Does Endogenous Immune Response Determine the Outcome of Surgical Therapy for Metastatic Melanoma?

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Background: Although the presence of tumor cells in the blood of patients with metastatic melanoma suggests widely disseminated disease, many of these patients enjoy prolonged survival or cure after surgical resection. Our previous study of adjuvant vaccine therapy after complete resection of metastatic melanoma revealed a strong correlation between postoperative survival and elevated antibody titers to a 90-kDa tumor-associated antigen (TA90) expressed by melanoma cells of the vaccine. We hypothesized a similar correlation between postoperative survival and endogenous anti-TA90 antibody titers induced by the patient’s melanoma in the absence of postoperative adjuvant immunotherapy.

Methods: From 1970 to 1996, 64 patients underwent complete resection of distant melanoma metastases and did not receive postoperative adjuvant immunotherapy. Serum collected within 4 months after surgery was tested in a coded and blinded fashion for anti-TA90 IgG and IgM by enzyme-linked immunosorbent assay, and for total IgG and IgM (controls) by radial immunodiffusion.

Results: Median follow-up for the study population was 19 months (range, 3–147 months). There was no significant correlation between anti-TA90 IgG titer and total IgG level (P = .4785), or between anti-TA90 IgM and total IgM (P = .0989). Univariate analysis showed that postoperative anti-TA90 IgM titer as a continuous variable was significantly associated with overall survival (OS); i.e., the higher the anti-TA90 IgM titer, the longer the OS. Using an established cutoff titer of 800, median OS was 42 months for patients with high anti-TA90 IgM titers (n = 28) vs. 9 months for patients with low titers (n = 36) (P = .0001). There was no significant correlation between total IgG/IgM and survival (P = .4107 and .4044, respectively). Multivariate analysis identified anti-TA90 IgM as the most significant independent variable influencing OS after complete resection of distant melanoma metastases (P = .0001).

Conclusions: We conclude that the endogenous immune response to metastatic melanoma determines the outcome after surgical therapy. Enhancement of this specific immune response may prolong the survival of patients with distant melanoma metastases.

Key Words: Melanoma—Endogenous immune response.
the serum of melanoma patients contains cytotoxic antibodies to cultured melanoma cells.\textsuperscript{12-14} Our group previously reported prolonged survival among AJCC stage III melanoma patients who had a high in vitro complement-dependent cytotoxicity response after adjuvant immunotherapy with a whole-cell melanoma vaccine (CancerVax).\textsuperscript{15} In addition, we have documented a significant correlation between survival and elevated titers of antibody to a 90-kDa glycoprotein antigen (TA90) in patients receiving adjuvant CancerVax immunotherapy after complete resection of AJCC stage IV melanoma.\textsuperscript{16} In the present study, we hypothesized a direct correlation between survival and the endogenous humoral immune response to melanoma cells remaining after clinically complete resection of stage IV melanoma. Our results confirmed a strong correlation between high postoperative anti-TA90 IgM antibody titer and prolonged overall survival (OS). This correlation suggests that adjuvant active immunotherapy for metastatic melanoma should attempt to elicit an anti-TA90 IgM immune response.

### PATIENTS AND METHODS

**Patients**

Our study population was drawn from patients treated for AJCC stage IV melanoma by members of the staff of the John Wayne Cancer Institute between January 1, 1972, and December 31, 1996. Of the 282 patients who did not receive active specific immunotherapy at any point during their treatment, 114 underwent complete resection of metastatic disease, i.e., resection that left no clinical or radiographic evidence of disease. Of the 114 patients, 64 had serum samples collected and cryopreserved within 4 months postoperatively. These 64 patients represented our study group.

In each case, the patient’s postoperative clinical status was recorded prospectively until time of death or the last follow-up date, and data were transferred to the melanoma database stored in our statistical coordinating unit. All study patients had given informed consent for postoperative blood collection, and all blood samples were processed, serum- aliquoted, and stored at $-35^\circ$C. Before use in the antibody assays, cryopreserved blood samples were thawed; all sera were coded and tested in a blinded fashion.

Patient demographics are shown in Table 1. The median follow-up time was 19 months (range, 3–147 months). Most (67%) of the study population were male. Eighty-six percent of the study patients had one site of metastases and 61% had visceral organ involvement.

### TABLE 1. Patient demographics

<table>
<thead>
<tr>
<th>Sex</th>
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<tbody>
<tr>
<td>Female</td>
<td>21</td>
<td>33</td>
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<tr>
<td>Male</td>
<td>43</td>
<td>67</td>
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<table>
<thead>
<tr>
<th>Primary site</th>
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<tbody>
<tr>
<td>Extremity</td>
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<tr>
<td>Head and neck</td>
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<tr>
<td>Trunk</td>
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<td>Unknown</td>
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<table>
<thead>
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<th>Age at diagnosis</th>
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<tbody>
<tr>
<td>&lt;60 y</td>
<td>47</td>
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<tr>
<td>$\geq 60$ y</td>
<td>17</td>
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<table>
<thead>
<tr>
<th>Number of metastases</th>
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<tbody>
<tr>
<td>1</td>
<td>55</td>
</tr>
<tr>
<td>$\geq 2$</td>
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<table>
<thead>
<tr>
<th>Site of first metastases</th>
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<tbody>
<tr>
<td>M1a</td>
<td>25</td>
</tr>
<tr>
<td>M1b</td>
<td>39</td>
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<table>
<thead>
<tr>
<th>Disease-free interval</th>
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<tr>
<td>&lt;60 mo</td>
<td>15</td>
</tr>
<tr>
<td>$\geq 60$ mo</td>
<td>49</td>
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</tbody>
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M1a, metastasis to skin, soft tissue, and distant lymph nodes; M1b, metastasis to visceral sites including bone, brain, gastrointestinal, and lung.

**Anti-TA90 IgG and IgM Assay**

TA90 was purified from urine of a melanoma patient.\textsuperscript{17} The purity with respect to protein heterogeneity was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis before using this antigen as a target in an enzyme-linked immunosorbent assay (ELISA). ELISA was performed according to standard procedures reported elsewhere.\textsuperscript{18} In brief, TA90 was first adsorbed to polystyrene wells of 96-well ELISA plates at 120 ng/well. Serum samples at dilutions of 1:100, 1:200, 1:400, 1:800, and 1:1600 were added to the wells. Subsequently, the bound immunoglobulins were reacted with the alkaline phosphatase-conjugated Fab fragment of goat anti-human IgG (at 1:500 dilution) or IgM (at 1:1000 dilution) (Sigma Chemicals, St. Louis, MO). The unreacted conjugate was then washed off and p-nitrophenyl phosphate (1 mg/ml) in 10% diethanolamine buffer was added. Absorbance at 405 nm was assessed in the microtiter plate reader (Titertek Multiscan; Dynatech, Alexandria, VA). Controls on each ELISA plate consisted of nonspecific binding of a patient’s immunoglobulins and the goat anti-human IgG or IgM to the polystyrene plate, and nonspecific binding of the goat anti-human IgG or IgM to the TA90 antigen. The antibody titer was defined as the reciprocal of the highest dilution resulting in an absorbance of 0.05 optical density at 405 nm after subtracting the absorbance values of the controls.