The Effects of MP Virus Infection in Lymphoma

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INTRODUCTION

Transient tumor regressions have been noted to occur after several types of virus infections (6, 13, 14). Molomut and Padnos (10) isolated the MP virus (named for their initials) from a filtrate of the supernatant fluid of Ehrlich ascites tumor cells. It behaves biologically and serologically as a member of the LCM group (E. Hotchin, unpublished data). It has caused inhibition of growth of both spontaneous and transplanted murine tumors (8) as well as canine lymphosarcoma (9). Webb noted some clinical benefit in patients with lymphoma and undifferentiated carcinoma (7). The purpose of this study was to determine the tolerance, toxicity, and effectiveness of the virus in patients with lymphoma and Hodgkin’s disease.

MATERIALS AND METHODS

Patients with far-advanced lymphoma resistant to standard forms of treatment were studied under the care of Dr. John Horton and Dr. Kenneth B. Olson at the Albany Medical College Clinical Research Center and the Veterans Administration Hospital, Albany, N. Y. All patients gave their fully informed, written consent. Careful isolation precautions were taken. The virus was originally isolated from association with Ehrlich carcinoma cells in 1963 (10). It was serially cultivated in HeLa cell spinner culture on Hanks’ essential basal medium supplemented with 5% fetal calf serum containing 100 units of penicillin and 1 μg of streptomycin/ml. Spinner cultures harvested 6 days postinoculation, centrifuged free of cell debris, sterile filtered, and aliquoted in 1-ml ampuls were stored at -196° in liquid nitrogen. Cultures were free of pleuropneumonia-like organism contamination as determined by broth culture agar plate assay and were free of lactic dehydrogenase virus (11). Potency was determined with the production of pleural or peritoneal fluid 7 to 10 days after virus inoculation in Swiss mice as the criterion for determining 50% infectivity end points (MIDso) according to the method of Reed and Muench (12). The virus was given i.v. in a dose of 1 ml of tissue culture fluid (approximately 107 mouse infectious doses) as a single injection. The inoculum supplied by Dr. N. Molomut, Waldemar Medical Research Foundation, Inc., Woodbury, N. Y., was the same batch as that used by Dr. Webb (7).

Viral and serological studies were kindly performed by the staff of the Division of Laboratories and Research, New York State Department of Health. Virus titrations were done by infecting serial 10-fold dilutions on LCM-infected BHK cells with the indirect Coons technique (1). Neutralizing antibody levels were determined in mice with the footpad inoculation method (4). CF antibody levels were obtained by inoculating 0.03-ml aliquots of serial 10-fold dilutions into each of 5 Swiss mice. End points were determined by mortality with the Reed and Muench calculation method (12) and were expressed as MIDso/g or ml. FAB levels were determined by testing serial 2-fold dilutions on LCM-infected BHK cells with the indirect Coons technique (1). Neutralizing antibody levels were determined in mice with the footpad inoculation method (4). CF antibody levels were obtained with LCM antigen prepared in this laboratory from infected chick embryos (5).

RESULTS

The 3 patients treated are described below.

Patient 1. R. G., a 61-year-old man, was discovered to have lymphosarcoma by node biopsy 14 years before admission. Previous treatments included radiation, nitrogen mustard, cyclophosphamide, vinblastine, vincristine, and Natulan. He had received no treatment during the previous 6 months but had had serum hepatitis 1 year before admission. His febrile clinical course is summarized in Chart 1. Death on Day 14 was primarily the result of pulmonary failure.

Patient 2. P. W., a 47-year-old man, was discovered to have Hodgkin’s disease by node biopsy 8 years before admission. Previous treatment included radiation, nitrogen mustard, cyclophosphamide, vinblastine, cytotoxic arabinoside, and BSO. He was taking prednisone, 10 mg daily, during treatment. His previous course had been complicated by frequent bacterial infections of the lung and inner ear as well as by herpes zoster. The fever shown in Chart 2 reflects not only the effect of virus infection but also those of the superimposed bacterial pulmonary infections, which caused death on Day 24.

SUMMARY

Three patients with far-advanced lymphoma were treated i.v. with MP virus, a strain of lymphocytic choriomeningitis virus. Probably because of severe depletion of their immune capacity, the infection was not controlled in two of them. No evidence of antitumor effect was observed.

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The abbreviations used are: LCM, lymphocytic choriomeningitis; MIDso, median mouse infectious dose; FAB, fluorescent antibody, CF, complement-fixing.

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Patient 3. G. H., a 22-year-old man, had a 1-year history of progressive pulmonary metastases from a reticulum cell sarcoma of bone despite treatment with radiation, cyclophosphamide, vincristine, prednisone, and Natulan. He was severely dyspneic before injection. He had a mild febrile viral illness (Chart 3) but died on Day 45 as a result of progression of the disease.

All 3 patients were at a very advanced stage of their disease at the time of treatment. All were anergic to tuberculosis, coccidioidomycosis, blastomycosis, mumps, histoplasmosis, and candida skin test antigens. No tumor shrinkage occurred.

Two patients were mildly leukopenic at the start of treatment, but no definite changes in levels of circulating neutrophils or lymphocytes were noted. Two patients had studies of cerebrospinal fluid before and during treatment. No cytological changes occurred.

Blood virus titers are shown in Charts 1 to 3. These had become positive by Day 5 in all 3 patients. Levels of $10^5$ MID$_{50}$ were reached in Patient 1, and these persisted until death. Intermediate levels ($10^3$) were found in Patient 2. These also persisted until death. Patient 3 showed a blood titer that reached $10^5$ on Day 10 only and was suppressed by Day 21. This patient was the only to exhibit FAB, which was first detected on Day 17 and rose to a maximum titer on Day 25. No neutralizing antibody could be detected, but the final serum specimen had a CF antibody titer of 1/16 for LCM virus.

Studies for virus in urine, feces, and throat washings were consistently negative. No virus was found in the cerebrospinal fluid of Patient 1, taken at Day 9.

Virus titers of organs obtained at autopsy of Patients 1 and 2 are shown in Table 1.

**HISTOLOGICAL CHANGES**

The appearance of the originally biopsied lymph node of Patient 1 was of a uniform replacement by lymphocytes. At autopsy, the nodes showed a more nodular arrangement with increased vascularity, sinusoidal dilation and histiocytosis, darker staining of the lymphocytes, and a more varied cell content. Changes in the lung were those of acute pneumonia with edema and marked pleuritis. The alveolar exudate contained a mixture of polymorphonuclear leukocytes and mononuclear cells. Many of the latter were nucleated but were not of the "heart failure" type. The liver showed cirrhosis, and the spleen showed vascular congestion. There was no appreciable loss or destruction of lymphocytes.

A cervical node biopsy obtained just before treatment in Patient 2 showed lymphocyte-depleted Hodgkin’s disease. The nodes at autopsy showed more advanced fibrosis and a suggestion of more eosinophils and mature lymphocytes. No hemorrhage, exudate, or marked histiocytic or macronuclear reaction was produced. The lung showed an extensive confluent bacterial bronchopneumonia with a marked polymorphonuclear exudate.

There was no definite histological evidence that the virus infection caused any specific alteration of the tumor in either patient studied. Autopsy was not performed on Patient 3.

**DISCUSSION**

The responses of the 3 patients to MP virus fell into 2 markedly different categories, which bear some similarity to responses of humans and mice to LCM virus. In the 1st type (Patient 3), viremia was short lived (ca. 15 days), the virus was suppressed, and FAB was produced. This situation corresponds to inapparent human infection with airborne LCM virus (J. Hotchin, unpublished data) and to the result of peripheral (s.c. or footpad) inoculation of mice (2, 3). The more severe illness...
suffered by the other 2-patients, with prolonged viremia and absence of FAB production, appears to correspond to the protracted viremia and chronic, often fatal, disease that frequently follows i.p. or intracerebral inoculation (2, 3). However, in mice this situation is accompanied by high titers of FAB in spite of continuing viremia. The absence of FAB in the 2 patients who died with viremia give additional evidence of severe depletion of their immune capacity. It is not surprising that 2 patients developed no neutralizing or CF antibodies, since these tend to develop considerably later than FAB in the course of disease (3). In spite of the fact that MP virus reacts immunologically and pathologically as a strain of LCM virus, none of the patients showed any evidence of meningitis, although 1 (Patient 2) had a high brain virus titer. Patients with fatal systemic LCM infection may exhibit no meningitis, but pneumonia similar to that occurring in Patient 2 has been described (2). It is likely that the meningitis of LCM arises only during a severe immune response to cerebral infection with the virus and would be absent in the anergic patient.

Lymphocytopenia during MP viremia has been noted in mice (10) and man (7). It is possible that its absence in our patients may have been related to their rather low initial peripheral lymphocyte counts. On the other hand, no cytotoxic or lymphocytolytic action was evident in the microscopic appearance of the tumor at autopsy. It seems likely that any lymphoid or neoplastic destruction induced by this virus is a manifestation of a cellular immune response against the virus-infected cells. In this light, the lack of a competent cellular immune response may obviate normal virus suppression and any virus-induced enhancement of tumor rejection.

REFERENCES

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