

## Osteogenic sarcoma. Immunologic parameters before and during immunotherapy with tumor-specific transfer factor.

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*J Clin Invest.* 1975;55(3):487-499. <https://doi.org/10.1172/JCI107955>.

### Research Article

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# Osteogenic Sarcoma

## IMMUNOLOGIC PARAMETERS BEFORE AND DURING IMMUNOTHERAPY WITH TUMOR-SPECIFIC TRANSFER FACTOR

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**ABSTRACT** 18 patients with osteogenic sarcoma were followed by serial measurements in vitro of tumor-specific cell-mediated cytotoxicity and of "active" and total rosette-forming T-cells. 13 of these patients have had or are currently receiving injections of osteogenic sarcoma-specific dialyzable transfer factor derived from healthy donors. In three patients with very small lesions, cytotoxicity was high before amputation and decreased within 2 mo after removal of tumor. Cytotoxicity was low at time of diagnosis in all patients with large tumor masses. The cytotoxicity of the patients' lymphocytes increased after administration of tumor-specific transfer factor in all patients so treated. Patients receiving nonspecific transfer factor showed evidence of declining cell-mediated cytotoxicity.

Tumor-specific transfer factor may produce an increase in cell-mediated cytotoxicity to the tumor in patients with osteogenic sarcoma. This possibility is suggested by the pain and edema that occurred in the area of the tumor in patients who had metastatic disease when therapy was started and by lymphocytic infiltrates in the tumor, as well as by the increase in cell-mediated cytotoxicity and the increase in percentage of active rosette-forming cells from subnormal to normal. Serial measurements of cell-mediated cytotoxicity are helpful in monitoring the efficacy of transfer factor and other modes of therapy in these patients, and these measurements are the best available criteria for selection of donors of tumor-specific transfer factor.

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*Received for publication 17 October 1973 and in revised form 4 November 1974.*

## INTRODUCTION

Dialyzable transfer factor was first described in 1955 by Lawrence (1), who subsequently conducted a series of meticulous studies of its properties (2). In 1970 our group reported the therapeutic use of transfer factor in a patient with Wiskott-Aldrich syndrome, a genetically determined cellular immune deficiency (3). Since 1970 transfer factor has been used to treat a variety of diseases (4-7); we have used it in more than 200 patients.

The possible relationship of immunologic defenses to survival of patients with malignant disease has been the subject of many reports (8). It is currently believed that antibody-mediated responses to tumors may, in some instances, enhance growth of tumors and, in others, be toxic to malignant cells; cell-mediated immunity usually inhibits growth of tumors (9, 10). Transfer factor can enhance cell-mediated immunity, but has no effect on humoral immunity (3); therefore, it appeared useful to investigate its use in the treatment of malignant neoplasms unresponsive to other measures.

In patients with osteogenic sarcoma, surgical excision, chemotherapy, and radiation therapy have produced a survival rate of only about 20%. Because some household contacts of patients with this tumor have cell-mediated immunity against the tumor (11) and can serve as a source of tumor-specific transfer factor, this investigation of the immunologic status of 18 patients with osteogenic sarcoma was undertaken. In 13 of these patients, clinical results of treatment with tumor-specific transfer factor, obtained from carefully selected donors and used as an experimental adjunct to conventional

TABLE I  
Clinical Status of Patients with Osteogenic Sarcoma\*

Patient	Age	Sex	Phase at diagnosis	Circulating antitumor antibodies	Received transfer factor	Length of follow-up	Status
1	16	F	I	IgG-kappa	Yes	<sup>mo</sup> 15	Treatment started with patient in phase II; now healthy and clinically tumor-free
2	18	M	I	No	Yes	11	Treatment started with patient in phase II; now healthy and clinically tumor-free
3	17	F	I	No	Yes	13	Treatment started with patient in phase II; now healthy and clinically tumor-free
4‡	17	F	II	No	Yes	27	No prophylactic immunotherapy; developed metastatic lesions unresponsive to chemotherapy and radiation; lesions resected; transfer factor, begun in phase IV, produced lymphocytic infiltrates in lesions; doing well 4 mo after surgery
5	10	F	II	No	No	6§	Disease progressed through phase IV; did not respond to chemotherapy, surgery, or radiation
6‡	15	F	III	No	Yes	23§	Inoperable primary tumor in ilium; after 9 mo of immunotherapy, donor lost cytotoxicity and metastatic lesion developed; remained stable with single metastatic lesion during 14 mo of additional treatment with transfer factor; died with massive pulmonary involvement 7 wk after voluntary discontinuation of immunotherapy
7‡	15	M	III	IgG-kappa	Yes	3§	Developed lesions in lung after irradiation of primary; transfer factor resulted in pneumonitis and pleural effusion; chemotherapy ineffective
8	16	F	III	No	Yes	24	Became pregnant during treatment with transfer factor; CI and T-E <sub>a</sub> both dropped, but recovered after elective termination of pregnancy; remains healthy and clinically free of tumor
9	12	F	III	IgM-kappa	No	11	Receiving prophylactic chemotherapy
10‡	29	F	IV	No	Yes	28	Single metastatic lesion, present at diagnosis, remained stable during 20 mo of treatment with transfer factor; second lesion resected and irradiated; patient again receiving transfer factor; now clinically stable
11	20	M	IV	No	Yes	11	No prophylactic immunotherapy; solitary lung lesion, which developed 2 mo after amputation, resected; patient now receiving transfer factor; clinically stable and free of tumor
12‡	13	F	IV	No	Yes	17	Primary tumor irradiated; did not appear to respond to transfer factor; did well for 14 mo with chemotherapy, then developed massive pulmonary metastases; patient is now terminally ill

TABLE I—(Continued)

Patient	Age	Sex	Phase at diagnosis	Circulating antitumor antibodies	Received transfer factor	Length of follow-up	Status
13†	21	M	IV	No	Yes	12 <sup>mo</sup>	Lung lesions appeared to be unresponsive to chemotherapy and to immunotherapy, but increase in size of lesions may have resulted from lymphocytic infiltrates
14†	16	F	IV	IgG-kappa	Yes	14	Appeared unresponsive to transfer factor; now receiving chemotherapy, but lesions are growing and increasing in number
15	17	M	III?	No	No	6§	Lost to follow-up after amputation; not treated
16	51	F	?	No	No	34 yr	Diagnosis and amputation occurred at age 17; malignant thyroid tumor removed at age 31; now clinically healthy with low CI to osteogenic sarcoma
17	17	M	II	IgG-kappa and IgM-kappa	Yes	7	Receiving prophylactic immunotherapy; presently clinically tumor-free
18	14	F	II	IgG-kappa	No	8	Receiving prophylactic chemotherapy; presently clinically tumor-free

\* A detailed report on each patient is provided in the Appendix. Unless otherwise stated, each patient's primary tumor was removed by amputation.

† Clinically obvious primary or metastatic tumor was present when transfer factor was begun.

§ Deceased.

therapy, are correlated with the changing state of cell-mediated immunity.

## METHODS

*Clinical studies.* Clinical histories of 14 patients and notes on 4 more are given in the Appendix. After informed consent was obtained, specimens were taken at the time of biopsy or amputation from patients with osteogenic sarcoma, and both benign (skin or muscle) and tumor tissues were placed in long-term culture.

Before immunotherapy was initiated, the tumor mass was reduced as much as possible by conventional therapy. The primary tumor was removed by amputation whenever feasible, and metastatic lesions were either removed by segmental resection or, if not resectable, treated by chemotherapy or localized radiotherapy of affected areas. Immunotherapy was then started, with transfer factor injected subcutaneously into the deltoid region until tumor-specific cell-mediated cytotoxicity (12) increased to 25 and active rosette-forming cells (T-E<sub>a</sub>)<sup>1</sup> increased to more than 20%. Transfer factor was then administered at 2-4-wk intervals. All patients were monitored for cell-mediated cytotoxicity and T-E<sub>a</sub>, and by monthly roentgenograms of the chest and semiannual tomograms.

<sup>1</sup> *Abbreviations used in this paper:* CI, cytotoxicity index; DTIC, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; PPD, purified protein derivative; T lymphocyte, thymus-derived lymphocyte; T-E<sub>a</sub>, active rosette-forming cells; T-E<sub>t</sub>, total rosette-forming cells.

Healthy potential donors of transfer factor (usually adult members of the patients' families) were screened for tumor-specific cytotoxicity; only those with cytotoxicity indices (CI) greater than 50 (range 50-90) on osteogenic sarcoma lines and less than 19 on control cell lines (carcinoma and fibroblast) were used as donors. Transfer factor was prepared by one of us (A. S. L.) by a modification of the method of Lawrence (13). Briefly, white blood cells of the donor were collected by leukapheresis. The cells were rinsed from the collection bag with normal saline and counted before being frozen and lyophilized. The lyophilized material was reconstituted in 10 ml of distilled water and dialyzed against two changes of 500 ml of pyrogen-free distilled water. The dialysis bag was then discarded and the water and dialysate were lyophilized. The lyophilized material was then reconstituted in water and divided into doses equivalent to 10<sup>6</sup> leukocytes per dose.

*Tumor cell lines.* Osteogenic sarcoma cell lines TE-85 (passages 17-19), and TE-418 (passages 10-12), and TE 415, the matching fibroblast line to TE 418, were provided by Dr. R. McAllister. Control cell lines included those from mammary carcinoma, hypernephroma, rhabdomyosarcoma, and matching fibroblast cell lines (12). Additional osteogenic sarcoma cell lines derived from tumors and skin of 11 of our patients were isolated and purified by the differential trypsinization technique of Owens (14). For the studies reported here, requiring a simple assay highly reproducible over a long period of time, TE 85 was chosen as a marker line for osteogenic sarcoma because donors of transfer factor and patients whose cells reacted to autologous

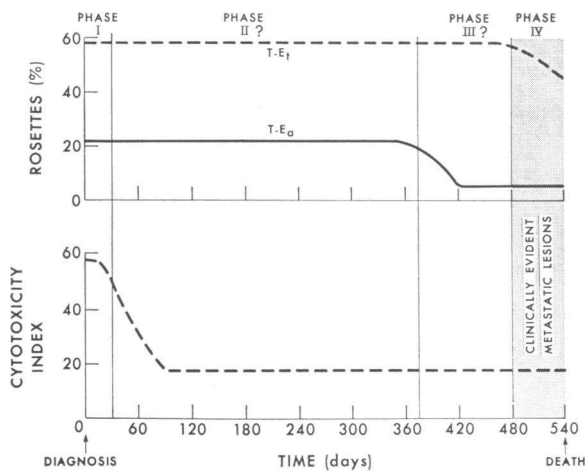


FIGURE 1 Postulated natural history of osteogenic sarcoma. Although the elapsed time varied, the pattern suggested has been observed by us in all patients reported here who received conventional therapy (amputation, resection, radiation, chemotherapy) and who died with metastatic lesions. Transfer factor appeared to alter the natural course of the disease.

and other osteogenic sarcoma cell lines also reacted to TE 85. The hypernephroma line A 498, passages 23-26, and breast carcinoma lines ALAB 496 and BT 20, together with a rhabdomyosarcoma line and hypernephroma line A49B, were used as specificity controls (12). All lines were stored in dimethyl sulfoxide, the cells were maintained in antibiotic-free medium, and limited passage levels were used to prevent genetic drift. Lines were harvested just at confluence for use in the assay.

Tumor-specific cell-mediated cytotoxicity of the patients' cells was assayed against cultured autologous tumor cell lines when available and against homologous osteogenic sarcoma cell lines, as well as against control cell lines as described above from mammary carcinoma, hypernephroma, rhabdomyosarcoma, and matching fibroblast lines.

**Rosette-forming cells.** Circulating thymus-derived (T)-lymphocytes were evaluated for their capacity to form rosettes with sheep erythrocytes. The numbers of T-cells capable of rosette formation were quantitated by the technique of Wybran, Levin, Spitler, and Fudenberg (15, 16). The lower limits of normal (mean  $\pm$  2 SD) are 16% for T-E<sub>a</sub> and 55% for T-E<sub>t</sub> (total rosette-forming cells).

**Fluorescence microscopy.** Antitumor antibodies were classified by immunoglobulin class and light-chain types by direct and indirect immunofluorescence. Indirect immunofluorescence studies were conducted with the serum obtained from each patient before amputation and during treatment; autologous tumor cells were used to characterize the immunoglobulin class and light-chain type of all antitumor antibodies (17).

**Skin tests.** Skin-test reactivity to six marker antigens, *Candida*, *Coccidioides*, mumps, purified protein derivative of tubercle bacillus (PPD), Varidase (streptokinase/streptodornase, Lederle Laboratories, Div. of American Cyanamid Co., Pearl River, N. J.), and trichophyton, was also followed.

## RESULTS

13 patients with osteogenic sarcoma were treated with tumor-specific transfer factor derived from household contacts who had specific cell-mediated cytotoxicity against the tumor. Their case histories are described in the Appendix and summarized in Table I.

Serial assays of cell-mediated cytotoxicity, T-E<sub>a</sub> and T-E<sub>t</sub>, and serial assessments of clinical states suggest that the natural history of osteogenic sarcoma can be described in four phases (Fig. 1).

During the first phase of the disease (patients 1-3 when first seen) primary tumors were small, and were usually detected on roentgenograms taken for incidental trauma. Metastatic lesions were not present. Before amputation, these patients had high cytotoxicity (CI greater than 30) and normal T-E<sub>a</sub> and T-E<sub>t</sub>.

Patients first seen during the second phase of the disease (patients 4 and 5) had large primary tumors, but no metastatic lesions could be located. These patients had

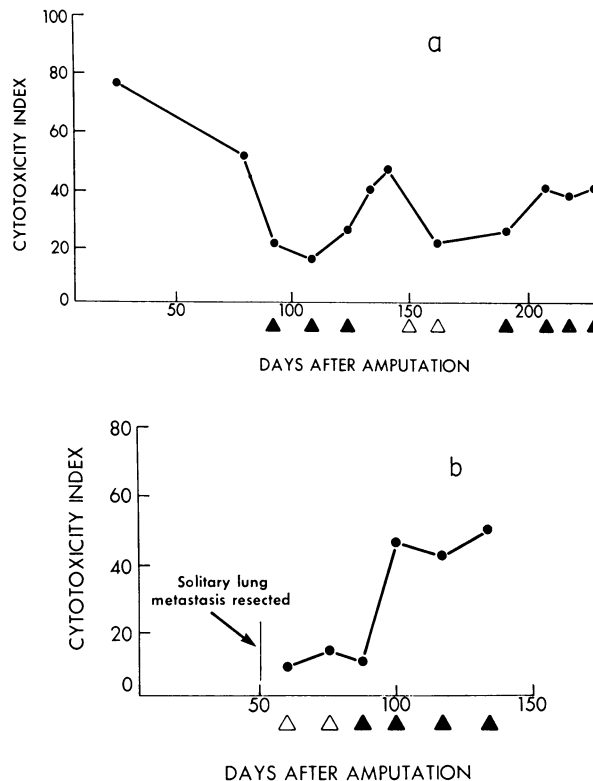


FIGURE 2 Tumor-specific cytotoxicity in patients with osteogenic sarcoma before and after administration of transfer factor. ▲, one dose of tumor-specific transfer factor; △, one dose of nonspecific transfer factor. a, patient 1; b, patient 11. These results are representative of those observed in every patient who received both specific and nonspecific transfer factor.

normal T-E<sub>a</sub> but low cell-mediated cytotoxicity (CI less than 30).

During the third phase (patients 6-9) primary lesions were large, but metastatic lesions were still not demonstrable. These patients uniformly had low T-E<sub>a</sub> and low cell-mediated cytotoxicity, but T-E<sub>t</sub> were within normal limits.

During the fourth phase (patients 9-14), patients had large primary tumors and metastatic lesions, low T-E<sub>a</sub>, and low cell-mediated cytotoxicity. As metastatic lesions grew, T-E<sub>t</sub> declined. Reactivity to skin tests also disappeared during the terminal phase. For convenience in presenting our results, patients are discussed in order of the apparent phase of the disease at diagnosis.

**Tumor-specific cell-mediated cytotoxicity.** The three patients who had small primary tumors and no detectable metastatic lesions when first seen (patients 1, 2, and 3) had high cytotoxicity against osteogenic sarcoma. When CI showed a significant decrease (usually within 3 mo after amputation), each patient began receiving bi-weekly injections of 1 U of tumor-specific transfer factor (1 U is the amount prepared from 10<sup>9</sup> leukocytes and contains 9-12 mg of RNA and 1 μM of protein). Cytotoxicity increased dramatically in all three patients (Fig. 2a). The same rise in cytotoxicity occurred after injection of tumor-specific transfer factor in patients who had low cytotoxicity when first seen (Fig. 2b). These patients were either clinically tumor-free after amputation of a large primary tumor (patients 8 and 17) or after removal of a primary lesion and surgical or radiologic treatment of small isolated metastasis (patients 10 and 11).

Administration of nonspecific transfer factor was followed by a drop in cell-mediated cytotoxicity to tumor in the patients to whom it was given (patients 1, 2, 10, 11). In patient 10, a sudden drop in cytotoxicity occurred 90 days after the administration of nonspecific transfer factor was begun; about 90 days thereafter, a new metastatic lesion was noted (Fig. 3). Although therapy with tumor-specific transfer factor was begun immediately, cytotoxicity did not increase until the lesion was surgically removed. The increased cytotoxicity to osteogenic sarcoma seen after resection was not accompanied by increased cytotoxicity to the control carcinoma cell lines.

**Rosette-forming cells.** The percentage of T-E<sub>t</sub> is considered to represent the percentage of T lymphocytes among the whole population of lymphocytes found in peripheral blood. T-E<sub>a</sub> appear to be a subpopulation of T lymphocytes closely linked to the capability of the host to mount an immune response to antigen. This population is seen to drop approximately 6 wk before clinical evidence of metastatic lesions in patients with cancer.

Patients who, when osteogenic sarcoma was diagnosed, had high cell-mediated cytotoxicity against their tumor (CI greater than 30) also had normal T-E<sub>a</sub>. These patients usually had small primary tumors and no metastatic lesions (patients 1, 2, and 3; phase I). After amputation, tumor-specific cytotoxicity decreased, but T-E<sub>a</sub> remained within normal limits. In patients in whom T-E<sub>a</sub> was low when they were first seen, or in whom it dropped later in the disease, tumor-specific transfer factor increased T-E<sub>a</sub> to normal levels. Patients not treated prophylactically after amputation of the primary tumor (patients 4 and 5) lost tumor-specific cell-mediated cytotoxicity before T-E<sub>a</sub> began to decline. There was usually an abrupt drop in T-E<sub>a</sub> approximately 6 wk before a metastatic event occurred.

**Humoral immunity.** Concentrations of immunoglobulins IgG, IgA, and IgM were within normal limits in all patients before and during therapy. Antitumor antibodies were detected before therapy in 5 of 18 patients (patients 1, 7, 14, 17, and 18) by indirect immunofluorescence; four had IgG-kappa circulating antibodies; the other had plasma cells and lymphoid cells containing both IgG and IgM within the tumor mass, but no circulating antibodies. All of the immunoglobulin-containing cells stained with anti-kappa antiserum, but not anti-lambda. Serial samples taken during immunotherapy

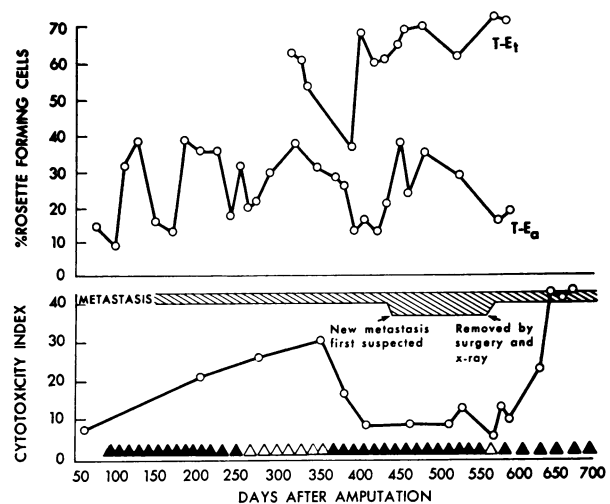


FIGURE 3 Serial measurements of the percentage of "active" and total rosette-forming cells and of osteogenic sarcoma-specific cell-mediated cytotoxicity in the peripheral blood lymphocytes of patient 10. The patient remained clinically stable and symptom-free for almost 1 yr after amputation, despite the presence of an inoperable metastatic lesion in her spine. Loss of cell-mediated cytotoxicity in the donor of transfer factor was followed by a drop in the patient's cell-mediated cytotoxicity; shortly thereafter, metastatic lesions were found in the clavicular region. ▲, tumor-specific transfer factor; △, nonspecific transfer factor.

showed no change in antibody class or titer attributable to tumor-specific transfer factor, and the presence or absence of antibody did not appear to correlate with clinical status.

*Skin test reactivity.* All patients, even those with clinically significant tumors, had positive skin-test reactions (5 mm of induration) to at least one of six skin test antigens. Patients 4 and 6, however, lost all reactivity to skin tests during the terminal phase of the disease. Transfer factor was capable of transferring skin test reactivity to marker antigens to all patients for whom such reactivity was present in donors. Because skin tests remained consistently positive until the terminal phase of the disease, such tests were not performed serially in all patients.

*Evidence in vivo for passive transfer of tumor immunity.* In patient 6 a large primary tumor remained static for 22 mo, and a single metastatic lesion in the lung did not increase in size for 14 mo during treatment with tumor-specific transfer factor (and, later, BCG).

Pain and edema, presumably caused by cellular immune reactions at the primary tumor site, were exacerbated by each injection of transfer factor. Immunotherapy was discontinued because the primary tumor did not regress. Pain and edema then subsided and new metastatic lesions appeared; the patient died 7 wk later with massive lung involvement. Patient 7, who had X rays suggesting many pulmonary lesions, developed chest pain, shortness of breath, fevers, and pleural effusions after the institution of transfer factor therapy. The pleural effusions were sterile but contained copious numbers of reactive leukocytes, primarily lymphocytes, and monocytes; no tumor cells could be detected. Patient 13, shortly after the institution of immunotherapy, developed shortness of breath and chest pains. After one dose of methotrexate and leucovorin (18), his pulmonary status improved dramatically and his lung shadows appeared to diminish in size. Despite further doses of methotrexate, the shadows did not further diminish in size. Other lesions grew and the patient died with massive lung involvement. Excisional

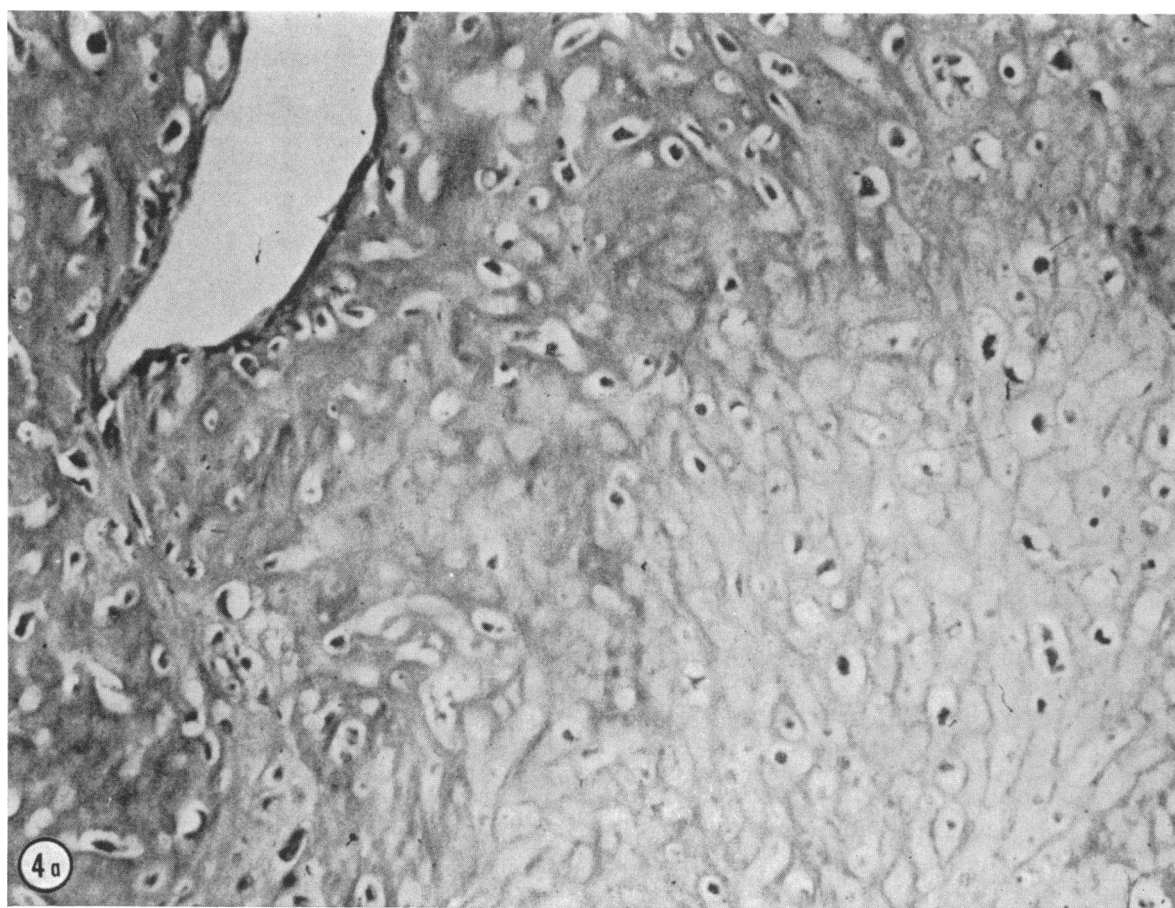


FIGURE 4a Biopsy specimen of tumor tissue from patient 4 before tumor-specific transfer factor (hematoxylin and eosin; original magnification,  $\times 250$ ).

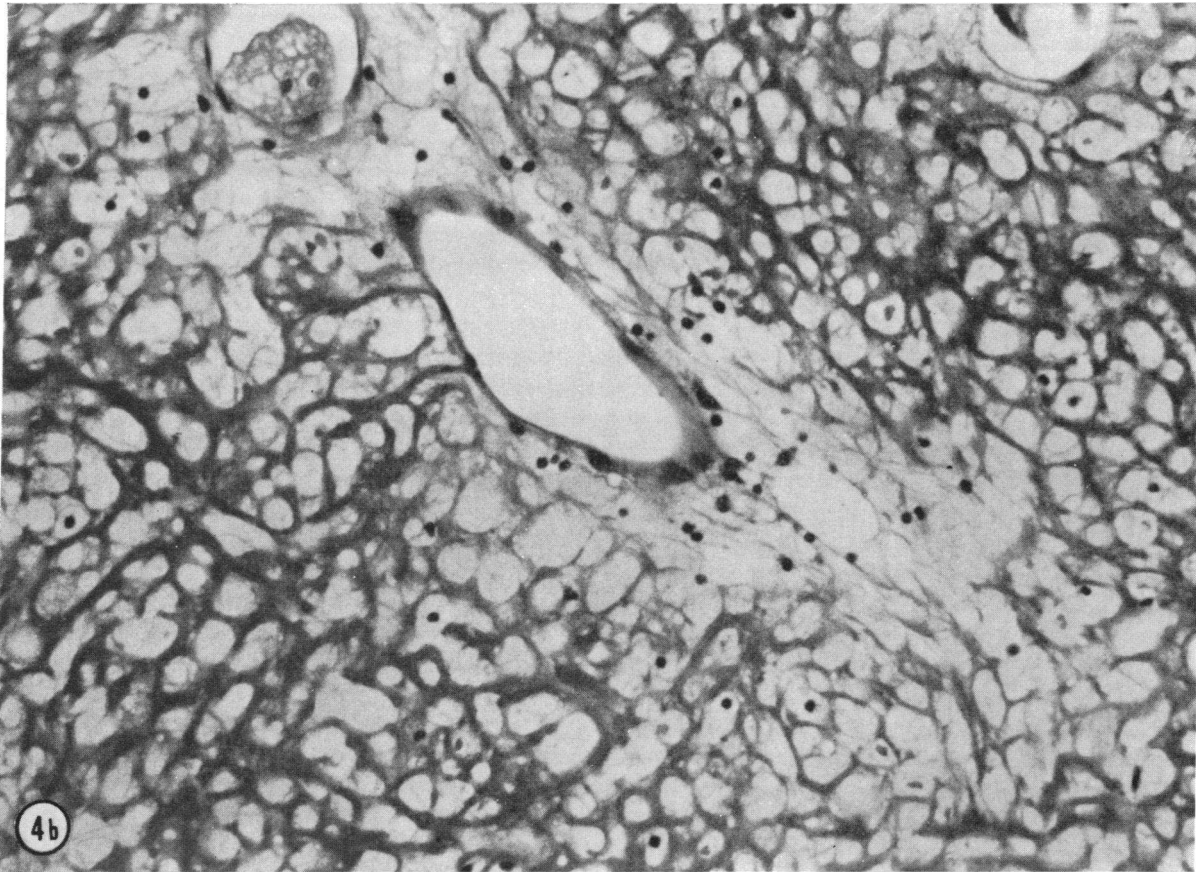


FIGURE 4b Biopsy specimen of tumor tissue from patient 4, 3 days after administration of transfer factor ( $\times 250$ ). Lymphocytic infiltrate and perivascular cuffing are clearly visible.

biopsies of the lesions of patients 4 and 10 were performed 3 days after administration of tumor-specific transfer factor. Each biopsy specimen contained massive lymphocytic infiltrates and also showed perivascular cuffing of lymphoid cells in their tumors. Biopsy specimens of both patients taken before therapy with tumor-specific transfer factor contained no lymphocytic reaction (Fig. 4).

#### DISCUSSION

Osteogenic sarcoma in children and young adults has a dismal prognosis: amputation of the primary tumor and resection, conventional chemotherapy, and radiation therapy of metastatic lesions offers a 2-yr survival rate of about 20%. In more than 50% of patients, metastatic lesions become clinically evident within 6 mo of diagnosis. The survival of patients with metastases is counted in weeks (19), and the incidence of spontaneous remissions is less than 1% (20). For these reasons, this tumor was chosen as the first to be treated with tumor-specific transfer factor. In the study of our first patient

(patient 6) treated with tumor-specific transfer factor, we found that two household contacts had specific cell-mediated cytotoxicity against this tumor; thus, one of these became our first donor (11). We have since shown that approximately 26% of household contacts of patients with osteogenic sarcoma have levels of cytotoxicity adequate for the preparation of transfer factor (12, 21).

Our treatment program attempted to retain the benefits of conventional therapy, while simultaneously providing specific immunotherapy to supplement residual latent host defenses. Before immunotherapy was initiated, T-E<sub>s</sub>, T-E<sub>t</sub>, cell-mediated cytotoxicity against the tumor, and antitumor antibodies were measured in each patient. Cytotoxicity was also measured in household contacts of the patients, and tumor-specific transfer factor was prepared from those who had CI greater than 50%. Subcutaneous injections of transfer factor were begun when either T-E<sub>s</sub> or cytotoxicity dropped below normal levels. Patients were serially monitored for cell-mediated cytotoxicity to tumor, T-E<sub>s</sub>, and antibodies to tumor during immunotherapy.



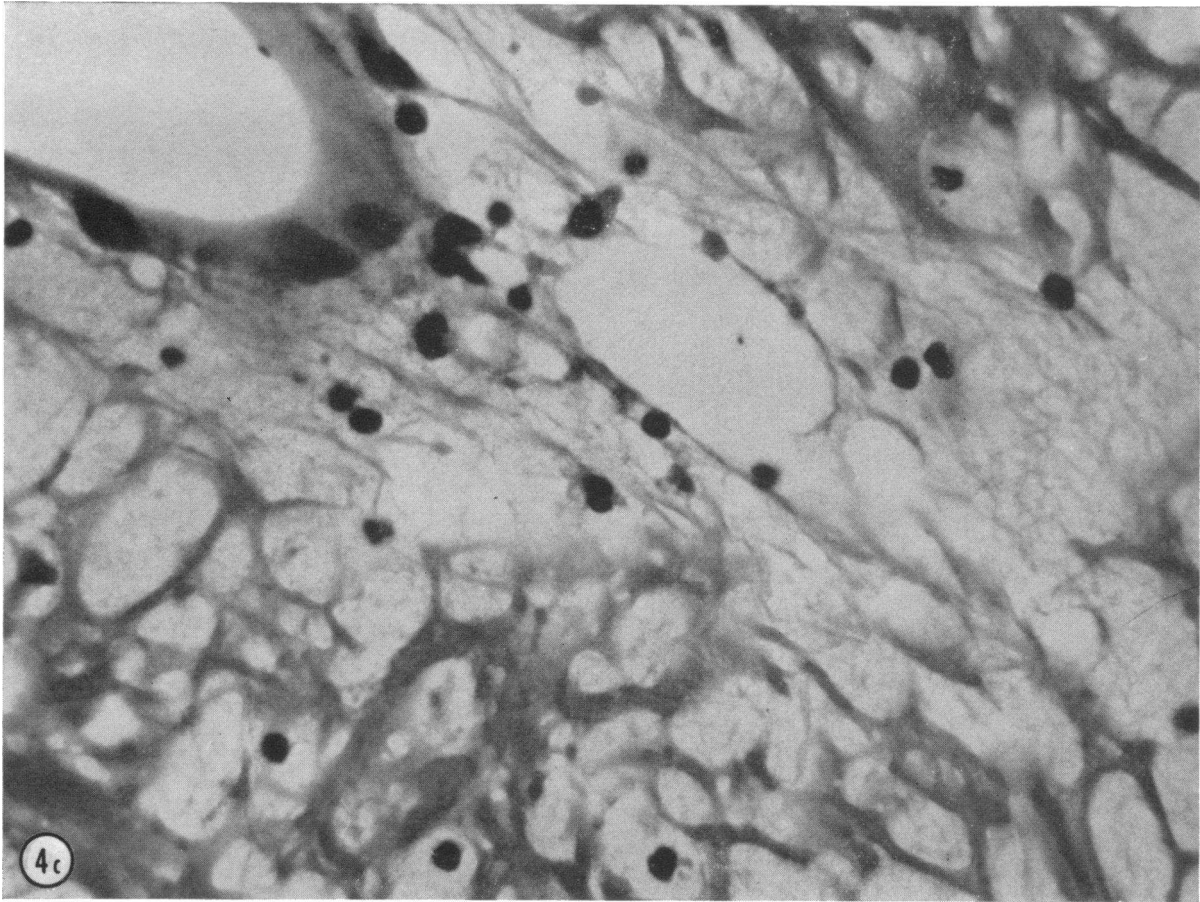


FIGURE 4c Biopsy specimen of tumor tissue from patient 4, 3 days after administration of transfer factor ( $\times 500$ ).

A suggested natural course of immunologic events in patients with osteogenic sarcoma, based on our studies of 18 patients, is depicted in Fig. 1. When the tumor was quite small, patients usually had high cytotoxicity, and T-E<sub>a</sub> and T-E<sub>t</sub> appeared to be within normal limits. Cell-mediated tumor-specific cytotoxicity decreased rapidly within 3 mo after amputation (we believe that it may decrease more gradually in nonamputee patients). The rest of the patients had low cytotoxicity at the time of discovery of tumor. About 90 days after the fall of cellular immunity, T-E<sub>a</sub> declined, followed in 6 wk by clinical evidence of metastatic lesions, a fall in T-E<sub>t</sub>, and death.

Administration of tumor-specific transfer factor appears to alter this course of events. In five patients who had inoperable primary or metastatic lesions at the time transfer factor was injected, pain and local inflammation in the area of the lesions, accompanied by transient febrile episodes, were noted 18–36 h after subcutaneous injection of tumor-specific transfer factor into the del-

toid region. In three patients, specimens obtained by biopsy or surgical removal of tumors during and after such an episode revealed substantial new infiltration of lymphocytes, evidenced by perivascular cuffing of lymphocytes and monocytes in the tumor tissue, not seen in the biopsy specimens taken before administration of transfer factor. Although these infiltrates suggest, they by no means prove passive transfer of tumor-specific immunity.

Suggestive evidence in vitro of the capability of transfer factor to enhance immunity is reflected by the following findings: an increase in T-E<sub>a</sub> is a consistent indicator of clinical response in immunotherapy of non-malignant states (16); a decrease in T-E<sub>a</sub> appears to be a harbinger of clinically or radiologically evident metastatic events (22). Therefore, active rosettes appear to measure the nonspecific capability of the patient to respond to antigen. T-E<sub>a</sub> rose in response to injections of tumor-specific transfer factor and fell after injections of nonspecific transfer factor in the patients studied. Cell-

mediated cytotoxicity (a measure of the patient's specific capability to respond against tumors) increased in all patients after injections of tumor-specific transfer factor with no indication of increased cell-mediated cytotoxicity against control tumor cell lines. Non-tumor-specific transfer factor, administered on several occasions to several patients, was uniformly associated with a decline in cell-mediated cytotoxicity in the patient; reinstitution of therapy with tumor-specific transfer factor was associated with a concomitant rise in cell-mediated cytotoxicity.

Any mode of therapy for malignant neoplasms in humans must be considered in the light of its differential toxicity, i.e., the "tumor-cell lytic" effect, as opposed to the "host-cell lytic" effect. A good example of this is radiotherapy. Perlmann, Perlmann, and Biberfeld have demonstrated that cell-mediated cytotoxicity against the tumor is transiently eliminated during radiotherapy of bladder carcinoma (23). We noted the same phenomenon in one of our patients with osteogenic sarcoma. It is highly unlikely that radiotherapy of a "radioresistant" tumor is advantageous for all patients, because radiotherapy, by virtue of its lympholytic effect, may reduce host defenses and lead to more rapid growth of tumor. The same is true of most lympholytic chemotherapeutic agents. Immunotherapy, although boosting the endogenous host responses against the tumor, is beneficial only if the host has the resources to respond.

In two patients, BCG was also used in an attempt to augment the T-cell immune response. This practice was abandoned because BCG appeared to decrease cellular immunity (as measured by T-E<sub>a</sub>) in one patient. Our evidence suggests that the effect of BCG on T lymphocytes differs from that of tumor-specific transfer factor (24).

Active transfer of immunity in solid tumors by vaccination is currently under study; data are too scant to allow conclusions at present, but there may be a slight trend toward prolongation of the disease-free interval in treated patients (25). More recently, passive transfer of cell-mediated immunity has been attempted with transfer factor (11). Lobuglio, Neidhart, Hilberg, Metz, and Balcerzak have also reported a patient with an alveolar soft part sarcoma treated similarly (26), with migration inhibitory factor as the laboratory parameter of change in cellular events. "The tumor neither regressed nor progressed during the 6-mo period following transfer factor therapy."

Three major drawbacks limit the efficacy of tumor-specific dialyzable transfer factor in patients with cancer. First, a large tumor mass may contain more cells than can be destroyed by the entire lymphocyte population of the host. Second, if a vital organ (such as the lungs), which could be significantly compromised by

an inflammatory reaction, contains large amounts of tumor (and hence tumor antigen), resection, chemotherapy, or radiotherapy is indicated to reduce the tumor mass before immunotherapy is initiated. Third, tumor-specific transfer factor treatment requires careful selection and constant monitoring of the donors and tedious monitoring of the recipient. At this time tumor-specific transfer factor treatment is far too arduous and expensive to be feasible in other than major research centers.

The results reported here indicate that patients with large inoperable tumors are poor candidates for immunotherapy; therefore, only patients who are clinically tumor-free should be considered for therapy with tumor-specific transfer factor. Our clinical goals are presently directed toward eliminating occult metastases, increasing the time interval between amputation and development of clinically evident metastases, and ultimately increasing the 5-yr survival rate.

The five patients in our study who were clinically tumor-free at the initiation of immunotherapy have all remained so to date (from 12 to 24 mo after diagnosis). Of the seven patients entering our program with clinically evident metastatic lesions, one died 7 wk after therapy with transfer factor was halted. She had survived for 23 mo with a solitary metastasis and without removal of the primary tumor. Two patients are presently stable with metastasis 27 and 28 mo after diagnosis. Transfer factor was halted in four patients after lung masses appeared to increase in size. Because there was no way to differentiate between tumor growth and lymphocytic infiltrate, all of these patients were started on chemotherapy.

## APPENDIX

The clinical courses of 14 patients are described below. Patients are presented in order of phase of disease at the time they were first seen. Brief clinical notes are included on four additional patients.

### Phase I

*Patient 1.* A roentgenogram obtained because of minor trauma revealed a 1-cm single tumor in the distal femur of this 16-yr-old girl. A biopsy specimen showed sclerosing osteogenic sarcoma. IgG-kappa antibodies directed against this tumor were found, and she had strong cytotoxicity against the homologous tumor line (CI of 77.7). Amputation was postponed by the patient because she had no symptoms. A second biopsy at the same site 2 mo later showed a friable tumor to which her original antibody did not react; however, she had strong T-cell-mediated cytotoxicity against this second autologous tumor along with the same homologous line. Amputation was performed, and the CI gradually dropped to 13.2 within 2 mo; T-E<sub>t</sub> and T-E<sub>a</sub> remained within normal limits (64% and 19%). Treatment with tumor-specific transfer factor induced a rise in CI to 50.3. The CI fell to 24.4 after two doses of nonspecific transfer factor, and rose to 46 after administra-

tion of tumor-specific transfer factor. Tumor-specific transfer factor has been given every 2 wk since, and the CI remains between 40 and 50. This patient is currently free of detectable tumor 15 mo after amputation.

*Patient 2.* This 18-yr-old boy developed osteogenic sarcoma in the proximal tibia. At the time of amputation, tumor-specific CI was 48, and T-E<sub>a</sub> and T-E<sub>t</sub> were within normal limits. Within 2 mo after amputation, CI fell to 16.2; T-E<sub>t</sub> remained within normal limits (58%), but T-E<sub>a</sub> were low. After two doses of tumor-specific transfer factor, the CI rose to 67.7; 1 mo after administration of non-specific transfer factor, the CI fell to 25.7. Subsequent treatment with tumor-specific transfer factor resulted in a rise in CI to 54; it has remained at this level. This patient is tumor-free 11 mo after amputation.

*Patient 3.* Osteogenic sarcoma was discovered in the proximal tibia of this 17-yr-old girl during inspection of an X ray taken as a result of incidental trauma. The leg was amputated. Cell-mediated cytotoxicity was high (CI of 46), and T-E<sub>a</sub> and T-E<sub>t</sub> were within normal limits. Within 3 wk of amputation, although T-E<sub>a</sub> and T-E<sub>t</sub> remained normal, the CI had fallen to 11.3. After two doses of tumor-specific transfer factor, CI rose to 31.2; however, T-E<sub>a</sub> had decreased to 12%. Subsequent therapy with tumor-specific transfer factor was associated with a rise in CI to 43 and of T-E<sub>a</sub> to 18. She is currently tumor-free 13 mo after amputation, and CI remains more than 40.

## Phase II

*Patient 4.* A 17-yr-old girl had osteogenic sarcoma in the proximal left tibia. There were no discernible metastatic lesions. An above-knee amputation was performed; immunologic parameters were measured serially but no prophylactic therapy was administered. 7 mo after surgery she had normal T-E<sub>a</sub> (23%) and T-E<sub>t</sub> (67%), with moderate cell-mediated cytotoxicity (CI of 28.6).

4 mo later, CI had declined to 5.1 and, although T-E<sub>a</sub> remained normal, she had developed metastatic lesions in the femur, tibia, and scapula. The patient received adriamycin and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC) (27) and tolerated therapy well for a brief period before the metastatic lesions began to grow. Local excision of several lesions was performed; histologic examination revealed classic osteogenic sarcoma with no evidence of lymphocytic infiltrates. Tumor-specific transfer factor was administered; 3 days after the third injection, all lesions were resected. The tumor specimens showed marked lymphocytic infiltration and perivascular cuffing of lymphocytes. 5 mo later she is receiving tumor-specific transfer factor; she is clinically stable and is attending a special school.

*Patient 5.* A 10-yr-old girl had osteogenic sarcoma in the proximal humerus. T-E<sub>a</sub> and T-E<sub>t</sub> were within normal limits (25% and 68%), but cell-mediated cytotoxicity was low (CI of 5). Forequarter amputation was performed. T-E<sub>a</sub> fell below normal limits (14%); cytotoxicity remained low (CI of 9). No prophylactic therapy was administered. 4 mo after amputation, approximately 6 wk after T-E<sub>a</sub> had fallen, five tumor nodules were observed in the lungs. At this time CI was 12. Actinomycin and radiation therapy were administered and the patient responded, but the lesions recurred within 1 mo after termination of radiotherapy. She died shortly thereafter with massive pulmonary metastases. Cell-mediated cytotoxicity, measured on three occasions during radiotherapy and chemotherapy, ranged be-

tween 8 and 11. T-E<sub>a</sub> measured on those occasions remained below 14%.

## Phase III

*Patient 6.* The first patient treated with tumor-specific transfer factor was a 15-yr-old girl who had had a bony protuberance in her right lower quadrant for 1 yr. Biopsy showed osteogenic sarcoma. Roentgenograms revealed an ellipsoid tumor mass with a 17-cm axis and a 12-cm diameter, protruding from the posterior lip of the right ilium. A bone survey revealed no other tumor masses, and no pulmonary metastases were seen on chest roentgenograms. The patient refused the only surgical procedure that offered a chance for cure, hemireporectomy. She received 5,500 R directed to the abdomen, but this radiation produced an estimated reduction in tumor mass of only 15%.

Transfer factor was prepared from the father and sister, both of whom had strong cell-mediated cytotoxicity against osteogenic sarcoma. Neither patient nor family members had antibodies against this tumor detectable either by immunofluorescence or by complement fixation.

Skin tests with six antigens (*Candida*, *Coccidioides*, mumps, PPD, streptokinase-streptodornase, and trichophyton) showed that one donor had a positive response to trichophyton; before immunotherapy, the patient had a negative response.

One dose of tumor-specific transfer factor was administered subcutaneously in the deltoid region each month. Trichophyton skin tests became positive after administration of transfer factor, suggesting that transfer factor of specific cell-mediated immunity had occurred. No growth of the primary tumor occurred for 7 mo. T-E<sub>a</sub> were approximately 30% at the initiation of therapy with transfer factor. (In this patient, T-E<sub>a</sub> and T-E<sub>t</sub> were not measured before immunotherapy.) After 7 mo of therapy, the patient had a severe viral flulike illness, and began to show signs of deterioration. She had an immediate decrease in T-E<sub>a</sub>, and 3 wk later skin reactivity decreased, followed by a depression of hematocrit and a loss of weight. 6 wk after the decrease in T-E<sub>a</sub> was seen, a single metastatic lesion was detected in the lung. A second series of tests for tumor-specific cytotoxicity at this time revealed that, concomitant with clinical deterioration, her tumor-specific cellular immunity had disappeared. More importantly, her donor had lost cell-mediated cytotoxicity to the tumor after repeated leukaphereses.

The patient was given one dose of BCG by scarification, followed by eight doses of tumor-specific transfer factor (derived from another donor with high cytotoxicity for osteogenic sarcoma) in 1 day. She then received alternating doses of transfer factor and BCG at biweekly intervals. T-E<sub>a</sub> again increased, skin-test reactivity became more intense, and clinical well-being returned. Measurements of T-E<sub>a</sub> were discontinued after the 12th mo because of logistic and psychological problems of the patient and family. After approximately 7 mo of treatment with both transfer factor and BCG (14 mo after the institution of transfer factor therapy), the child first noted edema in the right leg and pain in the area of the tumor; this was so exacerbated by injections of transfer factor that morphine was required for analgesia. Injections of BCG did not appear to produce the pain or edema. The patient was taking 45 mg of morphine sulfate intramuscularly daily together with large doses of other pain medications. 21 mo after the beginning of immunologic therapy, a chordotomy was performed to reduce pain. Immediately after surgery, the pain medica-

tion requirement was zero. 2 wk later, pain and edema returned within 24 h after an injection of transfer factor, and the patient again required medication for pain. After two injections of transfer factor (and one of BCG), the local physician decided to discontinue immunotherapy and to administer cyclophosphamide in low doses (50 mg orally each day). The pain and edema disappeared within 36 h. However, new lung metastases appeared soon after, and the patient died 7 wk after immunotherapy was stopped.

*Patient 7.* A 15-yr-old boy had painful swelling of the distal femur. Roentgenograms and biopsy established the diagnosis of osteogenic sarcoma. Chest roentgenograms revealed a single metastatic lesion in the lung. The first set of immunologic tests were done just before radiation was administered. His CI was high (88), although his T-E<sub>a</sub> were low (7%). After approximately 5,000 R had been directed to the leg, his CI was 10.5, and his T-E<sub>a</sub> remained low (6%).

The patient developed massive pulmonary metastases during radiotherapy. After radiotherapy was completed, palliative immunotherapy was administered, although there was no expectation of prolonging his life. After three weekly doses of tumor-specific transfer factor, the patient gradually developed severe chest pains with dyspnea. Roentgenograms revealed that the densities in the lungs were larger than before treatment. He then developed bilateral pleural effusions. The pleural aspirate was sterile fluid with copious numbers of leukocytes. The differential count was approximately 40% small mature lymphocytes, 25% monocytes, 20% polymorphonuclear leukocytes, and 15% pleural mesothelial cells that in gross appearance were benign. No tumor cells could be detected in the pleural effusion. Routine cultures for bacteria and fungi were negative, as were smears for acid-fast bodies and guinea pig cultures for tuberculosis.

The patient became moribund; his course was marked by prolonged fevers despite the fact that multiple blood cultures revealed no organisms. He was given 125 mg of hydrocortisone (Solu-Cortef, The Upjohn Co., Kalamazoo, Mich.) intravenously twice daily, in hope that its lympholytic effect would reduce the sterile pulmonary inflammation apparently caused by transfer factor. The patient responded dramatically within 24 h and became ambulatory. Adriamycin and DTIC (27) were started; soon afterward the chest lesions grew, and the patient died in respiratory failure.

*Patient 8.* A 16-yr-old girl had osteogenic sarcoma of the metaphyseal region of the left proximal tibia. An above-knee amputation was performed; T-E<sub>a</sub> were 10% at that time. Tumor-specific transfer factor was initiated. T-E<sub>a</sub> increased to 22%, and cell-mediated cytotoxicity gradually rose. She became pregnant, and both cytotoxicity and T-E<sub>a</sub> declined; the pregnancy was electively terminated. She is well and clinically tumor-free 24 mo after amputation. T-E<sub>a</sub> and cell-mediated cytotoxicity have risen again to prepregnancy levels.

*Patient 9.* This 12-yr-old girl had complained of pain in the upper third of the left tibia for approximately 2 yr after minor trauma. Roentgenograms revealed a large osteolytic lesion; biopsy revealed osteogenic sarcoma with much chondroblastic activity. At the time of amputation, T-E<sub>a</sub> were below normal (12%), and CI was 6.7. No metastatic lesions have been seen. The patient is now receiving prophylactic chemotherapy with methotrexate and leucovorin (23); immunotherapy has not been started. Follow-up assays are not available; however, the patient is maintaining normal activities 11 mo after amputation.

## Phase IV

*Patient 10.* A 29-yr-old woman had a pathologic fracture; osteogenic sarcoma was found in the proximal metaphyseal region of the right femur. Bone survey revealed a metastatic lesion at T-7. A roentgenogram of the hip taken 1 yr earlier for an unrelated problem was reexamined; the tumor had been present at that time. Hip disarticulation was performed. The metastatic lesion was treated with radiation therapy (3,500 R). Tumor-specific transfer factor (derived from her husband, who had high cell-mediated cytotoxicity to the tumor) and BCG were administered. During 14 mo of immunotherapy there was no evidence of new metastatic lesions, and no change in the lesion at T-7.

The percentage of T-E<sub>t</sub> remained stable at 60% or higher, except on one occasion, when it dropped to 37% concomitant with a severe virallike syndrome; T-E<sub>a</sub> were also subnormal at this time (13%). Cell-mediated cytotoxicity and T-E<sub>a</sub> gradually increased during therapy with tumor-specific transfer factor. A later drop in T-E<sub>a</sub>, 1 yr after amputation, coincided with loss of cell-mediated cytotoxicity by the donor of transfer factor after repeated leukaphereses. T-E<sub>a</sub> increased after administration of several doses of transfer factor and one of BCG. (It was noted retrospectively that the patient had had several significant decreases in T-E<sub>a</sub> and cell-mediated cytotoxicity after BCG. By then, based on observations in other patients, we decided that BCG may be detrimental as often as it is advantageous, and its use was discontinued in patients with osteogenic sarcoma.)

Approximately 18 mo after amputation, cell-mediated cytotoxicity began to decline; 3 mo later a significant decrease in T-E<sub>a</sub> was noted. 30 days later the patient complained of pain in the left clavicular region; after 2 mo several calcified lesions in the clavicular region became obvious by X ray. The patient received several doses of tumor-specific transfer factor from a new donor with high cell-mediated cytotoxicity and, although T-E<sub>a</sub> rose to the low normal region, cytotoxicity remained low. The metastatic lesions were surgically removed. Sections through the lesions showed necrotic tumor cells with extensive infiltration of lymphocytes. Radiation (5,000 R) was administered to the tumor region, and therapy with tumor-specific transfer factor was resumed. CI is 46 and T-E<sub>a</sub> is 22% 28 mo after amputation.

*Patient 11.* A 20-yr-old man developed osteogenic sarcoma in the proximal tibia; amputation was performed. 2 mo later a solitary metastatic lesion in the lung was resected. At that time, T-E<sub>a</sub> and T-E<sub>t</sub> were normal, but CI was low (13). CI remained low during the subsequent 8-wk period, and two doses of nonspecific transfer factor at 2-wk intervals did not change it. However, the first injection of tumor-specific transfer factor was followed 2 wk later by an increase in CI to 46. With injections of tumor-specific transfer factor every 2 wk, the CI remains over 40. The patient is clinically tumor-free 9 mo after resection of the lung lesion (11 mo after amputation).

*Patient 12.* A 13-yr-old girl had osteogenic sarcoma in the distal femur and many small metastatic lesions in the lungs. The primary tumor was irradiated. T-E<sub>a</sub> were subnormal (10%) and CI was 40. Two doses of tumor-specific transfer factor increased the CI to 82 and T-E<sub>a</sub> to 22%. 4.5 mo after diagnosis CI dropped to 23, T-E<sub>a</sub> dropped to 8%, and the lung masses became more dense on X-ray. The films could not differentiate between tumor growth and lymphocytic infiltrates around the pulmonary lesions, and the patient was not subjected to lung biopsy to define the

nature of the lesions. The decision was made to discontinue transfer factor and start chemotherapy. She was started on methotrexate and leucovorin. 16 mo after diagnosis she was noted to have recurrent metastatic lesions; she now has massive lung metastasis and pneumothorax 17 mo after diagnosis.

**Patient 13.** A 21-yr-old man developed osteogenic sarcoma of the distal femur, and his leg was amputated 1 yr before referral. He developed three large, rapidly growing lung lesions that were unresponsive to adriamycin and radiation. After administration of tumor-specific transfer factor, he developed chest pains and shortness of breath. Immunotherapy was stopped, and he received high doses of methotrexate and leucovorin. The pulmonary symptoms cleared after the first dose of methotrexate. Roentgenograms of the chest revealed that the lung densities were diminishing in size, but new ones were appearing. These increased in size during 1 mo despite the chemotherapeutic regimen.

Serial assessments of lymphocyte function during chemotherapy showed normal T-E<sub>a</sub> and normal responsiveness to phytohemagglutinin despite a drop in total lymphocyte count. The patient died with massive pulmonary involvement. The original response to chemotherapy (rapid diminution in size of the lung densities) may have been a result of the lympholytic action of methotrexate, although it is possible that lysis of more susceptible tumor cells occurred, leaving resistant tumor cells to grow and cause death.

**Patient 14.** This 16-yr-old girl had had a progressively growing osteogenic sarcoma in the proximal tibia for 8 mo before amputation; there were two metastatic nodules in the lungs. After amputation, tumor-specific transfer factor was administered. There was no significant increase in cell-mediated cytotoxicity, which was low and continued to decline slowly. The patient was considered as not helped by transfer factor, and she is on a regimen of adriamycin and DTIC 14 mo after diagnosis; the lung lesions are growing and increasing in number.

#### Other patients

**Patient 15.** A 17-yr-old man had a large osteogenic sarcoma in the proximal femur. T-E<sub>a</sub> were 7% at time of diagnosis. This patient was lost to follow-up; he died of metastatic disease 6 mo after amputation with no prophylactic therapy. He appeared to be in phase III of the disease at the time of diagnosis.

**Patient 16.** This patient is a 51-yr-old woman who had an amputation of the right leg for osteogenic sarcoma at the age of 17. At the age of 31, she developed carcinoma of the thyroid, which was surgically removed. She is now well and asymptomatic. Her CI is 20, T-E<sub>a</sub> are 19%, and T-E<sub>c</sub> are 62%.

**Patient 17.** This 17-yr-old man recently entered the protocol in phase II. He is receiving tumor-specific transfer factor and is clinically tumor-free 7 mo after amputation.

**Patient 18.** This 14-yr-old girl entered the protocol in phase II. She is doing well with a regimen of prophylactic chemotherapy 8 mo after amputation.

**Note added in proof.** All patients who began treatment with tumor-specific transfer factor when they had no clinically evident lesions remain healthy and tumor-free: patients 1 (19 mo), 2 (13 mo), 3 (15 mo), 8 (26 mo), 11 (13 mo), and 17 (9 mo). Patients 4 and 10, who had metastases, remain clinically stable 29 and 30 mo after diagnosis. Patient 18 is doing well on prophylactic chemotherapy 10 mo after diagnosis. Patients 13 and 14 have

died, and patient 12 is terminally ill; current follow-up is not available on the other patients.

#### ACKNOWLEDGMENTS

We thank Drs. Arthur R. Ablin and Joseph H. Kushner for referring their patients to us for study, and Mr. E. E. Bautista and Ms. Ann Merrill Knapp for their expert technical assistance.

This work was supported in part by the Elaine R. Shepard Cancer Research Foundation, and by U. S. Public Health Service Grants HD-05894 and AI-09145 and NIH Training Grant HL-05677.

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