In Vivo and in Vitro Studies of Immunotherapy of Nasopharyngeal Carcinoma with Transfer Factor

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Summary

Epstein-Barr virus, the apparent cause of infectious mononucleosis, may also be an etiological agent in nasopharyngeal carcinoma and Burkitt’s lymphoma. Lymphocytes from normal individuals with anti-Epstein-Barr virus antibody activity may be sensitized to Epstein-Barr virus and contain transfer factor with the potential to program and/or recruit other lymphocytes to react against the virus and/or viral antigens. A patient with nasopharyngeal carcinoma refractory to conventional therapy was treated with transfer factor obtained from normal, young adults with a previous history of infectious mononucleosis. Following immunotherapy, apparent slowing of tumor growth was observed, which was associated with intense lymphocytic infiltration of the tumor and reconstitution of delayed cutaneous hypersensitivity reactions to microbial recall antigens. A double-blind randomized clinical trial has been initiated to determine whether transfer factor immunotherapy is a useful adjunct to radiotherapy in the primary treatment of patients with nasopharyngeal carcinoma. If successful, a similar trial might be considered for African patients with Burkitt’s lymphoma.

Introduction

Recently, TF has been applied to the immunotherapy of patients with malignant disease, with some reports of tumor regression (1, 9, 13, 15, 19, 21, 25, 26). A major problem has been the identification of suitable donors of tumor-specific TF. The approach used here, and described previously (9), is based on the premise that individuals exposed to IM might serve as ideal tumor-specific TF donors in patients with either NPC or BL. The following assumptions are made: EBV is an etiological factor in all 3 diseases, IM, NPC, and BL; lymphocytes from patients with a past history of IM may be sensitized to EBV, and finally, TF or other lymphokines from those cells might have the potential to program and/or recruit uncommitted lymphocytes to react against EBV and/or viral antigens. This report describes a patient with NPC refractory to conventional therapy, who was treated with TF derived from normal adult donors with a past history of IM. The findings are discussed in relationship to in vitro studies in which transfer of CMI against NPC was demonstrated using TF from donors with EBV antibody activity (2).

Case Report

A 31-year-old Chin male from Hong Kong presented in November 1970 with right ear deafness and an enlarged lymph node in the right upper neck. On physical examination, a soft tissue mass was noted in the nasopharynx. No cranial nerve involvement was noted. A skull film showed a soft tissue mass in the right nasopharynx. On December 1, 1970, a biopsy of the nasopharyngeal tumor revealed infiltrating epidermoid carcinoma Grade 3 (Fig. 1A). The patient was treated with radiotherapy (6000 rads) to the nasopharynx and upper cervical region (Chart 1), resulting in hearing improvement and complete tumor regression.

In July 1971, hearing loss reappeared, along with a nasopharyngeal mass. On August 31, 1971, repeat biopsy showed infiltrating epidermoid carcinoma Grade 3 similar in appearance to that of the original tumor (Fig. 1B). Skin tests of delayed cutaneous hypersensitivity demonstrated a negative reaction to PPD (5 units) and Trichophyton rubrum and a weekly positive reaction (5 to 10 mm) to Candida albicans and mumps (Chart 1). The patient had been PPD-positive in 1960 on entrance to University and also on a repeat test in 1964.

On September 9, 1971, combination chemotherapy was instituted, following which rapid tumor growth was observed. Antibody against VCA was present at a titer of 1/512 (Chart 1). On October 5, 1971, as much tumor as possible was excised from the nasopharynx. The histological appearance of the tumor was unchanged; lymphocytic infiltration was not evident.

On October 7, 1971, immunotherapy with crude buffy coat extract from patients with a history of infectious mononucleosis was initiated. Approximately 5 to 10 ml of crude extract were injected i.m. weekly. Biopsy of the nasopharynx on November 3, 1971, showed persistence of carcinoma; however, a striking round-cell infiltration of the tumor was noted (Fig. 1C). The anti-VCA titer remained elevated at 1/512. Tests of delayed hypersensitivity now...
showed a positive response (>10 mm) to PPD and *T. rubrum*, as well as *Candida* and mumps (Chart 1). On December 17, 1971, the anti-VCA antibody titer had dropped to 1/64. Biopsy of the tumor in January 1972 showed persistence of the round-cell infiltration. The patient reported improved hearing acuity in the right ear.

On February 16, blood appeared in the right ear canal, a mass was noted in the nasopharynx, and immunotherapy with crude buffy coat extract was discontinued. A biopsy of the tumor showed epidermoid carcinoma Grade 3 similar to the original biopsy; there was little or no round-cell infiltration. An elevation of anti-VCA antibody titer to 1/256 was noted. Skin tests demonstrated positive responses to *Candida* and mumps but showed loss of reactivity to PPD and *T. rubrum* (Chart 1).

Crude buffy coat extract from IM donors might be expected to contain viable EBV and/or viral antigens that could elicit malignant transformation of other recipient cells and/or induce immune paralysis by virtue of antigen excess. For this reason, subsequent immunotherapy was performed with dialyzable TF, which eliminates molecules greater than 10,000 in molecular weight (16-18), thus excluding whole virus and, possibly, viral antigen.

On March 6, 1972, immunotherapy with dialyzable TF was initiated in which approximately 10^6 lymphocyte equivalents in 4 to 6 ml were given as a single i.m. weekly injection. On March 16, 1972, positive cutaneous reactions to PPD and *T. rubrum* had been restored (Chart 1). On March 27, 1972, nasopharyngeal biopsy showed epidermoid carcinoma with pronounced round-cell infiltration (Fig. 1D). Examination of the nasopharynx on May 9, 1972, showed no obvious tumor mass. However, biopsy disclosed residual epidermoid carcinoma once again with heavy round-cell infiltration (Fig. 1F).

The patient remained well until the end of June 1972, when he became symptomatic with evidence of involvement of the right cranial nerves V, IX, X, XI, and XII. An X-ray demonstrated a large soft tissue mass on the right side of the nasopharynx and destruction of the base of the skull. A biopsy showed infiltrating epidermoid carcinoma with little plasma cell or lymphocytic reaction. Skin tests revealed a loss of reactivity to PPD and mumps but positive responses to *T. rubrum* and *Candida*. The anti-VCA antibody titer remained stable at 1/256 to 1/512 (Chart 1). In view of the obvious tumor relapse, chemotherapy with bleomycin was started. July 8, and a total dose of 285 units was administered up to August 18, 1972. Immuno therapy with dialyzable TF was continued once weekly. The clinical status remained relatively stable. The patient received further radiotherapy and once-weekly TF in Hong Kong in August 1972. His condition was stable temporarily but then began to deteriorate; TF was discontinued and he died on March 12, 1974. At autopsy, no residual tumor was detected in the nasopharynx, but there was tumor invasion of the meninges, posterior cerebral fossa, and right cerebellar hemisphere. No distant metastases were noted.

### Materials and Methods

#### Selection of TF donors.
Normal, young adults, who had a history of IM supported by positive serology (Paul Bunnell or Mono spot test) 3 months to 6 years previously, were recruited from the nursing and medical staff to serve as TF donors. All individuals with a past history of hepatitis, malaria, or syphilis were excluded; donors were screened for hepatitis-associated antigen, and positive reactors were rejected.

#### Preparation of TF.
Crude buffy coat extract and dialyzable TF were prepared as described previously (2). This technique of preparing TF is more rapid than the prolonged dialysis method of Lawrence (16-18), and the reconstitution of delayed cutaneous hypersensitivity to PPD and *Trichophyton* in this case may attest to the biological activity of the preparation. Dialyzable TF, unlike the crude buffy coat extract, produced little pain on i.m. injection.

### Discussion

Evidence exists that EBV may be an etiological agent in NPC (5, 7, 11, 20, 23). Chinese and East African patients with NPC had high antibody titers to VCA relative to control patients with other head and neck neoplasms (3, 12). Antibodies to membrane-associated antigen and early antigen were also preferentially elevated in patients with NPC, compared with controls with lymphoproliferative neoplasms, carcinomas, or sarcomas (3, 4). The association of elevated titers with advanced disease does not differentiate between a passenger virus and a causal agent since, in either case, viral proliferation with antibody formation could accompany tumor growth (10, 12).

Using an anticomplementary fluorescent staining method to examine biopsy sections of NPC, zur Hausen (28) demonstrated a clearly positive fluorescence against the epithelial cell component of NPC tissue, using EBV antibody-positive sera from normal donors. The fluorescence depended on the concurrent presence in the test sera of antibodies against membrane-associated antigen. In addition, in hybridization studies, zur Hausen and Schulte-Holthausen (29) detected evidence for the presence of the EBV genome in the epithelial cell component.

Ggerber and Lucas (8), using thymidine incorporation as an index of CMI against EBV demonstrated reactivity only in EBV-seropositive subjects. Fimmel et al. (6), using PLMI,
also demonstrated CMI to EBV in normal individuals with anti-VCA antibody activity. Evidence that the reactivity was directed against the EBV genome, rather than membrane-associated antigen on BL cell lines, was that only cell extracts from EBV-producer cell lines but not that from the nonproducing Raji cell line provided a suitable source of antigen.

We reported previously (2) that the PLMI test has been adapted both to measure CMI against an antigen extract of NPC and as an assay for TF in normal individuals and patients with NPC. The study did not resolve whether the PLMI reactivity was directed against EBV genome or viral-associated antigens such as early antigen or membrane-associated antigen. TF from EBV-positive donor leukocytes converted the leukocytes in 7 of 9 EBV-negative normal subjects to a reactive state in vitro. TF also conveyed similar reactivity upon the leukocytes of 2 of 4 patients with NPC in relapse.

In this report, which preceded the above in vitro study, TF immunotherapy of a patient with NPC, on 2 occasions was associated with apparent slowing of tumor growth coincident with marked lymphocytic infiltration of the tumor and reconstitution of delayed cutaneous hypersensitivity to PPD and Trichophyton. It is possible that sampling error could influence the histological findings, or the observed changes might be interpreted as a biological variation of NPC, since many tumors of the nasopharynx have a heavy lymphoid component (10, 22, 24, 27). Several morphological studies support the concept that so-called lymphoepithelioma is not a separate entity but merely a variant of undifferentiated carcinoma and that lymphocytes are not an integral part of the tumor but represent a reactive infiltrate (22, 24, 27). Following immunotherapy, not only was there a marked round-cell infiltration of the tumor, but tumor cell degeneration was also observed.

It is conceivable that TF immunotherapy had no effect and the clinical and pathological changes observed were those of the natural course of the disease. Patients with NPC may survive up to 10 years with active disease without therapy (14). However, the apparent slowing of tumor growth and the associated finding of lymphocytic infiltration of the tumor and reconstitution of delayed hypersensitivity reactions suggest a favorable response to TF immunotherapy.

The literature regarding TF immunotherapy is replete with anecdotal case reports such as the one reported here (13, 15). A need exists for controlled clinical trials to determine whether TF immunotherapy of any disease is beneficial. As a result of our limited clinical experience with TF immunotherapy and our in vitro studies (2), a randomized, double-blind clinical trial is currently underway in Hong Kong, in collaboration with Dr. J. H. C. Ho, in which patients with NPC will be treated with conventional radiotherapy, and one-half of the group will also receive dialyzable TF from normal adults with a history of IM and/or with an anti-VCA antibody titer of 1/64 or greater. If successful, a similar trial of TF as an adjunct to chemotherapy might be considered in African patients with BL.

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Fig. 1. Photomicrographs of serial nasopharyngeal tumor biopsies. Except as stated, H & E, × 125. A, December 1, 1970. The initial biopsy on which a diagnosis of infiltrating epidermoid carcinoma Grade 3 was made. The predominant feature is undifferentiated carcinoma showing little evidence of lymphocytic infiltration. B, August 31, 1971. Tumor recurrence 7 months after radiotherapy, showing infiltrating epidermoid carcinoma Grade 3 similar to the original tumor. C, November 3, 1971. Nasopharyngeal tumor biopsy approximately 1 month after starting immunotherapy with crude buffy coat extract, showing marked round-cell infiltration of the tumor. D, February 22, 1972. Tumor progression concomitant with loss of delayed cutaneous hypersensitivity reactions to PPD and T. rubrum. The biopsy reveals epidermoid carcinoma with little evidence of lymphocytic infiltration and resembles the histological appearance of the original tumor (A). E, March 27, 1972. Nasopharyngeal tumor biopsy 3 weeks after starting immunotherapy with dialyzable TF showing reappearance of round-cell infiltration of the tumor; positive delayed hypersensitivity reactions to PPD and T. rubrum were obtained approximately 1 week before and 2 weeks after the biopsy. F, May 9, 1972. Nasopharyngeal tumor biopsy approximately 2 months after starting immunotherapy with dialyzable TF: nests of degenerating tumor cells are encircled by an intense lymphocytic and plasma cellular infiltrate. × 200.
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