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REVIEW ARTICLE

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Can spleen transplantation induce tolerance? A review of the literature

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Abstract In some rodent strain combinations, allogeneic spleen transplantation induces tolerance spontaneously to itself and to other donor-specific organs. In other combinations, a state of tolerance has been achieved in the weakened immune system of the recipient. The data indicate that if a balance can be achieved between host-versus-graft and graft-versus-host responses, tolerance develops, possibly due to the development of suppressor/regulatory cells. There have been a number of unsuccessful studies in outbred large animals, but none in MHC-defined donor-recipient pairs, and none in which the protocol specifically aimed at inducing tolerance. Spleen transplantation has been performed in approximately 50 humans for a number of reasons, however no clear immunologic advantage has been reported. Graft-versus-host disease (GVHD) was documented in at least 3 patients, and was lethal in one case, despite excision of the donor spleen. The advantages of tolerance over chronic immunosuppressive therapy are so great that a potentially tolerogenic approach such as spleen transplantation would seem worthy of further investigation in a suitable large animal model. Such a study is ongoing at our center.[1]

Keywords Allotransplantation · Human · Regulatory cells · Rodents · Spleen · Tolerance

Introduction

The induction of donor-specific transplantation (Tx) tolerance without long-term immunosuppressive therapy is the ultimate goal in both alloTx and xenoTx. The spleen has long been recognized as potentially tolerogenic and, in some species, can act as a source of hematopoietic cell restoration.

Carrel was probably the first to explore experimental spleen autoTx in 1910 [1]. The possibility of the transplanted spleen contributing to a state of neutral reactivity, a balance between the host-versus-graft reaction and the graft-versus-host (GVH) reaction, was emphasized by Simonsen in 1953 [2]. The concept of hematopoietic restoration by the intact spleen following whole body irradiation (WBI) finds its basis in the work of Jacobsen [3, 4], in which shielding of the spleen from lethal WBI resulted in hematopoietic cell restoration and clinical survival of rodents.

Spleen transplantation in rodents

Rats

Spontaneous development of tolerance

With the development of the techniques for spleen Tx in rodents [5, 6, 7, 8, 9, 10], Bitter-Suermann described spontaneous survival of vascularized spleen allografts in certain Major Histocompatibility Complex-incompati-

[1] * = contributed equally

ble rat strain combinations [11]. The spleen conferred a state of specific tolerance upon the recipient, as judged by subsequent skin and pancreas allograft survival [12, 13, 14]. Spleen grafts, which were usually accepted, were rejected if the donor had been sublethally-irradiated prior to Tx. The graft induced lethal graft-versus-host disease (GVHD) if the recipient had received a similar dose of WBI. This observation pointed to immunological competence of both graft and host as a prerequisite for the successful induction of this type of Tx tolerance [12]. The recipient would die of GVHD if the donor were to be actively pre-sensitized with injection of spleen and thymus cells from the recipient before spleen Tx, or if the recipient were to have undergone native splenectomy immediately before spleen Tx [14] (Table 1).

Although survival of a transplanted pancreas was prolonged when the spleen was included *en bloc* with the pancreas, 50% of the recipients developed GVHD and died [15, 16]. Pre-transplantation donor-specific blood transfusion and a 26-day course of cyclosporine therapy prevented GVHD and was followed by indefinite pancreas-spleen graft survival [16]. If a spontaneously-accepted WAG-to-AGUS rat spleen allograft was removed after 3–5 months, and a pancreas allograft was inserted at the same time, 68% of the grafts survived.

Subsequently, transplanted organs or tissues from the same strain as the spleen donor survived indefinitely if transplanted 6 weeks to 5 months after spleen Tx or together with the spleen graft. This proved to be the case even if the spleen graft had atrophied or had been surgically removed by this time [11, 17].

In studies by others, however, although the transplanted spleen was not rejected, its presence did not lead to prolonged survival (or tolerance) of a donor-specific skin graft and, indeed, in some studies there was evidence of sensitisation [18]. Early studies by Bitter-Suermann et al. showed no effect on solid organ survival by the injection of donor-strain splenocytes into the recipient [19]. This discrepancy with the results obtained following spleen Tx has been explained by the observation that the preparation of a single cell suspension not only disrupts the architecture of the organ but also alters the proportion of cellular elements to be transferred [7, 11]. There have been several studies by others with varying outcome [20, 21, 22], but these will not be reviewed here.

Induction of tolerance by immune manipulation

If AGUS recipients were pre-treated with a mixture of spleen, thymus, liver, and blood cells from PVG donors, administered intravenously 8–22 days before spleen Tx, 72% of the spleen grafts survived for longer than 4 months, in contrast to spleen allografts in unprepared recipients, which survived for approximately 3 weeks [23, 24]. Skin allografts in naïve recipients survived for

Table 1 Organ transplant survival after spleen transplantation in rats without immunosuppression. *P* parent, *F1* first offspring

First author	Rat strains	Organ Tx with spleen	Organ survival	Reference
Bitter-Suermann	PVG- > AGUS	skin	8 days	Nature 1974; 247:465–466
	AGUS- > VG	skin	> 3 months	
	Wistar- > AGUS	skin	> 4–5 months	
Taub	(LewisxBN)F1	kidney	5 months	Proc Eur Dial Transplant Assoc 1976; 12:423–428
Tilney	Aug- > (As x BN)F1	skin with presensitized	7 days	J Surg Res 1977; 22:54–58
	Aug- > (LewisxBN)F1	spleen isografts		
Bramis	(LewisxBN)F1- > Lewis	kidney	> 90 days	Transplant Proc 1977; 9:341–346.
		skin	> 72days	
Bitter Suermann	WAG- > AGUS	pancreas	indefinite	Transplantation 1978; 26:28–34
Blanchard	ACI- > Lewis	heart	9 days	J Microsurg 1980;1:381–386
	Lewis - > ACI	heart	20 days	
Shah	WAG - > AGUS	pancreas	4–10 months	Transplantation 1980; 30:83–89
Gugenheim	(LewisxBN)F1- > Lewis	skin	9 days	Transplantation 1983;36:470–471
Blanchard	ACI - > Lewis	kidney	17 days	J Microsurg 1985; 6:26–31
	Lewis - > ACI	kidney	93 days	
Gray	Lewis- > DA	islets	< 14 days (3)	J Surg Res 1986;40:77–84
			> 100 days (3)	
Schulak	Fischer- > Lewis	pancreas	18 days	Transplant Proc 1987; 29:1013–1014
	LBN- > Lewis		11 days	
	ACI- > Lewis		7 days	
Duncan	(WAGxAGUS)F1- > AGUS	heart (WAG)	> 100 days (3)	Transplantation 1987; 44:553–558
Wakely	Lewis- > BN	pancreas	> 12 days	Transplantation 1990;49:241–245
	BN- > Lewis		11days	
Pirenne	BN- > Lewis	small bowel	23 days	Transplant Proc 1993;25:1206
Suzuki	ACI- > DA	skin	indefinite	Transplantation 1997; 64:650–654
Takano	Wistar (P- > F1)	skin (P)	80% 1–2 weeks	Transplant Proc 1998; 30:2685–2686

Table 2 Organ transplant survival after spleen transplantation in rats with host immunosuppression or immunomodulation. *CyA* Cyclosporin A, *SpX* Host splenectomy, *WBI* Whole body irradiation, *DST* Donor-specific blood transfusion

First author	Rat strains	Organ Tx with spleen	Immunosuppression/ Immunomodulation	Organ survival	Reference
Tauber	Lewis- > ACI	heart	Delayed SpX day 3	indefinite	J Microsurg 1981; 2:261–268
Gray	Lewis- > DA	islets	CyA 10 mg/kg for 14 days	Indefinite	J Surg Res 1986; 40:77–84
Pollak	Lewis- > ACI	heart	Delayed SpX day 3	indefinite	Transplantation 1986; 42:528–531
Wakely	Lewis- > BN	pancreas	DST on day −7	10 days	Transplantation 1990; 49:241–245
			DST on day −7 CyA for 26 days	> 143 days	
Westra	BN- > Lewis	heart	CyA 25 on day 2	> 100 days	Transplantation 1991;52:952–955
Pirenne	BN- > Lewis	small bowel	CyA on day 2	52 days	Transplant Proc 1993; 25:1206
Suzuki	Lewis- > Fischer 344	skin	Tacrolimus for 14 days	indefinite	Transplantation 1997; 64:650–654
Hakamata	Wistar- > Lewis	pancreas	Tacrolimus for 14 days	21 days	Biochem Biophys Res Commun 2001; 286:779–785

approximately 8 days, whereas AGUS rats bearing successful long-term spleen allografts accepted subsequent skin allografts permanently. Even after removal or late atrophy, ('absorption') of accepted spleen allografts, the rats remained tolerant to skin allografts. Two rats accepted skin that had been grafted 4 months after the spleens had been 'absorbed' (after remaining viable for 5 months after grafting), demonstrating that persistence of a viable spleen graft was not essential for skin graft survival in this model. The mechanism of the prolonged survival of the spleens in these pre-treated rats was uncertain (Table 2).

Although confirmatory studies have been reported, not all subsequent investigators have confirmed the ability of a splenic allograft to induce tolerance or prolonged hyporesponsiveness.

Mice

In mice, the age of the spleen donor was not found to be of major influence on outcome of spleen Tx [25]. Spleen Tx has also been utilized to study the anemia of chronic inflammation [26].

Guinea pigs

Spontaneous development of tolerance

Based on Billingham's previous conclusions from bone marrow Tx, Bitter-Suermann et al. concluded from their rat studies that a spleen graft could launch and sustain GVH reactivity when (a) graft and host differed at major or minor histocompatibility loci, (b) the graft contained immunocompetent cells, and (c) the host was immunoincompetent, either for a genetic reason, from age, or because of exogenous immunosuppression [19, 27]. Bitter-Suermann and Shevach, however, observed that a majority of untreated guinea pigs (of strain 2) undergoing spleen Tx (from strain 13) succumbed to GVHD approximately 6 weeks after Tx, which was not in accordance with the third prerequisite above [19]. The recipients were immunocompetent and not immunosuppressed, but after receiving a spleen allograft, they behaved as if they were exogenously immunosuppressed. These authors believed this to be the only animal model in which an allograft induced lethal GVHD in a healthy immunocompetent host quite capable of rejecting an organ transplant from a third party donor.

Mechanism of tolerance induction

In guinea pigs with long-term surviving spleen grafts, the response against the donor was markedly suppressed in the mixed lymphocyte reaction (MLR). Furthermore, cells were present in the tolerant spleen recipients that were capable of suppressing the MLR response of normal host-strain cells towards donor-strain cells [28], although the period of MLR hyporesponsiveness was rather short-lived. Donor-strain cardiac allografts survived indefinitely in immunologically-uncompromised recipients that had received passive transfer of cells from guinea pigs with long-standing spleen transplants. The presence of the cardiac graft further enhanced MLR suppression, whereas most cardiac-allografted controls showed vigorous MLR responses to donor-stimulated cells after rejection of their cardiac transplants.

There was some discrepancy between in vivo and in vitro data. A number of recipients of spleen allografts had markedly suppressed MLR responses, and their cells were uniformly capable of transferring tolerance in vivo but did not show adoptive MLR suppression in vitro. In vivo tolerance could be transferred by cells taken from animals 4 weeks after spleen allografting, a time when the activity of the MLR 'suppressor' cells was not prominent in vitro [29, 30].

These experiments suggested the presence of regulatory cells capable of transferring MLR unresponsiveness and Tx tolerance to normal hosts, although it was not

Table 3 Spleen allotransplantation in dogs. *AZA* Azathioprine

First author	(*n*)	Immunosuppression	Outcome	Reference
Moore	29	none	19/29 early infarction, 1 spleen not rejected day 65	Ann Surg 1960; 152:374–387
Fisher	20	SpX	rejection < 10 days	Surg Gyn Obst 1961; 112:455–462
	24	WBI (300–500 cGy) at Tx	22/24 died	
	16	WBI (100–300 cGy) at Tx	1/16 died	
	12	WBI (300–500 cGy) prior to Tx	9/12 spleens grossly normal on day 14	
Dammin	43	none	rejection 7–9 days	Ann NY Acad Sci 1962;99:861–869
	?	WBI (250 cGy)	poor survival ?day	
	14	nitrogen mustard	6 died early < 5 days 7 died < 8 days 1 survived for 32 days no difference from teated	
Montague	18	nitrogen mustard SpX	2/18 died (hematopoietic depression) 4/18 died (early thrombosis spleen) 7/8 died after 5–8 days No protection to spleen rejection	J Surg Res 1962; 2:130–135
Wheeler	43	none	21/43 operative failure rejection < 1week	J Surg Res 1962;2:114–123
Fiscus	7	WBI (300 cGy)	death (6/7) < 10 days	Surg For 1963; 14:182–185
Jordan	36	none	11 died (early thrombosis), 25 rejection 5–12 days	Ann NY Acad Sci 1964; 120;612–625
	7	WBI (300 cGy) + SpX	GVHD, died < 10 days	
Marchioro	4	none	2–8 days survival	Ann NY Acad Sci 1964; 120:626–651
	4	WBI (150 cGy) on d-8 WBI (300 cGy) on d-1	4–7 days survival 5–7 days survival	
	7	AZA	2–11days survival	
Mahajan	57	26/57 successful	J Surg Res 1965;5:413–427	J Surg Res 1965; 5:413–427
	−12	none	rejection 5–8 days	
	−14	6-mercaptopurine	25% spleen graft survival 13–28 days	

Table 4 Spleen + kidney allotransplantation in dogs

First author	(*n*)	Immunosuppression	Outcome	Reference
Simonsen	1	none	Spleen rejected on day 4, accelerated kidney rejection	Acta Pathol Microbiol Scand 1953; 32 36–84
Kountz	9	none	Kidney no prolongation	Surg Forum 1962; 13:59–62
	9	SpX	Kidney 7 days prolongation wasting disease	
Marchioro	7	none	3 died (pneumonia), 4 survived for 25 days, prolonged kidney survival for 9 days	Ann NY Acad Sci 1964; 120:626–651
	12	WBI (100–450 cGy)	Survival for 6–32 days, hemolysis (9), spleen rejection < 10 days, accelerated kidney rejection	
	11	AZA	Survival for 6 days-7.5 months, kidneys protected from acute rejection	
Dubois	29	none	spleen rejection < 10 days, kidney no prolongation	Biomedicine 1976; 25:221–223
Hoffman	29 + autologous retransplantation	none	15/29 Successful, kidney no prolongation	Eur Surg Res 1984; 16:40–46

determined whether the cells involved were of recipient or graft origin. The state of unresponsiveness, favouring the survival of second donor-strain allografts, appeared to be weak during the first few weeks after spleen Tx. Skin allografts, for example, did not survive if placed earlier than 6–8 weeks after spleen Tx [13, 31].

Following spleen Tx in rats, unresponsiveness developed as early as 1 week [32, 33, 34, 35]. T cells obtained from the recipient's lymph nodes and native spleen exhibited reduced MLR to donor-strain cells, but responded normally to third party cells. In contrast, T cells obtained from the spleen graft were unresponsive to both donor and third-party stimulators. Therefore, although donor-specific T-suppressor cells appeared in the recipient's lymph nodes and spleen within 1 week of spleen Tx, at this time antigen-nonspecific suppressor cells predominated in the spleen graft. Only minimal cytotoxic T cell activity could be detected in the spleen graft, with

the host spleen and lymph nodes being devoid of cytotoxic T lymphocytes. Sera obtained 1 or 2 weeks following spleen Tx did not contain cytotoxic antibodies, and only a very weak response could be detected at 1 month.

Spleen transplantation in large animals

Apart from one early report of implantation of an allogeneic spleen in the peritoneal cavity in the chicken [36], all reports are on dogs and pigs.

Allotransplantation in dogs

In the early 1960s, several groups performed spleen Tx in dogs, with comparable results (Table 3). Spleens transplanted into untreated recipients were rejected within a few days. Marchioro et al. documented a viable spleen graft after 200 days in a dog treated with azathioprine for only the first 125 days [37]. Preceding treatment of the recipient with WBI (150–300 cGy) protected the spleen from rejection [37], but produced a GVH response, with anemia, thrombocytopenia, and lymphocytopenia [38]. Protecting the spleen from lethal WBI did not result in survival, but an intravenous (unirradiated) homogenate of the native spleen did [39] (Table 3).

Effects of spleen transplantation on a concomitantly-transplanted donor-specific organ

In untreated dogs, the simultaneous Tx of spleen and kidney from the same donor prolonged survival to 20 days, compared with 11 days when the kidney was transplanted alone [37]. Azathioprine therapy could prolong graft survival for from 2 to 7.5 months at which time, although the kidneys showed signs of chronic rejection, the splenic architecture remained normal [37]. Administering WBI (100–450 cGy) to the recipient prior to spleen Tx resulted in hemolytic anemia in 8 of 12 dogs, which required repeated blood transfusion, and yet provided no protection to the renal allografts [37]. Although the survival of renal allografts was prolonged when combined with simultaneous native splenectomy and spleen Tx, these animals developed a wasting disease, characterized by anorexia, weight loss, occasionally bloody diarrhea, and anemia, suggesting a GVH reaction, several of them dying from infection. Others found spleen Tx not to be beneficial to survival of a second donor-specific organ (Table 4).

Hoffman et al. [40] reported that immunization of the donor with horse red blood cells before spleen Tx led to the development of anti-horse red blood cell antibodies in the recipient. Furthermore, immunization of the recipient (after spleen Tx) with sheep red blood cells resulted in the production of anti-sheep red blood cell antibodies in the original donor after autologous retransplantation of the spleen. These observations demonstrated a transfer of sensitized lymphocytes in the transplanted spleens.

Allotransplantation for hemophilia A

Experimental spleen Tx with the intention to cure hemophilia A has been carried out in a variety of dog models. Although it could not be clearly demonstrated that the level of factor VIII increased, satisfactory graft perfusion and survival were demonstrated for more than 8 months [41]. Webster et al. described successfully functioning spleen allografts in severe classical hemophilia; immediate correction of the hemostatic defect and improvement in blood coagulation was achieved [42, 43]. Others had varying results (Table 5).

Combined pancreas-spleen allotransplantation in Pigs

Two groups [44, 45] have investigated the immunologic effect of transplanting the spleen with the pancreas in pigs. None of the pigs undergoing pancreas Tx alone showed signs of rejection while receiving cyclosporine

Table 5 Spleen allotransplantation for hemophilia in dogs. *CS* Corticosteroids, *ALS* Anti-lymphocyte Serum

First author	(*n*)	Immunosuppression	Outcome	Reference
Webster	2	None	52–72 h survival, correction of hemostasis	North Carolina Med J 1967; 28:505–507
Norman	2	None	factor VIII 0% < 24 days	Surgery 1968; 64:1-16
	4	AZA + CS + ALS, + actinomycin C	prolonged factor VIII synthesis	
Marchioro	1 matched spleen	AZA + CS	viable on day 93, no factor VIII increase	Transplant Proc 1969; 1:316–320
	1 non-matched spleen	AZA + CS	rejection < 15d	
Marchioro	1	AZA + CS	viable 47days, no factor VIII increase	Science 1969; 10:188–190
McKee	8	?	7–8 months (2), no factor VIII increase	J Lab Clin Med 1970; 75:391–402
Groth	4	AZA + ALS	survival 3–8 days	Surgery 1974; 75:725–733

for 28 days, but several recipients of combined pancreas-spleen grafts rejected their transplant before this time, some as early as 1 week [44, 45], suggesting that the presence of the spleen was immunogenic; in no case did signs of GVHD develop (Table 6).

Human spleen xenotransplantation in pigs

There is one brief and limited report on the pig as a recipient of a human spleen [46]. The pigs were pretreated with a a single dose of cyclophosphamide (30 mg/kg i.v.) 4 days before Tx, and 2 of 5 of them developed transient GVHD-like signs 2–3 weeks later. The number of human hematopoietic cells detected in these pigs, particularly in the bone marrow, was reported to be higher than that of pigs that had undergone injection of human splenocytes only, but details were not reported. The human spleens, however, eventually appeared to have been rejected (Table 6).

Spleen transplantation in humans

Human spleen autoTx has been reported, but will not be reviewed here.

Allotransplantation

Clinical experience with spleen alloTx began in the early 1960s.

Spleen allotransplantation for hypogammaglobulinemia, hemophilia, or Gaucher's disease

(Table 7)

In one patient with hypogammaglobulinemia, it was hoped that the transplanted spleen, donated by the patient's mother, would alleviate a state of immune deficiency [7], but no rise in serum antibody levels was observed during the 3 months for which the spleen survived. One spleen was transplanted from father to son for hemophilia A [47]; factor VIII levels did not rise and, because of severe bleeding, the graft had to be excised after 4 days. The transplanted spleen was found to be markedly swollen and had ruptured.

More recent spleen transplantatations have been successful [48, 49]. In one case, factor VIII was increased and no spontaneous hemorrhage occurred during 2 years of follow-up [48]. In a case report of spleen Tx for Gaucher's disease, a spleen graft from an unrelated living donor resulted in temporary improvement in the patient's biochemical parameters, but was finally rejected by day 40 [50, 51]. Chimerism of erythrocytes was documented 3–6 weeks after spleen Tx, and the patient developed in vitro donor-specific hyporesponsiveness [52].

Spleen allotransplantation for malignant disease

In four patients with terminal malignancies, spleen Tx was to superimpose a state of altered immunologic

Table 6 Spleen allo- and xeno-transplantation in pigs. *CPP* Cyclophosphamide, *GVHD* Graft-versus-host disease

First author	(n)	Procedure	Immunosuppression	Outcome	Reference
Dafoe	8	Pancreaticoduodenal graft + spleen	CyA + CS	rejection 7–16d	Transplantation 1985; 40:579–584
Dafoe	5	Pancreaticoduodenal graft + irradiated spleen graft	CyA + CS	no effect, no rejection d28	Transplantation 1986; 42;686–687
Gänger	7	Pancreaticoduodenal graft + spleen	none	rejection < 1w	Eur Surg Res 1987; 19:323–328
Li	5	Human spleen graft	CPP	stable chimerism, GVHD, rejection < 2w	Transplant Proc 2000; 32:1103–1104

Table 7 Clinical spleen allotransplantation for hypogammaglobulinemia, hemophilia, and Gaucher's disease

First Author	(*n*)	Indication	Outcome/Complications	Graft Survival	Reference
Marchioro	1	Hypogammaglobulinemia	No gammaglobulin production	3 months	Ann NY Acad Sci 1964; 120:626–651
Hathaway	1	Hemophilia	Hemorrhage	7 days	Transplantation 1969; 7:73–75
Groth	1	Gaucher's disease, (irradiated spleen 300 cGy)	Hemolysis & cachexia, temporary benefit, Chimerism erythrocytes Donorspecific hyporesponsiveness	40 days	Lancet 1971; 1:1260–1264 Clin Exp Immunol 1972; 10:359–365.
Xia	1	Hemophilia (mother-to-son)	Clinical improvement	> 2 years	Chin Med J (Engl) 1992;105:609–611
Liu	3	Hemophilia	Clinical improvement (2)	< 5 years (2)	Arch Surg 1995; 130:33–39
Xiang	1	Hemophilia	Clinical improvement	> 5 months	Transplant Proc 2002; 34:1929–1931

reactivity and thus suppress the growth of the neoplasms [37]. In an effort to generate a GVH reaction against the tumor, potential spleen donors, who also had a terminal malignancy, were sensitized with tumor cell suspensions from the patient 12 to 56 days before Tx. Recipient splenectomy was performed in all patients, and immunosuppression with azathioprine was initiated several days before transplantation. If rejection of the spleen were suspected, prednisone and actinomycin C were added. None of these patients demonstrated cessation or regression of tumor growth, and hypersplenism was reported in 3 out of 4 patients. This was transient in two cases but sustained in one, when it was associated with hemolytic anemia, leukopenia, and thrombocytopenia. Bitter-Suermann suggested that the failure of the donor spleen to generate an immunologic response to the malignant disease (a graft-versus-tumor effect) may have been due to the use of immunosuppressive drug therapy that may have affected both graft and host reactions. Thus, the results "may have been masked, without invalidating the underlying concept of a transfer of cancer immunity" [13] (Table 8).

In a leukaemic patient, a spleen was transplanted from the recipient's healthy identical twin [53], and remained viable for 6 months without immunosuppression, however without beneficial effect on the leukaemia. Others, however, have reported shrinkage of hepatocellular carcinomas and reduced serum alpha-fetoprotein levels after spleen Tx [54].

Combined pancreas-spleen transplantation

In the 1980s, several groups included the donor spleen to improve the hemoperfusion of the pancreas graft when performing pancreas Tx. Starzl et al. reported the first four cases [55], immunosuppression comprising cyclosporine and corticosteroids. No immunologic benefit appeared to result from the presence of the donor spleen in any patient. In a blood group A recipient of a composite graft from a donor of group O, urgent splenectomy was necessary after 6 days for severe hemolytic anemia associated with a positive Coomb's test and the presence of anti-A isoagglutinins. After removal of the transplanted spleen, the isoagglutinins disappeared over 9 weeks (Table 9).

Deierhoi et al. [56] reported one case of lethal GVHD after HLA-mismatched composite pancreas-spleen Tx. One week after Tx, the patient developed progressive leukopenia, which persisted after discontinuing azathioprine therapy and irradiating the spleen (1000 cGy over 3 days). Twenty days after transplantation, donor splenectomy was performed. Over the next 2 weeks, severe cutaneous GVHD was followed by multi-organ failure. Human Leukocyte Antigen-typing of blood lymphocytes revealed 80–100% to be of donor type. Cyclosporine was discontinued, and high-dose treatment with corticosteroids was begun, but the patient succumbed from systemic candidiasis.

Two additional cases of GVHD after pancreas-spleen Tx have been reported by Gonwa et al. [57]. Both patients developed leukopenia and thrombocytopenia, which in one patient did not respond to therapy, necessitating excision of the spleen graft. Mixed hematopoietic cell chimerism was found, although the extent was not reported. The second patient, who also developed skin GVHD, responded well to irradiation of the spleen.

The largest and most recent study on composite pancreas-spleen Tx was reported by Booster et al. [58]. In order to prevent GVHD, in 11 of 12 grafts, during cold storage of the donor organs before Tx, the spleen alone was irradiated (600 cGy). No GVHD was observed, but no immunologic advantage of spleen Tx was identified. In four patients, a search was made for chimerism, and lymphocyte chimerism of 14–20% was detected in two of them.

Xenotransplantation

Although clinical xenogeneic spleen Tx does not appear to have been performed, several hundred patients, with a variety of disease states, have had their blood or plasma perfused through pig (or other animal) spleens [59, 60, 61, 62]. The clinical response to extracorporeal spleen perfusion in patients with septic conditions has been reported to be good. Details on this form of therapy are limited, but a careful investigation (Paradis et al. [63])

Table 8 Clinical spleen allotransplantation for malignant disease. *AFP* Alpha-fetoprotein, *GVL* Graft-versus-leukemia

First Author	(*n*)	Indication	Outcome/ Complications	Graft Survival	Reference
Marchioro	4	Terminal carcinoma	Hemolytic anemia (1), Leukopenia (1)	10 days-8 months	Ann NY Acad Sci 1964; 120:626–651
Raccuglia	1	Leukemia	No GVL effect	5 months, viable	Clin Exp Immunol 1973; 14:1-18
Liu	3	Hepatocellular carcinoma	Shrinkage of tumors, decrease AFP	5–11 months	Arch Surg 1995; 130:33–39

Table 9 Clinical combined pancreas-spleen allotransplantation

First Author	(*n*)	Indication	Outcome/Complications	Graft Survival	Reference
Starzl	4	Diabetes	Hemolytic anemia (1)	6 days-11 months	Surg Gynec Obstet 1984; 159:265–272
Gonwa	2	Diabetes	GVHD (2), Hemolytic anemia (1)	16–24 days	Transplantation 1985; 40:299–304
Sollinger	7	Diabetes	Less complications	?	Transplant Proc 1985; 27:360–362
Munda	4	Diabetes	No side effects	12 days-8 months	Transplant Proc 1985; 27:353–357
Deierhoi	1	Diabetes	Lethal GVHD	19 days	Transplantation 1986; 41:544–546
Peltenburg	1	Diabetes	Spleen graft rupture, Accelerated rejection	< 3d	Transplantation 1992; 53:226–228
Booster	12	Diabetes	Accelerated rejection (1)	2 days-9 months	Transplantation 1993; 56:1098–1102

into the possibility that these patients be infected with porcine endogenous retrovirus, documented that 23 of the 30 tested patients demonstrated pig microchimerism. None of these patients had been exogenously immunosuppressed at the time of the procedure, which lasted only for hours. Even though the patients had thereafter not undergone exogenous immunosuppression, microchimerism persisted for between 2 months to 8 years after spleen perfusion.

Comment

There is convincing evidence of a tolerogenic effect achieved by transplanting the spleen under various conditions in some, but not all, rodent models. Several studies have indicated that a balance between host-versus-graft and GVH responses can be achieved after spleen Tx, leading to long-term survival of both the spleen and a second donor-specific organ. There is evidence of the induction of tolerance associated with the development of regulatory cells. A donor-specific organ transplant can be protected from rejection by the transfer of cells from an animal with a long-surviving spleen graft.

In large animals and humans there has been no definite evidence that spleen Tx results in tolerance to itself or improves survival of a concomitantly-transplanted second organ. Indeed, the prevailing evidence is that the transplanted spleen is rejected, as is any other organ. However, no study in large animals has investigated the potential of this approach in a comprehensive way in a model in which knowledge of the Major Histocompatibility Complex of both donor and recipient was known. Furthermore, the immunosuppressive regimen, designed to chronically immunosuppress rather than to induce tolerance, may have an adverse effect on the induction of tolerance [13, 64]. Clinical spleen Tx shows that hematopoietic cell chimerism can result, and that a GVH response can be severe enough to cause lethal GVHD. This indicates the spleen's potential in counteracting the usual host response that occurs after organ Tx.

The benefits of inducing a state of tolerance in an allograft recipient, and the advantages of this state over chronic pharmacologic immunosuppressive therapy are considerable. Although our ultimate goal is to explore the potential role of spleen Tx in xenoTx, if successful, it would seem a realistic approach to clinical alloTx. If, for example, the spleen could be demonstrated to induce tolerance to a donor-specific heart or kidney, the spleen could quite easily be transplanted on the same occasion without unduly complicating or extending the operative procedure.

It therefore seemed worth investigating spleen Tx in a large animal model, such as the Massachusetts General Hospital Major Histocompatibility Complex-defined miniature swine Tx-model, in which there is considerable experience of the Tx of other organs. Our initial studies indicate that spleen Tx across minor [65] or MHC class I (Dor FJMF et al, manuscript in preparation) antigen barriers, with a short course of immunosuppression, induces tolerance to itself and to a subsequently transplanted donor-matched kidney. Spleen Tx over a full MHC barrier, with modest host immunosuppression for 45 days, induces multilineage hematopoietic cell chimerism with engraftment of donor progenitor cells in host bone marrow (Dor FJMF et al, manuscript in preparation). This state is associated with in vitro donor-specific unresponsiveness and evidence for the presence of regulatory cells, in the absence of features of GVHD. Subsequent donor-matched kidney Tx is ongoing.

To date, we have not found GVHD except for a mild skin rash, even in the presence of chimerism of > 40%. The reasons for the absence of this potential complication, particularly when compared to its incidence after bone marrow Tx, remain uncertain. It is unlikely to be associated simply with differences in the pre-Tx conditioning and post-Tx immunosuppressive regimens, since these differ little from current experimental bone marrow Tx protocols used at our center (Cina RA et al, manuscript in preparation). Factors that may play a role in the lack of GVHD include the possibility that the hematopoietic progenitor cells released from the spleen may be subtly different from those in bone marrow, and the observation that the release of these cells from the spleen occurs over a period of several weeks (rather than being infused in a large number on a single occasion). Further studies are required, however, to confirm that

GVHD does not occur following spleen Tx using the protocol we have developed.

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