Immunization of Melanoma Patients with BEC2 Anti-idiotypic Monoclonal Antibody That Mimics GD3 Ganglioside: Enhanced Immunogenicity When Combined with Adjuvant

Mary McCaffery, Tzy-Jyun Yao, Linda Williams, Philip O. Livingston, Alan N. Houghton, and Paul B. Chapman

Clinical Immunology Service, Department of Medicine [M. M., L. W., P. O. L., A. N. H., P. B. C.] and Department of Biostatistics [T.-J. Y.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Previous attempts to immunize melanoma patients against GD3 ganglioside have been unsuccessful because of the poor immunogenicity of GD3. BEC2, an anti-idiotypic monoclonal antibody that mimics GD3, can induce anti-GD3 IgG in rabbits. Since clinical trials with BEC2 in melanoma patients demonstrated that BEC2 alone is not highly immunogenic, we have carried out sequential clinical trials exploring the use of two immunological adjuvants, BCG and QS21, administered with BEC2. Melanoma patients free of disease after surgical resection but at high risk for recurrence were immunized either with BEC2/BCG (14 patients) or BEC2/QS21 (6 patients). All patients developed high-titer IgG antibodies against BEC2, demonstrating that both adjuvants effectively enhanced the immunogenicity of BEC2. Anti-GD3 antibodies were induced in 3 of 14 patients immunized with BEC2/BCG; no patient immunized with BEC2/QS21 developed detectable anti-GD3 antibodies. After a median follow-up of 2.4 years, 71% of the patients immunized with BEC2/BCG remain alive and 64% are free of disease. In patients immunized with BEC2/BCG, no apparent association was observed between class II HLA type and either development of anti-GD3 antibodies or survival. We are encouraged by the results with BEC2/BCG, which suggest that further enhancement of the immune response to BEC2 will result in more frequent anti-GD3 antibody responses among immunized patients.

INTRODUCTION

GD3 ganglioside is an appealing target for immunotherapy of melanoma for several reasons. It is expressed abundantly on virtually all melanoma (1), and antibodies to GD3 can both block melanoma cell attachment to surfaces (2, 3), an early critical step in invasion and metastasis, and can inhibit melanoma cell growth in vitro (4). In animal models, MAb1 to GD3 can inhibit outgrowth of melanoma tumors, and treatment of metastatic melanoma patients with the anti-GD3 MAb R24 can result in major tumor shrinkage (5-8).

As a result of these observations, it has been hypothesized that induction of an antibody response against GD3 in melanoma patients could have significant therapeutic effects. However, GD3 is poorly immunogenic, and attempts to immunize patients against GD3 using GD3+ cells, purified GD3, or GD3 conjugates have been unsuccessful (9, 10). Because of this, we have turned to the strategy of using a mouse anti-idiotypic MAb, designated BEC2, that mimics GD3 and can induce antibodies against GD3 in rabbits (11). We hypothesized that a xenogeneic protein, such as BEC2, would be more immunogenic than GD3, a carbohydrate self-antigen, in part because of the ability of protein antigens to provide T cell help required for maturation of the antibody response associated with class switching and increased antibody affinity.

We have previously reported our initial clinical experience with BEC2 in which we immunized metastatic melanoma patients with 2.5 mg BEC2 administered s.c. without an immunological adjuvant (12). We were surprised to observe a relatively low-titer antismouse immunoglobulin response in only 55% of patients. Consistent with this low level of immunogenicity, only 1 of 20 patients developed transient, low-titer reactivity against GD3.

In exploring strategies to enhance the immunogenicity of BEC2, we have turned to the use of immunological adjuvants and have chosen to test two adjuvants: BCG and QS21. BCG is an attenuated strain of Mycobacterium bovis and is a potent immunological adjuvant that has been used to enhance the immune response to gangliosides (13) and to anti-idiotypic MAb (14). QS21 is a saponin extracted from the bark of the South American soap bark tree Quillaja saponaria molina, with significant adjuvant activity (15-19).

We now report two sequential clinical trials in which melanoma patients who were free of disease after complete surgical resection but at high risk for recurrence were immunized with BEC2 mixed either with BCG (BEC2/BCG) or with QS21 (BEC2/QS21) using the same BEC2 dose and schedule used in our previous trials. The primary objective was to test the hypothesis that immunization with BEC2 and a potent immunological adjuvant will enhance the immune response to BEC2
and result in the induction of antibodies against GD3 ganglioside. A secondary objective was to determine the HLA class II genotype of immunized patients to explore whether immunological responses to BEC2 or clinical outcome correlated with a particular genotype.

PATIENTS AND METHODS

Patient Selection

The BEC2/BCG vaccine and the BEC2/QS21 vaccine were tested in two separate, sequential clinical trials carried out under separate treatment INDs held by Memorial Hospital and approved by the Memorial Hospital Institutional Review Board. Patients were eligible for these trials if they were free of disease between 4 weeks and 6 months after surgical resection for American Joint Committee on Cancer stage III or IV melanoma. Patients free of disease after resection of American Joint Committee on Cancer stage II were also eligible if the primary tumor was ≥4 mm deep. Other eligibility requirements were: normal WBC and differential counts, no previous chemotherapy, immunotherapy, or radiation therapy within the previous month, and no history of other malignancies within the past 5 years. Patients were excluded if they had significant underlying medical problems (e.g., New York Heart Association class III or IV cardiac disease, active infections requiring antibiotics, or active bleeding), required antihistamines, steroids, or nonsteroidal anti-inflammatory drugs, had an immunodeficiency or were asplenic, had a history of metastases to the central nervous system, or were pregnant or lactating. All patients signed written informed consent.

Vaccine Components

BEC2 was purified from virus-free lipoprotein-free cell culture supernatants by Celltech, Ltd. (Slough, England) using protein A-affinity chromatography and anion exchange chromatography. The final product was >95% pure as determined by SDS-PAGE. BEC2 was supplied in sterile PBS at a concentration of 2.5 mg/ml in single-dose vials.

BCG (TheraCys) was purchased from Connaught Laboratories (Swiftwater, PA) in vials containing 3.4 × 10^8 CFUs/vial in a freeze-dried form. Immediately before administration, BCG was reconstituted with 3.4 ml sterile diluent and then further diluted in normal saline if necessary. Vaccine was prepared by mixing 1 ml BEC2 (2.5 mg) with 0.1 ml of the appropriate dilution of BCG immediately before administration.

QS21, supplied by Cambridge Bioscience, Inc. (Worcester, MA), was extracted from Q. saponaria molina tree bark using silica and reverse-phase chromatography as previously described (15). It was supplied in 2.1-mg vials as a lyophilized powder and, once reconstituted with sterile PBS, was stored at 4°C for not more than 14 days. Vaccine containing QS21 was prepared immediately before administration by mixing 1 ml BEC2 (2.5 mg) with 100 μg QS21. The dose of QS21 was selected based on a previous Phase I clinical trial (20).

Immunization Protocols

All patients underwent a complete history and physical examination, complete blood count with differential, liver function tests, lactate dehydrogenase, and chest X-ray (and other appropriate radiographic tests) within 1 month of starting treatment to confirm that the patient was free of detectable melanoma. Blood tests were repeated at the time of each immunization, and patients underwent a physical examination at least monthly. At the completion of the course of immunization, chest X-rays and other appropriate radiographic tests were repeated, and the patient was followed up closely by the principal investigator or the patient’s local physician.

BEC2/BCG. The vaccine was injected i.d. into multiple rotated sites on weeks 0, 2, 4, 6, and 10; injections were given only into limbs in which the regional lymph node basin was intact. In patients who developed anti-GD3 antibodies, a sixth immunization was offered at a time when the anti-GD3 antibody titers had returned to baseline.

The initial immunization contained 2.5 mg BEC2 mixed with 1 × 10^7 CFUs BCG. In the setting of grade III local toxicity, subsequent doses of BCG were attenuated 3-fold (i.e., 3 × 10^6 CFUs, 1 × 10^6 CFUs, 3 × 10^5 CFUs, 1 × 10^5 CFUs, 3 × 10^4 CFUs, and 1 × 10^4 CFUs). Patients with a positive purified protein derivative (or a history of a positive purified protein derivative) were started at an attenuated BCG dose of 3 × 10^4 CFU.

BEC2/QS21. Vaccine was administered as a single s.c. injection using the identical schedule and dose of BEC2 as for the BEC2/BCG vaccine (weeks 0, 2, 4, 6, and 10). Each vaccine consisted of 2.5 mg BEC2 and 100 μg QS21; the QS21 dose was not attenuated. Patients who developed anti-GD3 antibodies were eligible for additional booster injections of BEC2/QS21.

Serological Evaluation

Serum was collected pretreatment, at the time of each immunization, and 2 weeks after each immunization and stored at −20°C. Serum was tested for the presence of anti-BEC2 antibodies and anti-GD3 antibodies using an ELISA as previously described (12). Internal standards were run with each assay to ensure adequate and reproducible sensitivity. The specificity of the anti-BEC2 IgG response was characterized by comparing the anti-BEC2 IgG response to the IgG response against MPC11, an isotype-matched mouse MAb (IgG2b) with framework sequences that are similar to the BEC2 framework sequences, but which has distinct complementarity-determining region sequences (21). Ab3 antibodies were detected by their ability to bind BEC2 but not MPC11. The degree of binding to BEC2 versus MPC11 was compared by ELISA using a serum dilution that resulted in submaximal binding to BEC2 (absorbance values of ~0.5 were used). This was done (a) to ensure that the assay was carried out at a serum dilution corresponding to the linear portion of the titration curve for each serum sample and (b) to standardize the assay, allowing the data to be analyzed according to treatment group. The presence of Ab3 antibodies was confirmed in select patients by exhaustively absorbing immune serum against agaroose beads (Affigel 10; Bio-Rad Laboratories, Hercules, CA) coated with MPC11 until reactivity against MPC11 was not detected. The absorbed serum was then assayed for binding to BEC2.

A patient was considered to have developed anti-GD3 antibodies as detected by the anti-GD3 ELISA if at least two posttreatment sera samples reproducibly demonstrated a 4-fold...
or greater increase in anti-GD3 reactivity as compared with the pretreatment sample. This strict criterion was established based on our serological studies in patients immunized with BCG alone to avoid confusion with transient, low-level, nonspecific reactivity that can be induced by BCG and other adjuvants. Attempts to characterize further the specificity of anti-GD3 responses employed mixed hemadsorption assays against GD3+ allogeneic melanoma cell lines (5) and immune thin-layer chromatography (11).

**HLA Class II Oligotyping**

DNA for class II oligotyping was isolated either from peripheral blood mononuclear cells isolated by Ficoll-Hypaque centrifugation and cryopreserved in liquid nitrogen, or from hair shaft roots. Oligotyping was performed as previously described (22), and allele frequencies were calculated using standard methods (23).

**RESULTS**

**Patient Characteristics.** Fourteen patients were immunized with BEC2/BCG, and six patients were immunized with BEC2/QS21. Patient characteristics are shown in Table 1. Although the two groups of patients were relatively similar, the patients immunized with BEC2/BCG had a male predominance compared with the BEC2/QS21 group and were slightly older. Also, 21% of patients immunized with BEC2/BCG had either stage III with more than four lymph nodes positive or had stage IV disease, whereas none of the BEC2/QS21 patients had these poor prognostic features.

**Sero logical Responses.** All patients in both trials developed IgG antibodies against BEC2 (Table 2). This is in marked contrast to our previous results using BEC2 without an adjuvant in which only 55% of patients developed anti-BEC2 antibodies (12). There was no meaningful difference between the median or range of anti-BEC2 IgG titers induced by BEC2/BCG and BEC2/QS21 (Table 2) nor in the number of immunizations required to induce anti-BEC2 IgG (data not shown). This suggests that both BCG and QS21 adjuvants are equally potent at augmenting anti-BEC2 antibody responses.

To determine whether a portion of the anti-BEC2 response represented an Ab3 response, immune sera showing high titers of anti-BEC2 IgG were tested for binding to MPC11, an isotype-matched mouse MAb (IgG2b) in which 75% of the framework sequences are identical to the BEC2 framework sequences.\(^4\) Preferential binding to BEC2, as demonstrated either by higher titer or higher absorbance readings, was observed in 11 (79%) of 14 patients immunized with BEC2/BCG and 5 (83%) of 6 patients immunized with BEC2/QS21, implying that in the majority of patients, immunization induced BEC2-specific IgG antibodies. Data from a representative patient (patient 13 immunized with BEC2/BCG) is shown in Fig. 1A.

To compare the relative specificity for BEC2 versus MPC11 for each treatment group, the absorbance readings for BEC2 binding were compared with the readings for MPC11 binding at a serum dilution that resulted in submaximal binding to BEC2 (absorbance at 405 nm approximately equal to 0.5). This standardized the assay and ensured that it was carried out at a dilution corresponding to the linear portion of the titration curve for each serum sample. Fig. 1B shows the mean absorbance readings against BEC2 and MPC11 for patients immunized with BEC2/BCG or BEC2/QS21. In both treatment groups, the mean absorbance against MPC11 was lower than the reading against BEC2 and again implies that immunization induced BEC2-specific IgG. To confirm the presence of specific antibodies against BEC2 (i.e., Ab3), selected sera were absorbed exhaustively against MPC11 and then tested for binding to BEC2. Fig. 1C shows the results of an experiment using the same serum specimen used in Fig. 1A. After absorption against MPC11, no anti-MPC11 reactivity remained, but substantial reactivity against BEC2 was still observed, demonstrating the presence of Ab3 antibodies.

Anti-GD3 antibodies were detected using ELISA in 3 (21%) of 14 patients immunized with BEC2/BCG (Table 2), whereas no patient immunized with BEC2/QS21 developed detectable anti-GD3 antibodies. The anti-GD3 antibodies were detected with secondary antibodies against human IgG but not IgM, suggesting they were of the IgG isotype. The titers of the anti-GD3 antibody responses are illustrated in Fig. 2 and ranged from 1:80 to 1:640. Attempts to characterize further the specificity of these antibodies by mixed hemadsorption assays or immune thin-layer chromatography were unsuccessful presumably because of the low titer and low affinity of these antibodies. Patient 11 received a sixth immunization with BEC2/BCG 6 months after the fifth immunization, but no boost in anti-GD3 titer was observed (data not shown).

**Disease Recurrence and Overall Survival.** Surviving patients immunized with BEC2/BCG have been followed for a median of 2.4 years (2.2–2.9), and 10 (71%) of 14 patients remain alive. Of these 10 patients, 8 never experienced recurrence, 1 experienced recurrence with a solitary metastasis that was resected, and 1 patient remains alive with metastatic disease. Fig. 3 shows that the overall survival rate has plateaued, suggesting that the disease-free status of the remaining patients may be durable. Two of the three patients who developed anti-GD3 antibodies, including one patient who had stage IV disease, remain free of disease.

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\(^4\) Unpublished observation.
The patients immunized with BEC2/QS21 have been followed up for a median of 2 years, and of the six patients immunized, three remain free of disease. The number of patients immunized with BEC2/QS21 is too small for formal analysis or comparison to other data sets.

**Toxicity.** All patients immunized with BEC2/QS21 experienced grade III toxicity at the sites of immunization characterized by inflammation, ulceration, and ooze. In one patient, the third immunization had to be delayed for 1 month to allow the previous vaccine sites to heal. All sites healed with minimal wound care, and there were no instances of BCGosis. Eight patients experienced grade II fever, three patients noted grade II fatigue or malaise, and one patient had a transient grade II elevation in glucose. Grade I toxicities included elevated liver function tests (six patients), hyperglycemia (three patients), fatigue (three patients), fever (one patient), and hypocalcemia (one patient).

All patients immunized with BEC2/QS21 also experienced inflammation at the sites of injection, although only of grade II severity and of shorter duration; there was no ulceration or ooze. Two of the patients also noted grade II fever. Grade I toxicities included fatigue (four patients), hyperglycemia (two patients), neutropenia (two patients), and diarrhea (one patient).

Overall, immunizations were well tolerated, and no patient was removed from the study because of adverse reactions.

**HLA Class II Oligotyping.** As an exogenous antigen, BEC2 may be taken up by antigen-presenting cells, processed to peptides, and help T-cell epitopes presented by HLA class II molecules. In this way, BEC2 may provide T-cell help for the B-cell response against GD3. Therefore, we were interested in knowing the HLA class II type of the patients to determine whether there was any correlation between HLA class II type and immune response to BEC2 or overall survival.

Class II oligotyping was performed in 12 of the 14 patients immunized with BEC2/QS21 (Table 3); samples were not available in the remaining two BEC2/QS21 patients. Oligotyping was not carried out in the BEC2/QS21 patients because of the small number of these patients. There was no apparent association between HLA class II genotype and induction of anti-GD3 antibodies, induction of anti-BEC2 antibodies, or survival.

We compared the corresponding serologically defined class II allele distribution among our patients with the distribution published for the Caucasian population in the United States in general (24). We used this reference group (n = 232) because it best reflected the group of 12 melanoma patients. Although there were no apparent differences at the DR locus, there was an apparent decrease in DQ1 frequency (43.7% versus 25%) with a corresponding increase in the frequency of DQ2 (22.9% versus 33.3%) and DQ7 (16.3% versus 33.3%) among the melanoma patients. However, a formal statistical test was not performed because of the small sample size. These data are consistent with the hypothesis that certain HLA class II alleles, or the loss of certain alleles, may be associated with an increased risk of melanoma.

### DISCUSSION

These clinical trials extend our previous observations in melanoma patients immunized with BEC2 alone. In a previous study in which patients were immunized with BEC2 administered s.c. but without an immune adjuvant, only 55% of the patients developed anti-BEC2 antibodies, and only 1 of 20 developed detectable anti-GD3 antibodies (12). In the current studies in which patients were immunized with the same dose and schedule of BEC2 mixed with either BCG or QS21 adjuvant, all patients developed anti-BEC2 antibodies and at a significantly higher titer than that seen in patients immunized without adjuvant. This difference was statistically significant (P = 0.001, Fisher’s exact test). In addition, 3 of 14 patients immunized with BEC2/QS21 developed anti-GD3 antibodies, whereas none of the 6 patients immunized with BEC2/QS21 developed anti-GD3 antibodies, although this difference was not statistically significant. Thus, both BCG and QS21 are potent adjuvants that enhanced the anti-BEC2 antibody response, but anti-GD3 responses were observed only in patients receiving BEC2/QBCG.

This contrasts somewhat with what we have observed previously with the MELIMMUNE anti-idiotypic MAb vaccine, which mimics the high-molecular weight melanoma-associated antigen. In that study, QS21 was superior to BCG in inducing anti-MELIMMUNE antibodies (25). However, given that none of the patients developed antibodies against high-molecular weight melanoma-associated antigen, and the anti-MELIMMUNE titers induced were 10 times lower than the anti-BEC2 antibody titers observed in the current study, it is likely that these results are due to differences in immunogenicity inherent in the two anti-idiotypic MAbs.

Although antibody responses to carbohydrate antigens, such as GD3, are generally of the IgM isotype, it is interesting to note that the anti-GD3 responses detected after immunization with BEC2 were IgG. This is consistent with what we have observed previously in rabbits immunized with BEC2 (11) and may be due to the fact that as a protein that mimics GD3, BEC2...
Fig. 1 (opposite) Induction of Ab3 antibodies in patients immunized with BEC2/BCG or BEC2/QS21. A, reactivity of immune serum from patient 13 immunized with BEC2/BCG against BEC2 (●) and MPC11 (■). After five immunizations, immune serum was diluted as shown and tested for binding to BEC2 and MPC11 as described in "Patients and Methods." B, mean reactivity of immune sera against BEC2 (●) or MPC11 (■) in patients immunized with BEC2/BCG (n = 14) or BEC2/QS21 (n = 6). Each serum sample was tested at a dilution that resulted in absorbance readings of ≈0.5 against BEC2-coated plates. Data are presented as means; bars, SD. C, binding of preabsorbed serum from patient 13 immunized with BEC2/BCG against BEC2 (●) and MPC11 (■). The same serum as shown in A (diluted to 1:100,000) was absorbed exhaustively against MPC11-coated agarose beads before the assay was performed.

Fig. 2 Anti-GD3 antibody titers induced by BEC2/BCG in three patients. Serological assays were performed as described in "Patients and Methods." Arrows, immunization with BEC2/BCG. In these patients, anti-GD3 reactivity was detected only with alkaline phosphatase-conjugated second antibody against human IgG.
Immunization with BEC2 Anti-idiotypic Vaccine

suggest that most of the recurrences have already occurred. It is This encouraging disease-free survival rate in the BEC2/

**Table 3** HLA class II genotyping of melanoma patients immunized with BEC2/BCG

<table>
<thead>
<tr>
<th>Patient</th>
<th>Oligotyping</th>
<th>Serologically defined alleles*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DR alleles</td>
<td>DQ alleles</td>
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<tr>
<td>1</td>
<td>B*0701</td>
<td>B1<em>1104 B4</em>0101 B3<em>02 B1</em>0201 B1*0301</td>
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<tr>
<td>2</td>
<td>B*0301</td>
<td>B1<em>1103 B3</em>0101 B3<em>02 B1</em>0201 B1*0301</td>
</tr>
<tr>
<td>3</td>
<td>B*07</td>
<td>B1<em>0801 B4</em>0101 B1<em>0201 B1</em>0402</td>
</tr>
<tr>
<td>4</td>
<td>B*0301</td>
<td>B1<em>1103 B3</em>0101 B3<em>02 B1</em>0201 B1*0301</td>
</tr>
<tr>
<td>5</td>
<td>B*1501</td>
<td>B1<em>1104 B5</em>0101 B3<em>02 B1</em>0602 B1*0301</td>
</tr>
<tr>
<td>6</td>
<td>B*1302</td>
<td>B1<em>1306 B3</em>02 B3<em>0301 B1</em>0603 B1*0605</td>
</tr>
<tr>
<td>7</td>
<td>B*1501</td>
<td>B1<em>04 B5</em>0101 B4<em>0101 B1</em>0602 B1*0605</td>
</tr>
<tr>
<td>8</td>
<td>B*1501</td>
<td>B1<em>0402 B3</em>0101 B4<em>0101 B1</em>0201 B1*0302</td>
</tr>
<tr>
<td>9</td>
<td>B*0102</td>
<td>B1<em>1104 B3</em>02 B1<em>0501 B1</em>0301</td>
</tr>
<tr>
<td>10</td>
<td>B*14</td>
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</tr>
<tr>
<td>11</td>
<td>B*0701</td>
<td>B1<em>1104 B3</em>02 B4<em>0101 B1</em>0503 B1*0301</td>
</tr>
<tr>
<td>12</td>
<td>B*0301</td>
<td>B3<em>0101 B1</em>0301</td>
</tr>
</tbody>
</table>

HLA class II oligotyping was performed as described in **"Patients and Methods."** DNA was extracted from peripheral blood mononuclear cells except in patient 1, in whom DNA was extracted from hair bulbs. DNA was not available from patient 2 or 9.

* Serological designations were derived from the oligotyping data and were not directly determined.

is potentially capable of providing T-cell help necessary for isotype class switching.

An intriguing observation is that, after a median follow-up of 2.4 years, 10 (71%) of 14 patients immunized with BEC2/BCG remain alive, and 9 (64%) of 14 remain free of disease. This compares favorably with the survival of stage III melanoma patients in general (26) and stage III patients rendered free of disease after surgery (27), and although the follow-up is relatively short, both previous observations (26) and the fact that no recurrences have been observed for the past 19 months suggest that most of the recurrences have already occurred. It is interesting to note that many long-term survivors did not have detectable serum antibodies against GD3 after BEC2 immunization. This may be due to inadequate sensitivity of the anti-GD3 serological assays; alternatively, it may be that non-antibody mechanisms play a role in the protective effect observed. Randomized (28) and nonrandomized trials (29) using BCG alone in melanoma patients have not demonstrated an effect of BCG on disease-free survival, and therefore it is considered unlikely that BCG alone is responsible for the effect of BEC2/BCG on survival.

This encouraging disease-free survival rate in the BEC2/
BCG patients contrasts with observations in patients from a previous study, in which a similar population of patients was immunized with BEC2 without adjuvant (30). In that study, none of the patients developed anti-GD3 antibodies and only 33% of patients are alive after a median follow-up of 2.8 years. This comparison must be interpreted with caution because these were not randomized trials and were not designed to detect improvement in disease-free survival. As a result, we cannot rule out the possibility that this favorable survival rate occurred coincidentally rather than as a result of immunization. However, these observations suggest that highly immunogenic formulations of BEC2 can induce anti-GD3 antibodies in patients and may contribute to prolonged survival. We are encouraged by the results with BEC2/BCG and are working to enhance further the immunogenicity of BEC2 by exploring alternative routes of immunization and the use of BEC2 conjugated to proteins expressing potent Tₘ epitopes.

We determined the HLA class II type in the patients immunized with BEC2/BCG and found no apparent association between class II genotype and serological response to BEC2 or to disease-free survival. However, the small sample size and the low percentage of patient relapses may have decreased our ability to identify an association. A previous report found that DQB1*0301 (corresponding serologically to DQ7) was more common among melanoma patients (56%) than among a healthy control group (27%), and that DQB1*0301 correlated with an increased risk of advanced disease (31). We found a similar incidence of DQB1*0301 among our patients (58%) and also noted that the DQ7 allele frequency among our patients was higher than the frequency published for Caucasians in the United States. The significance of these findings must be interpreted with caution given the small number of patients studied, and it remains unclear whether the incidence of DQB1*0301 (DQ7) differs significantly from a carefully matched control group. Despite this, it is of interest that the current study is now the second report of observations consistent with an increase in frequency of DQ7 among melanoma patients, and this may merit further investigation.

ACKNOWLEDGMENTS

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