-cell immune responses.[37] Ganglioside-specific T-cell responses have been described in multiple sclerosis patients,[38] in a non-vaccinated melanoma patient,[39] and in mice treated with anti-idiotypic antibodies mimicking GD2.[39] T-cell responses have not been assessed systematically in ganglioside-targeted clinical trials, however. We have now described an N-glycolyl-GM3–specific IFNγ response in 5 of 13 evaluated patients. Chapman et al[34] have also used ELISPOT to assess IFNγ secretion after stimulation with GD3 in patients sequentially treated with GD3 lactone-KLH/QS21 and BEC2/BCG. The fact that they detected no ganglioside-specific responses might be ascribed to the poor immunogenicity of GD3. Unlike the cited authors, we have delivered the ganglioside stimulus to PBMCs by way of autologous DCs pulsed with ganglioside-loaded liposomes. Such a technical procedure previously allowed us to detect a low frequency of T-cell responses to N-acetyl-GM3, a much less immunogenic ganglioside.[19]

The association between T-cell responses and clinical impact was not an objective of this study. Our finding of the unexpected frequent development of N-glycolyl-GM3–specific T-cell responses deserves larger studies to test their clinical benefit, however.

Taking all the results together, the 3 dose levels were immunogenic and equally safe. The lowest examined dose (ie, 0.5 mg) was thus selected for future trials. Patients with BC involving multiple lymph nodes face a substantial risk of relapse and death despite the use of adjuvant chemotherapy.[40] Immunomodulating strategies such as cancer vaccines are gaining increased interest as potential adjuvant therapies.[41] Hence, vaccine approaches like 1E10 are likely to be explored more extensively in addition to standardized chemotherapy regimens, especially in the high-risk BC population.

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