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Innate immunity and cardiac allograft rejection

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Abstract

The development of immunosuppressive drugs to control adaptive immune responses has led to the success of heart transplantation as a therapy for end-stage heart failure. However, these agents are largely ineffective in suppressing components of the innate immune system. This distinction has gained clinical significance as mounting evidence now indicates that innate immune responses have important roles in the acute and chronic rejection of cardiac allografts including cardiac allograft vasculopathy (CAV). Whereas clinical interest in natural killer (NK) cells was once largely confined to the field of bone marrow transplantation, recent findings suggest that these cells can also participate in the acute rejection of cardiac allografts and in the development of CAV. Stimulation of Toll-like receptors (TLRs), another important component of innate immunity, by endogenous ligands released in response to ischemia/reperfusion is now known to cause an inflammatory milieu favorable to graft rejection. Finally, new data indicate that activation of complement is linked to acute rejection and CAV. In summary, the conventional wisdom that the innate immune system is of little importance in whole-organ transplantation is no longer tenable. The addition of strategies that target TLRs, NK cells, and complement will be necessary to prevent CAV completely and to eventually achieve long-term tolerance to cardiac allografts.

Keywords

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

Although more than 85,000 heart transplants have been performed worldwide since 1982 and the transplant half-life has improved to 10 years,[1] the procedure remains a palliation rather than a cure. The need for lifelong immunosuppression with its attendant side effects of infection and malignancy, coupled with the high incidence of cardiac allograft vasculopathy (CAV)—43% by the end of the eighth post-transplant year[2]—contributes to a steady linear increase in mortality by 3.5% each year, continuing for more than 15 years after transplantation.

Increasing the understanding of the adaptive immune system has permitted the development of drugs to prevent or treat acute cellular rejection, leading to significant improvement in survival in the first year after transplantation.[2] However, critical events immediately after heart transplantation, including graft infiltration by natural killer (NK) cells, activation of Toll-like receptors (TLRs), and complement deposition may set the stage for the development of graft injury and chronic rejection, ultimately leading to graft loss.

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Understanding the innate immune response to the cardiac allograft is therefore critical to maximizing the therapeutic benefits of cardiac transplantation.

NK Cells

The principal cellular component of the innate immune system is the NK cell, a lymphoid cell that is capable of responding to target cells without previous sensitization. NK cells can be activated by the absence ('missing') of self major histocompatibility complex molecules on their cellular targets and by the stimulation of receptors capable of identifying a limited number of viral and tumor antigens. When the combination of stimulation and inhibition received by the NK cell leads to activation, the outcome is target cell lysis by perforin and initiation of inflammation by cytokine release, principally interferon-γ (IFN-γ). Until recently, NK cells were believed not to acquire adaptive memory or contribute to the rejection of solid allografts. However, this established paradigm has been challenged by a number of reports that suggest a more complex role for this lymphocyte subset in whole-organ transplantation.

It is clear that NK cells are active in the early stages of allograft rejection. In the first few days after allogeneic heart transplantation, the majority of infiltrating lymphocytes are NK cells.[3] In murine recipients of cardiac allografts, expression of the NK cell-activating receptor NKG2D and its ligands, including retinoic acid early inducible (RAE-1) and minor histocompatibility antigen H60, is upregulated from 3 to 5 days after transplantation.[4] Expression increases with time as rejection develops, and only a modest effect is seen in the recipients of syngeneic grafts, suggesting that this receptor–ligand interaction could have a role in stimulating rejection.

Early evidence that NK cells could have a role in the acute rejection of cardiac allografts originated with studies using co-stimulation-deficient (CD28−/−) mice. Although blockade of the CD28-B7 co-stimulatory 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.[5] CD 28−/− mice can, however, be made to accept cardiac allografts by depletion of $NK1.1^+$ T cells.[6] In CD28−/− mice, a subpopulation of NK cells homes to allogeneic (but not syngeneic) grafts after transplantation. Antibody-mediated blockade of the activating receptor NKG2D prolongs the survival of cardiac allografts in CD28−/− mice from 21.3 to 70.1 days.[7] This finding suggested that NK cells could facilitate antigen-specific $CD8^+$ T-lymphocyte proliferation leading to graft rejection, either through the direct action of secreted cytokines or by promoting dendritic cell maturation.[8]

Although studies in CD28−/− mice suggest that NK cells participate in acute rejection by promoting the effects of alloreactive T lymphocytes rather than by some intrinsic capability of NK cells to directly reject solid organ allografts (reviewed in Kitchens *et al.*[9]), under the right conditions, mice lacking T and B lymphocytes (Rag−/−) are able to reject allogeneic skin through an NK-mediated process.[10] When skin allografts were placed on Rag−/− mice, NK cells infiltrated the graft but did not lead to rejection. However, when the animals were treated with interleukin (IL)-15, a powerful stimulator of NK cells, the allografts were rejected as the NK population expanded. In Rag−/− γc −/− animals, which also lack NK cells, no rejection occurred. Finally, when IL-15 was withdrawn, the ability of NK cells to reject the graft was lost. These studies counter conventional wisdom and suggest that IL-15-activated NK cells have the capability to reject major histocompatibility complex disparate allografts directly, without contributing to the adaptive immune system.

NK cells have also been implicated in the development of CAV in experiments using a parental-to-F1 strain combination. Transplanting mouse heart from parental donors to F1 hybrid recipients avoids activation of the adaptive immune system and isolates responses

mediated by the innate immune system.[11] Solid organs transplanted in this manner were accepted indefinitely without immunosuppression as expected, as there is no host T- or B-cell response against the donor. However, when parental allografts were removed 56 days after transplantation, 19 of 22 had developed advanced CAV without evidence of acute rejection. IFN-γ-deficient recipients of parental-to-F1 hybrid transplants did not develop CAV. These data strongly suggest that NK cells can contribute to the formation of vascular lesions of CAV through an IFN-γ-dependent pathway.

In a global sense, previous studies suggest that the time-honored distinctions between the adaptive immune system and innate immune system are beginning to blur. This is best exemplified by recent studies showing that NK cells can exhibit immunological memory, the *sine qua non* of the adaptive immune system. Sun *et al.*[12] expanded mouse NK cells carrying a receptor specific for cytomegalovirus (Ly49H) by infecting them with cytomegalovirus. They demonstrated that a portion of these expanded Ly49H-positive cells can mount a secondary expansion after a repeated stimulus. 'Memory' NK cells retain this property after adoptive transfer to a naive animal. There is also evidence for antigen-independent development of an NK memory-like subset. NK cells can be expanded *in vitro* with IL-12 and IL-18, with IL-15 as a survival factor and adoptively transferred into Rag−/− mice, which lack T and B cells.[13] These expanded NK cells resemble host NK cells, but result in higher levels of IFN-γ than do naive NK cells in response to a second stimulus 1–3 weeks later, although they do not have enhanced cytotoxicity. The discovery of memory-like NK suggests that CAV-free acceptance of cardiac grafts may require therapies to block the development of NK memory or deplete existing populations of previously sensitive NK memory cells.

There is only limited evidence for a central role for NK cells in acute rejection of cardiac allografts in human patients. One recent report [14] compared NK cell numbers in peripheral blood and endomyocardium in 20 patients with acute cellular rejection (grade 3a) with that of 19 stable patients (grade 0). A marked reduction in NK cells was detected in the blood of rejecting patients with an associated increase in CD16+ NK cells in graft biopsies, raising the possibility that during rejection NK cells travel from the blood to the graft.

TLRs

TLRs are among the best-studied components of the innate immune system. This ancient group of transmembrane proteins is expressed on epithelial cells, dendritic cells, macrophages, and T and B lymphocytes. TLRs recognize conserved ligands such as lipopolysaccharide on Gram-negative bacteria and similar structures on other pathogenic microbes. There is evidence that TLRs are also activated by endogenous ligands,[15] some of which are released following ischemia/reperfusion (I/R) injury.

There is now evidence that the I/R injury, which inevitably accompanies transplantation of a cold-preserved cardiac allograft, is associated with the development of accelerated atherosclerosis.[16] This process may be mediated by TLRs. For instance, tissue damage releases endogenous proteins such as the extracellular matrix polysaccharide hyaluronan,[17] which activates dendritic cells by binding to TLR4. Messenger molecules, including high-mobility group box 1 and heat-shock cognate protein 70, are also released from the heart after I/R injury, which in turn initiate a TLR4-dependent inflammatory response.[18] In uninjured mouse hearts, heat-shock cognate protein 70 is confined to the cytoplasm but is released into the coronary vessels after a period of warm ischemia and reperfusion. Proinflammatory molecules tumor necrosis factor (TNF)-α, IL-1, and IL-6 are increased by I/R and by recombinant heat-shock cognate protein 70. This effect is diminished by antibody blockade of heat-shock cognate protein 70 and is absent in TLR4-deficient mice. A similar

phenomenon is observed with high-mobility group box 1, which is released within 30 min of ligation of the left anterior descending artery. Inhibition of high-mobility group box 1 limits I/R injury, whereas treatment with recombinant high-mobility group box 1 leads to an enhanced inflammatory response.[19] Elevations of TLR4 mRNA, TNF-α, and IL-6 are also seen after I/R injury in rats.[20]

The inflammatory response triggered by I/R-mediated TLR4 impairs myocardial function. Wild-type mice display significant increases in TNF-α and IL-1 after I/R injury, which may be responsible for the associated depression of left ventricular diastolic pressure and dP/Dtmax.[21] This theory is supported by the fact that deficiencies in TLR4, TNF-α, and IL-1 are cardioprotective and that treatment with exogenous TNF-α and IL-1 restores the harmful effects of I/R in TLR4-deficient mice. Syngeneic heart transplants in TLR4-deficient mice are associated with reduced neutrophil infiltration and lower graft and serum levels of inflammatory cytokines including TNF-α, IL-6, monocyte chemoattractant protein-1, and IL-1.[22]

The effects of TLR activation on the cardiac allograft may be long lasting and may contribute to the development of CAV. In a series of human heart transplant recipients, expression of TLR4 (and its targets IL-12 and TNF-α) was significantly elevated in patients with allograft endothelial dysfunction, which is a predictor of subsequent development of CAV.[23]

TLR activation inhibits the development of tolerance to cardiac allografts. B6 mice spontaneously develop tolerance to cardiac grafts from major histocompatibility complex class II-disparate bm12 donors without immunosuppression, but rejection is restored by treatment with the TLR agonist CpG. CpG induces Th1 differentiation of naive T cells and attenuates suppression by Tregs.[24] Tolerance can be induced experimentally by blockade of CD28-B7 co-stimulation with anti-CD154 antibody, but treatment with CpG permits rejection to occur and inhibits the accumulation of Tregs in the cardiac allograft.[25]

Finally, there is a link between NK-cell effects and TLRs. The activation of NK cells through the NKG2D receptor–ligand interaction may be initiated by TLRs. Various TLRs are found on T and B lymphocytes, dendritic cells, macrophages, and epithelial cells, and bind to both exogenous and endogenous ligands.[15] On exposure to lipopolysaccharide, monocytes and macrophages produce NKG2D ligands including RAE-1[26] and major histocompatibility complex class I-related chain A.[27] Monocytes thus activated can, in the presence of IL-2, stimulate NK cells to secrete IFN-γ. When dendritic cells are depleted, NK cell activation by the TLR ligand CpG is inhibited.[28]

Complement

When acute cellular rejection is present, there is increased expression of complement genes by graft-infiltrating leukocytes. Analysis of samples from human heart transplant recipients demonstrates 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, IFN-γ, perforin, and granzyme B.[29] Deficiency of decay-accelerating factor, a naturally occurring inhibitor of complement activation, leads to antibody-independent acceleration of graft rejection, possibly through increased proliferation of alloreactive CD8 + T cells.[30]

I/R injury causes host complement proteins to bind the graft endothelium, possibly initiating a process of inflammation, coagulation, and permanent tissue damage. A series of cardiac graft biopsy specimens in the first several weeks after transplantation demonstrated that deposition of C4d and C3d was associated with histological evidence of peritransplant

ischemic injury; patients with complement deposition were more likely to demonstrate repeated episodes of rejection on later biopsies.[31] C4d deposition has recently been linked to the development of CAV in the first year after transplantation.[32]

Defects in complement activation may protect against cellular rejection. A 'minibody' antagonist of C5 given before cardiac transplantation in rats leads to lower levels of TNF-α and reduced cardiomyocyte death.[33] In humans, a naturally occurring defect in mannose-binding lectin, a protein involved in complement activation, is associated with a decrease in the number of episodes of cardiac allograft rejection.[34]

Together, these findings demonstrate a newfound importance for complement as a mediator of both the acute and chronic rejection of cardiac allografts.

Conclusion

Although conventional immunosuppressive drugs, including antiproliferative agents and calcineurin inhibitors, effectively suppress the adaptive immune system, they have little, if any, effect on components of the innate immune system. Recognition that the innate immune system has a major role in acceptance or rejection of a cardiac allograft demands that strategies aimed at blocking the activation of NK cells, the response to TLRs, and the deposition of complement be incorporated into protocols aimed at preventing CAV and inducing long-term tolerance to cardiac allografts.

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