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Human-to-Baboon Bone Marrow Transplantation After Conditioning With Nonlethal Irradiation

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THE INCREASING shortage of human donors critically limits allogeneic transplantation as a treatment of choice for end-stage organ failure. As a result of this problem, there has been a resurgence of interest in xenotransplantation. It has been universally recognized that to make transplantation across discordant species a reality, many formidable physiological barriers have to be triumphed. On the contrary, nonhuman primates (eg, chimpanzee, baboon, etc), by sharing many physiologic and genetic characteristics with humans, offer a more reasonable alternative as donors for xenogeneic organ transplantation. These observations, however, did not translate favorably in clinical trials, for attempts at transplantation across concordant species were met with an undesirable outcome,¹⁻³ prompting many clinicians and researchers to look for or attempt to create a more suitable donor for future xenotransplantation.

Our group is interested in modifying the donor's immune system prior to organ transplantation, and as such an attempt was made to create humanized baboons by infusing human bone marrow cells into a sublethally irradiated donor (baboon). We report the incidence of chimerism and in vitro immune reactivity in human-to-baboon bone marrow chimeras. The use of such chimeric donors for future xenotransplantation is also discussed.

MATERIALS AND METHODS

Animals

Three healthy juvenile baboons (*Papio annubis*) were obtained from the Southwestern Primate Reserve, San Antonio, Texas and were housed in an American Association for Accreditation of Laboratory Animal Care (AAALAC) approved facility at the Montefiore Hospital, University of Pittsburgh. They were rigidly screened for viral and microbial infections before and after arrival at Pittsburgh. Under light anesthesia, two animals received total lymphoid irradiation, (TLI; 7.5 Gy) sparing the lungs, liver, head, and neck and appendages. One additional animal received total body irradiation (TBI; 7.5 Gy). Immediately after irradiation, 6×10^6 unmodified cryopreserved human cadaveric bone marrow cells/kg body weight were infused into each animal through an intravenous catheter placed in the femoral vein, and the vital signs were monitored for 2 to 3 hours after infusion. Human cadaveric vertebral bodies were used as a source of bone marrow, the isolation and cryopreservation of which is described elsewhere.⁴ Each animal also received prophylactic therapy against *Candida*, herpes, and gram-negative infections. The surviving animals were sacrificed 18 months after bone marrow infusion.

Posttransplant In Vitro Monitoring:

Detection of Chimerism. The presence of human cells in the baboon's peripheral blood mononuclear cells (PBMCs) was determined by flow cytometry using mouse-antihuman major histocompatibility complex (MHC) class I monoclonal antibodies (specific for the bone marrow donor). This analysis was carried out monthly for the first 6 months and every 6 months thereafter. Probes directed against the bone marrow donor's choriogonadotrophin gene (CG) were also used to detect chimerism by polymerase chain reaction (PCR), in the baboon's PBMC every 6 months posttransplant and additionally in all tissues at the time of sacrifice.

In Vitro Immune Testing. All in vitro immune testing was carried out at the time of sacrifice (18 months posttransplant). A constant dose of PBMCs obtained from the two surviving baboons was used as a responder in a one-way allo- and xenogeneic mixed lymphocyte reaction (MLR). For xenogeneic MLR, γ -irradiated cryopreserved human PBMCs (donor and third-party specific) were used. Controls included incubation of responders and stimulators in RPMI 1640 alone and coculture of syngeneic stimulators and responders. The cells were cultured for 5 days at 37°C in 5% CO₂ in air, and for the last 18 to 24 hours 1 μ Ci of [³H] thymidine was added per well. The cells were harvested onto glass filtermats and radioactive thymidine incorporation was determined by liquid scintillation counting.

Clonogenic Progenitor Cell Assays. Bone marrow was obtained from the baboons' vertebral bodies at the time of sacrifice, and 2×10^5 cells were cultured in each tissue culture plate (35 mm) containing Terry Fox medium for 14 days at 37°C in 5% CO₂ in air. At the end of this period, colonies of burst-forming-unit erythroid (BFU-E), colony-forming-unit granulocyte and macrophage (CFU-GM), and colony-forming-unit granulocyte, erythrocyte, macrophage, and megakaryocyte (CFU-GEMM) were individually scored and harvested for blind PCR detection of donor DNA.

RESULTS AND DISCUSSION

Although the infusion of bone marrow was uneventful in all baboons, the animal receiving TBI (n = 1) died within 14 days of irradiation due to aplastic marrow. Therefore, this

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animal will not be discussed any further. The other two animals that received sublethal TLI conditioning prior to human bone marrow transplantation survived for the duration of this study (18 months). Donor cells were not detectable by FACScan at any time after bone marrow infusion in the PBMCs of both animals. However, PCR detection of donor-specific CG gene yielded a positive result in the PBMCs of one animal for up to 18 months after bone marrow transplantation; whereas in the other animal, donor DNA was detectable during the first 6 months, which dwindled thereafter to an undetectable level.

The presence of donor cells was also analyzed by PCR in different baboon tissues which were harvested at the time of sacrifice. Donor DNA was present in the bone marrow and liver of both animals, whereas the other tissues are currently being analyzed. Clonogenic progenitor cell assays carried out on baboons' bone marrow harvested at the time of sacrifice produced colonies of BFU, CFU-GM, and CFU-GEMM in both animals which, when tested for the pres-

ence of donor DNA by PCR, yielded positive results. This suggests that the human bone marrow has fully engrafted. Because *in vitro* immune testing was only done at the time of sacrifice, it is rather difficult to extrapolate the results of this analysis. Nevertheless, both animals exhibited comparatively similar responses against the donor and third-party stimulators as well as against allogeneic cells.

The use of nonlethal irradiation followed by reconstitution with recipient-type bone marrow cells might offer a reasonable alternative to modify potential donors for future xenotransplantation.

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