

The therapy of malignant melanoma with transfer factor

A preliminary report

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Much indirect evidence indicates that immunologic factors may play a role in malignant melanoma. Antibodies against melanoma cells have been shown to be present in serum in a high percentage of patients with melanoma, especially those with localized disease.^{1, 2} Hellström et al³ have shown that lymphocytes of patients with melanoma, of their close relatives, and of a surprisingly large number of Blacks have a cytotoxic effect on cultured melanoma cells. Clinical remissions have been induced by cross-transplantation of melanoma tumor and sensitized lymphocytes.⁴ Spontaneous regressions have been documented in malignant melanoma.⁵ There have been scattered reports that blood transfusions from patients whose melanoma has undergone regression sometimes induce a tumor response in other patients with melanoma.⁶ Studies have also shown a common tumor antigen on melanoma cells.^{1, 7}

Of the two types of immunity, humoral (mediated by bursal equivalent derived B cell lymphocytes) and cellular (mediated by thymus derived T cell lymphocytes), it seems that the cellular or T cell response is the tumoricidal one in

malignant melanoma.^{8, 9} Thus, it is conceivable that induction or increase in cellular immunity might increase the host's ability to destroy the tumor.

In 1954 Lawrence¹⁰ noted that the constituents of disrupted lymphocytes were capable of transferring delayed cutaneous hypersensitivity in man. The active principle responsible for this phenomenon is called transfer factor. It is soluble, dialyzable, lyophilizable, and has a molecular weight of less than 10,000.¹¹ It is not an immunoglobulin, nor is it immunogenic. Transfer factor is able to convert normal lymphocytes (probably T type) to the antigen-responsive state *in vitro* and *in vivo*, and is thereby capable of transferring cellular immunity of the donor to the recipient. Since transfer factor is not immunogenic, it does not provoke an immunologic reaction on the part of the host that intact sensitized lymphocytes may, thereby eliminating the need for determining H-LA compatibility between donor and recipient.

Materials and methods

Six patients with malignant melanoma were treated with transfer factor. Two had cutaneous and visceral disease, one had cutaneous and regional lymph node involvement, and three had only cutaneous disease as best we could determine after extensive study. Informed consent of the patient was obtained in all six cases. The three patients with advanced disease all preferred to be treated with transfer factor before chemotherapy.

Donors were chosen from patients who had recovered from malignant melanoma, close relatives, and Blacks, whose lymphocytes were cytotoxic to cultured melanoma cells *in vitro*, and

whose cellular immunity was preferably different from that of the patient as shown by the results of skin testing. Informed consent was obtained from the donors.

Delayed cutaneous hypersensitivity was measured by a battery of skin tests as follows: PPD .0001, histoplasmin, candida 1:100, varidase, mumps, stock respiratory pathogen, and in one case, stock enteric pathogen. Lymphocytes were obtained from donors by means of a blood cell separator (Celltrifuge, American Instruments). The lymphocytes were extracted with a granulocyte and monocyte contamination of approximately 20%. Transfer factor was prepared by the method of Lawrence and Al-Askari,¹² which consists of freeze-thaw lysis of the lymphocytes, dialysis of the transfer factor against distilled water, and lyophilization of the final product. One unit of transfer factor was defined as that obtained from 1×10^9 lymphocytes.

The transfer factor was administered intramuscularly twice weekly for 3 weeks, and the patients were checked periodically for lymphocytic cytotoxicity and blocking activity. The patients were skin-tested again after receiving the transfer factor.

Measurement of cellular inhibition (the ability of the patient's lymphocytes to be cytotoxic to cultured melanoma cells) and blocking activity (the ability of the patient's serum to inhibit lymphocyte cytotoxicity) was accomplished by using the multiwell technique described by the Hellströms.¹³ Essentially, 40 to 200 cultured melanoma cells were placed in each well and 1×10^5 lymphocytes to be tested were added to the wells, except for control wells. After 48 hours incubation, the cells were stained with crystal

violet and counted against the original number; hence the percent of cellular inhibition. Assay for blocking activity consisted of the above, plus the addition of 0.1 ml of inactivated (56 C for 30 minutes) patient's serum to be tested to the same wells.

Results

One patient developed cellular inhibition, and in another patient cellular inhibition was greatly increased following the administration of transfer factor. This was not associated with clinical improvement however. In two patients cellular inhibition was lost while receiving transfer factor. Blocking activity developed in two patients receiving transfer factor and was associated with a rapidly worsening clinical course. Blocking factor was present before giving transfer factor to one patient who died soon after from far advanced disease. Delayed skin test hypersensitivity was transferred in three patients. One patient with far advanced disease died before re-skin testing, one patient had the same skin test battery as the donor, and one patient failed to demonstrate any transfer of skin test hypersensitivity. All patients except one showed advancing clinical disease, and he is currently receiving treatment with azacytadine and BCG with significant clinical improvement.

Discussion

Six patients have been treated with transfer factor prepared from donors whose lymphocytes exhibited cytotoxic activity against melanoma cells *in vitro*. Three had advanced disease and three had only cutaneous disease. Despite the transfer of delayed cutaneous hypersensitivity and induction of cel-

lular inhibition, there was no demonstration of clinical improvement in any of the patients. The development or demonstration of blocking activity seemed to portend a poor clinical course, as has been reported by the Hellströms.¹³ There were no side effects associated with transfer factor administration.

Recently, Spitler et al¹⁴ reported on nine patients with malignant melanoma treated with transfer factor. One patient demonstrated regression of the cutaneous metastases which was the initial extent of her disease for 1½ years. The other eight patients showed no response to transfer factor.

We are not sure why transfer factor was of no clinical benefit. Perhaps one should not expect to see a response using immunotherapy in patients who have as much as 1×10^9 tumor cells or 1 gram of tumor tissue. Our patients, even those with only cutaneous disease, had tumor masses in excess of this amount. We really cannot quantitate transfer factor accurately, and the amount of transfer factor may have been too small to effectively sensitize lymphocytes against melanoma cells; vast amounts of this substance may be required for it to be effective. An insufficient quantity or a deficit in the quality of the patient's lymphocytes may be responsible for their inability to evoke an *in vivo* tumoricidal response, even if they were adequately sensitized. Nevertheless, transfer factor is an important material, and further studies regarding its clinical usefulness and mechanism of action will be of great interest.

Summary

Six patients with malignant melanoma were treated with transfer fac-

tor but none showed a clinical response. *In vitro* studies demonstrated transfer of lymphocyte cytotoxicity in one patient, enhancement of lymphocyte cytotoxicity in another, and no effect in four. Delayed cutaneous hypersensitivity was transferred in three of the six patients.

References

1. Morton DL, Moderator, NIH Conference: Immunologic aspects of neoplasia; a rational basis for immunotherapy. *Ann Intern Med* 74: 587-604, 1971.
2. Lewis MG, Ikonopisov RL, Nairn RC, et al: Tumour-specific antibodies in human malignant melanoma and their relationship to the extent of the disease. *Br Med J* 3: 547-552, 1969.
3. Hellström I, Hellström KE, Sjögren HO, et al: Destruction of cultivated melanoma cells by lymphocytes from healthy black (North American Negro) donors. *Int J Cancer* 11: 391-396, 1973.
4. Brandes LJ, Galton DA, Wiltshaw E: New approach to immunotherapy of melanoma. *Lancet* 2: 293-295, 1971.
5. Summer WC, Forackes AC: Spontaneous regression of human melanoma; clinical and experimental studies. *Cancer* 13: 79-81, 1960.
6. Symes MO, Riddell AG, Immelman EJ, et al: Immunologically competent cells in the treatment of malignant disease. *Lancet* 1: 1054-1056, 1968.
7. McCarthy WH, Cotton G, Carlon A, et al: Immunotherapy of malignant melanoma. A clinical trial. *Cancer* 32: 97-103, 1973.
8. Holm G, Pearlman P: Cytotoxic potential of stimulated human lymphocytes. *J Exp Med* 125: 721-736, 1967.
9. Stewart TH: The presence of delayed hypersensitivity reactions in patients toward cellular extracts of their malignant tumors. 2. A correlation between the histologic picture of lymphocyte infiltration of the tumor stroma, the presence of such a reaction and a discussion of this phenomenon. *Cancer* 23: 1380-1387, 1969.
10. Lawrence HS: The transfer of generalized cutaneous hypersensitivity of the delayed tuberculin type in man by means of the constituents of disrupted leukocytes. *J Clin Invest* 33: 951, 1954.
11. Lawrence HS: Transfer factor. *Adv Immunol* 11: 195-266, 1969.
12. Lawrence HS, Al-Askari S: The preparation and purification of transfer factor, pp 531-546, *In In Vitro Methods in Cell-Mediated Immunity*. Edited by BR Bloom, PR Glade, New York, Academic Press, 1971.
13. Hellström I, Hellström KE: Colony inhibition and cytotoxicity assays, pp 409-422, *In In Vitro Methods in Cell-Mediated Immunity*, Edited by BR Bloom, PR Glade, New York, Academic Press, 1971.
14. Spittler LE, Wybran J, Fudenberg HH, et al: Transfer factor therapy of malignant melanoma. *Clin Res* 21: 654, 1973.