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Liver Transplantation-Mediated Transfer of Immunity: Accelerated Rejection of a Skin Graft from a Sensitized Donor

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CLINICAL BONE marrow transplantation requires complete ablation of the recipient's immune system by lethal total body irradiation and subsequent replacement by donor marrow. Reconstitution of the recipient's immune system happens within several weeks. Adoptive transfer of immunity against a variety of infectious antigens like parasites (Ancylostoma caninum),[1] bacteria (*Streptococcus pneumoniae*, *Pseudomonas aeruginosa*)[2,3] or different viruses (tetanus, herpes simplex, hepatitis B)[4,5,6] from bone marrow donor to recipient has been observed after experimental and clinical bone marrow transplantation. Shouvan and Ilan[7] demonstrated in a systematic study anti HBV-antibodies in a previously negative recipient after transplanting bone marrow from a previously immunized donor. In a recent case report[8] they showed even ablation of persistent hepatitis B virus infection through adoptive transfer of immunity from a hepatitis-immune donor to a hepatitis B patient.

In this study we wanted to examine whether donor immunity can also be transferred by a solid organ graft. As a model, we chose a spontaneously tolerant rat liver allograft model and sensitized the liver donor by a third party skin graft. The successful transfer of immunity was assessed by determination of skin graft survival and analysis of anti-skin donor specific antibodies.

MATERIALS AND METHODS

Animals

Male brown Norway (BN, RT1^n) rats, ACI (RT1^a) rats, and Lewis (Lew, RT1^l) rats weighing 180 to 230 g were used as liver and skin donors respectively. Male Lewis rats, weighing 200 to 250 g were used as recipients. All animals were obtained from Harlan-Winkelmann. The operative procedures were done under methoxyflurane anesthesia. Throughout the experiments the animals were maintained behind barriers under controlled environmental conditions. Animal housing and procedures were carried out according to the German Animal Welfare Legislation.

Experimental Design

All experiments were carried out in the spontaneous tolerant rat liver transplantation model BN-to-Lewis. The sensitization protocol consisted of giving an ACI skin graft to either donor or recipient 2 weeks prior to liver transplantation. One control group consisted of transplanting a liver from an untreated BN-donor to an untreated Lewis-recipient. In the other control group the liver of an untreated BN-donor was grafted into a ACI-skin sensitized Lewis recipient. In the experimental group, untreated Lewis recipients received liver grafts from previously ACI-skin sensitized BN-liver donor. Two weeks after liver transplantation the liver graft recipient was challenged with 3 test skin grafts of different donor strains. ACI-skin was grafted to test the sensitization status of the recipient, BN skin was given to demonstrate the induction of donor-specific tolerance after liver transplantation as a function of the recipient's immune system, and the Lewis skin graft was used as technical control for the skin graft procedure.

Liver Transplantation

Orthotopic whole liver transplantation was performed by the cuff technique without arterial reconstruction according to the widely accepted original protocol of tolerance induction.[8] The suprahepatic IVC was anastomosed first by a suture technique with 7–0 prolene. Next, the portal cuff of the donor was inserted into the recipient portal vein, and the clamps were removed. Anastomosis of the infrahepatic IVC was completed in the same way. The anhepatic procedure did not exceed 15 minutes. Bile duct anastomosis was done by insertion of the secured silicone rubber tube into the bile duct of the recipient. All recipients were weighed regularly and monitored by daily inspection.

Skin Transplantation

Full-thickness skin grafting was carried out as described by Billingham and Medawar.[9] Skin grafts were placed on the back or thorax of the recipient, fixed with 6 to 8 stitches and secured by a circular tape. Grafts were inspected daily after removal of the bandage between days 5 and 7 and scored as rejected on the first day of 90% epithelial necrosis.

Statistical Analysis

The significance of differences in graft survival time between the groups was assessed using the Mann–Whitney U test. The result was considered significant when the P value was below .05.

Histological Studies

Animals were sacrificed either when experiencing severe rejection or in case of tolerance induction after more than 150 days post liver

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transplant. Liver samples for histologic evaluation were formalin fixed and embedded in paraffin. Sections were cut at 4 μm and stained with hematoxylin and eosin. Each sample was coded and then evaluated in a masked fashion for infiltration by inflammatory cells, bile duct proliferation, necrosis, congestion, edema, and hemorrhage.

Complement-dependent Cytotoxicity Assay (CDC)

The levels of lymphocytoxic antibodies in liver graft recipients, sensitized recipients, and sensitized donors were determined by a two-stage microcytotoxicity assay as described by Terasaki.[10] Lymphocytes were obtained from cervical lymph nodes, adjusted to a concentration of 5 × 106 cells/mL and 1 μL was plated to each well of a Terasaki plate. After 30 minutes incubation of the target cell with the serial dilutions of serum from donor and recipient, guinea pig complement was added. They were incubated for 30 minutes at 37°C. The percentage of cells stained with trypan blue was calculated.

RESULTS

Transplantation of a liver graft from previously skin sensitized BN-liver donors to Lew-recipients led to accelerated rejection of ACI-test skin grafts (median of 10 d, n = 11) compared to a median of 13 days in the control group (n = 6; P = .004729). Similar results were achieved after recipient sensitization (median rejection time of the test skin graft 10.0 d, n = 6). BN-skin grafts used as marker for the tolerogenicity of the BN-liver graft were prolonged to the same extent, including permanent acceptance, in all three groups.

Histologic evaluation of the liver grafts showed no or only very little portal infiltrate after BN-Lew liver transplantation. Neither donor nor recipient sensitization affected graft survival and no signs of rejection were detected in either group.

Determination of the anti-ACI antibody titer of the BN-donor just before liver transplantation shows a titer of up to 1:131,072. Lew liver graft recipients never had an anti-ACI-titer at the time of the liver transplantation. As early as 1 week after liver transplantation, they developed an anti-ACI titer of up to 1:32 to 1:128. Similar levels were found in the second week after liver transplantation.

DISCUSSION

This is the first systematic approach to demonstrate donor-specific immune functions in a liver graft recipient most likely explained by transfer of donor derived lymphocytes.

Recipient sensitization led to significantly accelerated rejection of an ACI-test skin graft in 10 days (median) compared to a graft survival time of 13.5 days (median) in a nonsensitized recipient. The ACI test skin graft was significantly faster rejected after liver transplantation from a previously skin-sensitized donor to an untreated recipient (median graft survival time 10 days), indicating the transfer of a liver donor specific immune function to the liver graft recipient. Preliminary experiments using ACI-hearts as test grafts are pointing in the same direction.

In order to study the humoral immune response we measured the anti-ACI antibody titer in the recipients of a liver graft from a ACI-skin sensitized donor and we found a low but significant specific titer between 1 and 2 weeks after liver transplantation in a previously negative Lewis recipient.

In all 3 groups, BN-skin graft survival time was prolonged if not permanently accepted. Thus liver donor specific tolerance induction was not influenced by the sensitization procedure. Histologic examination of samples from long-term surviving animals after liver transplantation showed almost uniformly no infiltrate. The recipient-specific development of liver graft induced tolerance was not impaired; leading to observation of both, donor and recipient specific immune properties in the liver graft recipient, thus pointing towards merging of donor and recipients immune system.

The Lewis skin grafts were permanently accepted in all animals, demonstrating technical success of the skin graft procedure.

After clinical and experimental organ transplantation donor cell microchimerism was first described in 1992 by Starzl[11] and later repeatedly by different groups.[12,13,14] In a historic observation of Starzl's group in 1964[15] the association between the success of a transplantation and the adoptive transfer of cellular immunity in kidney transplant recipients has been described (1964).[16] A panel of skin test studies had been performed on kidney recipients and their living donors. The majority of the skin reactions that had been positive preoperatively in the donor but not in the patients crossed over the previously negative recipients, along with the transplanted kidney. When this did not occur, it meant that the kidney transplant had failed. Combining these two observations, the concept of microchimerism as a prerequisite of tolerance induction emerged and initiated many studies dealing with the relevance of microchimerism for tolerance induction and producing contradictory results.

Up until now, there has been no systematic examination of the functional relevance of these donor-derived cells in the recipient. The aim of this study was to test systematically whether donor's immune function can be transferred to the recipient by solid organ, that is, by liver transplantation. Adoptive transfer of a donor's immunity to the recipient by the means of bone marrow transplantation due to the resulting donor cell chimerism, has been recently demonstrated clinically and experimentally by Shouval and Ilan[11] in 1995.

In order to induce an immune status, which could be possibly transferred, the liver donor was sensitized by an ACI-skin graft and the liver recipient was tested for accelerated rejection of an ACI test skin graft. Significant acceleration of test skin graft rejection after both sensitization of either donor or recipient, indicates the successful liver transplantation-mediated transfer of immunity from liver donor to recipient. Acceleration of rejection can only be explained by transfer of viable lymphocytes including memory cells from the liver donor to the liver recipient.

Induction of liver donor specific tolerance in all 3 groups is interpreted as unimpaired function of the recipient's immune system. The animals in the experimental group are exhibiting functions of the donor's (accelerated ACI-skin graft rejection) and recipient's (prolonged BN-skin graft survival) immune system, thus pointing towards a merging of donor and recipient immune system as postulated by Starzl in 1993.[14]

Further studies are needed to clarify the relationship between organ acceptance and "take" of donor immune system. Little is known about the relevance of merging the 2 immune systems from donor and recipient. More detailed investigations are needed to evaluate the possible benefits of organ transplantation mediated transfer of immunity. One could envision using Hepatitis B-immune liver donors for recipients with Hepatitis B cirrhosis, thus treating end-stage liver disease with the transplantation of the parenchymal component and preventing recurrence by enhancing the anti-hepatitis immune response by the immune component of the graft.

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