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Hematopoietic chimerism following allotransplantation of the spleen, splenocytes or kidney in pigs

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ABSTRACT

Background: Mixed chimerism is associated with donor-specific tolerance. Spleen or splenocyte allotransplantation (Tx) is recognized as potentially tolerogenic. There is no definitive report comparing chimerism levels following spleen and splenocyte Tx in a large animal model. We have compared chimerism after spleen, splenocyte, or kidney Tx in pigs.
Methods: Outbred (n = 5) and MHC-defined miniature (n = 1) pigs underwent orthotopic spleen Tx. Outbred pigs received splenocytes through a systemic vein (n = 1) or the portal vein (n = 3). Kidney Tx (n = 2) or concomitant Tx of spleen + kidney (n = 2) was carried out. All except one recipient pigs were irradiated (700 cGy thymic and 100–125 cGy whole body) on day − 2. Cyclosporine or tacrolimus was administered for 42 days. All donors were males and all recipients were females; chimerism in the blood was determined by Quantification-PCR for the donor Y chromosome. Mixed lymphocyte reaction (MLR) was performed before and after Tx.
Results: One week after spleen Tx in outbred and MHC-defined pigs, chimerism ranged between 0.8 and 22.5%, and 5.4–20.1%, respectively, and remained between 17.7 and 67.4%, and 2.2–7.4%, respectively, until day 28. One week after splenocyte Tx, chimerism ranged between 0.1 and 8.5%, and decreased to 0.1–0.8% at 3–4 weeks. There was no detectable chimerism 14 days after kidney Tx. The response on MLR of all recipient pigs to donor cells was decreased after Tx, except in one case of splenocyte Tx, indicating that this pig might have become sensitized. After discontinuation of immunosuppression, most isolated spleen or kidney grafts were not rejected, but the kidney was rejected after concomitant spleen + kidney Tx.
Conclusions: There was a significantly higher level of blood chimerism following spleen Tx compared to splenocyte or kidney Tx. However, concomitant Tx of spleen + kidney may be associated with accelerated kidney graft rejection.

1. Introduction

Immunological tolerance would eliminate the need for, and avoid complications of, chronic immunosuppressive drug therapy [1]. Successful spleen allotransplantation (Tx) in rodents can result in specific tolerance toward donor-matched organ allografts in the absence of exogenous immunosuppressive therapy [2]. These studies indicated that, following spleen Tx, there exists a delicate balance between the donor and recipient immune responses that, if manipulated properly, can lead to a state of tolerance. The potential of a spleen to reconstitute the bone marrow of a lethally-irradiated animal (in the absence of bone marrow Tx) has also been demonstrated [3], providing evidence of its potential to result in hematopoietic cell chimerism under the correct circumstances.

In humans, a spleen allograft in an immunosuppressed host can cause graft-versus-host disease (GVHD) [4–7]. This observation indicates the spleen's potential in counteracting the host's immune response to obtain balanced immunity between donor and recipient that may result in a chimeric and/or tolerogenic state.

Rejection of organ allografts is currently prevented by chronic non-specific immunosuppressive therapy. This therapy must balance the risks of immunosuppression (e.g., infection, lymphoproliferative disease, and other drug-related complications) against those of rejection. Induction of specific Tx tolerance across complete MHC barriers would permit organ Tx from deceased or living donors without the need for long-term immunosuppression [1,8]. Since there are differences in the response to vascularized organ allografts between small and large

Abbreviations: GVHD, graft-versus-host disease; MHC, major histocompatibility complex; MLR, mixed leukocyte reaction; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; Tx, transplantation; WBI, whole body irradiation.

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animal species with regard to tolerance induction [9,10], if a tolerance-inducing protocol is to be practicable for human organ Tx, then it must at least be successful in a large animal model across a full MHC barrier. From Dor's initial studies, it would seem that spleen Tx is much less likely to induce GVHD than is bone marrow Tx [11,12]. Spleen Tx, therefore, offers potential as an approach to the induction of allograft tolerance in the clinic.

Dor, Gollackner and their colleagues have previously reported that heterotopic spleen Tx in miniature swine is technically feasible [13], and that, in appropriate models, it results in hematopoietic cell chimerism and tolerance to the transplanted spleen [13]. Pigs with functioning spleen grafts had multi-lineage chimerism in blood, thymus, and bone marrow for >6 months, without GVHD, and developed in vitro donor-specific hyporesponsiveness. The chimerism results because the spleen in adult pigs and primates has been demonstrated to be a relatively rich source of very primitive hematopoietic progenitor cells [14]. In two pigs tolerant to the spleen graft, subsequent donor MHC-matched kidney grafts survived for >4 and >7 months in the absence of exogenous immunosuppression. In contrast, in two asplenic pigs, kidney grafts were rejected on days 4 and 15 [11].

We have continued these studies by measuring the extent of hematopoietic cell chimerism after spleen or kidney Tx in pigs. Furthermore, if spleen Tx is to play any role when organs are to be procured from a deceased donor, we have investigated the clinical outcome after *concomitant* Tx (rather than the *sequential* Tx) of the spleen and kidney. We have also investigated the effect on the immune system of the intravenous (i.v.) infusion of donor splenocytes.

2. Materials and methods

2.1. Animals

Spleen, splenocyte, and kidney donors (n = 17) and recipients (n = 17) were selected from either (i) outbred Landrace large white pigs (n = 24; Wally Whippo, Enron, PA), or (ii) a herd of specific pathogen-free partially-inbred, MHC-defined Yucatan miniature swine (n = 10; Sinclair, St. Louis, MO). Donors and recipients were matched for weight (approximately 20–25 kg). The Yucatan miniature swine donors and recipients were reported to be fully-mismatched for MHC class I and class II antigens [15].

All animal care procedures were in accordance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86–23, revised 1996). All protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee (Protocol #0409338-2).

2.2. Surgical procedures

All recipient pigs had catheters inserted into jugular veins for blood draw and drug infusion [16]. Procurement of the spleens and kidneys was by standard techniques [13,16]. The donor spleen was procured with a Carrel patch of aorta for ex vivo perfusion using histidine–tryptophan–ketoglutarate (HTK) solution (Essential Pharmaceuticals, Newtown, PA) (Fig. 1A). Heterotopic spleen Tx was carried out as previously described [11,13]. In addition, in some cases orthotopic (rather than heterotopic) spleen Tx was carried out, as it was hypothesized that this approach might be beneficial in that leukocytes migrating from the donor spleen would initially pass through the recipient liver, where they are possibly more likely to induce a state of tolerance [17]. This involved end-to-end anastomoses between the donor and recipient splenic arteries and the donor and recipient splenic veins, using an operating microscope (Fig. 1B).

The technique of kidney Tx involved end-to-side anastomoses between the cuff of donor aorta/renal artery to the recipient abdominal aorta, and between the cuff of donor inferior vena cava/renal vein to the recipient inferior vena cava [16]. The donor ureter was implanted into the wall of the recipient bladder using a 'tunnel' technique, using an extravesical ureterovesical anastomosis (according to Lich–Gregoir [18]). One native kidney was excised and the ureter of the other kidney was ligated. In order to identify donor hematopoietic cells (by detection of the Y chromosome) in the recipient, all transplants were from male donors into female recipients. All recipient pigs in all groups underwent native splenectomy.

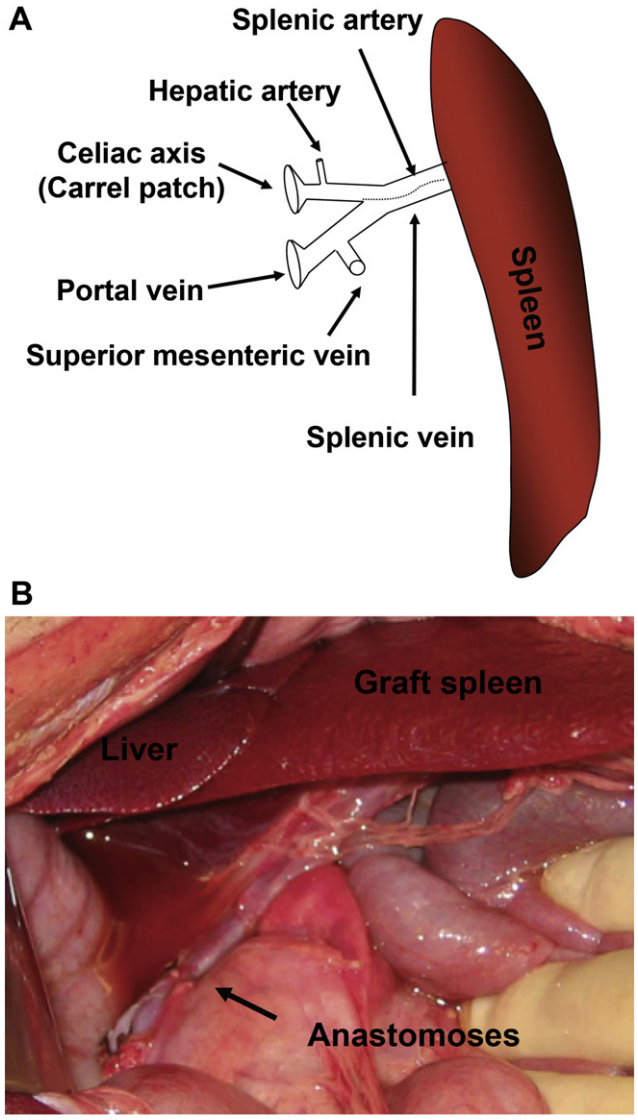

Fig. 1. Technique of orthotopic spleen Tx in the pig. (A) The spleen graft included celiac axis to perfuse HTK solution ex vivo. (B) Orthotopic spleen Tx was carried out by end-to-end anastomoses between the donor and recipient splenic arteries and the donor and recipient splenic veins. After reperfusion, the color of the spleen graft was similar to that of the liver.

2.3. Splenocyte preparation and infusion

After perfusion with HTK solution, the spleen was cut into small pieces, and mashed. Splenocytes were sequentially passed through meshes (100, 70 and 40 μm, Fisher Scientific, Waltham, MA), and washed with PBS (Invitrogen, Carlsbad, CA). In some experiments, mononuclear splenocytes were isolated. Diluted whole splenocytes with PBS were placed onto Ficoll-Paque PLUS (Amersham Bioscience, Piscataway, NJ) followed by centrifugation (600 g, 30 min). The buffy