Antibody Response to Murine Anti-GD2 Monoclonal Antibodies: Correlation with Patient Survival

Nai-Kong V. Cheung, Irene Y. Cheung, Adela Canete, Samuel J. Yeh, Brian Kusher, Mary Ann Bonilla, Glenn Heller, and Steven M. Larson

Departments of Pediatrics, Biostatistics and Radiology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Clinical strategies which modulate the human anti-mouse antibody response (HAMA) in patients may have a profound influence on the idiotype network inducible by murine monoclonal antibodies (MoAb). Prior to myeloablative chemotherapy (ABMT), 9 patients with Stage IV neuroblastoma were imaged with 131I-3F8, a MoAb specific for the ganglioside GD2. Their serum HAMA, anti-idiotypic, anti-GD2, and anti-anti-idiotypic antibodies were assayed by enzyme-linked immunosorbent assay prior to, and at 3 and 6 months postimaging. HAMA and anti-idiotypic levels remained low, in contrast to the high levels in 10 patients imaged with 131I-3F8 without ABMT. Five of the 9 patients are long-term survivors; all had elevated anti-GD2 and anti-anti-idiotypic levels, significantly higher than those who died of disease. Although 131I-3F8 imaging prior to ABMT detected abnormal sites in 4 of 9 patients, 3 of the 4 patients have relapsed. Patient survival'...
plus local radiotherapy: cisplatin 120 mg/m² on day −19; radiation 150 cGy/fraction, 2 fractions/day × 7 days (total, 2100 cGy); carbustine 200 mg/m² on day −9; melphalan 2 × 30 mg/m²/day on days −8, −7, −6, or thiopeta 300 mg/m²/day on days −8, −7, −6; and etoposide 300 mg/m²/day on days −5, −4, and −3. Histologically tumor-free bone marrow was harvested by aspiration from iliac crests and purged with 4-hydroperoxy cyclophosphamide at 100 μmol/liter before cryopreservation.

All 9 patients imaged with 131I-3F8 prior to ABMT were diagnosed from 1986 to 1988. Biological parameters were not uniformly measured at the time of the diagnosis. Patients 1–5 were survivors, and patients 6–9 died of progressive disease (Table 1). Four of 5 survivors had high ferritin levels (>142 ng/ml) consistent with high risk neuroblastoma, compared with 2 of 3 non-survivors. For patient 7, a serum ferritin level was not obtained at diagnosis. Only 4 of 9 patients had their tumors studied for N-myc amplification, and all 4 (patients 3, 5, 7, and 8) had single copy. Serum lactate dehydrogenase was determined or with a mixture of IgG3 (FLOPC21), IgG3 (Y5606) myelomas from Sigma Chemical Co, St. Louis, MO, and NS.7 (IgG3 MoAb specific for sheep red blood cells) obtained from American Type Culture Collection, Rockville, MD, each at 100 μg/ml (final concentration) for 1 h at 37°C to inhibit nonspecific IgG3 binding. A 50-μl quantity of the above sera was added to the wells containing 3F8 fragments with antigenic determinants for Ab2 quantitation, while unincubated (diluent alone without IgG3 myelomas) sera would be added to wells coated with intact 3F8 IgG (for HAMA determination) and allowed to react for 3 h at 37°C. A high-titer serum was used as the reference (assigned 10⁶ units/ml). In each assay plate, a standard curve was constructed using serial 5-fold dilutions of the reference serum, i.e., 20, 4, 0.8, 0.16, and 0.032 units/ml. After PBS wash, a peroxidase-conjugated goat anti-human IgG (heavy and light chains) from Jackson Immunoresearch Laboratories (West Grove, PA) was added at 100 μl/well and incubated for 1 h at 4°C. Upon washing with PBS, the plates were reacted with o-phenylenediamine at 0.5 mg/ml and 0.03% H₂O₂ in 0.1 M citrate phosphate buffer at pH 5 for 30 min in the dark. The reaction was quenched with 5% sulfuric acid. The absorbance of the reaction was measured at 492 nm using a Dynatech ELISA plate reader. The absorbances of the reference serum were used to derive the best-fit standard curve which was then used to determine the levels (titers) of HAMA of AbA or AbB (units/ml) in unknown samples. Titers in the presence of myeloma IgG3 inhibitors reflect the patient’s Ab2, whereas titers in the absence of inhibitors reflect the patient’s HAMA. Greater than 90% of the binding to irrelevant mouse IgG3 was inhibited by soluble myeloma inhibitors. The inclusion of 3 different IgG3 myelomas assured removal of all irrelevant reactivities including those against light chains of both κ- and λ-class. It should be noted that although the quantities of HAMA and Ab2 were both expressed in units/ml, these units were not directly comparable because of the differences in the assay antigens.

Since the HAMA response is expected to be greatly suppressed by myeloma-blative therapy, a sensitive in vitro HAMA assay is essential for this study. A direct comparison was carried out between the ELISA developed in our laboratory and a commercial kit (ImmuNoStrip HAMA IgG kit; Immunomedics, Warren, NJ) using a panel of 26 HAMA-positive sera from 3F8-sensitized patients. Twelve were obtained from patients shortly after (primary response) 3F8 treatment; 14 samples were obtained either after 3F8 rechallenge (secondary response) or long (late response) after first 3F8 exposure. The binding of HAMA to 3F8 in our ELISA was stronger than its binding to the mouse IgG in the kit. This preferential binding was most obvious for sera obtained during the secondary/late response as evidenced by the regression equations:

\[ \text{Ln } y = 2.23 (\text{In } x) - 17.3 \quad (r = 0.91) \quad \text{primary response} \]
\[ \text{Ln } y = 4.11 (\text{In } x) - 38.4 \quad (r = 0.88) \quad \text{secondary/late response} \]

where \( y \) was the HAMA level in ng/ml (measured by the kit) and \( x \) in ELISA units/ml referenced to a known standard in our assay. The limit of detection achieved by the commercial kit was 40 ng/ml serum, equivalent to 7,860 and 28,223 units/ml based on the 2 regression equations, respectively. In comparison, the limit of detection of our ELISA assay was 16 units/ml. We conclude that our ELISA had adequate sensitivity to detect low anti-3F8 antibody level, a measurement crucial for testing our hypothesis.

### Quantitation of AbA and AbB by ELISA

**Ganglioside-binding Antibody.** Ganglioside-binding antibody activity in the serum was measured by ELISA; 96-well flat bottomed polystyrene microtiter plates were coated with ganglioside G₂₀ purified from neuroblastoma (4 ng G₂₀/well), diluted in ethanol, and dried at room temperature overnight; 0.1% BSA in PBS was used as filler protein to saturate unbound sites. Control wells contained the same ganglioside fraction treated with neuraminidase and formic acid to destroy the G₂₀ moiety. Patient serum diluted in PBS containing 0.5% BSA and 1% skim milk was allowed to react with the antigen plates at 37°C for 3 h. A standard curve was constructed using serial dilutions of chimeric 3F8. After washing with PBS, the wells were reacted with peroxidase-conjugated affinity purified goat-anti-human IgG (heavy and light chains) antibody (Tago, Burlingame, CA) diluted to 1:1000 in PBS containing 0.5% BSA and 1% skim milk at 4°C for 1 h. After washing, the standard color reaction was performed. The absorbance was measured by an ELISA plate reader. Based on the fitted regression curve of chimeric 3F8, the antibody activity of samples was calculated in units/ml of binding activity.

**Idiotype-specific Ab3.** To measure idiotype-specific Ab3, 96-well flat bottomed polystyrene microtiter plates were coated with 20 μg/ml of protein-G (Pharmacia, Piscataway, NJ) affinity-purified rat IgG1 anti-idiotypic antibodies Idio-2, C4E4, C2H7, A1G4, and A2A6 specific for 3F8 (12). E6, a rat

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at diagnosis</th>
<th>Status at ABMT</th>
<th>3F8 scans</th>
<th>Survival</th>
<th>Progression-free survival</th>
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<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>VGPR</td>
<td>—</td>
<td>64+</td>
<td>64+</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>CR</td>
<td>—</td>
<td>60+</td>
<td>60+</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>PR</td>
<td>+</td>
<td>63+</td>
<td>63+</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>VGPR</td>
<td>—</td>
<td>60+</td>
<td>60+</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>VGPR</td>
<td>+</td>
<td>24+</td>
<td>24+</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>VGPR</td>
<td>—</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>7</td>
<td>29</td>
<td>VGPR</td>
<td>—</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>5.2</td>
<td>CR</td>
<td>—</td>
<td>16</td>
<td>12</td>
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<tr>
<td>9</td>
<td>3.3</td>
<td>VGPR</td>
<td>—</td>
<td>18</td>
<td>15</td>
</tr>
</tbody>
</table>

* VGPR, very good partial remission, same as complete remission except for bone scan, which was improved but not completely negative; CR, complete remission; PR, partial remission.

Except for MIBG, marrow examinations and radiological studies were normal at the time of ABMT.
IgG1 specific for mouse IgG2-Fc, was also included for background subtraction. Human sera were first centrifuged and filtered (0.2 μm) using Spin-x from Costar (Cambridge, MA) at 14,000 rpm for 5 min. They were then diluted at 1:100 and 1:500 in 0.5% BSA in PBS containing 10 μg/ml 2E6, and were incubated at 37°C for 1 h to remove nonspecific reactivities. The samples were added to antigen plates, together with varying concentrations of a chimeric 3F8 standard. After a 3-h incubation at 37°C, the plates were washed with PBS, and a peroxidase-conjugated goat anti-human IgG (Fc specific) was added and allowed to incubate at 4°C for 1 h. After washing with PBS, the o-phenylenediamine color reaction was run. Based on the chimeric 3F8 standard, sample absorbances were used to calculate titers in units/ml. The lower limit of detection was 3 units/ml. Although anti-GD2, Ab2 and Ab3 assays were similar, their units of measurement were not equivalent.

Statistics. The HAMA, Ab2, anti-GD2, and Ab3 serum levels of the 4 groups of subjects were compared. These included the control group, those with long term post-ABMT survivors, those who died of progressive disease after ABMT. For HAMA response, the Mann-Whitney rank sum tests (medians) for independent samples were used. Since our hypothesis predicted a higher Ab2/anti-GD2/Ab3 levels among the survivors, we used the one-tailed Mann-Whitney rank sum tests.

#### RESULTS

**HAMA/Ab2 Antibody Levels.** Patient characteristics are summarized in Table 1. All 9 patients had Stage IV neuroblastoma diagnosed at more than 1 year of age with bone and bone marrow metastases (Table 1). Their serum HAMA and Ab2 levels were measured by ELISA. Ab2 was defined as antibody binding to 3F8-F(ab’2)2 fragment uninhibitable by irrelevant mouse myelomas. More than 90% of reactivity against irrelevant IgG3s was removed by the inclusion of 3 myelomas, FLOPC21, Y5606, and NS.7 (data not shown). As shown in Table 2, sera from 28 controls without prior exposure to murine MoAb had levels of 151±31 units/ml. For the 10 patients who were imaged with 131I-3F8 but received no myeloablative therapy, the HAMA and Ab2 responses generally peaked rapidly by the third month after 3F8 exposure. The means of the HAMA and Ab2 levels were 16,044 (range, 266–61,407) and 1,128±5 (1,096–45,499), respectively. In marked contrast, the mean ± SD of the HAMA and Ab2 levels among the 9 patients who underwent 131I-3F8 imaging and ABMT were significantly lower whether tested at 3 or 6 months after 131I-3F8 injection. Although the mean HAMA and Ab2 levels were low, they peaked at 3 months (data not shown). However, they were not significantly different from those of the control group.

**Anti-GD2/Ab2 IgG Antibody Levels and Patient Outcome.** The pre-3F8 mean anti-GD2 antibody level among survivors was higher than that of nonsurvivors. One survivor had a preimaging level of 1745 units/ml. After 3F8 imaging, anti-GD2 increased to levels significantly higher than those of controls (P = 0.053) or nonsurvivors (P = 0.037; Table 2). However, an increase in anti-GD2 antibodies did not necessarily imply the induction of the idiotype network. In order to determine whether the anti-GD2 response was indeed Ab3, anti-idiotypic antibodies that recognized unique epitopes on the anti-GD2 antibody 3F8 were used to capture Ab3 by ELISA (12). Ab3 antibody was defined as human IgG that reacted selectively with rat anti-idiotypic antibody, not inhibitable by rat IgG. Using a chimerized (mouse-human) anti-GD2 antibody as standard, each unit of Ab3 was equivalent to 10 ng of chimeric IgG. The limitation of detection for human IgG binding to Gd2 was 8 ng/ml using immuno-thin-layer chromatography, equivalent to 0.8 unit/ml by Ab3 ELISA, whereas the limit of sensitivity of Ab3 ELISA was 0.032 unit/ml (data not shown). The same 28 controls without prior exposure to murine MoAb were tested for serum IgG antibody binding to these anti-idiotypic reagents. Except for A1G4 idiotope, the levels among control cancer patients and normal volunteers were low (Table 3). For A1G4 idiotope, 18 cancer patients had no detectable levels, while 3 of 10 normal volunteers had levels of 20, 24, and 82 units/ml, respectively.

The progression-free survival durations were significantly different between the survivor and nonsurvivor groups (P < 0.016, Table 1). Compared to the controls, the Ab3 levels among nonsurvivors (Table 3) were low prior to, and at 3 and 6 months after 3F8 imaging except for the A2A6 idiotope. In contrast, Ab3 levels were significantly higher (P < 0.02) among survivors than among controls for the idiotopes A1G4 and A2A6 pre-3F8, as well as at 6 months post-3F8 for the idiotopes Idio2, C4E4, A1G4, and A2A6 (Table 3). When

## Table 2 Mean HAMA, Ab2, and anti-GD2 levels in units/ml (ranges) in controls and in patients within 3 months after 3F8 imaging

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>HAMA (16–388)</th>
<th>Ab2 (16–338)</th>
<th>Anti-GD2 (0–1980)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28</td>
<td>116 (16–388)</td>
<td>136 (16–338)</td>
<td>578 (0–1980)</td>
</tr>
<tr>
<td>Imaged (no ABMT)</td>
<td>10</td>
<td>16,044a (266–61,407)</td>
<td>11,285a (1,096–45,499)</td>
<td>Not done</td>
</tr>
<tr>
<td>Imaged (plus ABMT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>4</td>
<td>148 (69–280)</td>
<td>109 (16–192)</td>
<td>342 (158–525)</td>
</tr>
<tr>
<td>Survivors</td>
<td>5</td>
<td>169 (36–408)</td>
<td>119 (16–394)</td>
<td>857 (509–1745)</td>
</tr>
</tbody>
</table>

* Comparison with control group, P < 0.001 by Mann-Whitney U 1-tailed test.

## Table 3 Ab3 levels in units/ml (mean ± SD) among controls and in patients undergoing ABMT

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Time</th>
<th>Idio-2</th>
<th>C4E4</th>
<th>C2H7</th>
<th>A1G4</th>
<th>A2A6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>28</td>
<td>3.1 ± 0.42</td>
<td>3.1 ± 0.38</td>
<td>3.3 ± 1.3</td>
<td>7.2 ± 16</td>
<td>3.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>ABMT patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>4</td>
<td>Pre-3F8</td>
<td>3.0 ± 0</td>
<td>4.5 ± 2.4</td>
<td>3.0 ± 0</td>
<td>4.0 ± 2.0</td>
<td>8.8 ± 8.3</td>
</tr>
<tr>
<td></td>
<td>6 mo post-3F8</td>
<td>4.8 ± 3.5</td>
<td>4.0 ± 0</td>
<td>4.8 ± 3.5</td>
<td>3.0 ± 0</td>
<td>7.5 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Survivors</td>
<td>5</td>
<td>Pre-3F8</td>
<td>4.6 ± 2.3</td>
<td>3.8 ± 1.3</td>
<td>3.0 ± 0</td>
<td>13 ± 14</td>
<td>19 ± 8.8</td>
</tr>
<tr>
<td></td>
<td>6 mo post-3F8</td>
<td>13 ± 11</td>
<td>14 ± 14a</td>
<td>3.4 ± 0.89</td>
<td>19 ± 9.4a</td>
<td>58 ± 50a</td>
<td></td>
</tr>
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</table>

* Comparison with nonsurvivor group, P < 0.05 by Mann-Whitney U 1-tailed test.
Fig. 1. This patient was diagnosed with Stage IV neuroblastoma at 15 months of age, with a left adrenal primary, orbital, liver, bone, and marrow metastases. After N5 induction chemotherapy, local radiation, and surgery, she was imaged with $^{131}$I-3F8 prior to ABMT (A). Uptake in her spine and right shoulder (arrows) was consistent with residual neuroblastoma. Prior to ABMT, her MIBG also showed faint uptake in the right shoulder. At 54 months after her first immunoscintigraphy, she received a reinjection of $^{131}$I-3F8, which showed overall improvement but residual uptake in her right shoulder but no new focus of uptake (B). M, iodine 131 marker; S, stomach.

compared to nonsurvivors, the mean pre-3F8 Ab3 levels among survivors were higher for the idiotopes A1G4 and A2A6 ($P = 0.056$), and at 6 months post-3F8 for Idio2, C4E4, A1G4, and A2A6 (Table 3).

**Immunoscintigraphy.** Four of the 9 patients showed abnormal $^{131}$I-3F8 uptake by immunoscintigraphy prior to ABMT. Three of these 4 patients have survived 24+ to 63+ months since ABMT, and all 3 mounted anti-GD2 and Ab3 antibody responses. Fig. 1A shows one of these patient’s $^{131}$I-3F8 image prior to ABMT, and Fig. 1B shows an improved scintigram 54 months after ABMT. The areas of abnormal uptake have disappeared from the spine, but persisted in the right shoulder. Compared to the $[^{131}]$MIBG scan pre-ABMT, the $[^{131}]$MIBG at 54 months post-ABMT showed persistent but improved disease in the right shoulder. In this patient, urinary dopamine levels have remained in the abnormal range.

**DISCUSSION**

To test the hypothesis of whether myeloablative therapy could modulate the idiotype network response to murine MoAb, patient serum was assayed for HAMA, anti-idiotypic (Ab2), anti-GD2, and Ab3 antibodies by ELISA. HAMA and Ab2 levels among 9 patients given $^{131}$I-3F8 plus ABMT were low to nondetectable, in contrast to the 10 patients given $^{131}$I-3F8 but no ABMT who developed approximately 100-fold higher mean levels. Despite the suppression of FIAMA and Ab2 responses, anti-GD2 and Ab3 antibody responses were detectable in 5 of these 9 patients. These 5 patients were all long-term survivors (4 past 60 months, one 24 months from ABMT) and their mean Ab3 levels at 6 months post-3F8 (Idio2, C4E4, A1G4, A2A6) were higher than those who died of disease progression. This lack of Ab3 response persisted despite
rechallenge with 3F8 in 2 nonsurvivors. While one patient failed to mount any Ab3 response, the other had a transient level. Even though 4 of the 9 patients had residual disease by $^{131}$I-3F8 immunoscintigraphy, this adverse prognostic variable did not affect the final outcome in 3 of the 4 patients negatively. These 3 patients have remained progression-free, and all 3 carried detectable anti-GD2 and Ab3 antibodies. Given the small study size and the retrospective nature of such analysis, it is very possible that patients survive because of the biological character of their cancer and not the host immune response to the murine antibody. Interestingly, the presence of a slightly higher anti-GD2 level prior to 3F8 imaging among survivors suggests that a pre-existing immune response towards the ganglioside may be sufficient to delay disease progression or to facilitate the development of the idiotype network.

Murine antibodies are highly immunogenic in patients. HAMA can generally be detected by 1.5 months and peak by 3 months after initial 3F8 exposure. These antibodies can neutralize the antitumor activity of the MoAb, and if persistent will preclude multiple courses of MoAb therapy. The frequency of HAMA reported in various clinical trials of MoAb depends on the sensitivity of the assay used. Our ELISA was able to detect anti-3F8 antibody with a much higher sensitivity than the commercial generic-HAMA assay. This is expected since the commercial kit was standardized with pooled mouse IgG myelomas, while our patients were tested preferentially sensitized to the mouse IgG3 3F8. In addition, HAMA from sensitized patients contained anti-idiotypic antibodies which could not be measured by the generic assay. The observation that HAMA from secondary antibody response showed more preferential binding to 3F8 than the primary response (see “Materials and Methods”) reflects the selection of anti-idiotypic specificities with higher affinities after boosting with the MoAb 3F8.

Cyclophosphamide, a commonly used antitumor and immunosuppressive agent, and total lymphoid radiation were not effective in suppressing the HAMA response to murine MoAb (2, 4). New immunosuppressive agents currently being evaluated include deoxypergualin, a metabolite of Bacillus laterosporus, which was found to suppress the antibody response to an immunotoxin in mice (3). In our study, the combination of high-dose alkylators and etoposide was able to suppress the HAMA response effectively. HAMA level was kept low to undetectable, allowing reimaging or retreatment with 3F8. Nevertheless, it should be noted that in this study, the imaging dose of antibody was small (1.5 mg). Only one of the survivors received a higher dose of $^{131}$I-3F8 (6 mCi/kg, 1.2 mg/kg of 3F8) prior to ABMT. Thus, the ability of ABMT to suppress the immune response to a larger dose of murine MoAb remains to be determined.

We measured Ab2 response by ELISA where the binding to 3F8 F(ab')2 was carried out in an excess of IgG3 myelomas. Inhibition experiments using human anti-murine IgG3 antibodies have shown that 100 μg/ml of myelomas could almost completely inhibit the binding of public specificities, such as those against the framework regions of F(ab')2 or Fc. Ab2 response generally peaked before 3 months after first 3F8 exposure. In contrast, Ab3 response peaked beyond 3 months. This temporal relationship of Ab3 peaking later than Ab2 response was consistent with the hypothesis that Ab2 formation precedes Ab3 induction. It also made it unlikely that all the detected Ab3 activity was due to cross-reactivity of HAMA with rat immunoglobulins.

Jerne’s (23) network hypothesis postulated that immunization with an antibody (Ab1) specific for an antigen will activate B lymphocytes, thereby producing Ab2 with variable regions bearing the internal image of Ab1 and mimicking the original antigen. Induced Ab2 can theoretically induce Ab3, which can cross-react with the original target tumor antigen. As immunogens, Ab2 have advantages over native gangliosides because glycolipids tend to be poorly immunogenic (24). Ab2 can induce better T-cell help, and thus stronger antibody response. In addition, Ab2 have other important attributes not possessed by native antigen, namely: (a) the immune response to Ab2 may be less genetically restricted to certain immune response genes than the tumor antigen; and (b) it can potentially induce cell-mediated (besides humoral) immunity, especially when introduced into class I pathways of antigen processing and presentation (25); (c) while infants and young children are unresponsive to most polysaccharide antigens, anti-idiotypes may bypass this tolerant state; (d) the epitope on ganglioside responsible for protective immunity expressed on tumor cells could be sparsely distributed, obscured by highly antigenic epitopes, or otherwise inaccessible to the immune system; and (e) the internal image of a nonimmunogenic antigen carried by the Ab2 in a different immunological milieu may trigger an immune response.

Our findings were not entirely consistent with Jerne’s (23) hypothesis because we failed to detect significant Ab2 response among the survivors. To bridge this gap in the idiotype network, we postulate that while myeloablative therapy suppressed Ab2 response, it did not completely eliminate it, even though the response was below the threshold of our detection. High levels of circulating Ab2 could hamper the study of Ab3 by: (a) interfering with its detection in vitro; and/or (b) inhibiting Ab3 formation. This might explain why in previous clinical studies where the existence of Ab2 was clearly demonstrated, Ab3 response has been difficult to document. If the failure to detect Ab2 or Ab3 was due to inadequate sensitivity of the assay, more sensitive methods of measurement (e.g., biotin-streapavidin for multistage amplification or chemiluminescence instead of colorimetry) or direct cloning of B-cells within the idiotype network may offer solutions. However, if Ab2 inhibits Ab3 formation, clinical strategies may need to be devised to facilitate Ab3 formation. For example, the profound effect of chemotherapy on Ab2/Ab3 response could be exploited by integrating antibody treatment into standard treatment protocols. Although anti-GD2 antibodies have shown clinical activities (15–17), the exact antitumor mechanism in vivo is not known and is likely to be complex. Anti-idiotypic network may be responsible for the antitumor effect in the late phase of response to antiganglioside antibodies, when Ab2 has significantly diminished and Ab3 response becomes possible. A better understanding of these mechanisms will be critical for manipulating immunity to gangliosides.

Since the immune response to Ab1 or immunization with Ab2 varies with the antigenicity of the MoAbs and likely involves multiple idiotypic circuits, it may be difficult to predict the patient’s response on the basis of data collected in preclinical animal models (26). To date, we do not know which animal model will best mimic the idiotypic repertoire of humans. Without a detailed analysis of each of the individual idiotype networks, it is not possible to predict their antitumor potentials. A previous study by Chen et al. (27) in a murine leukemia model found that survival was positively correlated with a specific idiotope produced after the implantation of tumor cells. In our study, A1G4 and A2A6 idiotopes, which correlated with patient survival, were found at low levels prior to exposure to Ab1. In contrast, the idiotope C2H7 was not detectable either before or after Ab1 injection. Between the 2 ends of the spectrum, C4E4 and Idio2 represented idiotopes that became detectable only after exposure of patients to Ab1. Analogous to the murine model (27), correlation of survival with specific idiotope may provide a useful tool to select disease-protective anti-idiotypic antibodies for vaccine trials. Since the number of patients in this pilot study was small, our findings and conclusions need to be confirmed in a much larger series.
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