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Transplantation of spleen cells in patients with hemophilia A

A report of 20 cases

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Abstract It has been reported that coagulation factor VIII (F. VIII) is produced in the spleen and other organs. Transplantation of splenic whole organ and spleen cells may, therefore, be used to treat patients with hemophilia A. The donor spleen from brain-dead donors was used to prepare spleen cell suspension for transplantation. Twenty-two spleen cell transplantations were performed on 20 patients suffering from severe hemophilia A at our institutes. Two of them underwent a second infusion of spleen cells since there was no increase in plasma F. VIII activity after the first transplantation. All but two patients showed a marked clinical improvement. Increased plasma F. VIII activity was observed in 18 of 20 cases. The peak plasma F. VIII activity in these recipients rose to 10%–15% posttransplantation in 14 cases and to over 15% in 4 cases from pretransplant levels of 0%–3%. Generally, the elevation of plasma F. VIII activity could be detected 4–7 days following transplantation of spleen cells and this lasted from 22 to 58 weeks. Four patients whose peak plasma F. VIII activity was greater than 15% experienced an uneventful course after transplantation. The patients with plasma F.VIII activity over 10% showed less frequent bleeding and prolonged intervals between bleed as well as improvement in hemophilic arthrosis. Two patients who had interval hematuria before transplantation did not have any relapse for up to 2 years after infusion of the spleen cells. These results indicate that spleen cell transplantation may be a promising method for the management of patients with hemophilia A.

Key words Spleen cell transplantation, hemophilia A
Hemophilia A, spleen cell transplantation / Factor VIII, spleen cell transplantation

Introduction

Hemophilia A, also known as classical hemophilia, is an X-linked genetic disorder [9]. This disease is characterized by spontaneous hemorrhages or bleeding following minor trauma and hemorrhagic arthropathy related to the lack of, or deficiency in, coagulating factor VIII (F. VIII). Thus far, there is no ideal treatment and no cure this disease.

Several experimental studies have demonstrated that F. VIII is produced in the spleen, liver, and vascular endothelial cells [8, 9, 13]. Over the past years transplantation of splenic whole organ [2, 3, 5, 6, 12] and infusion of spleen cells [1, 7] have been attempted in order to manage the patient with hemophilia A, and rising plasma F. VIII activity has, indeed, been reported in patients after both. This paper presents the results of a large group of spleen cell transplantations in hemophilic patients, and several related aspects of F. VIII synthesis are also discussed.

Methods

General clinical data

From June 1986 to June 1991, 25 male patients with severe hemophilia A, whose diagnosis was confirmed by their medical history and serial determinations of plasma F. VIII activity, underwent 27 consecutive transplantations of spleen cells at our institutes. Five of them were excluded from this group because immunosuppressive agents were not given in the early stage of this study. In the final group, a total of 22 spleen cell transplantations were performed upon 20 hemophiliacs. Two of them underwent a second infusion of spleen cells since there was no increase in plasma F. VIII activity after the first transplantation. These patients ranged in age from 2 to 29 years (mean age 16 years).

Fourteen of the 20 recipients required multiple fresh blood transfusions or infusions of exogenous F. VIII concentrates before transplantation of the spleen cells. Three of the 20 patients had varying levels of specific antibodies against human F. VIII in their blood and they failed to respond to the strategies of fresh blood transfusion and infusion of exogenous F. VIII. Mean plasma F. VIII activity in all 20 patients varied from 0 % to 3 % prior to transplantation of the spleen cells.

Preparation of spleen cell suspension

Twenty-two spleens from adult brain-dead donors were procured to prepare spleen cells for transplantation. The donor spleen was immersed in a container with an ice-balanced salt solution at 4 °C. The splenic artery was catheterized and the spleen was flushed with cold Ringer's solution supplemented with heparin until the effluent of the splenic vein became almost red cell-free. The donor spleen was then preserved with Euro-Collins solution at 4 °C until its second perfusion. Warm ischemia time of the donor spleen was no more than 10 min. The mean cold ischemia time of these donor spleens was 6 ± 4 h.

Since the spleen is a blood reservoir with rich blood sinusoids and since its perfusion is much more difficult than that of the kidney, liver, pancreas, and other organs, a second perfusion of the spleen is usually required. The donor spleen was reperfused in vitro using 300 ml albumin-anticoagulant acid citrate dextrose (AACD) solution through the splenic artery before disruption of splenic tissue. The composition of AACD solution is summarized in Table 1.

Three steps were followed to prepare spleen cells for transplantation, as previously described [4]. In brief, the splenic capsule and vessels in the splenic parenchyma were removed in a sterilized operating room. The spleen was cut into fragments 0.3×0.3 cm in size. The splenic fragments were weighed and they were then divided into a unit per 100 g. In the same manner, 100 g of AACD solution ice-powder was considered as one unit. Two units of splenic fragments were mixed with one unit of ice-powder. The mixture was put into a sterilized homognizer and rotated at 700 *g* for 3–5 min. Finally, the splenic homogenate was diluted with cooled AACD solution in an appropriate ratio after stirring.

Three filtrations of the splenic homogenate were performed. The blood transfusion net was used for the first filtration of the splenic homogenate, and a stainless steel filter net with holes 180 μm in diameter was used for the second filtration of the spleen cell suspension; another stainless steel filter net with holes 120 μm in diameter was employed for the final filtration. Afterwards the spleen cells were resuspended and preserved in AACD solution at 4 °C until transplantation. Details on how the spleen cell suspension was prepared for transplantation are given in Fig. 1.

The vitality of spleen cells was determined using trypan blue staining under phase contrast microscopy. A mean vitality of 85 % was obtained. Spleen cell suspension was abandoned when its vitality dropped below 65 %. Spleen cells were infused through a large peripheral vein, and the infusion speed of spleen cell suspension was adjusted according to the recipient's cardiovascular status. The number of spleen cells infused in each patient ranged from 108×10^7 to 380×10^7 in child recipients and from 450×10^7 to 1200×10^7 in adult recipients. All infusions were performed in the intensive care unit (ICU). Examinations of smears for bacteria and bacterial cultures of the spleen cell suspension were done after the first transplantation in order to determine what if any, prophylactic antibiotics were needed. Two patients who failed to respond to the first transplantation of spleen cells in this group received a second infusion 2 months later.

Table 1 The composition of AACD solution

ACD solution	300 ml
Albumin	7.5 g
Gentamycin	150 mg
Dexamethasone	10 mg
	added before using

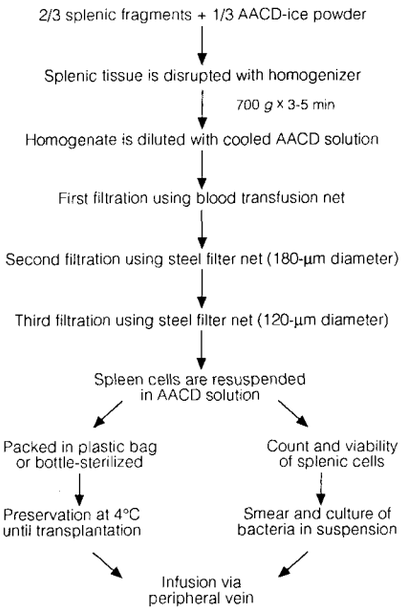

Fig. 1 Operating procedure used to prepare spleen cell suspension for transplantation

HLA matching

There was a mean of two HLA loci mismatch between donors and recipients. Donors and recipients were ABO-compatible in 16 cases and ABO-incompatible in 4 cases.

Immunosuppressive schedule

Azathioprine (2 mg/kg per day) and cyclosporin A (CyA, 10 mg/kg per day) were used as the immunosuppressive pretreatment in recipients 3 days prior to transplantation. On the day of spleen cell

Table 2 Comparative data on plasma F. VIII activity and bleeding frequency, the amount of blood transfused and F. VIII concentrates before and after spleen cells transplantation in 20 hemophiliacs (*BF* Bleeding frequency)

Pretransplantation						1st year posttransplantation			2nd year posttransplantation	
Patient no.	Age (years)	Blood transfused (units)	F. VIII[a] (units)	BF	F. VIII[b] activity (%)	Spleen cells infused (10^9)	BF	F. VIII[c] activity (%)	BF	F. VIII[d] activity (%)
1	17	2	200	4	< 1	5.5	–	14.5	1	6
2	10	–	–	2	1	3.0	–	12	–	7
3	14	1	400	5	2	4.5	–	14	2	6
4	7	2	–	3	1.5	1.08	1	9.6	2	3
5	21	1	–	3	< 1	8.5	–	22	–	15
6	29	1	600	4	1.5	4.6	1	10	3	4
7	16	–	–	2	2	7.6	–	18	–	12
8	10	1	–	2	2	2.8	–	13	–	8
9	19	–	700	5	< 1	5.5	2	11	4	4
10	22	1	400	4	< 1	8.4	–	14	–	11
11[e]	29	2	1200	6	0	6.9	7	2	8	< 1
12	19	1	–	3	2	8.2	–	15.5	–	9
13	10	1	–	2	3	3.0	–	16	–	7
14	19	–	200	3	< 1	7.7	1	12	2	5
15	13	–	400	2	2	4.9	2	11.7	3	6
16	8	–	–	2	2	3.8	–	14	–	8
17	22	2	400	5	2	7.7	2	11.8	3	4
18[e]	27	2	1400	6	0	12.0	6	3	7	< 1
19	2	–	–	1	3	3.0	1	10	2	4
20	16	–	200	3	< 1	7.5	–	15	–	9
								$p < 0.01$		

[a] Exogenous F. VIII concentrates
[b] Plasma F. VIII activity before transplantation
[c] Peak plasma F. VIII activity after transplantation
[d] Maximal plasma F. VIII activity of two follow-up studies in the 2nd year after transplantation
[e] Patients who underwent a second transplantation of spleen cells

transplantation, 200 mg hydrocortisone succinate in 200 ml normal saline were administered intravenously, followed by infusion of the splenic cell suspension. Ten milligrams of dexamethasone were added to the 300-ml spleen cell suspension to prevent potential hypersensitivity or allergy during infusion. Prednisolone (0.5 mg/kg per day) and CyA (8 mg/kg per day) were administered for 4 weeks after transplantation, after which they were tapered off.

Estimation of PT, aPTT, and F. VIII

Prothrombin time (PT) and activated partial thromboplastin time (Automated aPTT; Oranon Teknika, Belgium) were assayed before and after transplantation. Thromboelastographic (TEG) monitoring was performed by means of the thromboelastograph D (Hellige, Freiburg, Germany). The serial determinations of plasma F. VIII activity were conducted using the standard, two-stage method described by Pool and Robinson [10].

Statistical analysis

Plasma F. VIII activity and bleeding frequency before and after transplantation are presented as mean ± SD. Student's *t*-test was used to evaluate their statistical significance; a p value less than 0.05 was considered to be statistically significant, with one less than 0.01 being highly significant.

Results

Plasma F. VIII activity after transplantation

All but two patients (90%) showed remarkable clinical improvement. Elevated plasma F. VIII activity could be detected as early as 4–7 days after transplantation. Eighteen of the 20 recipients showed a significant increase in plasma F. VIII activity, and this lasted anywhere from 22 to 58 weeks (Table 2, Fig. 2).

The peak plasma F. VIII activity rose from 0%–3% pretransplantation to 10%–15% posttransplantation ($p < 0.01$ compared to the mean value pretranspantation) in 14 hemophiliacs. Plasma F. VIII activity above 15% ($p < 0.01$ compared to the mean value pretransplantation) was seen in four recipients (Table 2, Fig. 2). No evidence of the increment in plasma F. VIII activity was found in two patients who were unresponsive to the first transplantation of spleen cells, even after a second infusion of spleen cells was carried out.

TEG and the level of plasma aPTT

A significant decrease in aPTT and improved TEG were observed in four patients whose peak plasma F. VIII activity was above 15%. There was only a slight improvement

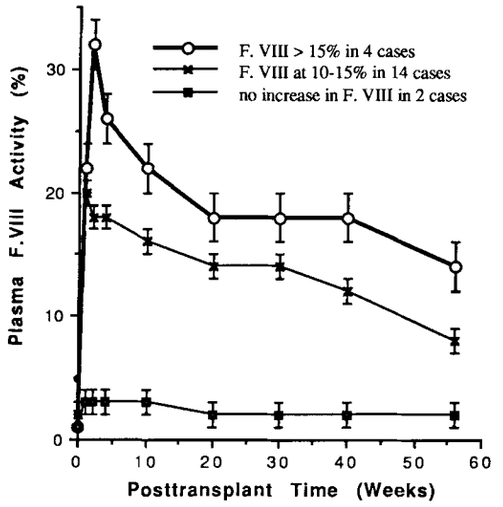

Fig. 2 Changes in plasma factor VIII activity in 20 patients with hemophilia A before and after transplantation of spleen cells. Values represent mean ± SD in each subgroup

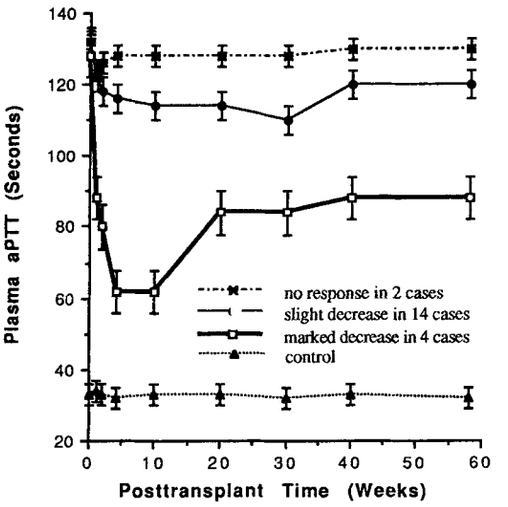

Fig. 3 Plasma aPTT levels in 20 hemophiliacs before and after transplantation of spleen cells. Values represent mean ± SD in each subgroup

in the aPTT and TEG values of 14 patients whose plasma F. VIII activity rose to between 10% and 15%. No change in the value of plasma aPTT could be seen in two patients whose plasma F. VIII activity failed to respond to spleen cell transplantation (Fig. 3).

Follow-up

All patients were followed up for an average of 2 years posttransplantation. Four patients whose peak plasma F. VIII activity was above 15% experienced an uneventful course after transplantation. Patients with plasma F. VIII activity above 10% had less frequent bleeding and prolonged intervals between bleed (Table 2), as well as improvement in hemophilic arthrosis. Two patients who had interval hematuria before transplantation did not have any relapse for up to 2 years after transplantation. Additional data, including the correlation between plasma F. VIII levels, the number of transplanted spleen cells, and bleeding frequencies are shown in Table 2.

Rejection and GVHR

Neither hypersensitivity during infusion of spleen cells nor acute rejection or graft-versus-host reaction (GVHR) following transplantation of spleen cells was noted in these patients. Complications, including temporary cardiospasm in two cases and respiratory distress in one case, were observed during the infusion of spleen cells but they were successfully managed.

Discussion

Over the past years, allotransplantation of splenic whole organ and infusion of spleen cells have been successfully attempted in patients with hemophilia A. Allografting of the whole spleen in hemophiliacs has been reported by Hathaway et al. [2] Shumakov et al. [12], and Liu and coworkers [3, 5]. Liu and his colleagues reported that plasma F. VIII activity in hemophilic patients undergoing whole spleen transplantation rose to 35% posttransplantation from 1% pretransplantation. The grafts, however, maintained their function for only 4 months after transplantation due to strong, acute rejection and GVHR [5].

In 1969, Desai et al. [1] reported on the infusion of spleen cells in four hemophilic patients and they claimed that plasma F. VIII activity in these recipients significantly increased for 60–90 days. Mehta et al. [7] described four patients with hemophilia A who underwent spleen infusion and who had elevated plasma F. VIII activity from 1 to 52 weeks. The results from our 20 hemophiliacs who underwent spleen cell transplantation are consistent with those reported by these other authors.

It is well known that a classical strategy for the physician trying to manage the severe hemophilic patient is the repeated use of blood transfusions or infusions of exogenous F. VIII concentrates. Unfortunately, these palliative therapies gradually become unresponsive to the cycle of medical therapy. Multiple blood transfusions and infusions of exogenous F. VIII concentrates may, on the other hand, induce anti-F. VIII autoantibodies or increases the patient's risk of contacting hepatitis or AIDS. Although gene therapy has recently been reported to be a new tendency for dealing with the hemophilic patient, at present

it is still an experimental treatment that has the potential to produce mutants in clinical practice. Therefore, it is necessary to develop new strategies, such as the transplantation of spleen cells into such patients.

It is worth pointing out that in the present study, the time interval between bleed was significantly prolonged and the bleeding frequency markedly curtailed for up to 2 years after transplantation in most of the patients, although their plasma F. VIII activity was only maintained for a year. We therefore, believe, that transplantation of spleen cells may be a promising method in the management of patients with hemophilia A who do not have positive titers of pretreatment antibodies against F. VIII.

To obtain high vitality of spleen cells, all patients in this group received a fresh spleen cell suspension. Our data demonstrate that there may be a correlation between the number of infused spleen cells and increased plasma F. VIII activity. To some degree, the larger the number of transplanted spleen cells, the higher the vitality of the spleen cells, and the better the increase in plasma F. VIII activity. When the total number of infused spleen cells is below 100×10^7 in a child recipient or below 300×10^7 in an adult recipient, there seems to be no striking increment in plasma F. VIII activity. A high plasma F. VIII level was detected in the younger recipients who had fewer previous fresh blood transfusions or infusions of exogenous F. VIII concentrates and who were free of pretreatment antibodies against F. VIII.

Retrospective analysis reveals a number of possible reasons for the failure of spleen cell transplantation in two patients in our group. These include the present of preexisting positive autoantibodies against F. VIII in the blood of these recipients, the lower number of transplanted spleen cells, and individual genetic differences between donor and recipient. An insufficient immunosuppressive therapy in these patients could also be another reason. According to our preliminary experience, we feel that a young patient with hemophilia A who is free of pretreatment antibodies against F. VIII in his blood and with no history of reluctant hemophilic arthopathy may be a expected candidate for spleen cell transplantation.

There is still controversy over whether F. VIII is synthesized or stored in the spleen. Desai et al. has demonstrated that human lymphocytes in vitro can sythesize F. VIII [1]. Our clinical results and other experimental data support this theory that spleen cells may generate F. VIII rather than store it [1, 5, 8, 9, 12].

First, the peak of plasma F. VIII activity in these patients was observed 1–3 weeks after the transplantation of spleen cells and it lasted 22–58 weeks, which seems to indicate that the transplanted spleen cells produce F. VIII. Clearly, plasma F. VIII would be consumed rapidly following transplantation if the elevated plasma F. VIII was stored in the transplanted spleen cells. Secondly, it has been proven in an experimental model of cross-circulation between a hemophilic dog and a healthy one that the half-life of F. VIII activity is only 96 h [13]. The half-life of the infused exogenous F. VIII activity in a hemophilic patient was also reported to range from 8 to 12 h [11]. Therefore, if the elevated plasma F. VIII activity in these recipients is due to a release of tissue factor from the transplanted spleen cells as they are destroyed, it should be dramatically lowered around 2 days following transplantation. Conversely, the peak plasma F. VIII activity was detected in the responsive patients in the present series. Finally, we could not confirm the elevation of plasma F. VIII activity in two patients in our group, even though they received a second transplantation of spleen cells after the failure of the first transplantation. We postulate, therefore, that the transplanted spleen cells may synthesize F. VIII or that these cells may be the specific materials that activate other cell populations or enzyme systems to generate endogenous F. VIII in the host.

It is worth emphasizing that investigations into donor phenotype or chimera are of great significance in confirming the long-term survival of the graft in the recipient's body after transplantation. One may wonder whether there is direct evidence that the freshly-generated F. VIII is produced by the transplanted spleen cells. Unfortunately, we do not have the existing data in our hands. The precise mechanisms for this cause and effect remain to be investigated further.

If we compare transplantation of the whole spleen with the infusion of spleen cells, we find that the former is a more effective, but much more expensive, operative procedure. The patient also risks life-threatening complications, such as damage to the graft during the harvesting of the donor spleen, overwhelming acute rejection, and GVHR after transplantation. On the other hand, the latter is a moderately effective alternative therapy that is inexpensive and relatively safe, and it can be repeatedly applied in the hemophilic patient who is free of rejection or hypersensitivity.

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