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LYMPHAPHERESIS IN ORGAN TRANSPLANTATION: PRELIMINARY REPORT

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ABSTRACT

Reduction of lymphoid tissue by splenectomy and/or thymectomy has been used as a part of immunosuppression in organ transplantation (4). More recently Walker (7), Johnson (2), Franksson (1) and Starzl (5,6) and their associates have shown that chronic depletion of lymphocytes by thoracic duct drainage decreases the incidence of rejection and hence increases renal graft survival.

Mechanical removal of lymphocytes from circulation peripheral blood should theoretically achieve the same or similar effect on the immunity as thoracic duct drainage. Since September, 1979, five organ transplant recipients have received multiple lymphocytapheresis by IBM 2997 Blood Cell Separator as a mechanical pretransplant immunosuppression. The changes in cellular and humoral immunity and the clinical outcome are presented in this report.

MATERIAL AND METHOD

Case Material

Four patients with end state liver disease and one with end stage renal disease received lymphapheresis three to six times a week prior to transplantation. Sex, age and original disease are listed in Table 1. All four liver patients had massive ascites and, therefore, they were unsuitable for thoracic duct drainage prior to transplantation. One kidney patient had had an unsuccessful thoracic duct drainage before he was chosen for lymphocytapheresis.

Vascular Access

External arterio-venous shunt was created for liver patients using the posterior tibial artery and great saphenous vein at the ankle level. Arterio-venous fistula of the forearm for hemodialysis was used for a kidney patient.

Lymphapheresis

IBM 2997 Blood Cell Separator was used for lymphocytapheresis. Four thousand to 6,000 units of heparin were given intravenously prior to lymphocytapheresis and 100 to 300 units/min. of heparin were infused regionally throughout the procedure. On some occasions 2-3 ml/min. of ACD-A solution were also infused to prevent the blockage of the WBC line. The blood was processed at the rate of 80 ml/min. and centrifuged at 1150 rpm. Buffy coat was collected at the rate of 2-3 ml/min. through the WBC line. Each lymphocytapheresis lasted for 60 to 120 minutes, processing 5,000 to 11,000 ml of blood.

Immunological Monitoring

Peripheral lymphocytes were counted before and after each apheresis and the total number of leukocytes and lymphocytes removed by pheresis were measured each time. Peripheral lymphocyte activity was studied by PHA stimulation and also humoral immunity by ADCC by the method described by Sasaki et al (3).

TABLE 1.

Age, sex and Original Disease of Five Patients Who Received Long Term Lymphocytapheresis

Case	Age	Sex	Original Disease	Transplant
1	33	Male	Primary biliary cirrhosis	Liver
2	32	Female	Chronic active hepatitis	Liver
3	27	Female	Chronic active hepatitis	Liver
4	36	Female	Secondary biliary cirrhosis	Liver
5	42	Male	End-stage renal disease (?CGN)	Renal

RESULTS

A. Lymphocytapheresis

Each procedure lasted 60 to 120 minutes and 4,000 to 10,000 ml of blood was processed each time. Sixteen to 42 aphereses were performed during the period of 21 to 52 days. The total number of lymphocytes removed by a series of aphereses prior to transplant ranged from 14.0 x 10^9 to 103.7 x 10^9, WBC (including lymphocytes) ranged from 18.2 x 10^9 to 376 x 10^9, and lymphocyte-rich plasma from 3629 to 9839 ml in five cases (Table 2).

TABLE 2.

Total Number of Lymphocytes, WBC and Plasma Removed by Lymphocytapheresis

	Number of Apheresis	Period of Apheresis (Days)	Lymphocytes ($x10^9$)	WBC ($x10^9$)	Plasma (ml)
Case 1.	22	30	69.1	83.6	3629
Case 2.	21	35	25.5	39.0	4192
Case 3.	16	21	14.0	18.2	4424
Case 4.	35	52	103.7	376.0	7514
Case 5.	42	52	93.5	146.2	9839

The mean number of lymphocytes, WBC, and platelets removed by a single lymphocytapheresis are summarized in Table 3; lymphocytes ranged from 0.9 x 10^9 to 3.5 x 10^9, WBC from 1.1 x 10^9 to 10.7 x 10^9, platelets from 10.5 x 10^6 to 122.6 x 10^9 and lymphocyte-rich plasma 189 to 275 ml.

TABLE 3. Mean Number of Lymphocytes, WBC, Platelets and Plasma Removed by Each Lymphocytapheresis

	Lymphocytes ($X10^9$)	WBC ($x10^9$)	Platelet ($x10^6$)	Plasma (ml)
Case 1.	3.5 ± 2.0	4.5 ± 2.6	144.0 ± 14.3	189 ± 46
Case 2.	1.3 ± 0.7	2.0 ± 1.2	83.7 ± 11.3	196 ± 32
Case 3.	0.9 ± 0.4	1.1 ± 0.4	10.5 ± 1.7	275 ± 45
Case 4.	2.9 ± 3.6	10.7 ± 15.1	78.2 ± 10.0	208 ± 40
Case 5.	2.1 ± 1.5	3.5 ± 2.0	122.6 ± 9.9	239 ± 39

The mean changes in peripheral blood, WBC, lymphocytes, platelet count and hematocrit by a single lymphocytapheresis are summarized in Table 4; WBC and lymphocyte count decreased significantly in three of five cases ($p < 0.05$); the platelet count decreased in all five cases, and the hematocrit in four of five cases.

TABLE 4

Mean Changes in Peripheral Blood WBC, Lymphocytes, Platelet Count and Hematocrit Before and After Lymphocytapheresis

	WBC ($/mm^3$)	Lymphocytes ($/mm^3$)	Platelet ($x10^3/mm^3$)	Hematocrit (%)
Case 1.	-730(-10%) p<0.1	-180(-7%) n.s	-16(-10%) p<0.01	-3.2(-12%) p<0.001
Case 2.	-2500(-17%) p<0.01	-300(-37%) p<0.05	-33(-26%) p<0.001	-3.8(-17%) p<0.001
Case 3.	-500(-12%) n.s	-100(-14%) p<0.1	-13(-21%) p<0.001	-3.6(-13%) n.s
Case 4.	-2400(-16%) p<0.001	-500(-45%) p<0.001	-65(-13%) p<0.001	-3.4(-13%) p<0.001
Case 5.	-600(-7%) p<0.05	-200(-15%) p<0.01	-87(-35%) p<0.001	-2.8(-12%) p<0.001

By a series of aphereses, peripheral blood WBC count decreased significantly in one of five cases (Case 3). The lymphocyte count decreased in four of five cases (Cases 2, 3, 4, and 5). The platelet count decreased slightly in all of the five cases, and the hematocrit decreased also in all cases. In four of five cases, blood transfusion was necessary to improve the general condition of the patients.

B. Immunological Changes

Cellular immunity measured by PHA stimulation index (3) decreased significantly by a series of lymphocytapheresis in three of five cases (Cases 1, 2, and 4). Humoral immunity monitored by ADCC (3) decreased in three of five cases (Cases 2, 3, and 4). A strong antimitochondrial antibody present in Case 1 completely disappeared after a series of lymphocytaphereses.

C. Results of Transplant

Two of the four liver transplant recipients are alive and well (Cases 3 and 4), without any evidence of graft rejection. One liver recipient (Case 1) died after 10 days because of a poorly functioning graft and another recipient died of pulmonary sepsis after three weeks.

One kidney recipient is doing well with normal renal function in the third post-transplantation month.

Figures 1 and 2 illustrate the changes in peripheral blood counts and immunity by a series of lymphocytaphereses.

DISCUSSION

The number of lymphocytes removed by lymphocytapheresis largely depends upon the lymphocyte count and the hematocrit of pheripheral blood. Peripheral blood lymphocyte count of less than 500/μl in the presence of hypersplenism and the hematocrit of less than 20% make the yield of lymphocytapheresis minimal. The clumping of leukocytes and platelets in the centrifuge and in the tubing system can occasionally become a problem with heparin anticoagulation. Additional ACD-A anticoagulant does not always solve this problem. Improvement

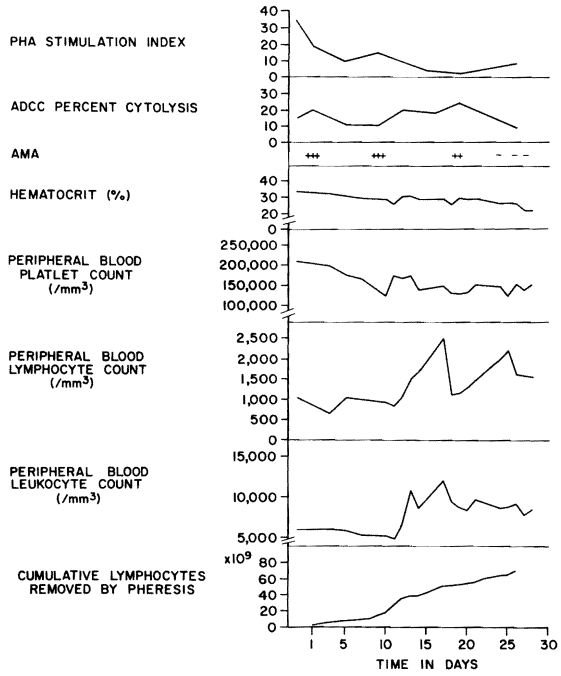

Figure 1. Case 1 is a 33-year-old male with primary biliary cirrhosis. The PHA stimulation index decreased rapidly by a series of lymphocytaphereses. Antimitochondrial antibody (AMA) disappeared after a three-week course of lymphocytapheresis.

of the tubing material, such as the Teflon coating of tubes and filters, may be helpful. Heparin seems to be better tolerated by the cirrhotics and the uremics than citrate anticoagulation. Fluid balance is very important for these patients, as fluid overload with priming saline to minimize the red cells and protein loss should be avoided.

Cellular immunity monitored by PHA stimulation and/or humoral immunity by ADCC decreased in four of five cases. These immunological alterations correlate with the numbers of lymphocytes removed by lymphocytapheresis on an individual basis. However, adequate immunosuppression was achieved by a relatively small amount of lymphocytapheresis in Cases 2 and 3. No effect was observed in Case 5 in spite of a large amount of lymphocytapheresis. A decrease in peripheral blood lymphocyte count did not always correlate with immunological suppression monitored with PHA stimulation and ADCC.

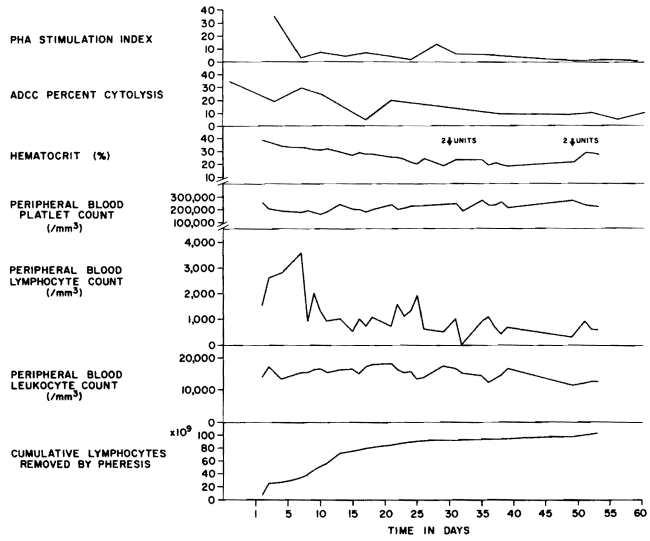

Figure 2. Case 4 is that of a 36-year-old female with secondary biliary cirrhosis. The PHA stimulation index and ADCC (% cystolysis) decreased rapidly by lymphocytapheresis. (↓ indicates blood transfusion).

Our limited clinical experience and primitive immunological studies in the five cases seem to indicate that lymphocytapheresis decreases cellular and humoral immunity and prevents rejection in organ transplant recipients. Detailed immunological analyses are being undertaken at present.

The disappearance of the antimitochondrial antibodies in Case 1 during lymphocytaphereses needs to be confirmed. Additional patients with primary biliary cirrhosis are now planned for lymphocytapheresis. The clinical significance of removing the antimitochondrial antibodies in these patients can only be discussed when more substantial data are available.

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