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Innate Immunity in heart transplantation

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Abstract

Purpose of review—Cardiac transplantation is the treatment of choice for end-stage heart failure but its efficacy is limited by the development of cardiac allograft vasculopathy. Although the adaptive immune system is efficiently suppressed by conventional drugs, the innate immune system is largely unaffected. The innate response may contribute both to stimulation of the adaptive response and to the future development of CAV.

Recent findings—Stimulation of Toll-like receptors by endogenous ligands released in response to ischemia/reperfusion causes an inflammatory milieu favorable to graft rejection and unfavorable to tolerance. New evidence suggests that natural killer cells have previously unknown memory-like features and are capable of graft rejection. Their role in rejecting the cardiac allograft has previously been underestimated. Complement deposition may also contribute to ACR and CAV.

Summary—The innate immune system is an important but neglected component of allograft rejection. Drugs that target TLRs, NK cells and complement may play an important role in preventing CAV and achieving tolerance to cardiac allografts.

Keywords

innate immunity; heart transplant; toll-like receptors; natural killer cells; complement

Introduction

In the fifty-five years since the first successful solid organ transplant, increasing understanding of the adaptive alloimmune response has led to steady improvement in rejection-free survival of graft recipients. Nevertheless, the therapeutic potential of cardiac transplantation is limited by the development of chronic rejection in the form of cardiac allograft vasculopathy (CAV), which is present in 43 percent of recipients by the end of the eighth year post-transplantation [1*].

The observation that peri-transplant ischemic injury contributes significantly to accelerated arteriosclerosis in an alloantigen-independent fashion [2] led to increasing interest in the effects of ischemic/reperfusion (I/R) on the cardiac graft, and in particular the response of the innate immune system to the injuries caused by the oxidative stress of I/R.

I/R injury activates the cellular (dendritic cells and innate lymphocytes) and humoral (natural IgM and complement) components of the innate immune system which combine to trigger the innate immune response. The innate response in turn collaborates in the initiation and

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amplification of the adaptive alloimmune response that leads ultimately rejection of the allograft.

Toll-like receptors

Among the best-understood actors in the innate immune system are the toll-like receptors (TLR), a family of transmembrane proteins expressed on epithelial cells, dendritic cells, macrophages and T- and B-lymphocytes (Table 1). One of the most ancient components of the immune system, TLRs bind to conserved ligands on microbial pathogens such as lipopolysaccharide on Gram-negative bacteria. TLRs are thought to be activated both by exogenous and by endogenous ligands [3*].

Release of endogenous ligands results from the I/R injury that follows global myocardial ischemia. Some of these ligands are the direct result of tissue injury—for example, the extracellular matrix polysaccharide hyaluronan [8] accumulates in the myocardial interstitium during allograft rejection and activates dendritic cells predominantly by binding to TLR4.

In addition to products of tissue injury, I/R leads to the release of actively secreted messenger molecules including heat-shock protein 70 and high-mobility group box 1 (HMGB1). Zou et al [9**] have demonstrated that I/R leads to the release of heat shock cognate protein 70 (hsc70) from the heart and that this leads to the TLR4-dependent inflammatory response. Isolated mouse hearts were subjected to 20 minutes of warm ischemia followed by 60 minutes of reperfusion. Prior to I/R injury, HSC70 was detectable within the cytoplasm of cardiomyocytes but not in the coronary effluent; after I/R, the protein was detectable in the coronary effluent and in the extracellular space. Expression of inflammatory cytokines TNF- α , IL-1 and IL-6 after I/R was reduced by antibody blockade of HSC70 and increased by treatment with recombinant HSC70 (rHSC70). In TLR4-deficient mice there was no up-regulation of inflammatory cytokines.

A role for HMGB1 in cardiac I/R injury has also recently been established in mice [10]. Ligation of the left anterior descending artery led to increased HMGB1 expression within 30 minutes with a peak six hours after reperfusion. I/R injury was worsened by treatment with recombinant HMGB1 and ameliorated by inhibition of HMGB1.

Activation of TLR4 initiates an inflammatory cascade that contributes to myocardial dysfunction after I/R injury. Cha et al [11**] demonstrated this effect by subjecting isolated mouse hearts to global ischemia. They found that, in comparison to wild-type animals, mice carrying mutations in TLR4 demonstrated significantly higher left ventricular diastolic pressure (LVDP) and dP/dtmax 40, 50 and 60 minutes after reperfusion. I/R was associated with a 6-8 fold increase in TNF- α and a 6-15 fold increase in IL-1 in wild type mice but not in TLR4 mutants, and there was a four-fold increase in NF- κ B DNA-binding activity seen only in wild-type mice animals. Inactivating mutations of TNF- α and IL1 were similarly cardioprotective. In TLR4-deficient mice, an exogenous source of TNF- α and IL1 restored the deleterious effects of ischemia and reperfusion.

A similar effect was reported in vivo in rats subjected to one hour of ischemia followed by reperfusion [12]. In the I/R group, TLR4 mRNA and protein levels increased with a peak one hour after reperfusion. There were significantly higher TNF- α and IL6 levels compared to a sham group. These data suggest an immediate harmful effect of TLR4-driven inflammation in the immediate post-transplant period.

Although these experiments were performed in models of myocardial infarction, a similar effect occurs after reperfusion of an ischemic, cold-preserved graft [13]. When syngeneic heart transplants are performed in TLR4-mutant mice after cold two hours of cold ischemia, serum

and graft levels of inflammatory cytokines (TNF- α , IL6, MCP-1, IL1 β) are significantly lower than when the same transplants are performed in animals with intact TLR4. In the mutant mice, there is less translocation of myocardial NF κ B and reduced neutrophil infiltration.

In addition to early myocardial dysfunction, TLR activation appears to promote subsequent development of CAV. Methe et al [14] analyzed expression levels of TLR4 and its downstream targets (B7, IL12 and TNF- α) in the circulating monocytes of human cardiac transplant recipients. In the 13/38 patients that developed allograft endothelial dysfunction, expression of TLR4 and secretion of IL-12 and TNF- α was significantly elevated compared to the remaining 25 patients. They observed a similar effect in mice receiving heterotopic heart transplants. Endothelial dysfunction is a highly sensitive and specific precursor to the development of CAV, suggesting that upregulation of TLR4 could be the first step in a chain of events leading to chronic rejection.

Activation of the innate immune system via TLRs can impair the development of tolerance [15]. In mice, transplanted organs that are protected from pathogenic stimuli (including the heart) are tolerated after blockade of the CD28-B7 costimulatory interaction using an anti-CD154 antibody. Those organs commonly exposed to antigens (lung, skin, bowel) are resistant to co-stimulatory blockade. When mice are treated with a TLR agonist, CpG, at the time of an allogeneic heart transplant, administration of anti-CD154 does not lead to tolerance and allografts are rejected acutely [15]. In anti-CD154-treated mice, FoxP3⁺ Tregs accumulate in the graft but this effect is not seen in TLR-stimulated mice.

Spontaneous tolerance to cardiac allografts is also impeded by TLR-activation [7**]. Although B6 and bm12 mice are MHC class II disparate, B6 mice accept cardiac allografts from bm12 donors without immunosuppression and the survival of skin grafts between these strains is prolonged more than 50 days by a short course of sirolimus and costimulatory blockade. When treated with the TLR-agonist CpG long-term acceptance is prevented. Treatment with CpG induces naïve precursor T cells to differentiate into Th1 effector T cells and interferes with Treg suppression [7**].

Lymphocytes of the innate immune system

The understanding of the cellular components of the innate immune system is also evolving rapidly. Natural killer cells are lymphoid cells that were thought not to require sensitization to lyse foreign target cells (Table 2). The “missing self” hypothesis postulates that NK cells are activated by the absence of self MHC molecules on target cells. Autoreactivity of NK cells is prevented by inhibitory receptors specific for self-derived MHC class I ligands. In addition, stimulatory receptors, most notably NKG2D, recognize antigens on virally infected or tumor cells. When the balance of stimulatory and inhibitory signals received by the NK cell favors activation, the result is cell lysis by perforin and initiation of inflammation by cytokine release, principally interferon-gamma. These cells were believed not to acquire immunologic memory or participate in the rejection of transplanted solid organs

The conventional wisdom about NK cells has been challenged by several recent findings that could have profound implications for cardiac transplantation: NK cells now appear able to acquire a memory phenotype and to reject a transplanted organ.

Mouse NK cells carrying a receptor specific for cytomegalovirus (Ly49H) are induced to expand by viral infection. Sun et al [16*] demonstrated that, after a period of contraction, a portion of these expanded Ly49H-positive cells lie dormant for 40-50 days and can mount a secondary expansion after a repeated stimulus. “Memory” NK cells retain this property after adoptive transfer to a naïve animal.

There is also evidence for antigen-independent development of an NK memory-like subset. Cooper et al [17*] expanded NK cells *ex vivo* using IL12 and IL18 with IL15 as a survival factor. When adoptively transferred into Rag -/- mice, which lack T- and B-cells, these expanded NK cells were phenotypically similar to host NK cells, but produced significantly more IFN- γ than naïve NK cells on re-stimulation 1-3 weeks later. The memory-like NK cells did not however, demonstrate enhanced cytotoxicity. The existence of memory-like NK cells raises the possibility that long-term cardiac allograft survival without CAV may require therapies that either inhibit the development of NK memory or deplete existing populations of previously sensitive NK memory cells.

In addition to acquiring a memory phenotype, NK cells are able to reject allogeneic skin grafts. Kroemer et al [18*] studied skin allograft rejection in Rag -/- mice, which lack T- and B-lymphocytes and Rag-/- γc -/- mice which additionally lack NK cells. Although skin grafts are heavily infiltrated by NK cells in the Rag -/- recipients, the grafts were not rejected in either group. When Rag -/- mice were treated with IL-15, the NK cell population expanded and rejected allogeneic (but not syngeneic) skin grafts. When IL-15 was withdrawn the NK population returned to a resting state and did not reject a subsequent MHC-disparate graft 30-40 days later.

Although blockade of the CD28-B7 costimulatory interaction with anti-CD154 leads to tolerance of cardiac allografts in mice, CD28-deficient mice remain able to reject cardiac allografts through a CD8-mediated process [19]. In these CD28-/- mice, a subset of NK cells are recruited to allogeneic (but not syngeneic) grafts after transplantation. These NK cells are able, either directly via cytokine release or indirectly via promoting DC maturation, to promote antigen-specific CD8⁺ T-lymphocyte proliferation leading to graft rejection [20]. Treatment of CD28-/- mice with a neutralizing antibody against NKG2D, an activating receptor expressed by NK cells, prolonged the survival of cardiac allografts from 21.3 to 70.1 days [21*].

NK-dependent rejection has recently been demonstrated to be important in rejection of cardiac xenografts. In a mouse heart-to-rat xenotransplantation model [22], rejection of xenogeneic tissue is associated with infiltration by macrophages and NK cells with significant IFN- γ production and relatively little T-cell infiltration. Treatment with cyclosporine has no effect on survival, whereas depletion of NK cells with anti-asialo-GM-1 led to significant prolongation of graft survival.

Evidence for a critical role for NK cells in acute rejection in human patients is limited. A recent report [23] compared number of NK cells in peripheral blood and endomyocardial tissue in 20 patients with acute cellular rejection (grade 3a) with 19 stable patients (grade 0). There was a significant depletion of NK cells in the blood of rejecting patients and an increase in CD16⁺ NK cells in graft biopsy specimens, suggesting that NK cells home to the graft during rejection. A similar finding has been reported in recipients of lung transplants, although in this case chronic rather than acute rejection was present [24*].

The importance of NK cells to the development of CAV was demonstrated in mice by transplanting hearts from parental donors to F1 hybrid recipients [25]. Solid organs transplanted in this fashion were accepted without immunosuppression; there was no host adaptive immune response and the organs did not develop acute cellular rejection. However, when hearts were removed at 56 days post-transplant, 19/22 had developed advanced CAV. IFN- γ deficient and T-cell-deficient recipients of parental-to-F1 hybrid transplants did not develop CAV.

The activation of NK cells may be facilitated by TLR-mediated interactions with dendritic cells and macrophages. Stimulation of monocytes and macrophages with LPS leads to production of several ligands of NKG2D, including retinoic acid early inducible-1 (RAE-1) [26] and MHC

class I-related chain A (MICA) [27]. In the presence of IL-2, TLR-activated monocytes were capable of stimulating NK cells to secrete IFN- γ . Hochweller et al [28] were able to deplete dendritic cells efficiently by creating a transgenic mouse in which the diphtheria toxin receptor is expressed only in DCs. They demonstrated that NK cells were activated by the TLR ligand CpG only in the presence of DCs, and that production of IL-15 by DCs is needed to maintain NK cell homeostasis.

Complement

Reperfusion exposes the graft endothelium to host complement proteins, potentially triggering a cascade leading to inflammation, coagulation and irreversible tissue damage. In a series of cardiac graft biopsy specimens 1-3 weeks after transplantation, deposition of C4d and C3d was histologically linked to peri-transplant ischemic injury and patients with complement deposition were more likely to demonstrate repeated episodes of rejection on later biopsies [29]. More recently, deposition of C4d has been strongly linked to the development of CAV in the first year after transplant [30].

There is increased expression of complement genes by graft-infiltrating leukocytes when acute cellular rejection is present. Analysis of samples from human heart transplant recipients patients demonstrated higher transcript numbers of complement factor B, C3 and properdin, and C3a receptor and C5a receptor as well as other genes potentially associated with rejection including CD3, interferon gamma, perforin, and granzyme B [31].

Complement activity in the cardiac allograft appears to play a role in triggering alloreactive CD8⁺ T-cells [32]. Complement activation is inhibited by decay-accelerating factor (DAF). When cardiac allografts from mice deficient in DAF are transplanted into wild-type hosts, graft rejection is accelerated in an antibody-independent fashion. In the absence of DAF, C3 production by graft cells leads to enhanced proliferation of alloreactive CD8-cells.

Impaired complement activation may be protective against rejection in both animal models and human patients. When rats receiving heart transplants were treated with a blocking “minibody” against C5 prior to transplantation, there was a reduction in cardiomyocyte apoptosis and necrosis and in levels of serum creatine phosphokinase and TNF- α compared to untreated controls [33*]. Mannose-binding lectin, a protein that is essential to the initiation of the lectin pathway of complement activation, is defective in 5-10% of patients. In a series of 90 heart transplant recipients, patients with MBL deficiency had fewer rejection episodes (6.3 $\pm$ 3.8%) than those with normal MBL (12.9 $\pm$ 11.6%) [34].

Conclusion

Conventional immunosuppressive drugs, including antiproliferative agents and calcineurin inhibitors, target the adaptive immune system; the innate immune system is largely unaffected even by heavy immunosuppression. Improved understanding of innate immunity suggests several potential therapeutic targets, including blocking the response of Toll-like receptors to I/R injury, depleting NK cells or blocking their activation and interfering with complement activation and deposition. Controlling the innate response may be the crucial missing piece in the puzzle of preventing CAV.

Abbreviations

ACR	Acute cellular rejection
CAV	Cardiac allograft vasculopathy

I/R	Ischemia/reperfusion
NK	Natural killer
TLR	Toll-like receptor
LVDP	left ventricular diastolic pressure

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Table 1

Toll-like receptors expressed in mammalian hearts and their immunologic significance

TLR	Exogenous agonists	Exogenous sources	Endogenous agonists	Significance to heart	References
TLR2	Lipoteichoic acid, Peptidoglycan	Gram positive bacteria	Hyaluronan	After I/R, TLR2-/- mice have smaller infarct sizes and reduced inflammatory response. TLR2-/- heart transplant recipients may have slightly prolonged survival of cardiac allografts.	[3,4]
	Lipopolysaccharide	Mycobacteriae	Hsp-60 Hsp-70		
	Lipopolysaccharide	Gram-negative bacteria	Fibronectin Fibrinogen Hyaluronic acid Hyaluronan Heparan sulfate		
TLR4			Hyaluronan	After I/R, TLR4-/- mice have maller infarct sizes, decreased cardiac expression of TNF, improved systolic function and reduced left ventricular remodeling. TLR4-/- heart transplant recipients appear to have similar graft survival to wild type animals.	[3,4]
TLR3	dsDNA	Viruses	mRNA	Significance to I/R and transplantation is unknown. After EMCV infection, TLR3-/- mice demonstrate impaired proinflammatory cytokine expression but interferon expression is normal.	[5,6]
TLR5	flagellin	Bacteria	Unknown	Unknown	[3]
TLR7	ssRNA	Viruses	Unknown	Unknown	[3]
TLR9	Unmethylated CpG DNA	Bacteria	Unknown	Treatment of cardiac allograft recipients with CpG abrogates tolerance after costimulatory blockade by impairing Treg function and promoting Th1 differentiation.	[7]

Table 2

Characteristics of lymphocytes of the adaptive and innate immune systems

	B-lymphocytes	T-lymphocytes	Natural killer cells
Function	Secretion of specific antibodies	Cellular immunity, costimulation of B-lymphocytes	Innate immunity against intracellular and viral infections
Receptors	Surface immunoglobulins with near-infinite repertoire due to somatic recombination	Near infinite repertoire of T-cell receptors (TCR), formed by somatic rearrangement	Germ-line encoded inhibitory and activating receptors for MHC class I
Requirements for activation	B-cell receptor engagement, costimulation by T cell (can also occur in T-cell independent fashion)	TCR engagement, costimulation via CD28-B7 interaction	Stimulation of activating receptors. Cytotoxicity increased by IFN- α and IFN- β , IL12, TNF α . Proliferation induced by IFN- α , IFN- β
Effector mechanism	Expansion after clonal selection, antibody secretion	Cytokine production, direct cytotoxicity	Direct cytotoxicity, proinflammatory cytokines (principally IFN γ)
Memory response	Long-lived memory B cells formed after initial stimulus	Memory T cells formed after initial stimulus	Recently discovered, not fully understood.
Role in allograft rejection	Antigen presentation, humoral rejection	Acute cellular rejection	Not well understood.