Immunological transformations in the recipient of grafted allogeneic human bone

Abstract Twenty-one patients received allogeneic human bone grafts following deep freezing according to various orthopaedic indications. The HLA antigens of all donors and recipients had been determined preoperatively, and grafting was performed without any respect to the HLA match. The immunological follow-up of the recipients was managed by two different methods: MLC (mixed lymphocyte culture) and MAILA (monoclonal antibody-specific immobilisation of lymphocyte antigens). No immunosuppression was performed. The follow-up lasted up to 6 years. Allogeneic grafting of human cancellous bone induces specific immunological reactions in the recipient. The consequences of these observations are: (1) allogeneic bone grafting may induce second-set reactions following subsequent blood transfusion, tissue grafting or organ transplantation; (2) transplantation of fresh, perfused, vascularised allogeneic bone or joint may become a therapeutic approach in the near future. Then the employment of standard immunosuppressive protocols will be mandatory in order to fight acute rejection of the graft.

Introduction

Allogeneic transplantation of human bone has been practised since the last century. There are different opinions regarding the immune reactions of the recipient against the allogeneic osseous grafts. Bone transplantation is performed without any histocompatibility matching and without subsequent immune suppression of the recipient. Our study centred on the antigenicity of the cryopreserved transplanted bone and the immunological reactions in the graft recipient. Different factors are investigated and discussed: underlying disease on transplantation, degree of HLA incompatibility between the donor and recipient, simultaneous blood transfusions, volume of the graft.

Materials and methods

Bone graft and donor cell procurement

Only multi-organ donors (MOD) were used (Fig. 1). After explantation of heart, liver, pancreas, kidneys and lungs, bone from the vertebral bodies and iliac crests was harvested. All donors were typed for HLA class I and class II antigens, serologically tested for human immunodeficiency virus (HIV) 1/2, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) and cytomegalovirus (CMV). Swabs were taken from the explantation site in order to exclude bacterial or fungal contamination. Positive test results for HIV 1/2, HBsAg and HCV as well as contaminated swabs were exclusion criteria for grafting the bone. Only positive test results for CMV were tolerated because the bone recipients received no drug immunosuppression. A small piece of the harvested bone was examined by pathology in order to exclude any generalized lymphoproliferative disease. During the perfusion phase of organ procurement, 1–2 l of donor’s blood was sampled using the central venous line and defibrinated with 1.0–2.0 ml heparin. Employing standard techniques (Ficoll-Isopaque gradient) donor lymphocytes were separated immediately following explantation and preserved at −196°C in liquid nitrogen for subsequent immunological monitoring. Thus, more than half a year after the donor’s death, vital donor-specific cells were available as target cells for a potential bone recipient. The vitality of all donor cells after unfreezing was tested employing the trypan blue method and estimated at better than 90%.

The bone banking technique and logistic described by the Boston group [10, 11] as modified by others [19, 37] was employed. Without any processing the material was frozen down to −80°C and stored for at least half a year. This quarantine time was necessary to await a second testing of HIV 1/2, HBsAg and HCV in the organ recipients from the same donor. This is the logistical management in our bone bank to reduce the risk of HIV or hepatitis B/C transmission to a minimum [14, 15, 17].

Allogeneic bone graft recipients

The underlying disease leading to bone loss, topographical localisation of the defects, amount of the grafted material, blood group and degree of donor-recipient HLA mismatch in 21 recipients are shown in Table 1.
The immunological follow-up is based on 20 of these patients. One patient (B.R., 30 years old, male) with a severe open luxation fracture of the proximal tibia and a combined vascular trauma (disruption of the popliteal artery) had to be excluded because of severe local infection complications.

For the recipients participation in this study was voluntary. Their HLA status had been determined preoperatively. Thus, all grafting procedures were performed electively. No emergency operations were included in this study. The donor-recipient combinations were planned to include all disparities in blood group and HLA match, only respecting the following criteria: availability of sufficient graft material from one donor; availability of enough donor lymphocytes and serum for complete immunological monitoring in the postoperative course; no grafting of material from a rhesus-positive donor to a young female rhesus-negative recipient. No immunological cross-match between donor cells and recipient serum was performed preoperatively.

Operative procedures were performed according to the AO/ASIF principles of operative fracture treatment [26]. A double-shot antibiotic prophylaxis was administered at the beginning and at the end of the surgical procedure. The clinical and radiological follow-up of the patients ranged from 3 to 71 months (mean 48.4 months).

Control group

Trauma patients (n = 42) undergoing comparable surgical procedures but without involving allogeneic bone grafting or blood transfusions served as a control group. Especially for the mixed lymphocyte culture (MLC), it was advisable to have two controls for every recipient in the study group to ascertain statistical genetic disparity.

Immunological monitoring

The immunological monitoring of the bone graft recipients was done with two methods: mixed lymphocyte culture (MLC) and monoclonal antibody-specific immobilisation of lymphocyte antigens (MAILA).

The MLC served as an in vitro model for the immunological in vivo mechanisms in the recipients of allogeneic bone grafts. Donor lymphocytes as the in vitro target cells express the same surface antigens (HLA class I/II) as the grafted bone cells. Therefore, the recipient's lymphocytes (R) reacted against donor lymphocytes (D) and against lymphocytes of two control persons (C), demonstrating stimulation or suppression of the recipient's specific immune response. Three MLCs were performed for every bone recipient (one day before operation, 14 days and 30 days following grafting) employing standard techniques as described in the literature [22].

The MAILA assay enables one to detect donor-specific alloantibodies (i.e., antibodies against defined HLA molecules) in the graft recipients. Even a differentiation of the alloantibodies against HLA class I (A, B, C) and HLA class II (DR, DP, DQ) is possible. The technique of this highly specific test has been described elsewhere [23–25]. It was performed 1 day before the operation and then at 2-day intervals until the 30th post-operative day and on days 60 and 90.

Results

Patients

The study is based on 20 completely monitored patients. Sixteen had defects following trauma, while in four patients different tumours were responsible for the defects. At the time of the last check (March 1995), 16 patients...