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LONG-TERM DISCORDANT XENOGENEIC (PORCINE-TO-PRIMATE) BONE MARROW ENGRAFTMENT IN A MONKEY TREATED WITH PORCINE-SPECIFIC GROWTH FACTORS[1]

TOMASZ SABLINSKI,[2,3] DAVID W. EMERY,[2] ROD MONROY,[4] ROBERT J. HAWLEY,[4] YUANXIN XU,[4] PIERRE GIANELLO,[2] THOMAS LORF,[2] TOMASZ KOZLOWSKI,[2] MICHAEL BAILIN,[2] DAVID K.C. COOPER,[2] A. BENEDICT COSIMI,[2] AND DAVID H. SACHS[2,5]

Transplantation Biology Research Center and Transplant Unit, Department of Surgery, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts 02129, and BioTransplant, Inc., Boston, Massachusetts

Background. **Mixed allogeneic hematopoietic chimerism has previously been reliably achieved and shown to induce tolerance to fully MHC-mismatched allografts in mice and monkeys. However, the establishment of hematopoietic chimerism has been difficult to achieve in the discordant pig-to-primate xenogeneic model.**

Methods. **To address this issue, two cynomolgus monkeys were conditioned by whole body irradiation (total dose 300 cGy) 6 and 5 days before the infusion of pig bone marrow (BM). Monkey anti-pig natural antibodies were immunoadsorbed by extracorporeal perfusion of monkey blood through a pig liver, immediately before the intravenous infusion of porcine BM (day 0). Cyclosporine was administered for 4 weeks and 15-deoxyspergualin for 2 weeks. One monkey received recombinant pig cytokines (stem cell factor and interleukin 3) for 2 weeks, whereas the other received only saline as a control.**

Results. **Both monkeys recovered from pancytopenia within 4 weeks of whole body irradiation. Anti-pig IgM and IgG antibodies were successfully depleted by the liver perfusion but returned to pretreatment levels within 12–14 days. Methylcellulose colony assays at days 180 and 300 revealed that about 2% of the myeloid progenitors in the BM of the cytokine-treated recipient were of pig origin, whereas no chimerism was detected in the BM of the untreated control monkey at similar times. The chimeric animal was less responsive by mixed lymphocyte reaction to pig-specific stimulators than the control monkey and significantly hyporesponsive when compared with a monkey that had rejected a porcine kidney transplant.**

Conclusion. **To our knowledge, this is the first report of long-term survival of discordant xenogeneic BM in a primate recipient.**

The insufficient supply of donors organs is a major problem in organ transplantation today. Xenotransplantation is a possible solution to this increasing need. Miniature swine have several potential advantages as a donor species, including excellent breeding characteristics, a size similar to humans, and physiological similarities (*1, 2*). Pigs are, however, immunologically discordant to humans, and therefore several obstacles have to be surmounted before clinical xenotransplantation is likely to be successful.

Induction of donor-specific tolerance to swine xenoantigens by hematopoietic tissue transplantation would have significant advantages over chronic pharmacologic immunosuppressive therapy and is being investigated (*3*). The successful induction of mixed chimerism would allow graft acceptance while maintaining otherwise normal immune responses (*4*). Our laboratory has previously demonstrated that mixed lymphohematopoietic chimerism provides an effective means of inducing long-term tolerance to skin grafts across full MHC barriers in mice (*5, 6*) and, more recently, to kidney allografts in cynomolgus monkeys (*7*). The efficacy of the mixed chimerism approach in producing long-term survival of xenografts in concordant rat-to-mouse combinations has also been established (*5, 8*). Tolerance can also be achieved in the pig-to-mouse combination (*9*), but the additional transplantation of the porcine stromal environment is necessary for T-cell reconstitution in severe combined immunodeficient mice (*10*). However, this requirement may not be universal for all species combinations.

There are few data of survival of bone marrow (BM*) cells in a discordant xenogeneic environment. As the establishment and long-term survival of donor BM cells in the host is believed to be a prerequisite for achieving tolerance to a transplanted organ, the aim of the current study was to determine whether the engraftment of discordant xenogeneic BM could be achieved in the clinically relevant pig-to-monkey combination. We present here evidence of long-term hematopoietic engraftment in a monkey that received porcine BM and concomitant recombinant pig hematopoietic growth factors (stem cell factor [SCF] and interleukin [IL]-3).

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[2] Transplantation Biology Research Center and Transplant Unit, Department of Surgery, Massachusetts General Hospital/Harvard Medical School.

[3] Current affiliation: Novartis Pharmaceuticals, East Hanover, NJ.

[4] BioTransplant, Inc.

[5] Address correspondence to: David H. Sachs, MD, Transplantation Biology Research Center, Massachusetts General Hospital, MGH-East, Building 149-9019, 13th Street, Boston, MA 02129.

* Abbreviations: BM, bone marrow; FITC, fluorescein isothiocyanate; IL-3, interleukin 3; mAb, monoclonal antibody; MLR, mixed lymphocyte reaction; PBL, peripheral blood lymphocyte; PCR, polymerase chain reaction; SCF, stem cell factor.

MATERIALS AND METHODS

Animals. Male cynomolgus monkeys weighing 5–8 kg (Charles River Laboratories, Wilmington, MA) were used as recipients. MHC class I and class II inbred minipigs of two haplotypes (SLA aa and dd) and an outbred pig (Yucatan) served as donors of cells and/or organs (*11*). All surgical procedures and postoperative care of the animals were carried out in accordance with the *NIH Guide for Care and Use of Laboratory Animals* and approved by the Subcommittee for Research Animal Care of the Massachusetts General Hospital.

Two cynomolgus monkeys (M6593, experimental; and M6093, saline control) were followed long-term after porcine BM transplantation (Table 1). In addition, three monkeys (M4393, naive; M1294, sensitized to pig antigens after rejection of a porcine renal xenograft; and M1693, which had undergone a conditioning regimen followed by renal allografting) (Table 1) provided peripheral blood lymphocytes (PBLs) and sera for colony-forming unit (CFU) assays, mixed lymphocyte reaction (MLR), and monkey anti-pig antibody assays.

Monkey conditioning and immunosuppression. Whole body irradiation (300 cGy) from a ^{60}Cobalt source was administered in two fractions of 100 cGy on day 6 and one fraction of 100 cGy on day 5. Cyclosporine (Sandimmune; Novartis, Basel, Switzerland) was administered intravenously from days 0 to 27 at a dose of 15 mg/kg/day, and 15-deoxyspergualin was administered intravenously from days 0 to 13 at a dose of 6 mg/kg/day.

Depletion of monkey anti-pig antibodies. The pig liver used for hemoperfusion/immunoadsorption was isolated through a midline incision, perfused with cold Ringer's lactate until pale, excised, and maintained at 4°C. Monkeys were anesthetized as previously described (*12*) and central venous and arterial cannulae were placed. Splenectomy was performed immediately before pig liver hemoperfusion. The monkey's blood was perfused for 60 min through the pig liver to remove anti-pig antibodies, as previously described (*12*). The antibody level was measured before and after liver hemoperfusion and then daily.

Measurement of monkey anti-pig antibodies. Serum titers of monkey anti-pig IgG and IgM antibodies were measured by flow cytometry. Briefly, freshly isolated pig PBLs were incubated for 45 min with monkey plasma at 4°C, washed twice, and incubated for an additional 45 min with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human IgG or IgM monoclonal antibody (mAb; Zymed, San Francisco, CA). Phenotyping of monkey blood for detection of pig cells was performed using the following mAbs: (i) 36–7-5 (mouse anti-mouse) (class I K^k) IgG2b and 12–2-2 (mouse anti-mouse class I K^k IgM) as isotypic controls, (ii) W6/32 (mouse anti-human class I MHC) cross-reactive with 100% monkey PBLs, and (iii) 1030H-1–19 (mouse anti-pig leukocyte, IgM) as a sensitive detector of a low number of pig cells in monkey PBLs. Goat anti-mouse-FITC (Zymed) was used as a secondary reagent. Flow cytometry was performed

TABLE 1. Experimental design

Monkey	Induction therapy[a]	Liver hemoperfusion (anti-pig antibody immunoadsorption)	Porcine bone marrow administered (number of cells×10^8/kg)	Porcine growth factors administered (SCF and IL-3 10 μg/kg/day)	Porcine cell survival (days)[b]
M6093	WBI	Yes	2.3	No	<208
M6593	WBI	Yes	2.5	Yes	>300
M1693[c]	WBI, TI, ATG	No	—	—	—
M1294[d]	—	Yes	—	—	—
M4393[e]	—	No	—	—	—

[a] Abbreviations used in table: ATG, horse anti-human thymocyte globulin 50 mg/kg/day i.v. on three consecutive days (−2, −1, and 0); TI, thymic irradiation of 700 cGy on day −1; WBI, whole body irradiation of 300 cGy total dose in three fractions on days −6 and −5.
[b] Detectable by PCR.
[c] Allogeneic bone marrow and renal transplant recipient.
[d] Porcine renal xenograft recipient, rejected on day 7.
[e] Naive unmodified control.

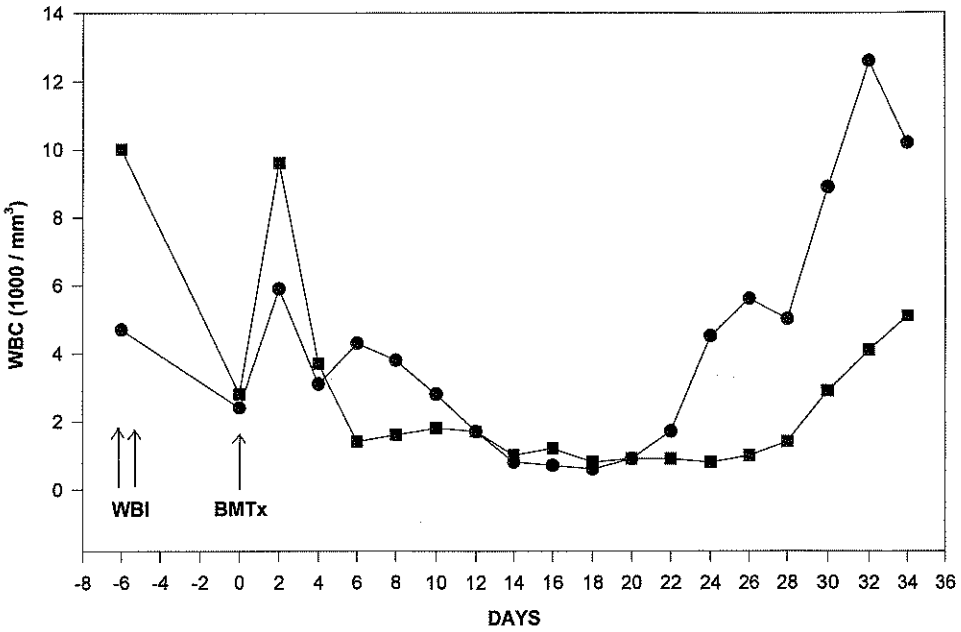

FIGURE 1. BM reconstitution (as documented by total white blood cell counts in the peripheral blood) in M6593 (●) and M6093 (■).

using a Becton Dickinson FACScan, and data were analyzed with Lysis II software (Becton Dickinson, San Jose, CA).

Porcine BM transplantation. BM was harvested from the donor pig as previously described (*13*). Immediately after processing, the BM was infused on day 0 approximately 3 hr after completion of liver hemoperfusion at doses of 2.3 and 2.5×10^8/cells/kg, respectively, in the two monkeys (M6093 and M6593).

Porcine growth factors. Two recombinant pig cytokines—SCF and IL-3 (*14, 15*)—were administered, each at a dose of 10 μg/kg/day by continuous intravenous infusion, except for short periods when other medications were delivered through the same intravenous line.

Progenitor colony assay. Using an established colony assay protocol (*16*), BM cells were suspended in plating medium consisting of Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY), 30% heat-inactivated defined fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, 10^{-4} M β-mercaptoethanol (Sigma, St. Louis, MO), antibiotics, 0.8% methylcellulose (Terry Fox Laboratory Media Preparation Service, Vancouver, BC, Canada), and cytokines. This preparation was then dispensed into 35-mm suspension culture plates in duplicate at 1.1 ml per plate, and incubated at 37°C. To estimate the frequency of pig progenitor chimerism, parallel cultures were supplemented with either recombinant swine IL-3, which is species specific (*17*), or the recombinant human IL-3/granulocyte macrophage-colony stimulating factor fusion cytokine PIXY321 (Immunex, Seattle, WA), which induces colony formation by both swine and primate progenitors (*17*). After 12–16 days, colonies were scored microscopically. To confirm the species origin of colonies grown under pig IL-3, colonies were picked and collected in normal saline either individually (for definitive colonies, ≥500 cells) or as pools (abortive colonies, ~100 cells each). After pelleting, DNA was prepared by the addition of 40 μl of lysis buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.45% Tween-20, 0.45% NP-40, 100 μg/ml proteinase K) followed by digestion for 60 min at 55°C. The proteinase K was denatured by an additional 10-min incubation at 100°C. Amplification was then carried out using Ampliwax beads (Perkin-Elmer, Norwalk, CT), as described by the manufacturer, and primers specific for the pig IL-3 gene. The final reaction mixtures contained 25 μl of DNA in 100 μl of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 100 μM each dNTPs, 3 mM $MgCl_2$, and 2.5 U of Amplitaq polymerase. The forward primer C1-F (5′-GAGCCAATCTCTATTGAGGAGG) and the reverse primer C1-R (5′-CAATTCAACAGAC TCCACAGG), both derived from the pig IL-3 gene (*14, 15*), were used at 200 nM. After 33 cycles of amplification, the reaction products were separated by gel electrophoresis, Southern blotted, and hybridized to an internal oligonucleotide probe for positive identification of the pig IL-3 gene product. All polymerase chain reactions (PCR) also contained 10 copies of an internal positive control template, comprised of the target pig IL-3 sequence containing a small insertion, to verify proper amplification in each sample.

Flow cytometry for detection of porcine cells. The method used involved two-color analysis to simultaneously detect pig and primate cells. Briefly, Peripheral blood or BM cells ($1–2\times10^5$) were separated by Ficoll density gradient and incubated with an anti-pig mAb (H-1–19, IgM isotype) that detects all pig cells and an FITC-conjugated anti-human class I mAb (W6/32; Harlan Sera-Lab, Sussex, England) for 30 min at 4°C. The cells were washed in phosphate-buffered saline/0.1% bovine serum albumin/0.1% azide, pelleted, and incubated with phycoerythrin-conjugated goat anti-mouse IgM (Southern Biotechnology Associates, Birmingham, AL) for 30 min at 4°C. The cells were washed again, resuspended in phosphate-buffered saline/bovine serum albumin/azide, and analyzed using the Becton Dickinson FACScan flow cytometer running Lysis II software with propidium iodide gating for dead cell exclusion.

MLR. After isolation of mononuclear cells from the peripheral blood, the cultures were prepared in 96-well plates (Costar, Cambridge, MA) with each well containing 4×10^5 responders and 4×10^5 irradiated (25 Gy) stimulators in 0.2 ml of AIM-V media (Gibco). Cultures were incubated for 6 days at 37°C in 8% CO_2 and 100% humidity, and were pulsed with [^{3}H]thymidine for the final 18 hr of incubation. Cultures were harvested with the Betaplate system (LKB-Wallac, Turku, Finland), and proliferation was assayed by the incorporation of [^{3}H]thymidine. Stimulation ratios were calculated by the equation:

$$\text{Stimulation ratio} = \frac{\text{Recipient anti-experimental stimulator response (cpm)}}{\text{Recipient anti-autologous stimulator response (cpm)}} \times 100$$

Each combination of cells was prepared in triplicate.

RESULTS

Porcine BM reconstitution. The two monkeys remained in good general condition, although both developed some peripheral edema, probably related to hypoalbuminemia. The white blood count reached a nadir of 0.4 and 0.7 thousand cells/mm^3 on days 18 and 19 in M6593 and M6093, respectively, and began to increase again shortly thereafter (Fig. 1). Recovery to the level of 1000 cells/mm^3 occurred on days 22 and 26, respectively. Both animals required several transfusions of packed red blood cells to maintain hematocrits >20% during the first 3–4 weeks. Similarly, thrombocytopenia necessitated two infusions of platelets in each monkey.

Monkey anti-pig antibody levels and the secondary antibody response. Sixty minutes of perfusion of monkey blood through a pig liver effectively depleted monkey anti-pig antibodies, as previously described (*12*). Anti-pig IgM and IgG returned to pretreatment levels between days 6 and 24 in both animals and remained at these levels for several months of follow-up (data not shown). The efficiency of extracorporeal liver perfusion in this model has been described previously in detail (*12*).

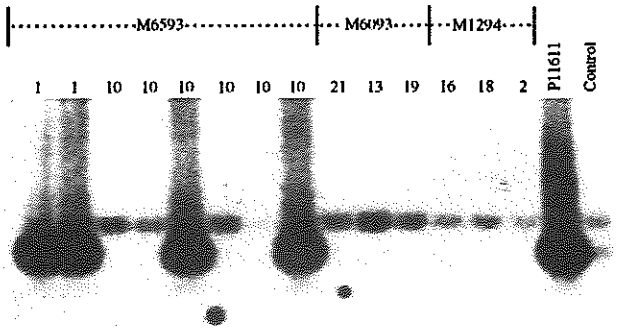

FIGURE 2. PCR identification of pig progenitor colonies. BM aspirates were obtained from M6593 (postoperative day 302; treated with pig cytokines), M6093 (postoperative 330, not treated with pig cytokines), and M1294 (untreated control) and plated in methylcellulose progenitor colony cultures with pig IL-3 (pig specific) or PIXY321 (pan specific). After 12–16 days, colonies were picked either as single large definitive colonies (typically >500 cells) or pools of smaller abortive colonies and assayed for the presence of the pig IL-3 gene by PCR using species-specific primers followed by gel electrophoresis, Southern blotting, and hybridization to a species-specific IL-3 oligonucleotide probe. The number or colonies per assay pool is indicated above each line. P11611 indicates PCR products from one large colony from the BM of this pig. Control indicates PCR product from 10 cell equivalents of purified pig DNA. All reactions contained a positive internal control template, which is amplified and detected by the same PCR and hybridization primers used to detect the authentic pig IL-3 gene (Int. Con.-upper band in each lane).

TABLE 2. Results of PCR for pig DNA in M6953 and M6093

Monkey	Day posttransplantation	Cytokine	Total cells plated ($\times 10^4$)	Total colonies[a]	Chimerism[b]
M6953	180	PIXY321	14	105	
		Pig IL-3	14	2	1.9%
	302	PIXY321	14	66	
		Pig IL-3	42	4	2.0%
M6093	208	PIXY321	14	78	
		Pig IL-3	14	0	ND[c]
	330	PIXY321	14	68	
		Pig IL-3	42	0	ND

[a] Only PCR-positive colonies reported for pig IL-3 cultures.
[b] Chimerism=(number of colonies/10^4 plated in pig IL-3)/(number of colonies/10^4 plated in PIXY321).
[c] ND, not detectable (limit of sensitivity assumes <1 colony detected).

TABLE 3. Results of MLR[a]

Responders	Stimulator 1 (SLA aa)	Stimulator 2 (SLA dd)	Stimulator 3 (YUC)	Stimulator 4 (human)
M6093	22.9	2.5	8.5	170
M6593	1.3	1.1	1.6	12
M1693[b]	19.0	1.4	10.3	120
M1294[c]	64.5	6.0	44.8	39
M4393[d]	17.8	3.0	9.8	346

[a] MLR responses are presented as stimulator indices.
[b] Allogeneic bone marrow and renal transplant recipient.
[c] Porcine renal xenograft recipient, rejected on day 7.
[d] Naive unmodified control.

Detection of chimerism. Using the mAb H-1–19 and flow cytometry, we did not observe the presence of cells expressing the cognate pig antigen in the peripheral blood or BM of either monkey. To detect chimerism at a more primitive hematopoietic level, we screened BM aspirates using progenitor colony assays and cytokines specific for both primates and pig (PIXY321) or just pig (recombinant pig IL-3) (*17*). Because there was some abortive colony formation by monkey BM in the pig IL-3 cultures, we independently confirmed the species origin of all of the colonies grown under pig IL-3 by PCR (for example, see Fig. 2). As outlined in Table 2, about 2% of the BM progenitors in recipient M6953 were of swine origin at 180 and 300 days after transplantation, whereas no swine progenitors were detected in the marrow of the control (no cytokine) animal at 208 and 330 days after transplantation (with an estimated limit of sensitivity of about 1.3% and 0.5%, respectively).

Cellular responses. Pretransplantation MLR was not performed in these studies, although previous studies have generally demonstrated a strong response in the pig-to-baboon combination. Posttransplantation MLR revealed hyporesponsiveness of M6593 to pig stimulators when compared to the response of M6093, to that of a naive monkey (M4393) and to that of an additional monkey that underwent similar conditioning and immunosuppression (M1693) (Table 3). The alloresponses on 6593 and 6093 both remain comparable to those of naive monkeys (with stimulation indices of 30–100-fold). These observations suggest functional hyporesponsiveness. In three separate MLR assays performed at 200, 250, and 270 days after BM transplantation, the responses of M6593 were lower to two of three pig haplotypes tested than were those of M6093. MLR against a different xenogeneic target (human) (Table 3) and allogeneic targets (data not shown) remained relatively strong in all animals, suggesting specific hyporesponsiveness to porcine antigen(s).

DISCUSSION

To our knowledge, this is the first evidence of long-term survival of BM in vivo in this discordant xenogeneic combination, and it encourages us in our goal of achieving tolerance induction through chimerism. Although we are fully aware of the very small number of animals in this study, certain aspects of the regimen that led to this result are worthy of comment.

The nonmyeloablative conditioning regimen used in this and our previously described studies (*12*) causes substantial aplasia and necessitates blood product support. Recently published reports indicate the possibility of achieving engraftment of allogeneic BM in hosts not receiving myelosuppressive conditioning (*18, 19*). Extrapolation of such protocols to xenogeneic BM transplantation would clearly make this approach more attractive for clinical application.

Extracorporeal liver hemoperfusion has prevented or delayed hyperacute rejection of pig kidneys in over 20 monkeys studied at our center (*12*). The side effects of liver hemoperfusion include significant trapping of red blood cells and platelets, nonspecific loss of proteins, and transient hemodynamic instability. The safety of this approach is confirmed, however, by limited but well-documented clinical studies in which the approach of extracorporeal pig liver hemoperfusion has been used to support patients in fulminant hepatic failure (*20, 21*). However, we have recently changed to the use of immunoaffinity columns of synthetic Galα1–3Gal oligosaccharides instead of pig livers, with equal efficiency (*22, 23*).

The induction of T-cell tolerance by the mixed chimerism approach may also result in the induction of tolerance at the

humoral level (*24*). Because anti-pig antibodies returned after pig liver hemoperfusion in M6593, higher levels of chimerism may be needed to achieve such B-cell tolerance. The return of antibody may be associated with the absence of chimerism in the blood of this animal. In addition to an insufficient level of chimerism, the lack of humoral tolerance may be related to the presence of "memory" B or plasma cells. 15-Deoxyspergualin, which has been used to prevent antibody-mediated rejection in patients with ABO-incompatible kidney transplants (*25*), is currently being tested in many experimental xenotransplantation models to suppress the rebound of xenoreactive antibodies previously depleted from the circulation (*26*). It is difficult to assess its effectiveness in the current study. Splenectomy is believed to disturb the proliferative response of circulating B lymphocytes (*27*) and is widely used in experimental models of xenotransplantation and in ABO-incompatible kidney transplantation (*28*), although no adequate control data are available. Splenectomy was included in our own protocol based on observations that it results in decreased levels of circulating IgM (*26*) and in an approximate 60% reduction in anti-pig antibodies in baboons (*23*).

The survival of porcine progenitor cells in the BM in the presence of anti-pig antibodies is intriguing. Possible explanations include a low immunogenicity of progenitors (with possibly low expression of αGal), although in vitro studies do not suggest this is so (D. Emery, unpublished data). Accommodation could take place within the BM, the progenitor cells may be "sequestered" in the BM, or the BM may act as an immunoprivileged site. This latter possibility may also explain the absence of pig cells in the blood. Pig cells leaving the BM may be susceptible to destruction by anti-αGal antibodies. Alternatively, it could be that the pig progenitors, although able to expand and differentiate to form colonies within the BM, proliferate inadequately in the blood in the absence of the long-term presence of pig-specific cytokines.

Using BM progenitor colony assays and conformational PCR, we were able to document hematopoietic chimerism in the cytokine-treated recipient at 180 and 300 days after transplantation but not in the untreated control animal. Although only 2% chimerism was detected in this animal (M6593), this level was consistently above the limit of detection, which we have estimated to be 1.3%. However, the relatively small difference between the limit of detection and the detected level makes it difficult to assess the true difference in chimerism between this animal and the control animal (M6093). To estimate the frequency of this chimerism, we used the human IL-3/granulocyte macrophage-colony stimulating factor fusion molecule PIXY321 to induce colony formation by progenitors of both swine and monkey origin, and pig IL-3 to preferentially induce colony formation by pig progenitors alone. Previous studies demonstrated that PIXY321 induces progenitor colony formation by pig progenitors essentially as well as pig IL-3 (*17*), and is known to be highly active among primates. Nevertheless, there may be subtle differences in the activities of these cytokines on the progenitors of the two species used in this study, so that the degree of chimerism measured here (1.9–2.0%) should only be considered an estimate. Chimerism at the level of mature hematopoietic cells was not observed in the peripheral blood or BM of either recipient based on flow cytometry with the mAb H-1–19, which is highly specific for an epitope on all pig blood cells. This may either be due to the limits of detection in this assay or to the lack of terminal maturation of the pig myeloid progenitors in the absence of a continual source of species-specific cytokines such as IL-3 or SCF. Precedence for this latter possibility comes from human-to-severe combined immunodeficient mouse BM transplantation studies in which peripheral chimerism could only be achieved after spiking the recipients with species-specific cytokines such as PIXY321 or SCF (*29*).

The results of MLR would suggest that (i) infusion of the pig BM did not cause an increase in the response to stimulation by porcine cells, and (ii) hyporesponsiveness to pig antigens in the chimeric monkey (M6593) might be related to the long-term presence of pig cells in the BM of the host. It cannot be excluded that exposure to the donor's antigens in the form of infused BM is sufficient to cause hyporesponsiveness in MLR by a mechanism similar to that of donor-specific blood transfusion (*30*). The interpretation of MLR should be made in the context of recent findings suggesting nondefective recognition of xenogeneic antigens (*31*), and indicating very strong human anti-pig MLR reactivity (*32*). The fact that anti-pig responses in M6593 were drastically diminished is, therefore, encouraging.

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REFERENCES

1. Sachs DH. The pig as a potential xenograft donor. Vet Immunol Immunopathol 1994; 43: 185.
2. Kirkman RL. Of swine and men: organ physiology in different species. In: Hardy MA, ed. Xenograft 25. Amsterdam: Excerpta Medica, 1989: 125.
3. Sachs DH, Sablinski T. Tolerance across discordant xenogeneic barriers. Xenotransplantation 1995; 2: 234.
4. Zinkernagel RM, Althage A, Callahan G, Welsh RM Jr. On the immunocompetence of H-2 incompatible irradiation bone marrow chimeras. J Immunol 1980; 124: 2356.
5. Ildstad ST, Sachs DH. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. Nature 1984; 307: 168.
6. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a nonlethal preparative regimen. J Exp Med 1989; 169: 493.
7. Kawai T, Cosimi AB, Colvin RB, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. Transplantation 1995; 59: 256.
8. Sharabi Y, Asksentijevich I, Sundt TM III, Sachs DH, Sykes M. Specific tolerance induction across a xenogeneic barrier: production of mixed rat/mouse lymphohematopoietic chimeras using a nonlethal preparative regimen. J Exp Med 1990; 172: 195.
9. Lee LA, Gritsch HA, Sergio JJ, Arn JS, et al. Xenogeneic swine thymus grafts generate functional murine T cells that are tolerant of pig antigens. Proc Natl Acad Sci USA 1994; 91: 10864.
10. Gritsch HA, Glaser RM, Emery DW, et al. The importance of non-immune factors in reconstitution by discordant xenogeneic hematopoietic cells. Transplantation 1994; 57: 906.
11. Sachs DH. MHC homozygous miniature swine. In: Swindle MM, Moody DC, Philips LD, eds. Swine as models in biomedical research. Ames: Iowa State University Press, 1992: 3.
12. Sablinski T, Latinne D, Gianello P, et al. Xenotransplantation of

pig kidneys to nonhuman primates. I. Development of the model. Xenotransplantation 1995; 2: 264.
13. Pennington LR, Sakamoto K, Popitz-Bergez FA, et al. Bone marrow transplantation in miniature swine. I. Development of the model. Transplantation 1988; 45: 21.
14. Hawley RJ, Abraham S, Akiyoshi DE, et al. Xenogeneic bone marrow transplantation. I. Cloning, expression, and species specificity of porcine IL-3 and granulocyte-macrophage colony stimulating factor. Xenotransplantation 1997; 4: 103.
15. Giovino MA, Hawley RJ, Dickerson MW, et al. Xenogeneic bone marrow transplantation. II. Porcine-specific growth factors enhance porcine bone marrow engraftment in an in vitro primate microenvironment. Xenotransplantation 1997; 4: 112.
16. Emery DW, Shafer GE, Karson EM, Sachs DH, LeGuern C. Retrovirus-mediated transfer and expression of an allogeneic major histocompatibility complex class II DRB cDNA in swine bone marrow cultures. Blood 1993; 81: 2460.
17. Emery DW, Sablinski T, Shimada H, et al. Expression of an allogeneic MHC DRB transgene, through retroviral transduction of bone marrow, induces specific reduction of alloreactivity. Transplantation 1997; 64: 1414.
18. Ramshaw HS, Rao SS, Crittenden RB, Peters SO, Weier HU, Quesenberry PJ. Engraftment of bone marrow cells into normal unprepared hosts: effects of 5-fluorouracil and cell cycle status. Blood 1995; 86: 294.
19. Sykes M, Szot GL, Swenson K, Pearson DA. Induction of high levels of allogeneic hematopoietic reconstitution and donor-specific tolerance without myelosuppressive conditioning. Nat Med 1997; 3: 783.
20. Norman JC, Brown ME, Saravis CA, Ackyroyd FW, McDermott WV. Perfusion techniques in temporary human-isolated ex vivo porcine liver cross circulation: possible adjunct in the treatment of hepatic failure. Surgery 1966; 6: 121.
21. Collins BH, Chari RS, Magee JC, et al. Mechanisms of injury in porcine livers perfused with blood of patients with fulminant hepatic failure. Transplantation 1994; 58: 1162.
22. Xu Y, Lorf T, Sablinski T, et al. Removal of anti-porcine natural antibodies from human and non-human primate plasma in vitro and in vivo by a Galα 1–3Gal β1–4 βGlc-X immunoaffinity column. Transplantation 1998; 65: 172.
23. Kozlowski T, Ierino FL, Fuchimoto Y, et al. Depletion of anti-Gal α1–3Gal antibody in baboons by specific αGal immunoaffinity columns. Xenotransplantation 1998; 5: 122.
24. Aksentijevich I, Sachs DH, Sykes M. Humoral tolerance in xenogeneic BMT recipients conditioned with a non-myeloablative regimen. Transplantation 1992; 53: 1108.
25. Takahashi K, Tanabe K, Ooba S, et al. Prophylactic use of a new immunosuppressive agent, deoxyspergualin, in patients with kidney transplantation from ABO-incompatible or preformed antibody-positive donors. Transplant Proc 1991; 23: 1078.
26. Leventhal JR, Flores HC, Gruber SA, et al. Evidence that 15-deoxyspergualin inhibits natural antibody production but fails to prevent hyperacute rejection in a discordant xenograft model. Transplantation 1992; 54: 26.
27. Foster PN, Trejdosiewicz LK. Impaired proliferative response of peripheral blood B cells from splenectomized subjects to phorbol ester and ionophore. Clin Exp Immunol 1992; 89: 369.
28. Alexandre GPJ, Squifflet JP, De Bruyere M, et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. Transplant Proc 1987; 19: 4538.
29. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. Science 1988; 242: 1706.
30. Fabre JW, Morris PJ. The effect of donor strain blood pretreatment on renal allograft rejection in rats. Transplantation 1972; 14: 634.
31. Alter BI, Bach FH. Cellular basis of the proliferative response of human T cells to mouse xenoantigens. J Exp Med 1990; 171: 333.
32. Yamada K, Sachs DH, DerSimonian H. The human anti-porcine xenogeneic T-cell response: evidence for allelic specificity of MLR and for both direct and indirect pathways of recognition. J Immunol 1995; 155: 5249.

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