Transplantation of Human Fetal Tissue in Hematology

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Intravenous transplantation of human fetal tissue, notably of the liver and thymus, is used in emergency hematological states: aplastic anemia and acute leukemia. The strong manifestation of "graft-versus-host" disease proved unexpected. Fetal donor hepatic cells stimulate hemopoiesis in the recipient. In some cases true cellular chimerism has been observed, specifically when a transplant of human fetal tissue (THFT) to a fetus with prenatal pathology was performed *in utero*. In gene therapy attempts have been made to infuse hemopoietic stem cells intravenously or to introduce the adenosine aminase gene into leukocytes of patients with a deficiency of this enzyme. Treatment using transplantation of human fetal tissue will help solve the problem of HLA-histocompatibility and will make gene therapy more widely applicable.

Key Words: *transplantation of human fetal tissue (THFT); treatment of hematological crises; gene therapy; transplantation of hemopoietic stem cells*

The wide use of fetal donor material in hematological practice has become possible due to understanding of the fine molecular and cellular hemopoietic mechanisms. Fetal material is used owing to its high plastic potential and to the absence of mature immunocompetent cells which provoke the development of "graft-versus-host" disease in the hemopoietic organs of the fetus. The human fetal liver is an almost ideal source of hemopoietic stem cells, and, most important, a favorable clinical effect is achieved even when allogeneic cell material is transplanted.

Robert Gale, summarizing his own findings and the results obtained by other researchers, noted that the treatment of aplastic anemia with cells of allogeneic fetal liver was successful in 66 patients out of 122 (54%) [2,3]. The material was obtained from 6-32-week donors. The total number of transplanted nucleus-containing cells of the fetal liver was 0.004-13.0×10. All patients received immunosuppressive premedication. In no case was "graft-versus-host" disease observed. Cell chimerism was established in just 4 patients. In the treatment of acute leukemia a short-term engraftment was obtained in 41% of patients (in all, in 39 patients). Weakly expressed signs of "graft-versus-host" disease were noted only in 2 patients. The life span of such patients following treatment was over 2 years. A long-term engraftment was obtained in 2 cases. A short-term engraftment was confirmed by chromosome analysis in 12 patients, by isoenzyme and antigenic typing of erythrocytes in 5 cases, by HLA typing in 2 cases, and by the synthesis of fetal hemoglobin in 3 cases. Since the patients of this group were subjected to intensive chemo- and radiotherapy, the effect of fetal tissue therapy cannot be interpreted simply [2,3].

These findings allow for some conclusions to be drawn. First, the actual possibility of at least a short-term take of donor hemopoietic cells was proven. This was most clearly demonstrated by the results of cytogenetic analysis performed after treatment of acute leukemia. The incidence of successful engraftment was markedly higher in leukemia

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(41%) than in aplastic anemia (4%). In 23 patients with leukemia who had received intensive immunosuppressive therapy pretransplantation, the efficacy of engraftment was 54%. These results are in accord with those obtained in experimental models on animals, corroborating the immunogenicity of fetal liver cells and the possibility of their rejection after transplantation to immunocompetent recipients. In patients with aplastic anemia a fully functional immune system is usually preserved, and therefore a high risk of rejection of fetal liver cells is to be expected. In patients with leukemia the likelihood of graft rejection is markedly reduced due to changes in the system of immunity associated with the main disease, as well as due to the use of high-dose chemo- and radiotherapy pretransplantation.

Graft rejection or its functional deficiency is the main obstacle to the use of THFT [1,3,10]. Several reasons for these processes may be determined. First, an insufficient number of transplanted hemopoietic cells; second, the absence or deficiency of T lymphocytes in the graft. The latter needs to be explained. As is well known, T cells may directly interact *in situ* with hemopoietic stem cells, supplying to them the necessary array of growth and differentiation factors. For instance, it is known that preliminary removal of T cells from bone marrow grafts increases the risk of donor cell rejection. At the same time, T lymphocytes which are present in the donor material are potential inducers of the "graft-versus-host" response and may be the source of excessive donor antigens, provoking a "host-versus-donor" response. Since mature T cells are absent in the fetal donor material used for transplantation, but the earlier T-lymphocyte precursors can be present, this does not promote the development of the "graft-versus-host" reaction (the cells become tolerant to the recipient's antigens). On the other hand, as T lymphocytes mature, they may provide for the full-value function of hemopoietic precursors.

Since true chimerism is rarely established in the case of transplantation of fetal liver cells, and a favorable clinical effect may manifest itself even under conditions of a short-term take of transplanted cells, this suggests that fetal donor liver cells are capable of stimulating hemopoiesis in the recipient. Many researchers are inclined to such an opinion [3,7-10,14]. Evidently, the therapeutic effect is attained via the production of growth factors stimulating the recipient's hemopoietic stem cells. According to this assumption, in the majority of cases repair of endogenous hemopoiesis should occur due to direct utilization of the factors supplied by the grafted biomaterial; thus, by definition such treatment should be termed fetal tissue therapy.

The rare cases where true cell chimerism has been stable are encouraging, since a favorable clinical effect cannot be expected in cases of a functional deficiency of endogenous hemopoiesis (hereditary diseases). In any case, the creation of a chimeric hemopoietic system is of primary importance in hemopoietic disorders, since this guarantees reliable correction of this defect.

In this context, the achievements of Jean Louis Touraine [16-18] in transplantation of fetal liver stem cells and fetal thymocytes *in utero* are of special interest. The cells were transplanted to 3 fetuses (2 cases of immunodeficiency and one case of sickle cell anemia). In one infant with the "bare lymphocytes" syndrome true lymphocyte chimerism and a vigorous T-cell antigenic response were established soon after birth. Clinical manifestations of immunodeficiency were entirely absent. In the other two fetuses partial recovery of disturbed functions was observed. No side effects of THFT on the maternal or fetal organism were noted in any of these three cases.

Touraine came to the conclusion that transplantation of fetal liver stem cells promotes engraftment, ensures ideal isolation of the patient (in the uterus), and provides the optimal microenvironment for donor cell development in the fetal host [18].

Advances in genetic engineering show promise of patient cure even in the case of complete dysfunction of certain specialized biological structures of the organism. The first successes in this field were achieved in the treatment of disorders of the hemopoietic system. Initial isolation of hemopoietic stem cells from the patient's organism, followed by transfection with a bona fide analog of the defective gene, seems to be the most promising. It was suggested that after multiplication of transfected cells *ex vivo*, they be transplanted to the same patient. In this connection novel experimental investigations performed at the Becton Dickinson Company are very promising. Two subclasses of pluripotent stem cells of human fetal bone marrow were characterized: a CD 34+ HLA-DR+ CD38- subclass capable of differentiating into progenitor cells of all hemopoietic strains, and a more primitive CD34+ HLA-DR- CD38- subclass, from which hemopoietic precursors and stromal cells, capable of maintaining differentiation of these precursors, originate [5]. The identification and isolation of the common precursor of hemopoietic and stromal cells are very encouraging from the practical viewpoint, since selective trans-

plantation of such cells after their target-directed gene modification may provide for complete restoration of hemopoiesis in patients with genetic defects. Malcolm Brenner was the first to prove the fundamental possibility of integrating elements of a xenogeneic genome with human hemopoietic stem cells without disturbing their functional activity [15].

The first attempt at the treatment of hereditary diseases by the method of gene therapy was made by Michael Blaese, who transplanted the adenosine aminase gene to leukocytes of two children with a deficiency of this enzyme. Disturbed function of the immune system was completely restored [15].

Thus, powerful new tools have emerged in modern hematology, making it possible to improve the methods of treatment of severe diseases. The spectrum of therapeutic procedures can be quite broad - from classical THFT, the efficacy of which is undeniable but which has certain limits, to the transplantation of intact fetal cells *in utero* or of genetically modified hemopoietic precursors. The latter offers real promise for the correction of the most debilitating hereditary diseases and for the cure of malignant neoplasms in the hemopoietic system.

It should be mentioned that the described strategy of treatment of hemopoietic diseases is not the only suitable one, at least in relation to the problem of histocompatibility. Another approach involves the use of HLA-identical biological material. In this case, there is a real possibility of attaining true chimerism of hemopoietic cells, since an immune conflict in the donor-recipient system is ruled out. The perfected methods of HLA typing and the choice of appropriate biomaterial (for instance, the increasing use of hemopoietic stem cells derived from placenta [13]) are contributing to the development of this approach. However, this demands the creation of an extensive bank of HLA-typed cells. In addition, there is the problem of typing transplant antigens which are not encompassed by the HLA system [4,6,11,12].

Advances in fetal cell allotransplantation and the reality of gene modification of autologous cells offer hope that studies in these two directions will lend a strong impetus to the correction of disorders in the hemopoietic system, irrespective of developments in classical transplantology, which is mainly aimed at solving the problem of histocompatibility between donor and recipient.

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