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The Effects of Tolerance on Allograft Damage Caused by the Innate Immune System

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Abstract

Background—It is not known whether tolerance can be induced in a strong proinflammatory milieu or whether the induction of tolerance can prevent interferon (IFN)- γ -associated graft injury. To address these questions, we studied the effects of rIFN- γ infusion on porcine cardiac allograft survival.

Methods—Recombinant interferon (rIFN)- γ was continuously infused into the left anterior descending artery of hearts transplanted into major histocompatibility complex–inbred miniature swine treated with a 12-day course of cyclosporine A. Group 1 recipients received a nearly syngeneic heart, group 2 recipients received a class I disparate heart, and group 3 recipients were cotransplanted with a class I–disparate heart and kidney, a procedure demonstrated to induce tolerance to both grafts. A fourth group of animals were not transplanted but received intracoronary rIFN- γ infusion into the native heart.

Results—rIFN- γ perfusion not only accelerated the acute rejection of class I–disparate hearts (mean survival time, 19 ± 7.21 vs. 38 ± 8.19 ; $P=0.025$) but caused near-syngeneic heart transplants, which otherwise survived indefinitely, to reject within 35 days. In contrast, rIFN- γ perfusion had no demonstrable effects on hearts grafts in tolerant recipients or on autologous hearts.

Conclusions—These results suggest that tolerance induction can occur in the presence of IFN- γ -mediated inflammation, and that tolerance induction can prevent the tissue injury caused by the overproduction of IFN- γ . This suggests that the beneficial effects of tolerance may include protection from nonspecific inflammatory responses, such as those produced by ischemia-reperfusion injury and brain death.

Keywords

Tolerance; Inflammation; Cytokines; Heart; Swine

It is well established that interferon (IFN)- γ , produced by Th1 cells during the adaptive immune response to an allograft, plays an important role in acute (1–7) and chronic (8–10) allograft

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rejection. IFN- γ is also generated by cells of the innate immune system which contribute to tissue injury through inflammatory responses resulting from ischemia-reperfusion (11,12) and brain death (13,14), both of which adversely affect allograft survival (15–18).

The induction of immune tolerance after organ transplantation suppresses alloaggressive responses of the adaptive immune system and in doing so, prevents tissue damage by Th1-associated cytokines, including IFN- γ . However, it is not known how tolerance and IFN- γ produced by components of the innate immune system interrelate. For instance, can tolerance be induced in the presence of a constant and strong proinflammatory milieu such as that associated with IFN- γ overproduction, or can the induction of tolerance prevent the tissue damage associated with IFN- γ overproduction?

To address these questions, we administered recombinant interferon (rIFN)- γ directly and continuously into the coronary arteries of porcine cardiac allografts in recipients undergoing tolerance induction. Our results show that tolerance induction can occur in the presence of IFN- γ -mediated graft inflammation and that tolerance induction can prevent the local tissue injury caused by IFN- γ overproduction. These results suggest that the beneficial effects of tolerance induction may extend to the nonspecific inflammatory responses of the innate immune system associated with perioperative events such as brain death and ischemia-reperfusion.

MATERIALS AND METHODS

Animals

The MGH-inbred miniature swine used in this study have been described previously (19). Presently, inbred swine of three homozygous MHC haplotypes (swine leukocyte antigens [SLA]), SLA^a, SLA^c, and SLA^d are maintained. In addition, six intra-major histocompatibility complex (MHC) recombinant haplotypes (SLA^g, SLA^h, SLAⁱ, SLA^j, SLA^k, SLA^e) have been derived by spontaneous recombination events during the breeding of heterozygotes as part of the breeding program. Two MHC-identical SLA^{dd} animals (nos. 10172 and 10122) were chosen for further inbreeding based on their coancestry (a calculation that gives the probability that genes taken at random from each of the concerned individuals are identical), which was estimated at 58.2%. Brother-sister or father-daughter pairs were chosen from the first litter (termed G1) for further mating, with each successive generation termed G(N+1). This process has been conducted for the last 15 years, and the animals used in this study were G8-inbred miniature swine or eighth generation (after animals were already homozygous for MHC alleles). The coancestry has increased significantly with each generation, reaching a value greater than 94% in the G8 generation (20). All animal care and procedures were performed in compliance with both the Principles of Laboratory Animals Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Heterotopic Cardiac Transplantation and Heart-Kidney Cotransplantation

Heterotopic cardiac transplantation and heart-kidney cotransplantation were performed and allograft function monitored as previously described (21,22).

Osmotic Minipump and Intracoronary Catheter Insertion

After the donor heart was explanted, a vinyl catheter (outer diameter: 1.14 mm; Science Commodities Incorporated, Lake Havasu City, AZ) was inserted through the donor aortic wall into the ostium of the left main coronary artery and threaded down into the left anterior descending artery (LAD). It was sutured into position to prevent displacement. The end of the catheter was connected to an Alzet osmotic minipump (Alzet, Alza Corporation, Palo Alto, CA) that was filled with either porcine rIFN- γ or the saline carrier. The pump was positioned

in a subcutaneous pocket on the abdominal wall that was easily accessible for refilling of the pump reservoir. The pumps used in the experiments (Alzet Model 2ML) had a reservoir of 2 mL and nominal pumping rate of 10 μ L/hr. In the control group of native hearts that received rIFN- γ , a median sternotomy was performed and the animal placed on cardiopulmonary bypass. The aorta was crossclamped and cardioplegia administered into the aortic root to stop the heart. The cannula was inserted through the aorta and into the left anterior descending artery as above. The crossclamp was removed and the animal weaned from cardiopulmonary bypass.

Cytokines

According to the manufacturer's instructions, lyophilized porcine recombinant IFN- γ (BioSource International Camarillo, CA) was reconstituted in deionized water to 0.1 mg/mL and stored at -80°C . For each pump exchange, an aliquot was thawed and further diluted in 2 mL of phosphate buffered saline. This allowed the left anterior descending coronary artery to be continuously perfused with porcine rIFN- γ at a dose of 100 ng/d until graft failure or sacrifice. The rIFN- γ infusion began at the completion of the surgery.

Cyclosporine

Cyclosporine (CyA), generously provided by Novartis (Hanover, NJ), was administered intravenously to selected recipients from postoperative day (POD) 0 to POD 11 aiming for blood trough levels between 400 and 800 ng/mL on the basis of earlier results (21).

Alloantibody Detection

The presence of antidonor class I antibody (immuno-globulin [Ig] M and IgG) in the serum of recipient swine was detected by indirect flow cytometry using peripheral blood leukocytes (PBL) from SLA-recombinant swine to determine the SLA-binding specificity of the antibody as described previously (23).

Cell-Mediated Lympholysis

Cell-mediated lympholysis (CML) assays were performed as previously described (24). Briefly, mixed lymphocyte cultures were containing 4×10^6 responder peripheral blood mononuclear cell (PBMC) and 4×10^6 irradiated (2,500 rads) stimulator PBMC were incubated in 2 mL of CML medium in 24-well plates (Costar) for 6 days at 37°C and 6% CO_2 . On day 6, effector cells were harvested and tested for cytolytic activity against ^{51}Cr -labeled PHA (Life Technologies) targets in a 5.5-h ^{51}Cr release assay. Supernatants were harvested using the Skatron collection system (Skatron, Sterling, VA), and ^{51}Cr release was determined on a gamma counter (Micromedics, Huntsville, AL). The results were expressed as the percent specific lysis, calculated as % specific lysis = $[(\text{experimental release} - \text{spontaneous release}) / (\text{maximum release} - \text{spontaneous release})] \times 100$.

Histology and Immunohistology

Open biopsies specimens of the transplanted heart were obtained by using a Tru-Cut needle (Baxter, Deerfield, IL). On POD 100 or on the day of graft failure animals were sacrificed and grafted. Coded histology specimens were stained by using hematoxylin and eosin, Masson's trichrome, and/or periodic acid-Schiff staining and scored under light microscopy by a blinded observer to determine the severity of acute rejection. Scoring of acute rejection in the heart allograft was based on the International Society for Heart and Lung Transplantation (ISHLT) classification (24,25). Class II expression was assessed as described (26) using the mouse anti-class II mAb ISCR3 (clone Sp-345) which has interspecies crossreactivity with human, porcine, and rodent class II antigens (27).

Statistical Analysis

Graft survival times were analyzed using a log-rank test. Value of $P < 0.05$ was considered statistically significant.

RESULTS

Intracoronary rIFN- γ Perfusion Increased MHC Class II Expression on Coronary Endothelium

It has shown previously that recombinant porcine IFN- γ upregulates MHC class II expression on pig endothelial cells (28). To confirm the delivery and biological activity of the rIFN- γ in our experimental animals, we compared the expression of endothelial MHC class II antigens in cardiac allografts perfused with rIFN- γ to class II antigen expression in untreated controls. Both treated and nontreated recipients had received class I disparate heart and kidney allografts previously and were tolerant to donor antigen. Figure 1 shows that allografts locally perfused with rIFN- γ exhibited a substantial increase in MHC class II expression on endothelial cells when compared to untreated controls (Fig. 1A and B). This finding confirmed that our rIFN- γ delivery system was effective and that the dose of cytokine infused was biologically active.

Intracoronary rIFN- γ Accelerates Acute Rejection of Isolated, MHC Class I–Mismatched Cardiac Allografts

Hearts with implanted intracoronary catheters were transplanted into MHC class I–mismatched miniature swine treated with a 12-day course of CyA. Porcine rIFN- γ was continuously infused into the LAD of allografts in three animals, while three control allografts were perfused with normal saline. Another historical control group had no catheters placed. We have previously shown that cardiac allografts transplanted into MHC class I disparate swine treated with a 12-day course of CyA were rejected between 38 and 55 days (Table 1, Group 2) (29,30). When the coronary arteries of class I disparate hearts were continuously perfused with saline, the tempo of the rejection process was not significantly different (Table 1, Group 3). However, intracoronary infusion of rIFN- γ significantly accelerated acute cellular rejection with complete graft failure occurring between 13 and 27 days (Table 1, Group 4). Severe cellular rejection was seen even while recipients had therapeutic serum levels of CyA, which is very unusual in this transplant model (31). Indirect flow cytometry demonstrated that antidonor antibody production was also accelerated in INF- γ -treated animals compared to saline controls (Fig. 2). This effect was most prominent for serum IgM levels which increased up to 3-fold by POD 9 as compared to only a slight increase in IgM levels by POD 28 in saline control animals (Fig. 2A). The effect of rIFN- γ treatment on the production of IgG followed a similar pattern, but was less pronounced with serum levels of IgG generally starting to rise at or near the time of graft rejection (Fig. 2B).

Intracoronary rIFN- γ Induces Acute Rejection in Nearly Syngeneic Cardiac Allografts

Next, we tested the effect of intracoronary rIFN- γ infusion in nearly syngeneic cardiac grafts. In an earlier study, we demonstrated that hearts transplanted between highly inbred G7 miniature swine (90% consanguinity) survived more than 300 days without signs of rejection, despite the absence of immunosuppression (Table 2, Group 1) (20). However, when hearts were transplanted between the G8 miniature swine (94% consanguinity) and perfused with intracoronary rIFN- γ , the grafts were rejected on PODs 5, 17, and 32 (Table 2, Group 2) despite CyA therapy. At autopsy, all rIFN- γ -treated G8 heart grafts showed severe cellular infiltrates (Fig. 1C).

Intracoronary rIFN- γ Does Not Affect the Native Heart

Although nearly syngeneic, the G8 heart transplants probably express weak minor antigen differences, as evidenced by the fact that first set skin grafts between G7 animals were accepted

indefinitely, while second set skin grafts were rejected (20). We therefore performed an additional experiment wherein intracoronary rIFN- γ was administered into the native heart of naïve animals. The two nontransplanted animals receiving intracoronary rIFN- γ infusion were sacrificed on PODs 50 (due to a dislodged catheter) and 100, both with vigorously beating hearts (Table 2, Group 3). Histology revealed no evidence of a cellular infiltrate, endothelial activation or myocyte damage in either heart (Fig. 1D).

Tolerance Induction Prevents Tissue Injury in rIFN- γ Perfused Cardiac Allografts

We have developed an effective and reliable means of inducing tolerance to class I cardiac allografts in miniature swine by cotransplanting hearts and kidney allografts from the same donor with a 12-day course of CyA (22,32) (31). Cotransplantation of both the heart and kidney allografts results in the indefinite survival of both organs (Table 3) and prolongation of donor-specific skin grafts (22,31,32). To test the effects of tolerance induction on the actions of IFN- γ in vivo, recipients of combined class I disparate heart and kidney allografts were perfused with intracoronary rIFN- γ . In contrast to the acceleration of acute rejection observed in nontolerant recipients transplanted with an isolated heart allograft, intracoronary rIFN- γ perfusion had no demonstrable effects in recipients cotransplanted with heart and kidney allografts undergoing tolerance induction (Table 3). Function of the cardiac allografts (palpation scores and electrocardiographs) remained unchanged over the entire 100-day period of IFN- γ perfusion. None of the tolerant, rIFN- γ treated animals produced antidonor class I antibodies (Fig. 2). Furthermore, cell mediated lympholysis (CML) assays demonstrated that despite rIFN- γ treatment, all heart-kidney recipients developed donor specific unresponsive by POD 50 similar to nonperfused and saline perfused animals (Fig. 3). The function of the cotransplanted kidney grafts monitored, as monitored by serum creatinine and kidney biopsies, also remained unchanged throughout the 100-day period in both the control and experimental animals. Serial biopsies on heart grafts revealed only occasional cellular infiltrates that were indistinguishable from controls (Table 3) and at necropsy all hearts looked pristine (Fig. 1E). Of note, histological analysis revealed that two out of the four recipients of heart and kidney allografts developed cardiac allograft vasculopathy (data not shown).

DISCUSSION

Studying the effects of cytokines requires local delivery because cytokines are inactivated rapidly in vivo (33) and the systemic administration of cytokines such as IFN- γ in a dose sufficient to be locally active would be toxic (34). We have developed a model that allows rIFN- γ to be delivered continuously into the arterial tree of a vascularized allograft and that allows the biological effects (the observation of increased expression of class II antigens) of that treatment to be verified. MHC-inbred miniature swine were used because of their size and because we have previously established models of delayed rejection (class I-disparate transplantation with 12 days of CyA) and tolerance (class I-disparate cotransplantation of a donor-matched heart and kidney with 12 days of CyA) (22,30). The induction of tolerance in the heart and kidney co-transplantation model seems to be due to radiosensitive, thymus-dependent regulatory T cells generated by the co-transplanted renal allograft (35). Another advantage of using MHC inbred miniswine is the availability of a new, highly inbred line of miniature swine which allows for nearly syngeneic transplantation (20).

Our major findings were that 1) intracoronary rIFN- γ perfusion, which accelerated the rejection of both class-I mismatched and near-syngeneic hearts, did not prevent the induction of tolerance in our cotransplantation tolerance model; and 2) tolerance induction itself was protective against the normally detrimental effects of this proinflammatory cytokine.

The reason why tolerance induction was able to prevent rIFN- γ -mediated tissue damage is not clear. Other investigators have ascribed the ability of IFN- γ to promote inflammation and

rejection to its known capacity to up-regulate endothelial MHC class II expression (36–42). Based on this hypothesis, one could postulate that the induction of tolerance and/or the CyA treatment (43) itself may have suppressed the class II upregulation caused by IFN- γ and thus dampened the inflammatory response. However, our immunohistological studies showed that exogenous rIFN- γ treatment did in fact increase class II expression, despite CyA treatment and tolerance induction.

Alternatively, one could postulate that the production of anti-inflammatory cytokines during the process of tolerance induction counteracted the proinflammatory effects of IFN- γ . One candidate is interleukin (IL)-10, which is known to regulate Th1 responses (44,45) and reduce the antigen-presenting capacity of APCs (46–48). Indeed, previous work from our laboratory has demonstrated that CyA-based tolerance induction protocols in swine are accompanied by the upregulation of IL-10 in graft infiltrating cells (49). Furthermore, we have shown that IL-10 production is one mechanism of action of porcine regulatory T cells (Tregs) (50) and that Tregs play an important role in the heart/kidney cotransplantation model of tolerance (35,51,52). The generation of Tregs should not have been affected by the local infusion of IFN- γ .

Finally, it is possible that the early presence of IFN- γ tipped the balance of the immune response away from inflammation and toward regulation (53). Emerging evidence shows that the role of IFN- γ in an immune response, and inflammation in general, is more complex than once thought and paradoxically includes both proinflammatory and anti-inflammatory components (53–55). Although IFN- γ clearly plays an important role in the Th1-driven acute rejection response by promoting cytotoxic T cell (CTL) generation, antibody/cytokine production, and MHC antigen expression (1–7,56–59), it can also suppress inflammation and promote regulation (53–55). For instance, IFN- γ has been shown to inhibit CTL generation (60) and prevent early allograft necrosis in renal allografts (61). Furthermore, the long-term survival of murine skin and heart allografts induced by costimulatory blockade (58,62,63) and the spontaneous acceptance of murine liver allografts (64), are both highly dependent on the presence of IFN- γ and its receptor. Importantly, Sawitzki et al. (65) and others (66) have shown that IFN- γ is required for the functional activity of alloantigen-reactive regulatory T cells during the development of operational tolerance to donor alloantigens *in vivo*. Based on these and other data, Wood and Sawitzki (53) have suggested a model, where by the early presence of IFN- γ not only inhibits the activation and proliferation of IFN- γ R1 and IFN- γ R2-bearing alloaggressive T cells, but also indirectly prevents further T-cell activation by creating a microenvironment that influences the function of APCs. This influence is likely to be the result of IFN- γ -induced inducible nitric oxide synthase, indoleamine-2,3-dioxygenase and heme oxygenase-1 expression (53).

Another interesting finding in this study was that rIFN- γ infusion had no effect on native hearts but caused hearts in nearly syngeneic G8 donor/recipient pairs to reject acutely, even while on CyA. This was surprising because the G8 donor/recipient pairs used in this experiment were one generation more inbred than animals previously shown to accept reciprocal skin and heart grafts indefinitely *without* immunosuppression (20). One could argue that this result was still due to a small number of residual minor antigen differences between the G8 animals (20). However, a 12-day course of CyA usually prevents the immune response generated by minor antigens in kidneys (67), albeit less so in hearts (30). It is also possible that antigen-independent inflammation associated with ischemia/reperfusion injury related to the transplant procedure contributed to IFN- γ -induced rejection in the G8 animals. Indeed, studies in a rodent ischemia-reperfusion model demonstrated that IFN- γ produced within 48 hr after transplantation can amplify an MHC-independent inflammatory response (68).

We know that the tissue injury associated with antigen-independent or nonspecific inflammatory responses resulting from ischemia-reperfusion (11,12) and brain death (13,14)

is mediated in part by IFN- γ . Both phenomena accelerate acute rejection in experimental transplant models (17,18,69) and adversely affect allograft survival in humans (15,16,70). Although many approaches have been examined to prevent the inflammation and immune activation associated with ischemia-reperfusion injury and brain death, few have been tested clinically or have demonstrated beneficial effect (71,72). To our knowledge, no one has specifically investigated whether the induction of tolerance can ameliorate the consequences of ischemia-reperfusion injury and/or brain death.

After transplantation, the recipient's immune system localizes and intensifies the nonspecific inflammatory response manifested by upregulation of endothelial MHC expression and adhesion molecules, cytokine/chemokine release, and T-cell activation at the site of injury. These data suggest that the induction of tolerance may mitigate the immune-related exacerbation of the inflammatory process in the allograft and break, or at least dampen, the cycle of inflammation, immunity, and tissue injury. Likewise, the induction of tolerance may diminish the contributions of the innate immune system to allograft rejection by stabilizing T lymphocytes which, when activated, play an important role in the activation of macrophages and NK cells.

There is precedent for promoters of peripheral tolerance such as costimulatory blockade to have a beneficial effect on antigen-independent inflammation (73–75). Our findings lend credence to this hypothesis by demonstrating that the induction of tolerance prevented the tissue injury caused by the direct administration of rIFN- γ . Of course, other cytokines such as IL-1 and tumor necrosis factor- α are also dominant mediators of inflammation in ischemia-reperfusion injury and brain death (76) and will need to be investigated in this model.

In summary, our studies demonstrate that coronary perfusion with rIFN- γ promotes acute cellular rejection in MHC mismatched and MHC-matched heart grafts but that the induction of tolerance counteracts the deleterious effects of this cytokine. The fact that tolerance induction could overcome the combined effects of histoincompatibility, ischemia/reperfusion injury, and IFN- γ overproduction may imply that tolerance actively ameliorates the powerful nonspecific inflammatory effects associated with ischemia/reperfusion injury and brain death in human transplantation.

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FIGURE 1.

Histology of rIFN- γ perfused hearts. (A) MHC class II staining of allograft myocardium from the tolerant recipient no. 12125 transplanted with a class I disparate heart and kidney 86 days earlier but not treated with rIFN- γ (untreated control). (B) MHC class II staining of allograft myocardium from the tolerant recipient no. 14876 transplanted with a class I disparate heart and kidney 100 days earlier and treated with intracoronary rIFN- γ perfusion. (C) Hematoxylin/eosin stain of rIFN- γ perfused near-syngeneic heart graft of recipient no. 14757 on POD 35. (D) Hematoxylin/eosin stain of the native heart of animal no. 15053 treated with intracoronary IFN- γ for 100 days. (E) Hematoxylin/eosin stain of a rIFN- γ perfused MHC class I mismatched heart, 100 days after heart-kidney cotransplantation in recipient no. 14876.

FIGURE 2.

Antidonor antibody production in animals transplanted with class I mismatched hearts and treated with 12 days of CyA. At indicated time points, serum antidonor MHC class I antibodies were measured using indirect fluorescence-activated cell sorting as described in methods. Heart-kidney transplantation indicates tolerant recipients cotransplanted with heart and kidney allografts. Grafts were perfused with rIFN- γ or saline (carrier) as indicated in the legend.

FIGURE 3.

CML assays of tolerant animals treated or not treated with intracoronary rIFN- γ . Recipient nos. 17033 and 15258 were cotransplanted with MHC class I-mismatched heart and kidneys under a 12-day course of CyA. Recipient no. 15258 received intracoronary IFN- γ perfusion, whereas recipient no. 17033 did not. PBMCs of both animals were tested for cytotoxicity against donor (●) and third party (Δ) type targets as described in methods.

TABLE 1

Intracoronary rIFN- γ infusion in isolated MHC class I disparate cardiac allografts

Histology by ISHLT scoring system											
Treatment	Animal no.	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Graft survival (days)	Mean survival time±SD (days)
Group 1											
None	13384 ^a	4									7
	13896 ^a	4									7
Group 2											
CyA	11068 ^a	1b	1b	—	2	—	—	3a		45	46±6.06
	11749 ^a	0	0	—	1b	—	—	—	3b	55	
	11776 ^a	1a	1a	—	3a	—	—	4		47	
	12049 ^a	—	—	—	—	1b	—	4		46	
	12048 ^a	—	—	—	—	3a	4			38	
Group 3											
CyA+saline	14862	3a	—	1a	—	—	—	4		47	38±8.19 ^b
	14695	1a	—	3b	—	4				31	
	14921	3a	—	3b	—	4				36	
Group 4											
CyA+rIFN-γ	14784	3b	4							13	19±7.21 ^c
	14630	0	1b	—	4					27	
	14660	3a	—	4						17	

^aPreviously published (29,30).^b $P = 0.32$ compared to group 2.^c $P = 0.025$ compared to group 3.

TABLE 2
Coronary rIFN- γ infusion in near-syngeneic isolated heart transplants and native hearts

Group	Experiment	Treatment	Animal no.	Histology by ISHLT scoring system								Graft survival (days)
				Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	
1	G7 hearts ^a	None	14230	—	—	—	—	0	—	—	0	>331 ^b
			14250	—	—	—	—	1b	—	—	0	>318 ^b
2	G8 hearts	CyA+rIFN γ	14638	4								5
			14915	0	4							17
			14757	—	1a	1b	—	3b				35
3	Native hearts	CyA+rIFN γ	15053	—	—	—	—	—	—	—	0	100
			15474	—	—	—	—	—	—	0		50 ^c

^aPreviously published (20).
^bAnimals were sacrificed with beating hearts.
^cAnimal pulled the coronary catheter on POD 50 and was sacrificed on the same day.

TABLE 3
Coronary rIFN- γ infusion in tolerant recipients of class I-disparate heart and kidney grafts

Coronary perfusion	Animal no.	Histology by ISHLT scoring system				Graft survival (days)
		Week 4	Week 7	Week 11	Week >14	
None ^a	12125	1a		0		>86 ^d
None ^a	11982	1b	1b	—	1a	>100
None	17033	—	0/4	—	1a/4	>100
None	16946	—	1a/4	—	0/4	>100
None ^a	12333	0	0	—	0	>100
Saline ^b	15508		1a		0	>100
rIFN- γ ^c	14876	0	0	0	2	>100
rIFN- γ ^c	15004	0	0	0	1a	>100
rIFN- γ ^c	15258	0	—	0	0	>100
rIFN- γ ^c	15137	0	—	0	0	>100

^aThe results of these control animals have been reported previously.

^bIntracoronary infusion of normal saline as described in Methods (22).

^cIntracoronary infusion rIFN- γ 100–200 ng/day.

^dAnimal died from unknown reason and was graftectomized on the day of death.