The Effect of Transfer Factor Therapy on Tumor Immunity in Alveolar Soft Part Sarcoma

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A young man with alveolar soft part sarcoma and his identical twin were studied in terms of immunologic response to the patient's tumor homogenate. The lymphocytes from both twins underwent lymphoblastic transformation to tumor homogenate but only the healthy twin's lymphocytes released demonstrable migration inhibition factor (MIF) to the tumor preparation. Transfer factor was prepared from the healthy twin and administered to the tumor-bearing twin. A total dose of transfer factor equivalent to $45 \times 10^6$ lymphocytes given in three separate doses produced a persistently positive MIF assay in the patient. The tumor neither regressed nor progressed during the 6-month period after transfer factor therapy.

Lawrence has described a dialyzable substance, transfer factor, which is present in the lysate of lymphocytes (1). This unique material had the capacity to transfer specific cellular immunity from a sensitive donor to a nonsensitive recipient within 24-48 hr after injection (2). The clinical efficacy of this material in the treatment of such chronic infectious disorders as mucocutaneous candidiasis (3, 4) and such immunologic deficiency states as the Wiskott-Aldrich syndrome (5) suggests that it is a potent clinical tool for improving cellular immune resistance. Preliminary clinical trials of transfer factor therapy in patients with cancer have had encouraging results (6, 7). These studies, however, have not included in vitro assays to characterize the cellular immune reactivity to tumor antigens in the donors of transfer factor or in the recipients before and after therapy. In vitro assays will be important in the development and evaluation of this form of immunotherapy since little is known regarding selection of donors, dosage, or frequency of treatment.

This report describes studies of tumor immunity in a patient with alveolar soft part sarcoma and his unaffected identical twin. This tumor is an unusual, slow-growing sarcoma affecting primarily young adults. It usually pursues an indolent course with a 50% survival rate of 6-7 years despite the presence of metastasis (8). Little information exists regarding response to chemotherapy, but case reports suggest that the tumor is resistant to radiotherapy (9). Histogenesis of the tumor remains ob-

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scure despite several efforts to define the tissue of origin (10). Several investigators have described tumor specific antigens in the more common types of sarcoma (11) but no immunologic studies have been done with alveolar soft part sarcoma.

This report further describes the effect of transfer factor therapy on this patient’s tumor immunity as measured by the migration inhibition factor assay. The source of transfer factor was the patient’s healthy twin brother who was immune to the patient’s tumor preparation.

CASE REPORT

G. Mill., a 33-year-old white male, was well until January 1970 when he noted a mass on the lateral aspect of the left thigh. Over the next 9 months, the mass gradually increased in size and was associated with local pain. In October 1970 because of a nonproductive cough of 2 months’ duration, a chest X-ray was taken and showed bilateral metastatic nodules. Biopsy of the thigh mass revealed alveolar soft part sarcoma. Chemotherapy with actinomycin D and cyclophosphamide was administered intermittently between January and August 1971 without any regression of the primary or metastatic lesions. In August 1971 he was referred to Ohio State University Hospital at which time prominent symptoms were dyspnea on two block exertion, nonproductive cough, pain in the thigh mass, and frontal headaches. No hemoptysis, weight loss, fever, bone pain, or focal neurological symptoms were present. Family history revealed the patient’s mother had metastatic carcinoma of the colon and a maternal aunt had died of a sarcoma.

Physical examination revealed mild tachypnea, bilateral coarse rhonchi, and occasional wheezes, and an 8.0 × 10.0-cm mass on the lateral aspect of the left thigh unattached to underlying bone. There was no lymphadenopathy, organomegaly, or other masses. The neurologic examination was normal.

Laboratory data. Hemoglobin was 12.7 g/100 ml, hematocrit 36%, platelets 385,000/mm³ with a normal differential. Urinalysis and levels of serum creatinine, lactic dehydrogenase, alkaline phosphatase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase were normal. Liver scan and bone films showed no evidence of metastases. Chest film revealed 15–20 metastatic nodules in the lower lung fields bilaterally. Pulmonary function studies were within normal limits. Spinal fluid was normal.

Chemotherapy was discontinued and immunological studies were instituted. The mass on the left thigh was excised for studies described below. Review of chest X-rays taken from time of diagnosis until August 1971 showed slow progression of disease with increase in the number of pulmonary metastases.

D. Mill., twin brother of G. Mill. had been in excellent health and completely asymptomatic. He had no evidence of neoplastic disease on either physical examination or radiographic studies.

METHODS

H1-A typing was performed using lymphocyte cytotoxicity (12) and 22 antisera in a tissue-typing tray series 02 obtained from the Tissue Typing Laboratory, N.I.H. Red cell typing was performed by the Blood Grouping Laboratory, Boston, MA.

Skin test reactivity was determined using commercially obtained preparations of PPD (intermediate strength), histoplasmin, dermatophytin, and mumps. Reactions were read at 24 and 48 hr.
Mixed leukocyte cultures were performed by the method of Bach (13). All cultures were done in triplicate and thymidine uptake was expressed as dpm/10^6 lymphocytes. Significant stimulation was determined statistically using the Student t test. Stimulation ratios were calculated as the ratio of dpm in cultures with heterologous stimulating leukocytes to those containing autologous stimulating leukocytes. Lymphoblastic transformation to tumor preparations was tested using the same culture systems described above for mixed leukocyte culture except that stimulating cells were either 5 x 10^6 homogenized tumor cells or 5 x 10^6 homogenized autologous leukocytes. Lymphoblastic transformation to phytohemagglutinin (PHA) was tested by established techniques (14). The tumor homogenate was obtained at the time of operation and used for the migration inhibition factor assays as described in the preceding article (15).

Transfer factor was prepared from leukocytes by the method of Lawrence (1). The leukocytes were harvested from the healthy twin (D. Mill.) using an Amincom blood cell separator. Doses of transfer factor were expressed in terms of the number of lymphocytes used in the preparation of the material to be injected.

RESULTS

Confirmation of monozygosity. The twins were phenotypically identical and had identical reactivity in leukocyte HL-A typing (HL-A 1 and 4-C positive) as well as identical reactions for 22 red cell antigens. Table 1 outlines the mixed leukocyte culture testing. HL-A identity was confirmed by MLC since neither twin’s lymphocytes were affected by the other twin’s stimulating leukocytes.

General immunologic reactivity. Both twins had strongly positive skin tests to histoplasmin, dermatophytin, and mumps. Lymphocytes from the tumor-bearing twin had a normal response to PHA with a stimulation of 58,132 cpm and a control of 613 cpm for a stimulation ratio of 95. In addition, lymphocytes from both twins were able to respond well in mixed leukocyte culture with stimulating cells from an unrelated leukocyte donor (Table 1). Thus, despite the presence of metastatic

<table>
<thead>
<tr>
<th>Responding cells</th>
<th>Stimulating cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy twin (D. Mill)</strong></td>
<td><strong>D. Mill.</strong></td>
</tr>
<tr>
<td>dpm/10^6 lymphocytes</td>
<td>2,136; 2,952; 705</td>
</tr>
<tr>
<td>Mean</td>
<td>1,931</td>
</tr>
<tr>
<td>Significance</td>
<td>--</td>
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<tr>
<td>Stimulation ratio</td>
<td>--</td>
</tr>
</tbody>
</table>

| Tumor-bearing twin (G. Mill) | | |
|-------------------------------|-------------------|
| dpm/10^6 lymphocytes | 1,664; 1,544; 1,096 | 2,146; 365; 1,767 | 16,047; 19,461; 11,047 |
| Mean | 1,435 | 1,426 | 15,581 |
| Significance | NS | -- | P < 0.001 |
| Stimulation ratio | 1.0 | -- | 11.1 |

* X = Unrelated donor.
* NS = Not significant.
TABLE 2
LYMPHOCYTE RESPONSE TO TUMOR ANTIGENS

<table>
<thead>
<tr>
<th>Responding cells</th>
<th>Stimulating cells</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Autologous leukocytes</td>
<td>Tumor</td>
</tr>
<tr>
<td>Healthy twin (D. Mill)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dpm/10^6 lymphocytes</td>
<td>14,921; 21,057; 16,280</td>
<td>37,474; 34,968; 35,658</td>
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<tr>
<td>Mean</td>
<td>17,419</td>
<td>36,033</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulation ratio</td>
<td>—</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Tumor-bearing twin (G. Mill)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dpm/10^6 lymphocytes</td>
<td>14,778; 14,979; 16,915</td>
<td>59,979; 56,876; a</td>
</tr>
<tr>
<td>Mean</td>
<td>15,557</td>
<td>58,423</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulation ratio</td>
<td>—</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Third tube broken in processing.

disease, the tumor twin did not have a grossly impaired cellular immune system as determined by these studies.

**Lymphoblastic transformation to tumor homogenate.** As outlined in Table 2, the tumor-bearing twin’s lymphocytes were significantly stimulated by tumor cell preparations (P < 0.001). Similarly, the healthy twin’s lymphocytes were stimulated by tumor cells (P < 0.01) despite the fact that he had no evidence of disease. The homogenized tumor cell preparations alone demonstrated no uptake of tritiated thymidine.

**Migration inhibition factor response to tumor homogenate.** As reported in the previous paper, the healthy twin’s lymphocytes repeatedly produced MIF in re-

![Figure 1](image_url)

**Fig. 1.** The MIF response to tumor homogenate (alveolar soft part sarcoma) in the tumor-bearing twin (G. Mill.) during the administration of transfer factor from his identical twin. The dosage of transfer factor was equivalent to 15 X 10^6 lymphocytes on the three dates illustrated. Significant MIF response is greater than 20% inhibition.
response to a homogenate of his twin's tumor with assays of 42, 39, and 22%. In contrast, the tumor-bearing twin's lymphocytes did not produce MIF to his own tumor homogenate despite their ability to undergo transformation to tumor homogenate.

**Effects of transfer factor therapy.** The effect of transfer factor therapy on the ability of the patient's lymphocytes to make MIF in response to his autologous tumor homogenate is depicted in Fig. 1. An apparent cumulative dose response to transfer factor was noted in that the MIF assay remained negative after the first dose, was transiently positive after the second dose, and was strongly positive 3 days and 3 weeks after the third dose. Further *in vitro* testing was curtailed by lack of adequate amounts of tumor homogenate.

The patient's clinical course in the 6-month period after transfer factor therapy was characterized by a 6-lb weight gain and no change in the number or size of the pulmonary metastases. He is fully ambulatory and has continued to have cough, dyspnea on exertion, and headache. He has not received chemotherapy during the period of study (August, 1971–June, 1972).

**DISCUSSION**

These studies have demonstrated that a patient with alveolar soft part sarcoma and his healthy identical twin had immunologic reactivity to the patient's tumor homogenate. This is the first demonstration that this unusual form of sarcoma has tumor-specific antigens and may have immunologic responses similar to the more common types of sarcoma (16). The presence of tumor immunity in the healthy twin as well as the high incidence of tumor immunity in family members of patients with the more common sarcomas (11) suggest that these family members had come into contact with a tumorigenic agent (? virus) and developed an immunologic response to tumor antigens with no subsequent clinical disease. Whether this immunologic reactivity is responsible for the lack of disease is conjectural but this interpretation is compatible with the concept of immunologic surveillance (17).

The immunologic studies in the patient prior to transfer factor therapy were of great interest in that his lymphocytes were able to recognize some antigen in the autologous tumor preparation and undergo lymphoblastic transformation. They failed on several occasions, however, to make detectable MIF in response to tumor preparations. This failure of *in vitro* response did not seem to be explained on the basis of immunologic suppression due to chemotherapy or advanced cancer since he had not received any chemotherapy in the 3 months prior to study and other parameters of immunologic responsiveness, including skin tests' reactions, *in vitro* lymphocyte response to phytohemagglutinin and mixed leukocytes culture were normal. This dichotomy of immune response to an antigen has been noted in patients with chronic mucocutaneous candidiasis whose lymphocytes may undergo transformation but not produce MIF to candida antigen (4). MIF production correlates with *in vitro* immunity since responsiveness to skin testing with candida is often lacking as well. Clinical response of these patients to transfer factor from donors immune to candida correlates with the development of MIF response to candida antigen (4, 18). In addition, clinical response to transfer factor in patients with Wiikott–Aldrich syndrome has correlated with the development of positive MIF assays to antigens and not with lymphoblastic transformation (5). These observations suggested that transfer factor therapy from a donor known to be immune to the patient's tumor might improve his immunologic response to tumor antigen *in vitro* as measured by
the MIF assay. It also suggested that the development of a positive MIF assay might be indicative of clinical benefit in terms of tumor resistance but this might be dependent on a number of variables including size of tumor mass, strength of immunologic reactivity, and presence or absence of blocking factors.

The use of transfer factor as an immunotherapeutic agent has one distinct advantage over other approaches. This material can produce cellular immune reactivity to an antigen without causing inflammatory or humoral antibody responses (2). This therapy should, therefore, not cause increases in immunoregulatory alpha globulins (19) or “blocking” antibody (20). It represents a potential means of selectively stimulating that portion of the immune response thought to be important in tumor resistance and minimizes the danger of enhancing tumor growth. A major problem in the use of transfer factor in cancer therapy is the selection of transfer factor donors. Lawrence (21) has suggested three sources of donors: normal individuals; patients with cancer who have been cured by surgery or irradiation; or patients or normal volunteers actively immunized with tumor preparations. We have selected a fourth alternative, i.e., contacts of patients who have immunity to tumor antigens, an immunity presumably acquired during the development of cancer in the patient. This type of immunity can be interpreted as naturally occurring and possibly protective in that the immune individual did not develop cancer. A second problem in transfer factor therapy relates to dosage and frequency of administration. In order to determine clinical efficacy, in vitro assays are necessary to determine the immunologic potency of transfer factor preparations as well as to determine if enough transfer factor has been given to improve the patient’s immunologic response to tumor antigens. For example, this patient’s initial transfer factor injection was derived from $15 \times 10^8$ lymphocytes from a donor known to be immune to his tumor antigens. This represents approximately five times the amount demonstrated by Lawrence to transfer immunity from a donor very sensitive to PPD to a nonsensitive recipient (2). This dose did not change the patient’s in vitro MIF response to tumor antigen and the dose was repeated on two subsequent occasions before consistent improvement in immunologic response to tumor antigens could be demonstrated.

This MIF response to tumor antigens in our patient represents the first documentation that transfer factor can induce immunologic responsiveness to tumor antigens. The apparent dose response seen in the patient suggests that rather large amounts of transfer factor may be necessary to produce an improvement in tumor immunity. The dose requirement will depend on the strength of immunologic reactivity in the donor and the degree of immunologic responsiveness of the cancer patient. This patient, who had extensive metastasis required an amount of transfer factor equivalent to $45 \times 10^8$ lymphocytes. This represents the lymphocytes from approximately 3 liters of peripheral blood and suggests the dosage range which may be necessary to produce objective improvement in immunologic reactivity in a cancer patient whose transfer factor donor has immunologic reactivity but of unknown potency. These observations also support the need for a blood cell separator to obtain adequate numbers of lymphocytes for the production of transfer factor. The blood cell separator can produce, in a 6-hr period, an average yield of $75-100 \times 10^9$ lymphocytes from a normal donor. This allows repeated administration of relatively large doses until an in vitro response is documented.

The clinical state of this patient after transfer factor has been characterized by no progression or regression of disease over a 6-month period of observation. The eventual efficacy of transfer factor therapy will have to be judged in terms of clinical
response but a great deal of information regarding donors, dosage, and regimens will have to be developed before clinical response rates can be evaluated.

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