BRIEF REPORT

Infusion of Isologous Immune Plasma in Chronic Lymphocytic Leukemia

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THE USE OF ANTILYMPHOCYTE serum for the treatment of leukemia has been explored intermittently during the past forty years. In 1927 Lindstrom concluded that antiserum was approximately as effective as the method of radiation therapy then in use. Most of the early studies were conducted using hetero-antisera, such as those now being used, to suppress the response to transplanted tissues. From studies in dogs and rats, it is known that these highly potent sera can be dangerous and may cause nephritis, anaphylaxis or even overwhelming infection if the immune suppression is complete. It will be shown in this paper that these hazards have not been encountered following the use of iso-immune plasma in selected patients having chronic lymphocytic leukemia and that a marked temporary fall in the lymphocyte count can be achieved with minimal risk to the patient.

Two types of antisera can be used to produce lympholysis in leukemic subjects: those produced specifically against cells from leukemic subjects, or those produced against normal lymphocytes which cross-react with isoantigens of the leukemic cells. There are serious ethical problems associated with the development of specific leukemic cell antisera, although direct cellular transmission of leukemia has not been produced in man. For this reason, it was decided to test the effects of isoantibodies on patients having chronic lymphocytic leukemia (CLL) by infusing plasma obtained from volunteers who had been immunized against normal lymphocytes. We have previously shown that cells from patients with CLL show a normal or even an exaggerated response to isoantisera in vitro, and it seemed reasonable to attempt to achieve cytotoxicity of leukemic cells by means of in vivo infusion of these antisera.
INFUSION OF PLASMA IN CHRONIC LYMPHO CYTIC LEUKEMIA

CELLS FROM CLL PATIENT AND FAMILY COMPARED TO 79 NORMAL SUBJECTS

Fig. 1.—Lymphocyte typing using the in vitro cytotoxicity test. Lymphocytes from 79 normal subjects were tested against 45 antisera at approximately the same time as Patient 1 and his family were being studied.

METHODS

Leukemic lymphocytes were isolated and grouped against 45 antisera by the method described in a previous paper. Anti-lymphocyte plasma was prepared by immunizing normal subjects against normal lymphocytes injected intradermally on three or more occasions at two to four week intervals. Donors were selected providing that their hyperimmune plasma was cytotoxic to more than 75 per cent of the patients' cells when tested in vitro. Plasma was obtained by plasmaphoresis under sterile conditions.

IgG globulin was separated from dialyzed plasma on a DEAE Sephadex column using Tris buffer at pH 8.0. Iodine label (I$^{125}$ or I$^{131}$) was attached using one of the following procedures: Sodium nitrate, Iodine monochlonide, or chloramine T.

IgG isoantibody-containing globulin was prepared from selected sera by chromatography on A-50 DEAE Sephadex. Thrice dialyzed 5.0 ml aliquots of sera equilibrated with pH 8.0, 0.01 M Tris Cl buffer was chromatographed by starting buffer development on 1.2 × 50 cm. columns. The wash-through peak was collected, concentrated to the initial serum volume by vacuum evaporation at 4 C, and analyzed for purity by microimmunoelectrophoresis and IgG concentration by radial diffusion. Maintenance of cytotoxicity in the chromatographed fraction was assayed as above. Aliquots of the fractionated IgG was radiolabeled with I$^{125}$ or I$^{131}$ (IBS-3 Amersham, England) using one of three methods, sterilized by ultrafiltration and assayed for sterility and pyrogenicity prior to parenteral use.

RESULTS

For the purpose of these investigations, three patients with chronic lymphocytic leukemia who had failed to respond to standard chemotherapeutic drugs were selected. An illustration of the typing of normal lymphocytes and of leukemic lymphocytes from patient 1 is shown in Fig. 1. There was a bimodal
distribution of the frequency of positive reactions among 79 normal subjects with a mode of 40 per cent. Lymphocytes from the wife and children of the patient fell within the normal frequency distribution. By contrast, the patient’s leukemic cells reacted with 82 per cent of the test sera; a degree of reactivity well beyond the normal distribution. This increased frequency of reactivity against normal lymphocyte antisera was also found in other patients having chronic lymphocytic leukemia.

Patient 1 is a 48 year old Negroid male laborer with a three-year history of chronic lymphocytic leukemia found to be unresponsive to trials of chlorambucil, prednisone, methotrexate, streptonigrin and 6-mercaptopurine. During that time, he had been hospitalized on six occasions for major infections, such as septicemia, abscess and osteomyelitis. Hospitalization had been required for five of the twelve months prior to this study.

Against this background of illness, the patient was willing to assume the risk attendant to administration of iso-immune plasma. Fig. 2 shows the experience gained from five infusions of non-hemagglutinating cytotoxic plasma obtained from three hyper-immunized donors and two infusions of normal plasma from two normal donors. Rapid administration of iso-immune plasma over a 30-minute infusion period resulted in a fall in the white counts with an associated decrease in lymph node diameters of 20–50 per cent and with no significant change in the platelet count, hemoglobin or hematocrit. The percentage of abnormal lymphocytes remained at 92–98 per cent throughout. No chills, fever or other signs of systemic toxicity were noted. Infusion of normal plasma did not result in a fall in leukocyte count, decrease in lymph node size, or any measurable toxicity. During the two years of these plasma in-
fusion studies, the patient required a total of five months of hospitalization, two months of which were attributable to recurrent infection and three months for these elective studies. With the exception of the infectious complications, the patient’s performance status rose from 50 per cent to 80 per cent. During the course of these studies, the typing of the recipient’s lymphocytes was unchanged and he failed to develop isoantibodies against the gamma globulins of the donors.

The second patient treated with immune plasma was in a near-terminal state with refractory chronic lymphocytic leukemia and extensive bronchopneumonia at the time of the study. He was a 75 year old man who had been treated with P2, chlorambucil and prednisone over a period of eight years. Transfusions were required to maintain his hemoglobin and his platelet count was 30,000/cmm. His white blood count had been increasing and was 70,000/cmm. with 98 per cent lymphocytes at the time of infusion of 160 ml. of isoimmune plasma. After an initial rise in white count to 180,000/cmm., the white count fell within 24 hours and remained at 20,000/cmm. for the remaining three weeks of his life, when he expired of continued infection. His spleen had decreased markedly in size concomitant with the fall in white blood count. This patient did experience a brief episode of chills and fever to 101 F lasting for two hours following the infusion.

The third patient, a 68 year old Negroid male, had chronic lymphocytic leukemia and Laurence-Moon-Biedl syndrome. His white blood count was 80,000/cmm. with 91 per cent abnormal lymphocytes, hemoglobin was 10.6 Gm per cent and platelets were 85,000/cmm. After plasma infusion, his white count decline reached 53,000 at 12 hours, but returned to preinfusion levels at 36 hours. This patient experienced no constitutional signs of toxicity and lived for a year after the study. In none of these patients was there any significant effect on the percentage of lymphocytes in the differential count or on the platelet counts following the infusion. There was no change in the hemoglobin and no change in liver or kidney function. A single patient in the blastic crisis of acute granulocytic leukemia was treated with immune plasma after failing to respond to 6-MP, cytoxan, prednisone, methotrexate and thioguanosine. The plasma had no effect on his blood counts or differential, and he experienced a slight chill and fever to 100.6 F one hour following the infusion.

The results of labeled globulin disappearance in patient 1 are shown on Fig. 3. Labeled globulin activity is presented in terms of the fraction of total dose administered per liter of plasma. The initial volume of distribution extrapolated to time zero varied. This may reflect the precision of standardization of the labeled dose, variability in the amount administered or in the technique in placing the label on the IgG globulin used. The closed circles and squares compare the rate of distribution and survival of labeled normal IgG and IgG-containing isoantibody when administered together with large doses of plasma and isoantibody containing plasma. In this paired label experiment, the normal globulin was given and allowed to equilibrate for six days prior to administration of 200 cc. of immune plasma containing the labeled iso-
Fig. 3.—Disappearance of labeled normal IgG and IgG containing isoantibody. The open circles and squares represent labeled globulin administered in 1 ml of plasma and the solid circles and squares represent globulin given with 200 ml of normal or iso-immune plasma.

antibody. The different distribution curve of the activity of this label suggests that the fraction of globulin containing the labeled isoantibody had a distinctly different fate than the control globulin infusion. Note also the effect of the administration of isoantibody on the non-specific globulin distribution value. Shortly after administration of the isoantibody, the activity of the equilibrated labeled globulin diminished briefly and then resumed its general path of catabolism. The open circles and squares compare the results of similarly labeled low doses of labeled human gamma globulin (IgG portion) with labeled IgG containing isoantibody. At these low concentrations, the fractional catabolic rate of isoantibody globulin was similar to normal IgG, at approximately 12 per cent per day.

DISCUSSION

To date, there have been seven infusion studies using five different cytotoxic plasma preparations administered to three patients having chronic lymphocytic leukemia. A prompt fall in leukemic lymphocytes was observed in each instance within 24 hours following the infusion. In some, this effect was transient and in others it appeared to be maintained for weeks. The fall in white count was associated with a decrease in lymph node size, but was without significant change in the differential cell count or platelet count. A single
study in the patient having the blastic phase of acute granulocytic leukemia failed to lower the white count or otherwise modify the disease.

From these studies, it seems that plasma selected for its cytotoxicity in vitro does cause a cytotoxic reaction of lymphocytes in vivo as measured by the fall in white count, decrease in lymph node size, and rapid disappearance of the antibody from the plasma. Control plasma selected for its lack of cytotoxicity in vitro failed to cause any of the changes attributed to cytotoxic plasma. The in vitro cytotoxicity test, therefore, predicts the presence or absence of biologic effects of plasma infused in vivo. There are doubtless modifying factors, such as the level of complement, the presence of blocking antibodies, antibody binding on sites other than lymphocytes and other unidentified factors. Instances of prolonged lymphopenia might be due to antibody re-utilization or to stimulation of host mechanisms such as corticosteroid production. Preliminary experiments of the binding of globulins to lymphocytes suggest that there is a thousandfold increase in the binding of normal globulin following the infusion of large quantities of isoantibody. This relationship is under further study.

The apparent lack of serious side effects of isoantibody infusion in these immunologically deficient patients was of particular interest. It seems likely that the magnitude of the leukopenic effects depends not only on the potency of the antibody, but also on the quantitative relationship between the amount of antigen and the amount of antibody available for binding. From the antibody titers in vitro, it could be calculated that each of these experiments involved the condition of marked antigen excess, and that, unless antibody units were re-utilized, a vast number of cells in lymph nodes and extravascular sites would not have the opportunity to interact with cytotoxic antibody. Our present approach to this problem is to deplete the total lymphocyte mass with the use of chemotherapeutic agents and then to administer immune plasma in an attempt to eradicate the small number of remaining cells.

**SUMMARY**

This report describes the results obtained by the infusion of cytotoxic isoantibodies into patients having chronic lymphocytic leukemia. Each study was followed by transient lymphopenia and decrease in lymph node size. The fractional catabolic rate of large amounts of labeled isoantibody (IgG) was more rapid than of normal IgG.

**SUMMARIO IN INTERLINGUA**

Es describite le resultatos obtenite per le infusion de cytotoxic iso-anticorpore in patientes con chronic leucemia lymphocytic. Le studios individual eseva sequite de transiente lymphopenia e de un declino in le dimensiones del nodos lymphatic. Le coefficiente del catabolismo fractional de grande quantitates de marcate iso-anticorpore (IgG) eseva plus alte que illo de IgG normal.

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