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Transplantation of stem cells of embryonic liver in a patient with severe combined immunodeficiency

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A patient had severe combined immunodeficiency syndrome and x-chromosomal recessive heredity. Since the parents and siblings were not suitable as HLA-compatible bone marrow donors, stem cells from embryonic liver were transplanted intravenously in 3 stages (6×10^6; 3.5×10^6, and 9×10^7). Transplantation was tolerated well; there were no signs of a graft-versus-host reaction. Examination of the immunological condition after transplanation showed evidence of T-cell reconstitution, immunohistochemistry revealed beginning immune globulin production. The child died at the age of 5 months due to respiratory failure.

Severe combined immunodeficiency (SCID) is a partly genetically determined lethal T and B cell defect which was originally thought to be caused by deficiency of lymphatic stem cells. Today SCID designates a heterogeneous group of congenital T-B-cell defects of differing pathogenesis. Apart from the conventional "Swiss type" of SCID with autosomal recessive heredity, there is a type with x-chromosomal recessive inheritance.

The preferred therapy for SCID is allogeneic bone marrow transplantation from an HLA-identical, MLC-compatible sibling donor. This method has so far given the best results with regard to long lasting and complete immunological reconstitution [1, 2, 3]. Recently there has been a number of papers reporting on successful bone marrow transplantation from related or non-related donors who were not fully identical [11, 12, 19], but in these cases a graft-versus-host reaction is to be expected.

As an alternative treatment for SCID patients for whom histocompatible bone marrow donors are not available, transplantation of vital embryonic liver has been proposed [4, 5, 8, 20]. We have carried out transplantation of stem cells of embryonic liver in an infant suffering from SCID with x-chromosomal recessive heredity, at the age of 12 weeks. The present paper reports on this therapeutic approach.

Genealogy

Under the authors' care is a family from which over two generations a total of 11 male descendants originated. Of these, 8 had died in early

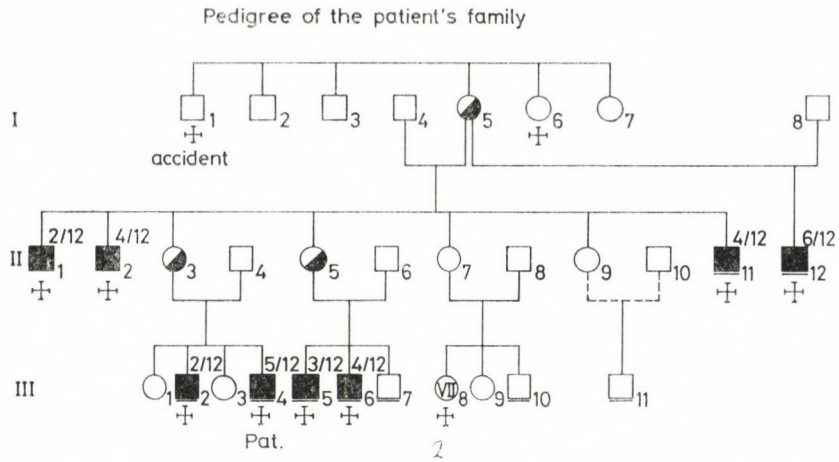

FIG. 1. Genealogical tree of the family afflicted with SCID

infancy (Fig. 1). The grandmother (I 4) of the family had had 3 brothers and 2 sisters who had all reached adult age.

The marriage of I 4 had produced a total of 7 children, 3 boys and 4 girls, plus a boy born out of wedlock (II 11). The boys had died between 2 and 6 months of age.

In the meantime, 3 daughters of I 4 have married (II 3, II 5, II 7). The oldest daughter (II 3) first gave birth to a healthy girl, followed by a boy (III 2) who was the first for whom the diagnosis "Severe combined immunodeficiency" was put forward and verified in immunological and histological terms. He died from generalized cytomegalovirus infection and giant-cell pneumonia. The third child of II 3 is a clinically healthy girl.

The second daughter (II 5) of I 4 has so far had 3 boys, of whom 2 died in our hospital due to immunodeficiency. In the third boy (III 7) from this family immunodeficiency was excluded both immunologically and histologically. He is developing normally. Healthy boys have been born to II 7 and II 9 whose transmission status therefore remains unclear. On the whole, there can be no doubt about the x-chromosomal heredity of SCID in this family as it has affected only boys and, in addition, half brothers from different fathers in one generation.

Report of a Case

Another boy (III 4) was born in this family in March 1981 who was admitted to our hospital at the age of 4 weeks. SCID had been diagnosed because of a reduced and disproportionate transformation response of peripheral lymphocytes to non-specific T and, later on also to B cell mitogens, as well as of a continuous decrease in serum immunoglobulins after the 4th week of life.

Histological examination of an extirpated lymph node revealed an immature structure without characteristic B and T

regions and without secondary and tertiary follicles. Immunohistochemistry showed no IgA and IgG production, while IgM, $\varkappa$ and λ were detected in small amounts. Other findings which supported the diagnosis were negative skin test reactions, the absence of a thymic shadow and the absence of plasma cells in the bone marrow. ADA activity in plasma and erythrocytes was normal. At the age of 12 weeks stem cells of embryonic liver were transplanted three times during a period of 12 days (Table I). The patient received a total of 1×10^8 cells (i.e. 2×10^7 cells per kg of body weight) intravenously. A maximum of 90 min elapsed between removal and the intravenous application of stem cells. Transplantation was tolerated well, and subsequently there was no clinical or morphological (skin excision) evidence of any graft-versus-host reaction. The patient died 8 weeks after transplantation, at the age of 5 months, due to respiratory failure. A few days earlier 100 ml human gamma globulin had been given intravenously.

Autopsy revealed a SCID and a dysplastic thymus anlage without lymphocytes, generalized cytomegalovirus infection and marked hyaline membranes in the lung where immune complexes were detected by means of immunohistochemistry.

Methods

HLA typing was done according to a modified NIH technique using the lymphocytotoxic test on Terasaki plates.

Lymphocyte transformation test (LTT). 1×10^6 mononuclear cells from heparinized blood (free of phenol) which had been enriched by glass adhesion were cultivated in Parker's medium with antibiotics and 20% autologous plasma at 37°C for between 3 and 7 days, in the absence (control) or presence of different mitogens: phytohaemagglutinin (PHA) 20 μl/ml, concanavalin A (Con A) 20 μg/ml, pokeweed mitogen (PWM) 20 μl/ml (3 and 7 days, and lipopolysaccharide from *E. coli* B) 44 0111 (LPS). Evaluation was carried out morphologically by counting of transformed cells.

Mixed lymphocyte culture (MLC). In view of the expected non-reactivity of the potential recipient, the response of the potential donor toward the cells of the recipient was tested, with the donor cells acting as the responding cells and the recipient (patient's) cells as the stimulating cells. The lymphocytes of the potential donor and the potential recipient were separated from heparinized blood using Ficoll-Paque, and suspensions of 1×10^9 lymphocytes/l in RPMI 1640 medium were prepared adding Hepes, L-glutamine, antibiotics and 20% inactivated AB serum. The responding cells were subjected to no further treatment. Stimulating cells were incubated with mitomycin C (Serva) 25 μg/10^7 cells for 30 min at 37°C. After washing they were readjusted to 1×10^9 lymphocytes/l. Equal aliquots of both responding and stimulating cell suspensions were pipetted on round-bottom plates (100 μl = 1×10^5 cells each), gassed with CO_2, incubated for 6 days at 37°C

Table I

Transplantation of stem cells from embryonic liver

Uterus — TE	Weeks of gestation	Transplanted cells
Uterus myomatosus	8	6×10^6
Carcinoma in situ	6	3.5×10^6
Carcinoma in situ	12	9×10^7

and labelled 16 hours before the termination of cultivation with 37 KgBq/well ^{3}H-thymidine (spec. activity 333 KBg/mmol). The cells were harvested using a Flow cell harvester and radioactivity was measured in LKB liquid scintillation counter.

Immunoglobulin determination was done according to Mancini using conventional LC plates (Behring Corp).

Stem cell preparation (Table I). Indication for extirpation of the uterus during pregnancy was in one case a myoma, and in two others in-situ carcinoma in 40/42-year-old women who wanted no further children. After removal of the uterus the entire amniotic sac was removed under sterile conditions and the liver dissected in a laminar air box. The liver was cut into small pieces with scissors, suspended in the medium and passed through cannulas up to S 20. The medium consisted of an electrolyte solution containing infesol, tromethamol, calcium gluconate, cyanocobalamine, folic acid, streptomycin and penicillin. Cell counts were estimated and one vitality test and several sterility tests were performed.

Results

HLA typing showed an antigen difference in the A-locus between the father and the patient, and uncertain homozygosis in the B-locus. MLC gave a threshold index and therefore no full compatibility for the D-locus. The other family members had only one compatible haplotype each, so that a suitable donor for allogeneic bone marrow transplantation was not available.

At no time was the patient suffering from lymphopenia. The number of lymphocytes was always above 2.0 Gpt/l. Transplantation of stem cells from the embryonic liver was followed by a marked increase with a maximum of 6.8 Gpt/l 6 weeks after transplantation (Fig. 2). The LTT during the 4th week of life showed slightly reduced T cell functions of peripheral lymphocytes. At the same time an extreme increase in the detection of B cell properties and spontaneous activation of lymphocytes was observed. In the 10th global T cell function, measured by PHA response, was reduced (PHA transformation 0.23, standard 0.79), although certain T cell subpopulations and especially suppressor and helper cells, continued to be stimulated well (Con A, PWM three-day value). There was a slight increase of B cells in the circulation which could be autonomously stimulated by LPS. Shortly before and one week after liver cell transplantation almost no T or B cells could be stimulated, except for a T cell subpopulation stimulated by PWM after 3 days. On the other hand, subnormal T-cell PHA transformation was verified 6 weeks after transplantation, with the Con A transformation (suppressor cells) higher than the PHA transformation. This indicated an incomplete but clear T cell reconstitution (Fig. 2). The concentration of immunglobulins in the serum which was still normal at the age of 4 weeks, continuously dropped despite the transplantation of stem cells of embryonic liver and substitution with human gamma globulin.

The serum IgM content had been in the lower standard range before

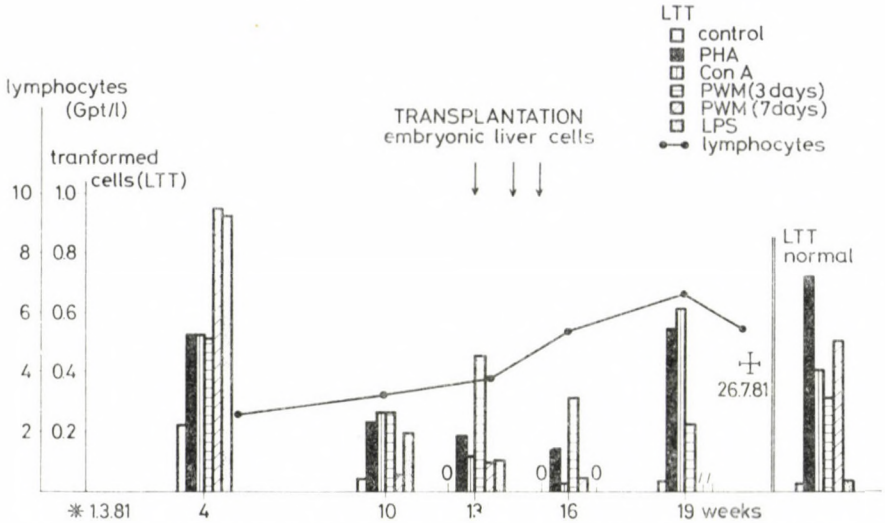

FIG. 2. Absolute lymphocyte number and results of the lymphocyte transformation test before and after transplanation of stem cells from embryonic liver

transplantation and rose very slightly 5 weeks after transplantation. The immunohistochemical staining (PAP technique) of cytoplasmic IgM, IgD, kappa and lambda in the lymph node, spleen and appendix (IgG in isolated cases) also pointed to an initial immunoglobulin secretion of lymphoplasmocytoid cells.

DISCUSSION

In view of the genealogy, the immunological, histological and immunohistochemical findings and the development of the disease, the diagnosis of SCID with x-chromosomal recessive heredity could not be doubted. No satisfactory explanation could be offered for the fact that clearly functional T cells were found at the age of 4 weeks (PHA, Con A and PWM transformation in the LTT, Fig. 2). This may have been caused by the intrauterine transmission of maternal cells since maternal lymphocytes which pass the placental barrier may persist in children suffering from SCID and contribute to the development of chimerism without a graft-versus-host reaction [13, 17, 18]. Similarly, antigenic stimulation, for example by vaccines, may induce lymphoproliferation in cases of SCID [1].

In our patient bone marrow transplantation was impossible because there was no HLA identity and no MLC compatibility of the other family members. In addition, no one was ready for bone marrow donation. We decided to transplant stem cells from embryonic liver in view of the fact that thymus transplantation alone had brought only short [16], and thymus factors no, success whatever

[7, 22]. Until the 12th week of gestation the embryonic liver contains no mitogen-reactive lymphocytes [14] and is therefore suitable for allogeneic transplantation without regard to histocompatibility antigens. The development of graft-versus-host disease with fetal liver over 12-weeks remains a theoretical consideration, especially if the applied cell-mass is so low as in our case. Liver cell transplantation is designed to give the recipient a population of lymphatic precursor cells which have only minimal potency for triggering a graft-versus-host reaction. The qualitative function of these cells concerning their suitability for transplantation and proliferation seems to differ widely, depending on the stage of gestation and the particular case, and therefore no correlation between the number of cells transplanted and the therapeutic success seems to exist [12]. In animal experiments approximately 2×10^8 cells per kg of body weight have been required to restore the immunocompetence and to develop a donor cell population [9]. However, stem cells from embryonic liver of this order of magnitude can be obtained only from fetuses older than 16 weeks where serious graft-versus-host reactions are to be expected. Successful transplantation or auto-restauration [21] has been achieved in humans with fewer cells [4, 20].

We have transplanted a total of 2×10^7 embryonic liver cells from the 6th, 8th and 12th week of pregnancy (calculated from the last menstruation) in three sessions, over a period of 12 days (Table I). The haemopoietic cell composition of the 6-week liver remarkably differs from that of the 12-week one, including differences in lymphocyte and lymphoid cell content [10].

The functional reconstitution of the immune system after liver cell transplantation develops gradually and takes several months [9]. No significant population of donor cells has so far been detected earlier than 6—10 weeks after transplantation [14]. Our patient showed a marked T cell mitogen response of the peripheral lymphocytes and a simultaneous rise in the absolute number of lymphocytes as early as 6 weeks after transplantation (Fig. 2). No increase in the serum immunoglobulins was found, with the exception of a slight rise in IgM (Fig. 3). IgM has also been detected immunohistochemically in the form of cytoplasmic IgM in lymphocytes of lymph nodes, spleen and gut. On the other hand, IgG-IgA secretion was not clearly demonstrable by the time the patient died at the age of 21 weeks, 8 weeks after transplantation.

At least some of the SCID patients suffer from a primary alteration in differentiation of the epithelial thymus anlage so that the hormone output of the thymus is impaired and the bone marrow stem cell does not develop into a differentiated T cell. In most cases, therefore, complete immunological reconstitution has been achieved only after simultaneous or subsequent thymus trans-

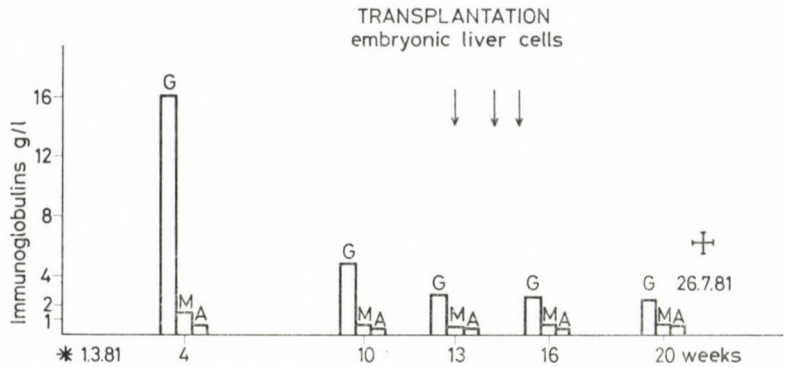

FIG. 3. Immunglobulins (g/L) before and after transplantation of stem cells from embryonic liver

plantation [15] or grafting of cultivated thymus epithelium [6]. The results were particularly favourable in cases where the lymphocytes of SCID patients after co-cultivation with normal thymus epithelium had differentiated into functional T lymphocytes [6].

Transplantation of stem cells from embryonic liver does not influence thymus function, nor the rise ot the thymic hormone level [14] and, in our patients, did not induce homing of the patient's thymus with T lymphocytes. Unfortunately, in our case the time of observation from transplantation to death was not long enough for an assessment of whether the stem cells from embryonic liver alone would have caused full and lasting immunological reconstitution. Moreover, we were not able to document true transplantation, and that is why auto-restauration could not be excluded [21].

According to our clinical observations, the intravenous application of human gamma globulin seems to have initiated the fatal phase of the disease. It is possible that the human gamma globulin produced a local immunocomplex reaction. The antibodies of the human gamma globulin preparation may have reacted with bacterial as well as antigens of the patient and led to massive excretion of immune complexes which induced the alteration in pulmonary diffusion. Breakdown of these immune complexes was obviously impossible because of a reduced capacity for phagocytosis of the macrophage system due to the generalized cytomegalovirus infection.

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