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The Effects of Tolerance Induction on the Actions of Interferon- γ on Cardiac Allografts

Ruediger Hoerbelt^{*}, Louis C. Benjamin^{*}, Tsuyoshi Shoji^{*}, Douglas R. Johnston^{*}, Ashok Muniappan^{*}, Dax A. Guenther^{*}, James S. Allan^{*,†}, Stuart L. Houser^{*}, and Joren C. Madsen^{*,†}

^{*}*Transplantation Biology Research Center, Department of Surgery, Massachusetts General Hospital/Harvard Medical School, Boston, MA 02129, USA.*

[†]*Division of Cardiac Surgery, Cardiac Transplantation, Massachusetts General Hospital / Harvard Medical School, Boston, MA 02114, USA*

[‡]*Division of Thoracic Surgery, Massachusetts General Hospital / Harvard Medical School, Boston, MA 02114, USA*

Abstract

It is well known that Interferon- γ (IFN- γ) not only plays a critical role in antigen-dependent but in antigen-independent tissue injury, but it is not clear how tolerance induction affects the actions of IFN- γ in the transplant setting. To address this question, we compared the effects of IFN- γ on porcine recipients of syngeneic, rejecting and tolerant heart transplants. IFN- γ was perfused continuously into the left anterior descending artery of hearts transplanted into three groups of MHC inbred miniature swine, each treated with a 12-day course of CyA. Group 1 recipients received a class I disparate heart, Group 2 recipients received a near syngeneic heart and Group 3 recipients were cotransplanted with a class I disparate heart and kidney, which uniformly induces tolerance to both grafts. An additional, group of animals were not transplanted but received intracoronary IFN- γ infusion into their native hearts. IFN- 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 survive indefinitely, to reject within 35 days ($n=3$). In contrast, IFN- γ perfusion had no demonstrable effects on either interstitial rejection, the development of vascular lesions or graft survival in tolerant heart plus kidney allograft recipients ($n=4$) or in autologous hearts ($n=2$). These results suggest that tolerance induction mitigates the damaging effects of IFN- γ itself and that the beneficial effects of tolerance induction on acute and chronic rejection may extend to antigen-independent factors like ischemia/reperfusion injury.

Introduction

The Effects of Tolerance Induction on the Actions of Interferon- γ on Cardiac Allografts

Interferon- γ (IFN- γ) has been known to play a critical role not only in antigen-dependent but also in innate immunity. IFN- γ produced by activated CD4⁺ and CD8⁺ T cells and NK cells stimulates the production of various chemokines such as IFN- γ -inducible protein 10 kD (IP10)

Corresponding author: Joren C. Madsen, M.D., D.phil., Department of Surgery, Massachusetts General Hospital, Boston, MA 02114, USA E-mail address: madsen@helix.mgh.harvard.edu.

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and monokine induced by IFN- γ (MIG) (1). These, in turn, promote the infiltration of polymorphnuclear leukocyte (PMN) and ultimately, tissue necrosis. Other functions of IFN- γ during acute rejection include facilitating immunoglobulin (Ig) class switch to IgG3 (2), increasing the expression of Fc receptors by monocytes/macrophages and NK cells (3) and the induction of major histocompatibility class (MHC) II (4). Given that IFN- γ plays a central role in both the antigen dependent and independent components of allograft rejection we hypothesized that the induction of tolerance exerts some of its beneficial effects on allograft survival by mitigating the proinflammatory effects of this critical cytokine. To test this hypothesis, we administered IFN- γ directly into the coronary arteries of transplanted porcine hearts which avoided the confounding effects of systemic administration.

Methods

Recombinant porcine IFN- γ (200 ng per day) was continuously infused into the left anterior descending artery of heart grafts using osmotic mini pumps attached to vinyl catheters. The heart grafts were heterotopically transplanted into three groups of MHC inbred miniature swine, each treated with a 12-day course of cyclosporine A (CyA; trough levels 400 - 800 ng/ml). Group 1 recipients received a MHC class I disparate heart. In Group 2 transplants were performed between highly inbred animals (G8; co-ancestry > 0.92) (5) referred to as near-syngeneic. Group 3 recipients were cotransplanted with a MHC class I disparate heart and kidney, which uniformly induces tolerance to both grafts (6). An additional group of animals received intracoronary IFN- γ infusion into their native hearts. Heart grafts of control animals were perfused with carrier alone. Study endpoints were graft failure or postoperative day (POD) 100.

Results

Unmanipulated MHC class I mismatched cardiac allografts (Group 1) were rejected between 38 and 55 days and coronary perfusion with carrier alone did not alter the time course of rejection ($p=0.32$). In contrast, perfusion of MHC class I disparate cardiac allografts with IFN- γ significantly accelerated acute cellular rejection with graft failure occurring between POD 13 and 27 days ($p=0.025$). Indeed, severe cellular rejection was seen while recipients had therapeutic levels of CyA (> 400 ng/ml; Fig. 1). Near-syngeneic grafts in Group 2 recipients were uniformly long-term accepted, whereas coronary perfusion with IFN- γ led to rejection on POD 5, 17 and 35 (Fig. 1). On histology, these grafts showed interstitial cellular infiltrates accompanied by graft necrosis typical for acute rejection. In contrast, tolerance induction by cotransplantation of MHC class I mismatched heart plus kidney and a short course of CyA (group 3) led to uniform acceptance of carrier- and IFN- γ perfused heart transplants ($n=4$). On histology there was no evidence of rejection, which was similar to controls ($n=4$; Fig. 1). Coronary IFN- γ perfusion in two native hearts ($n=2$) had no demonstrable affect on cardiac dysfunction, EKG or tissue histology (data not shown).

Discussion

This study was undertaken to evaluate the effect of tolerance induction on the actions of continuous coronary IFN- γ perfusion of cardiac allografts. Coronary perfusion with IFN- γ significantly accelerated acute rejection of MHC class I mismatched heart grafts. The earliest rejection occurred as early as five days after transplantation while the recipient was still on high dose immunosuppression. Similar to MHC-mismatched recipients, IFN- γ perfusion facilitated rejection in near-syngeneic recipients. Although there were minimal minor antigen differences in G8 animals (5), that may have been unmasked by the IFN- γ perfusion, the rapidity with which heart grafts were rejected suggests that antigen-independent mechanisms were responsible. Likewise, recent studies by El Sawy and colleagues showed that CD8+

memory T cells produced IFN- γ within 48 hours after transplantation - and before T cell priming occurred -, which amplified an antigen-independent inflammatory response (7). The fact that no inflammation was observed in IFN- γ perfused native hearts may indicate that some tissue injury like ischemia-reperfusion is needed in addition to the IFN- γ . Given the rapid rejection of near-syngeneic grafts we were surprised that no rejection occurred in IFN- γ perfused, MHC class I mismatched heart grafts of heart-kidney recipients. According to historical experiments, transplantation of a MHC class I disparate kidney combined with a 12-day course of high-dose CyA induces stable tolerance not only to the kidney but also to the co-transplanted heart (6). Studies investigating the mechanism of tolerance in this model revealed regulatory T cells (8) and upregulation of interleukin (IL) 10 (9) in tolerant heart-kidney recipients. Interleukin 10 has been known to counteract some of the actions of IFN- γ such as macrophage activation and MHC class II induction (10), and we therefore speculate that IL 10 may have inhibited inflammation and rejection of IFN- γ perfused heart grafts. In conclusion, our results suggest that tolerance induction mitigates the damaging effects of IFN- γ itself and that the beneficial effects of tolerance induction on acute and chronic rejection may extend to antigen-independent factors like ischemia/reperfusion injury.

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Figure 1.

Graft survival of IFN- γ - or carrier perfused heart grafts in rejecting (Ht-Tx), near-syngenic (G8-Ht-Tx) and tolerant (Ht+Kd-Tx) recipients. All animals received a 12-day course of high-dose CyA.