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Humoral immune response to intralymphatic immunotherapy for disseminated melanoma: Correlation with clinical response

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Peter C. Jones, M.D., Guy Juillard, M.D., Denise J. Roc, M.S., and
Donald L. Morton, M.D., Los Angeles and Sepulveda, Calif.

Since September 1979, 44 stage III melanoma patients treated with intralymphatic immunotherapy (ILI) with an oncofetal antigen (OFA-I)–enriched tumor cell vaccine (TCV) had evaluable humoral immune responses and clinical follow-up. Seventeen patients (39%) had stabilization or regression of tumors or remained free of resected disease. The median survival was 17 months, compared with 6 months for controls \((P < 0.061)\). Humoral immune responses were monitored by immune adherence using an OFA-positive human melanoma cell line, M14, as target. Alloantibodies were removed by absorption with L14 lymphoblasts autologous to M14. Twenty-two patients (50%) developed elevated antibody titers within 4 months, and 12 of the 22 (55%) had no disease progression. In contrast, 20 of 22 patients (91%) who failed to develop elevated titers had disease progression \((P < 0.01)\). The median titers were significantly higher during the first 4 months in the group whose disease did not progress \((P < 0.04)\). This study demonstrated that ILI with allogeneic OFA-I–enriched TCV can induce objective tumor regression and prolonged survival in patients with disseminated melanoma. Furthermore, because the specific humoral immune response correlates with clinical results, immunotherapy efficacy can be monitored within a short period of time, which should aid future efforts to achieve optimal immunotherapy.

From the Departments of Surgery/Oncology, Radiation Oncology, and Biomathematics and the Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, and the Surgical Services, Veterans Administration Medical Center, Sepulveda, Calif.

Morton demonstrated in 1970 that 90% of dermal melanoma metastases regressed after intramuscular injections of bacille Calmette Guérin (BCG). Furthermore, 15% to 20% of noninjected distant nodules also regressed. This procedure remains the single most effective and reproducible form of tumor immunotherapy to date. As a direct extension of this work, we have been investigating ways to improve immunotherapy results, especially for patients with regional or disseminated melanoma.

In 1976 Irie et al. identified a tumor-associated membrane antigen in melanomas that cross-reacted with fetal brain tissue. The antigen was named oncofetal antigen (OFA). Subsequent studies indicated that OFA was immunogenic in humans, and it was renamed OFA-I. On further characterization, Irie et al. found that 80% of biopsied melanoma specimens contained cells with OFA-I and that anti–OFA-I in the presence of complement induced lysis of melanoma cells in vitro.

The results of a clinical trial with stage II melanoma patients treated with intradermally administered BCG and an irradiated, trivalent allogeneic tumor cell vaccine (TCV) rich in OFA-I revealed a significant correlation between elevated IgM–anti-OFA-I and a prolonged disease-free interval and survival. However, despite a small tumor burden, only 14 of 40 (35%) of these stage II patients achieved significantly elevated IgM–anti-OFA-I levels. Clearly, a more efficient vaccination method was needed.

In 1978 Juillard et al. reported a clinical immuno-
therapy trial of 21 patients with various advanced malignancies who received intralymphatic injections of irradiated autochthonous or allogeneic tumor cells. Although specific humoral immune responses were not measured, the study demonstrated the feasibility and safety of intralymphatic immunotherapy. Furthermore, 5 of 19 patients with evaluable disease achieved objective tumor regression.

These promising results and the need for a more efficient immunotherapy modality and an effective systemic therapy for melanoma prompted us to administer the OFA-I-rich TCV by the intralymphatic route. We reasoned that this immunization modality, which places the tumor cells in direct contact with lymphoid tissue, might stimulate an increased immune response to OFA-I and other melanoma-associated antigens.

Accordingly, in September 1979 we began a pilot clinical trial of intralymphatic immunotherapy (ILI) for patients with stage III (disseminated) melanoma. A preliminary report of the clinical and humoral immune responses of patients treated during the first 15 months of this trial appeared earlier. A detailed analysis of the clinical results is forthcoming. In this article we describe the humoral immune response and its correlation to the clinical progress of the patients to date.

MATERIAL AND METHODS

From September 1979 through September 1981, 51 patients with disseminated melanoma were treated with ILI composed of irradiated trivalent allogeneic TCV rich in OFA-I. Seven patients were excluded from the study—three were lost to clinical follow-up, two had tumor regression but received other concomitant treatment, one had tumor regression with autologous TCV before receiving the vaccine used in this study, and one had regression but antibody data were unavailable. Humoral immune responses and clinical progress were evaluable for 44 patients.

The TCV was produced from three cultured melanoma cell lines (UCLA-SO-M7, M14, and M20) established in our laboratory. All cell lines were known to contain OFA-I as previously described by Irie et al. Prior to use, the TCV was irradiated with 10,000 rads for 2 seconds—a dose/time schedule that maintains cell viability but prevents cell proliferation. TCV was administered as described by Juillard. The sites selected for lymphatic cannulation were the dorsal

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**Table 1. Correlation of elevated titers within 4 months and no disease progression**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Progression</th>
<th>Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated titer</td>
<td>22</td>
<td>12† (55%)</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>No elevated titer</td>
<td>22</td>
<td>2‡ (9%)</td>
<td>20 (91%)</td>
</tr>
</tbody>
</table>

*P < 0.01.

†Six regressions, four stable, and two NED.
‡One regression and one NED.
Table II. Comparison of prognostic factors in ILI and control patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>ILI</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>44</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>45</td>
<td>47</td>
<td>0.65</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>33/11</td>
<td>19/6</td>
<td>1.00</td>
</tr>
<tr>
<td>Site(s) of disease*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>15 (34%)</td>
<td>5 (20%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Skin</td>
<td>16 (36%)</td>
<td>8 (32%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Lymph node</td>
<td>7 (16%)</td>
<td>5 (20%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Brain/nasopharynx</td>
<td>4 (9%)</td>
<td>6 (24%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Bone</td>
<td>3 (7%)</td>
<td>4 (16%)</td>
<td>0.25</td>
</tr>
<tr>
<td>GI tract/liver</td>
<td>6 (14%)</td>
<td>2 (8%)</td>
<td>0.70</td>
</tr>
</tbody>
</table>

*At time stage III disease was diagnosed.

Prior to initiation of ILI, each patient had a physical examination, complete blood count, blood chemistry tests, chest x-ray examination, and other appropriate baseline or staging radiographic studies. These studies were repeated at monthly intervals and as needed.

The clinical results were defined as follows:

Regression: A ≥ 50% reduction of tumor mass and no new lesion for at least 3 months

Stable: No measurable growth or <50% reduction of tumor mass and no new lesion for at least 3 months

NED: No evidence of recurrent disease for at least 3 months in patients who, by surgical resection just prior to ILI, were free of measurable disease

Progression: Measurable growth of tumor or development of new lesions within 3 months of the first treatment

Blood samples were obtained by peripheral venipuncture on the day of and 1 week after each treatment. The serum was extracted and stored at −35°C. Before testing, serum samples were heated to 56°C for 30 minutes to inactivate complement.

Serum antibody determination was made by the immune adherence (IA) assay,4 which specifically detects complement-fixing antibody and is particularly sensitive for IgM. The target cell M14 was also used in the TCV and was known to be rich in OFA-I.9 Each serum sample was absorbed with autologous lymphoblasts to eliminate anti-HLA and other alloantibodies. As previously described, the antibody that remained
was known to be predominantly directed against OFA-I. Therefore, antibodies detected by this assay system were predominantly complement-fixing, IgM, melanoma-associated anti-OFA-I. Titers of 1:8 or greater and at least fourfold increases over pretreatment titers were considered positive responses to ILI. The interval from diagnosis of stage III disease until the death of ILI patients was compared with that of 25 patients who had disease recurrences in a previous stage II randomized trial. These control patients had surgery only for their stage II disease but received chemotherapy, radiation therapy, heat therapy, and/or nonintralymphatic immunotherapy for their stage III disease. The relevant prognostic factors (age, sex, and site of recurrence) were compared to eliminate known differences between the two groups of patients. Although this control group was not randomized, it did allow comparison of survival rates in similar groups of patients.

The median survival for ILI and control patients was estimated with the Kaplan-Meier estimate. The differences in survival distribution were tested using the log rank statistics. The comparability of the two groups of patients for known prognostic factors was tested with Fisher’s exact test and Student’s t test. The association of humoral immune response and clinical results was tested with Fisher’s exact test.

RESULTS

The ages of the 33 men and 11 women ranged from 22 to 88 years (mean 45 years). Thirty-eight patients had measurable disease, and six had no evidence of disease (NED) after resection of their measurable disease 1 month before ILI. Disease sites were lung in 23 patients, skin/subcutaneous layer in 20, lymph node in 7, brain/nasopharynx in 5, bone in 3, and gastrointestinal tract/liver in 6.

Thirty patients (68%) had progression of their disease, and 14 (32%) did not (Table I). Seven (18%) of the 38 patients had objective tumor regression for 18, 18, 5, 5, 12+, 12+, and 17+ months, respectively. Four patients (11%) had disease stabilization for 4, 3, 11, and 17+ months, respectively. Of the six patients without measurable disease, three (50%) had NED for 18+, 22+, and 23+ months, respectively.

The median survival time from diagnosis of stage III disease until death for these 44 patients was 17 months (range 4 to 47 months), whereas that of the comparable control group was 6 months (range 0 to 33 months). Twelve of the 44 ILI patients are still alive. The median follow-up is 16 months (range 11 to 35 months). The difference in the survival distributions for the ILI and control groups was statistically significant (P < 0.001) (Fig. I). The two groups were similar in age, sex, and tumor site, although the ILI group tended to have more lung metastases and fewer brain and bone metastases (Table II).

Twenty-two of the 44 (50%) patients in this study developed elevated antibody titers within 4 months (Table I and Fig. 2), and 12 (55%) had no progression of their disease. Ten patients (45%) had disease progression. In contrast, of the 22 patients who did not
have elevated antibody titers, only 2 (9%) had no disease progression. This association of elevated titers with clinical results was statistically significant \( P < 0.01 \) (Table I). Furthermore, during the first 4 months of treatment, the median antibody titers were significantly higher in the group of patients whose disease did not progress than in the group whose disease did progress \( P < 0.04 \) (Fig. 3).

**DISCUSSION**

This study demonstrated that objective tumor regression and prolonged survival can be achieved in patients with disseminated melanoma with ILI using allogeneic OFA-I–enriched TCV. Seven of 38 patients (18%) with measurable disease achieved tumor regression for 5 to 18 months. The median survival is nearly three times that of a comparable although nonrandomized group of stage III patients treated in our institution and others.3,7,11

In this trial the TCV used for ILI was selected because it contained an abundance of a tumor-associated antigen, the OFA-I. Humoral immune responses to this antigen could be monitored as an indicator of clinical response. Twelve of 14 patients (86%) whose disease did not progress developed elevated antibody titers within 4 months of initiation of immunotherapy, while only 2 of 22 patients (9%) who failed to achieve elevated titers within 3 months demonstrated no disease progression. This correlation of a specific humoral immune response with the clinical results permits monitoring of the immunization efficacy within a short period of time.

The immunotherapy in this study induced elevated antibody titers within 4 months in only half the patients. Improved immunization results may be possible with changes in vaccine dosage, treatment intervals, or changes in the vaccine itself. Other possibilities include improvement of the patients’ immunocompetence by tumor-debulking procedures, the addition of general immune stimulants (e.g., BCG, retinoids, or interferon), and the avoidance of any possibly immunosuppressive drugs.

Immunization efficacy also might be improved by use of this TCV only for patients whose melanoma contains OFA. Since OFA-I is not present in 20% of biopsied melanoma specimens,10 a TCV rich in OFA-I might not be appropriate for certain patients. Ten of 22 (45%) patients in this study had elevated antibody titers but had disease progression. Some of these patients may have had OFA-I-negative tumors and thus would not benefit from stimulation of anti-OFA-I. On the other hand, their tumor burden may have been too extensive for the amount of antitumor immunity induced, or blocking factors, either cellular or humoral, may have inhibited the effectiveness of the induced immunity. Furthermore, if tumor membrane antigen modulation occurred during immunotherapy, the current TCV would become inappropriate and ineffective.

If the TCV is indeed inappropriate for certain patients whose tumors are OFA-I negative or whose tumors undergo antigen modulation, then the identification and characterization of other antigenic systems that could be incorporated into a vaccine should also improve immunization efficacy. The two patients who did not have elevated antibody titers but whose disease did not progress (one had tumor regression for 5 months and the other has NED at 23 months) may have had antibodies against just such an antigenic system that was not or could not be detected by the IA assay used in this study. However, other factors such as cellular immunity may have been stimulated as well. In fact, one can postulate that anti-OFA-I itself may only be an indirect measure of other humoral or cellular immune factors that participated in the favorable course of the patients with elevated antibody titers.

Finally, since OFA-I is present in several other neoplasms,3 ILI with TCV rich in OFA-I has potential for use in future immunotherapy trials of other neoplasms. As other tumor-associated antigens are identified and characterized, the role of ILI with tumor-associated antigens may gain even broader applications.

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DISCUSSION

Dr. Hiram C. Polk, Jr. (Louisville, Ky.). I am particularly pleased to see this group moving toward more specific immunization. It has been an unfortunate tendency to lump very nonspecific immunization, with BCG or Corynebacterium parum for example, with much more well-defined material such as that studied by Seigler et al. (Ann Surg 186:1, 1977).

I am discouraged by the reference to historic controls, especially for an illness associated with frequent, so-called spontaneous regressions. On the other hand, the technique described is notable in that the measurable antibody levels correlated very well with progression or regression as the case may be.

Finally, in another vein, could you tell me whether you have attempted the use of any other specific adjuvant substances?

Dr. Mutaz B. Habal (Tampa, Fla.). I have a question about the survival of these patients. Have you had any correlation between the final survival and the initial lesion, whether it is stage I, II, or III, or any correlation with the histologic staging? They do vary toward the end, according to how they start initially.

Dr. Sutherland (New Orleans, La.). We use a somewhat similar system. We use autologous cell lines, and I would like to confirm your observation about the antimelanoma antibody. It is exactly the same in our laboratory. A value of 1:8 gives very good clinical results, and anything below that does not. I think it is interesting that both of us have come to exactly the same conclusions on that issue.

Dr. Shapiro (Boston, Mass.). Dr. Ahn, have you looked at any measurements of cellular immunity in these patients, either DTH or in vitro cytotoxicity?

Dr. Sam Ahn (closing). Dr. Polk, in response to your comments and questions, this group of patients was not necessarily a historic control. They were patients treated essentially simultaneously, although the group was not randomized. Also, the prognostic factors for the two groups of patients were very similar. There was no statistical difference in the prognostic factors that were looked at, including disease site, age, and sex. I might also add that the patients treated here had visceral metastasis, and most of the responses were seen in patients with visceral metastasis. Most of the spontaneous regressions, I believe, were in patients with cutaneous metastasis.

Yes, we are conducting adjuvant trials now, using this tumor cell vaccine (TCV). We have a prospective randomized trial of stage II patients following lymphadenectomy, to whom the TCV is administered intralymphatically.

Dr. Sutherland, I thank you for sharing your observations. It is good to hear that other people are getting similar results.

Dr. Habal, we have not correlated the original histologic studies with the results that we obtained.

Dr. Shapiro, we have looked at the cellular immune response, which we report separately. All the patients did undergo delayed hypersensitivity skin testing to the TCV, and there was a correlation of the cellular immune response by delayed hypersensitivity and clinical results. Unfortunately, the majority of patients treated did develop a positive skin test, and the skin test, I believe, really measures a very nonspecific parameter instead of the specific humoral immune response we noted here.

We are currently looking at the peripheral blood lymphocytes to examine cytoxicity on a cellular basis.