Proc. Nat. Acad. Sci. USA
Vol. 71, No. 6, pp. 2319–2323, June 1974

Effects of Dialyzable Transfer Factor in Patients with Breast Cancer

(immunology/delayed hypersensitivity)

H. F. OETTGEN*, L. J. OLD*, J. H. FARROW*, F. T. VALENTINE†, H. S. LAWRENCE†, AND L. THOMAS*

*Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N.Y. 10021; and † New York University Medical Center, 550 First Avenue, New York, N.Y. 10016

Contributed by Lewis Thomas, March 18, 1974

ABSTRACT **Five patients with advanced breast cancer were treated with pooled dialyzable transfer factor from healthy adult donors. The period of treatment ranged from 21 to 310 days, the total dose from 20 to 257 ml. Transfer factor did not elicit inflammatory or hypersensitivity reactions or detectable formation of antibody to itself, nor any hematological or biochemical abnormalities or other side effects. Three patients became responsive (by skin test) to tuberculin and/or streptococcal antigens. Marked partial regression of the breast cancer, lasting 6 months, was observed in one patient.**

One of the immunological methods that have been considered in the context of cancer therapy is the transfer of immunity with lymphocytes or lymphocyte extracts from deliberately immunized donors or from donors suspected of being resistant to the type of cancer in question. There are reports that laboratory animals with established tumor grafts can be successfully treated by transferring large numbers of lymphocytes from tumor-immunized syngeneic, allogeneic, or xenogeneic donors (1, 2). Human cancer patients have been treated with blood leukocytes from other cancer patients who had been inoculated with the recipients' tumor tissue (3). This was rarely effective. Because of possible risks associated with inoculating cancer cells into patients, and because of severe reactions produced by large numbers of foreign lymphocytes, we have used a procedure that does not involve deliberate immunization, and that does not provoke untoward reactions.

Our approach was prompted by (*a*) Thomas' suggestion of the evolutionary significance of cellular immunity as a defense against neoplasia (4), a homoiostatic function that Burnet termed immunologic surveillance (5); and (*b*) by increasing evidence that established dialyzable transfer factor (TF_d) as a potent immunologic agent that reconstitutes cellular immunity in patients with a variety of congenital (6, 7) and acquired (8–11) cellular immunodeficiency syndromes and with cancer (12). TF_d has also proved effective in restoring cellular immunity in patients with mucocutaneous candidiasis (7, 13–15) and disseminated coccidiomycosis (16), and this has had a beneficial effect on the outcome of these infections.

We report here attempts at immunologic reconstitution of five patients with advanced breast cancer. We used pooled TF_d collected and prepared from blood leukocytes of normal women over 40 years of age. The basic assumption was that healthy women in this age group have eliminated incipient breast cancers by immunologic mechanisms and thus have become sensitized to shared antigens of breast cancer cells. We found that TF_d could be given safely in large doses over long periods, that delayed hypersensitivity was augmented in cancer patients who received TF_d, and that one patient's tumor regressed temporarily. Portions of this work have been presented before (17).

Abbreviations: TF_d, dialyzable transfer factor; PPD, purified protein derivative of tuberculin; SK, streptokinase; SD, streptodornase.

METHODS

Blood was collected from healthy women, age 40–65 years, who had no history of cancer or hepatitis. Blood (375 ml) was drawn into an Ion Exchange Pack (JB-2, Fenwal Laboratories, Morton Grove, Ill.), 130 ml of 6% Dextran in saline (Grade H, Pharmachem Corp., Bethlehem, Pa.) was added, and the erythrocytes were allowed to settle for 1 hr at 37°. The supernatant plasma and leukocytes were then removed with a plasma extractor into a transfer pack (TA-2, Fenwal Laboratories) and centrifuged in the pack at 2400 rpm (1200 × *g*) in a refrigerated centrifuge (International PR2). All but 30–50 ml of the supernatant were removed, and the leukocytes mixed with the remaining plasma were then transferred into sterile centrifuge tubes in 8-ml aliquots and centrifuged at 1800 rpm (650 × *g*) for 30 min. The supernatant was removed, and a few drops of saline were added to the packed leukocytes, which were then transferred into glass vials and stored at −70°. The yield from one blood donation was 0.5–5.0 ml of packed leukocytes, including no more than 1.0 ml of saline.

For the preparation of TF_d, 5-ml aliquots (approximately 4×10^9 leukocytes) of the leukocyte preparations were thawed at room temperature and transferred into 50-ml centrifuge tubes. The vials were rinsed with 4 ml of sterile water for parenteral injection (Abbott Laboratories, North Chicago, Ill., no. 4156) and the rinse was added to the transferred material to give a total volume of 9 ml. This was mixed with 2 mg of deoxyribonuclease (2 × recrystallized, Worthington Biochemical Co., Freehold, N. J.) in 1 ml of water (see above) and with 2 drops of a 50% magnesium sulfate solution. The mixture was frozen (dry ice in alcohol) and thawed (water-bath 37°) 10 times and then dialyzed (dialysis tubing no. 8, Union Carbide Corp., Food Products Div., Chicago, Ill.) against 1000 ml of water (see above) at 4° for 16 hr. Great care was taken to prevent contamination of the outside of the dialysis tubing with the leukocyte preparation. The dialysate was lyophilized and the residue was dissolved in 5 ml of water (see above). Since TF_d has not yet been characterized chemically, the preparations were assayed for total solids, ash, biuret-positive material, and nitrogen, and subjected to

TABLE 1. *Results of chemical analysis of* TF_d

Batch	Total solids (mg)*	Ash (mg)*	Biuret-positive (mg)*	Nitrogen (mg)*
OAA	2306	660	260	130
ODA	2502		420	150
OGA	2800	736	408	157

* Yield from 100 mg of leukocyte concentrate.

spectrophotometric analysis to provide some indication of uniformity. By means of gel diffusion against antibody directed against whole human serum (Behringwerke, Marburg-Lahn, Germany) it was ascertained that the preparation was not contaminated with serum proteins (this antiserumn detected a minimum of 200 μg of albumin per ml). The dialysate was passed through a Millipore filter (pore size 0.22 μm) and stored frozen in sterile vials until used. TF_d was administered by subcutaneous injection into the upper arm (or, rarely, into the tumor) daily or three times per week, at a dose of 0.5–4.0 ml.

Five patients with advanced inoperable breast cancer of the "inflammatory" type were chosen as recipients of TF_d. This type of breast cancer spreads through the lymphatics of the skin; the accompanying erythema and induration allow a precise assessment of the extent of the lesion. As a rule, the prognosis is poor.

Clinical observation, as well as hematological and biochemical determinations, were carried out as is customary. Delayed hypersensitivity to microbial antigens was determined by intracutaneous injection of tuberculin purified protein derivative (PPD), (Parke Davis and Co., Detroit, Mich.), streptokinase–streptodornase (SK/SD) (Varidase, Lederle Laboratories, Pearl River, N.J.), mumps skin-test antigen (Eli Lilly Co., Indianapolis, Ind.), diphtheria toxoid (Massachusetts Public Health Biological Laboratories, Boston, Mass.), coccidioidin (Cutter Laboratories, Berkeley, Calif.), and

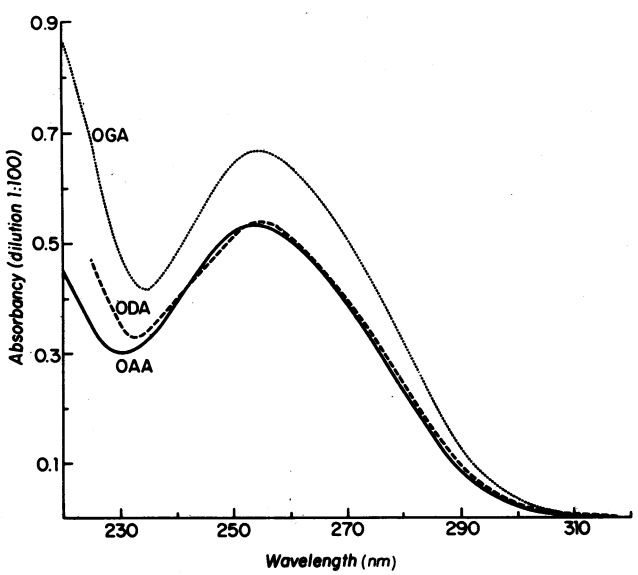


FIG. 1. Absorbancy spectrum of three TF_d preparations. Transfer factor SK 30203 was used.

Candida antigen (Hollister Stier Laboratories, Yeadon, Pa.). SH antigen and SH antibodies were assayed by hemagglutination inhibition and hemagglutination, respectively. The immunoglobulin concentration in the serum was measured by radial immunodiffusion on Immuno-Plates (Hyland Division, Travenol Laboratories, Inc., Los Angeles, Calif.).

RESULTS

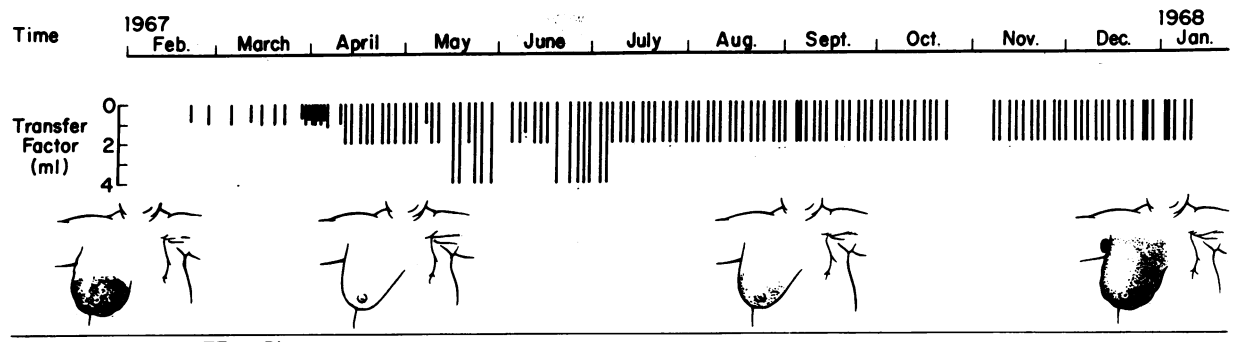
Properties of TF_d preparations

The results of chemical analysis of three batches of TF_d are summarized in Table 1. A fair degree of uniformity is evident, the mean being 2536 mg of total solids, 698 mg of biuret-positive material, and 146 mg of nitrogen for each 100 ml of leukocyte concentrate. Fig. 1 shows the results of spectrophotometric analysis of three of these batches. The absorbancy peak was at 253 nm in each case.

Case histories

B.B., a 59-year-old Negro woman, underwent left radical mastectomy on 23 July 1965 for infiltrating duct carcinoma, grade III, metastatic to axillary nodes at levels 1 and 2, followed by treatment with ThioTepa. In August 1966, recurrent disease of the chest wall was noted and irradiated with 5000 rads. In December 1966, the patient noted rapidly increasing redness of the skin and heaviness of the right breast. The clinical diagnosis of inflammatory carcinoma was confirmed by biopsy. The patient was treated with TF_d from 21 February 1967 to 10 January 1968. She received a total dose of 257 ml in 310 days (Fig. 2). Three weeks after the beginning of treatment, the tumor in the right breast became softer and smaller, the edge of the involved area of the skin receded, and the erythema was replaced by dark pigmentation. The skin, which had been distended and tense, could now be wrinkled easily, and the patient felt great relief and was once again able to sleep on her right side. This state of partial tumor regression was maintained for 6 months. Then, the tumor started to grow again and could no longer be controlled by treatment with TF_d (later batches of TF_d were prepared from the leukocytes of different donors). Subsequent hypophysectomy, radiation therapy, and chemotherapy with 5-fluorouracil had no beneficial effect, and the patient expired on 18 July 1968 with progressive metastatic disease.

R.V., a 40-year-old Puerto Rican woman, was first seen in in 1963 at another hospital with apparently inoperable carcinoma of the left breast. She was treated with radiation (5000 rads to left breast, supraclavicular area, and axilla), Methotrexate, and ThioTepa; she also received radiation (2000 rads) to her ovaries, which resulted in amenorrhoea. After another course of radiation therapy (2200 rads) to the left breast, palliation was attempted by simple mastectomy in July 1965 (widely infiltrating duct carcinoma, grade III, with invasion of lymphatics and metastases to multiple axillary lymph nodes), followed by radiation therapy (4200 rads) to the left supraclavicular area. In July 1966, more radiation therapy was given to the left side of the chest and the right axilla. In May 1967, the patient presented with inflammatory carcinoma (confirmed by biopsy) involving the right breast, right axilla, and entire left side of the chest. TF_d was given from 12 May to 28 September, a total dose of 162 ml. Initially, some flattening and softening of the margins of the involved skin were noted, and the patient felt pain in this area several hours after the injections. There was no clearly measurable



Skin Tests (mm diameter)		TF-Site	Distant Site				
PPD	0	22x33	24x36	16x16	15x18		15x17
Mumps	23x24	-	-	-	-		-
Candida	20x22	-	-	-	-		-
Coccidioidin	0	0	-	-	0		0
Diphtheria	0	-	0	0	0		-
SK-SD	0	11x16	8x12	17x20	35x40		30x35
Serological Tests							
Immunoglobulins (mg/100 ml)							
IgM	140	115		170		215	225
IgA	360	280		410		410	440
IgG	1350	1200		2100		1200	1350
C3(β_1C/β_1A) (mg/100ml)	220	155		220		230	200
Antibodies							
SH	-						-
Lung Ca	-	-		-		-	-
Melanoma	-	-		-		-	-
Mesothelioma	-	-		-		-	-
Breast Ca	+	+		+		+	+
CMV	+	+		+		+	+
HL-A (21)	-	-		-		-	-
SH-Antigen	-						-

FIG. 2. Course of TF_d treatment of patient B.B., a 59-year-old woman. *Ca*, cancer; *CMV*, cytomegaly virus.

tumor regression, however, and eventually the disease progressed. Subsequently, the patient was treated with 5-fluorouracil, Chlorambucil, arabinosylcytosine, L-asparaginase, hypophysectomy, Medrogestone, radiation therapy, and phenylalanine mustard. While none of these measures induced regression of the tumor, it grew very slowly; the patient died in December, 1970.

Three other patients received injections of 20 ml, 46.5 ml, and 48 ml of TF_d. This treatment did not alter the course of their disease.

Histological Findings. Ten biopsy specimens obtained at various times during therapy were compared with pretreatment specimens in patient B.B. Considerable infiltrates of lymphocytes and plasma cells surrounding nests of cancer cells were present before therpay, and this did not change during treatment.

Transfer of Delayed Hypersensitivity to Microbial Antigen. The first TF_d preparation given to patient B.B. was prepared from the leukocytes of only one donor (in contrast to all preparations used later) who was known to react strongly to PPD and SK/SD but not to coccidiodin or diphtheria toxoid. In this instance, the skin tests were performed 6 hr after the injection of TF_d. Both local and systemic transfer of sensitivity to PPD and SK/SD had occurred at that time, while skin reactions to diphtheria toxoid or coccidioidin could not be elicited. Although the donors whose leukocytes constituted the various pools were not tested, it was assumed that most of them were sensitive to SK/SD, and some to PPD. When we consider the large amount of TF_d given, and the large number of individual donors in each pool, it seems likely that each patient received components that effect transfer of sensitivity to SK/SD, and possibly PPD, in healthy recipients. The results of skin tests are summarized in Table 2. Two patients who had negative tuberculin tests and three patients who had negative (or very weakly positive) skin tests with SK/SD prior to treatment with TF_d developed strongly positive skin tests after treatment had been started.

Serologic Studies. IgM, IgA, and IgG were found in normal concentration in the patients' serum before treatment with TF_d, and no change was noted during treatment. Neither SH-antigen nor antibodies to SH could be detected. No antibodies to the human leukocyte antigens 1,2,3,9,10,11,W19,W28, or 5,7,8,12,13,W10,W22,W27,W14,W15,W17,W5,W18,W29, and Da(6), were demonstrable in the serum of B.B. and R.V., although they had received transfer factor in an amount equivalent to 128 and 81 blood transfusions, respectively.

DISCUSSION

The immunologic plight of patients with metastatic cancer resembles that of patients with disseminated infections caused by obligatory or preferentially intracellular microbes in that it reflects the association of a replicating antigen with a state of depressed cellular immunity. The options for the use of TF_d in attempts to reconstitute cancer patients immunologically are three: (*i*) to use TF_d with demonstrated

TABLE 2. *Delayed hypersensitivity to microbial antigens before and after treatment with* TF_d

Patient	Period of treatment	Date of skin test	Diameter (mm) of skin reactions: PPD 0.1 μg	PPD 5 μg	SK/SD 4/1 μg	SK/SD 10/2.5 μg	SK/SD 40/10 μg	Mumps	Diphtheria toxoid	Coccidioidin	Candida
B.B.	2/21/67–1/10/68	2/9/67	neg.	neg.	neg.	neg.		23 × 24	neg.	neg.	20 × 22
		2/21/67		24 × 26	8 × 12				neg.	neg.	
		3/23/67		15 × 8		15 × 20			neg.		
		5/10/67		16 × 16		17 × 20			neg.		
		6/21/67		15 × 18		35 × 40			neg.		
		1/3/68		15 × 17		30 × 35					
R.V.	5/12/67–9/28/67	5/8/67	neg.	neg.		neg.		30 × 30	neg.	neg.	20 × 24
		6/21/67		50 × 40		12 × 12			neg.	neg.	
M.H.	11/10/67–12/1/67	11/8/67	neg.	neg.	neg.	neg.	18 × 25	neg.	neg.	neg.	neg.
		11/27/67	neg.	neg.	neg.	neg.					
F.T.	2/2/68–3/27/68	1/30/68	neg.	neg.	neg.	neg.	30 × 18				33 × 30
		4/1/68		neg.							
L.M.	3/22/68–5/16/68	3/20/68	neg.	20 × 20	neg.		6 × 6	36 × 39		neg.	6 × 6
		4/17/68					70 × 72				

specificity for the antigens of the tumor in question with the intent of transferring tumor-specific immunity, (*ii*) to use TF_d of undetermined specificity in an effort to restore cellular immunity in general, and (*iii*) to use pooled TF_d from many donors assuming that it may serve both purposes. We have assessed options two and three.

Our experience has shown that TF_d can be given with impunity to cancer patients in large amounts over long periods of time. The complete absence of side effects is noteworthy and contrasts with fever and hypersensitivity reactions that are seen when viable leukocytes or homogenates of leukocytes are administered in similar amounts. Moreover, the risk of a graft-versus-host reaction in cancer patients whose cellular immune mechanism is frequently impaired, is eliminated. Evidently, the administration of TF_d is limited only by the availability of the leukocytes from which it is obtained. In addition to the absence of immediate side effects, it is noteworthy that hepatitis was not transmitted; there is, in fact, no reported instance of transmission of hepatitis with TF_d. This and the lack of sensitization to leukocyte antigens was also borne out by serological studies.

Although causing no toxic effects, TF_d was effective in transferring delayed hypersensitivity to patients with advanced cancer. That transfer of sensitivity to tuberculin and streptococcal antigens was not achieved in every case may have been due to the critical condition of some recipients (one patient was moribund when treatment was started) or to differences in the distribution of specificity in different pools.

After our initial investigation of pooled TF_d (17), other investigators have used what they considered to be tumor-specific TF_d in therapeutic trials in cancer patients. TF_d was obtained from donors who had been inoculated with the patient's tumor [malignant melanoma (18, 19)] or from family members of patients with malignant melanoma (20, 21) or sarcoma (22, 23), or from healthy unrelated individuals who were known to have responded immunologically to antigens that were also found in association with a certain type of cancer (24). In the latter case, the recipients of TF_d were patients with nasopharyngeal carcinoma, and the donors were individuals who had recovered from infectious mononucleosis, two diseases that are associated with high titers of antibodies to the Epstein–Barr virus in the serum (25, 26). Inflammatory reactions in tumor tissue, partial tumor regression, and the development of immunological reactivity to tumor antigens were observed in some cases. While these observations are of considerable interest, it has to be kept in mind that the specificity of tumor-associated immune reactions in these circumstances is still far from clear, and that transfer of cellular immunity to cancer-specific antigens of the cell surface (which make cells vulnerable to immunological attack) has not yet been proved.

We do not suggest, at this point, that pooled TF_d from healthy donors offers promise in the treatment of patients with breast cancer or any other type of cancer; an impressive response was seen in only one of five of these patients. It was, in fact, the concern that premature hopes might be raised that restrained us from reporting our experience at an earlier date. We feel, however, that publication is now both justified and timely, since TF_d is being used on such a wide scale to treat patients with a variety of syndromes that result from immunological deficiency. For physicians who treat such syndromes it is important to know that large amounts of TF_d can be given over long periods without risk to their patients. Moreover, we have now accumulated a large amount of pooled TF_d obtained from healthy donors so that more definitive studies of the type reported here can be undertaken. Recent advances in the development of *in vitro* tests for cell-associated immunity may eventually make it feasible to select the donors of TF_d on the basis of proved rather than assumed sensitization to cancer antigens. At the moment, we are more concerned to determine whether and how TF_d affects the changes of the immunological system that are induced by cancer or cancer therapy.

We thank Dr. A. M. Prince for testing sera for SH-antigen and SH-antibodies, and Dr. M. Fotino for HLA-antibody assays. This work was supported by grants from the National Cancer Institute (Ca 08748) and from the Fleischman Foundation.

1. Delorme, E. J. & Alexander, P. (1964) "Treatment of primary fibrosarcoma in the rat with immune lymphocytes," *Lancet* ii, 117–120.
2. Borberg, H., Oettgen, H. F., Choudry, K. & Beattie, E. J.,

Jr. (1972) "Inhibition of established transplants of chemically induced sarcomas in syngeneic mice by lymphocytes from immunized donors," *Int. J. Cancer* **10**, 539–547.
3. Nadler, S. H. & Moore, G. E. (1968) "Immunotherapy of malignant melanoma," *Geriatrics* **23**, 150–153.
4. Thomas, L. (1959) in *Cellular and Humoral Aspects of the Hypersensitive States*, ed. Lawrence, H. S. (Hoeber-Harper, New York, N.Y.), pp. 529–532.
5. Burnet, F. M. (1970) in *Progress in Experimental Tumor Research. Immunological Aspects of Neoplasia*, ed. Schwarz, R. S. (S. Karger, Basel), pp. 1–27.
6. Levin, A. S., Spitler, L. E., Stites, D. P. & Fudenberg, H. H. (1970) "Wiskott-Aldrich syndrome, a genetically determined cellular immunologic deficiency: Clinical and Laboratory responses to therapy with transfer factor," *Proc. Nat. Acad. Sci. USA* **67**, 821–828.
7. Levin, A. S., Spitler, L. E., Stites, D. P. & Fudenberg, H. H. (1971) "Molecular intervention in genetically determined cellular immune deficiency disorders," *J. Clin. Invest.* **50**, 59a.
8. Lawrence, H. S. & Zweiman, B. (1968) "Transfer factor deficiency response — a mechanism of anergy in Boeck's sarcoid," *Trans. Ass. Amer. Physicians* **81**, 240–247.
9. Lawrence, H. S. (1970) "Transfer factor and cellular immune deficiency disease," *N. Engl. J. Med.* **283**, 411–419.
10. Bullock, W. E., Field, J. & Brandriss, M. (1971) "Transfer factor therapy in lepromatous leprosy: an evaluation," *J. Clin. Invest.* **50**, 16a.
11. Brown, R. E. & Katz M. (1967) "Passive transfer of delayed hypersensitivity reaction to tuberculin in children with protein caloric malnutrition," *J. Pediatr.* **70**, 126–128.
12. Solowey, A. C., Rappaport, F. T. & Lawrence, H. S. (1967) in *Histocompatibility Testing*, eds. Curtoni, E. S., Mattiuz, P. L. & Tosi, R. M. (Ejnar Munksgaard, Copenhagen), pp. 75–78.
13. Rocklin, R. E., Chilgren, R. A., Hong, R. & David, J. R. (1970) "Transfer of cellular hypersensitivity in chronic mucocutaneous candidiasis monitored *in vivo* and *in vitro*," *Cell. Immunol.* **1**, 290–299.
14. Kirkpatrick, C. H., Chandler, J. W. & Schimke, R. N. (1970) "Chronic mucocutaneous moniliasis with impaired delayed hypersensitivity," *Clin. Exp. Immunol.* **6**, 375–385.
15. Schulkind, M. L., Adler, W., Altemeier, W. A. & Ayoub, E. M. (1972) "Transfer factor in the treatment of a case of chronic mucocutaneous candidiasis," *Cell. Immunol.* **3**, 606–615.
16. Graybill, J. R., Silva, J., Jr., Alford, R. H. & Thor, D. E. (1973) "Immunologic and clinical improvement of progressive coccidioidomycosis following administration of transfer factor," *Cell. Immunol.* **8**, 120–135.
17. Oettgen, H. F., Old, L. J., Farrow, J., Valentine, F., Lawrence, H. S. & Thomas, L. (1971) "Effects of transfer factor in cancer patients," *J. Clin. Invest.* **50**, 71a.
18. Brandes, L. J., Galton, D. A. G. & Wiltshaw, E. (1971) "New approach to immunotherapy of melanoma," *Lancet* **ii**, 293–295.
19. Morse, P. A., Jr., Deraps, G. D., Smith, G. V., Raju, S. & Hardy, J. D. (1973) "Transfer factor therapy of human cancer," *Clin. Res.* **21**, 71.
20. Spitler, L. E., Levin, A. S., Blois, M. S., Epstein, W., Fudenberg, H. H., Hellstrom, I. & Hellstrom, K. E. (1972) "Lymphocyte responses to tumor-specific antigens in patients with malignant melanoma and results of transfer factor therapy," *J. Clin. Invest.* **51**, 92a.
21. Spitler, L. E., Wybran, J., Fudenberg, H. H. & Levin, A. S. (1973) "Transfer factor therapy of malignant melanoma," *Clin. Res.* **21**, 221.
22. Neidhart, J., Hilberg, R., Allen, E., Metz, E., Balcerzak, S. & LoBuglio, A. (1972) "The effect of transfer factor on immunologic response to alveolar sarcoma," *Clin. Res.* **20**, 748.
23. Levin, A. S., Spitler, L. E., Wybran, J., Fudenberg, H. H., Hellstrom, I. & Hellstrom, K. E. (1972) "Treatment of osteogenic sarcoma with tumor specific transfer factor," *Clin. Res.* **20**, 568.
24. Goldenberg, G. J. & Brandes, L. J. (1972) "Immunotherapy of nasopharyngeal carcinoma with transfer factor from donors with previous infectious mononucleosis," *Clin. Res.* **20**, 947.
25. Old, L. J., Boyse, E. A., Oettgen, H. F., DeHarven, E., Geering, G., Williamson, B. & Clifford, P. (1966) "Precipitating antibody in human serum to an antigen present in Burkitt's lymphoma cells," *Proc. Nat. Acad. Sci. USA* **56**, 1699–1704.
26. Niederman, J. C., Evans, A. S., Subrahmanyan, L. & McCollum, R. W. (1970) "Prevalence, incidence and persistence of EB virus antibody in young adults," *N. Engl. J. Med.* **282**, 361–365.