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LETTER TO THE EDITOR

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No tolerance induction with cryopreserved bone marrow cells after allogeneic kidney transplantation and antilymphocyte globulin in rhesus monkeys

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Sir: Donor-specific tolerance without the need for chronic immunosuppression is the ultimate goal of organ transplantation. In one successful animal model, the infusion of fresh donor bone marrow cells after transplantation and after a course of antilymphocyte globulin (ALG) resulted in prolongation of graft survival [2, 3]. Also, in Rhesus monkeys treated with a 5-day course of antithymocyte globulin (ATG) and infusion of fresh donor bone marrow cells, long-term allograft survival was found [6]. The best graft survival in Rhesus monkeys was found when major histocompatibility complex (MHC) class II-depleted fresh bone marrow cells were infused; the mechanism of this improved survival is not known [7]. In 50 % of these animals, infusion of fresh donor bone marrow cells resulted in long-term graft survival (> 150 days).

In human cadaveric donor transplantation, no fresh bone marrow cells are available to infuse after a course of ALG. We wanted to find out if cryopreserved bone marrow cells could similarly prolong graft survival in this transplantation model.

Ten Rhesus monkeys received an allogeneic kidney graft (matched for one DR and, when possible, for one A and one B antigen), followed by a 5-day course of ALG (50 mg/kg body weight) subcutaneously (Fresenius, Oberursel, Germany). On day 5, five recipients received donor bone marrow cells (dosage $> 1 \times 10^7$ cells/kg body weight) that were cryopreserved after controlled freezing and MHC class II depletion with immunomagnetic beads (Advanced Magnetics, Cambridge, Mass., USA) after incubation with the monoclonal antibody L243 (Celltech, Berkshire, UK). The five monkeys in the control group did not receive donor bone marrow cells.

One monkey in the control group died of a technical complication (uremia due to urine leakage) and was not included in the analysis. Depletion of the bone marrow of MHC class II-positive cells was successful, and appropriate numbers of viable bone marrow cells were infused ($1.3–9.8 \times 10^7$/kg body weight) in the recipients of the experimental group. Facs analysis demonstrated CD2- and CD8-positive cells in the infused donor bone marrow. We found no prolongation of graft survival (median 20 days; 18 days in control group); all grafts were lost due to rejection.

The use of cryopreserved bone marrow cells in our study did not result in prolonged kidney graft survival compared with an ALG-treated control group. This failure to induce prolonged graft survival can not be explained by an insufficient number of (viable) infused bone marrow cells or by the infusion of the wrong type of cells. The number of infused bone marrow cells in the present study corresponded to the number of cells in other successful kidney transplant studies in Rhesus monkeys [6, 7]. Immunophenotyping of the infused bone marrow in our study showed CD2- and CD8-positive cells. This subset of bone marrow cells was thought to be responsible for the prolongation of graft survival in another ALG bone marrow study in Rhesus monkeys [7], so we assume that the right subset of bone marrow cells was infused. All our recipients shared at least one DR antigen with their donors, which should facilitate tolerance induction in the ALG/bone marrow cell infusion protocol [8].

The failure to induce tolerance might have been due to the cryopreservation of the bone marrow cells. The applied cryopreservation is suitable for bone marrow transplantation in Rhesus monkeys, but our data suggest that cryopreservation may be harmful for the (still uncharacterized subset of) tolerance-inducing cells. Another reason for not achieving tolerance in our study might have been the nature of the ALG. The ALG used in most animal experiments is not commercially available. We administered Fresenius rabbit ALG, which is made by immunization against T lymphoblasts. Because the lymphopenia in the blood of the recipients and the cytotoxicity of the serum at the moment of bone marrow infusion were comparable with those in the experiments of Thomas et al. [6], it is less likely that the use of this different ALG preparation explains the failure to induce tolerance in our study.

Ideally, we would like to have a second control group in which the recipients received fresh donor bone marrow after ALG. But because of the high costs of the experiment, we did not have the financial resources for this second control group.

Although the ALG/bone marrow protocol has been applied in human transplantation and microchimerism has been demonstrated in some

studies, so far no successful transplant survival has been reported without the need for chronic immunosuppression [1, 4, 5]. Before applying the ALG/bone marrow model to human organ transplantation, additional experiments need to be carried out to determine the efficacy of various methods of preservation of donor bone marrow cells in non-human primate models. In the human situation, it has yet to be determined whether the ALG/bone marrow model will lead to immunosuppression-free regimens after organ transplantation.

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