Th1 Adaptive Immune Responses in Cardiac Graft Arteriosclerosis
Deleterious or Beneficial?

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Heart transplants are not rejected in the absence of adaptive immune responses, eg, in transplantsations between genetically identical donors and hosts or to recipients with severe combined immunodeficiencies. Activation of adaptive immunity to an allograft causes destructive responses against donor parenchymal or vascular cells, called acute rejection. In the presence of adequate immunosuppression, acute rejection is suppressed; however, chronic rejection may occur, manifesting as conduit arterial lumen loss resulting from expansion of the tunica intima and remodeling of vessel size, called graft arteriosclerosis.

The differences in pathological findings and in sensitivity to immunosuppressive agents suggest that chronic graft failure arises from different mechanisms operative in separate allograft compartments than in acute graft rejection. The precise pathogenesis of graft arteriosclerosis is unknown, although there is broad consensus that it is, at least in part, an alloimmune process in that the arteriosclerotic changes are limited to the vascular bed of the allograft and spare the host vasculature. Adaptive immunity may activate a variety of effector mechanisms that cause chronic graft rejection in murine transplantation models. Although it is not the only factor that can cause graft arteriosclerosis, the evidence for the signature Th1 cytokine, interferon (IFN)-γ, is particularly compelling. In mouse transplantation models, serological neutralization or the genetic absence of IFN-γ reduces intimal expansion of graft arteries. Moreover, in a chimeric human-mouse model, antibody neutralization of IFN-γ prevents allogeneic T cell-mediated endothelial dysfunction, intimal thickening, and outward vascular remodeling; administration of IFN-γ accelerates these effects; and exogenous IFN-γ in the absence of leukocytes is sufficient to cause the arteriosclerotic changes. However, definitive evidence for a pathogenetic role of IFN-γ in clinical cardiac transplantation is lacking.

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In this issue of Circulation, van Loosdregt et al performed a comprehensive inventory of cytokines, chemokines, and chemokine receptors expressed in human arteriosclerotic versus referent nontransplanted arteries. They demonstrate a Th1-type immune response within the artery wall of chronically rejecting hearts characterized by the expression of IFN-γ, IFN-γ–inducible chemokines (eg, Mig, IP-10, I-TAC, RANTES, and fractalkine), and corresponding chemokine receptors expressed preferentially by Th1 cells such as CXCR3, CCR5, and CX3CR1. The induction of chemokine transcripts demonstrates that the IFN-γ–producing immune responses are functional and provide a mechanism for the recruitment of further IFN-γ–secreting T cells. Activation of Th1 and Tc1 cells in human graft arteriosclerosis is selective; production of the Th2 cytokine, interleukin (IL)-4, is barely detectable, and that of the Th3 cytokines, IL-10, and transforming growth factor-β is constitutive. These patient-related findings demonstrating a correlation of Th1-type immune responses with graft arteriosclerosis have become the “gold standard” in the field against which experimental models must be validated.

Previous clinical studies have reached a similar conclusion with regard to the predominance of Th1-type immune responses in graft arteriosclerosis by analyzing patient samples other than diseased arteries. Expansion of circulating Th1 cells is associated with endothelial dysfunction after cardiac transplantation, a predictor of graft arteriosclerosis. The propagation of Th1 cells from endomyocardial biopsies correlates with the subsequent development of graft arteriosclerosis. Similarly, induction of IFN-γ- and IFN-γ–inducible chemokine transcripts in endomyocardial biopsies precedes the development of graft arteriosclerosis. Although these types of studies provide insight into the relationship between acute and chronic rejection, they may not necessarily be relevant in the pathogenesis of graft arteriosclerosis because immune responses may differ in peripheral, parenchymal, and arterial compartments. Relatively few analyses of alloresponses have examined arteriosclerotic coronary arteries procured at necropsy or retransplantation, such as the limited immunohistochemical studies demonstrating an increased expression of RANTES, I-TAC, and CXCR3.

Shortcomings of the study by Loosdregt et al include a limited number of patient samples; a graded analysis according to disease severity could not be performed. Instead, an all-or-none definition of graft arteriosclerosis was used. This is contrary to what we know about the disease. The incidence of graft arteriosclerosis is ≈50% at 5 years by angiography, which underestimates disease, whereas it is present to some extent in almost all grafts after 1 year by postmortem examination. The more sensitive technique of intravascular...
ultrasound (IVUS) has demonstrated arterial remodeling in many grafts within the first year of transplantation. It may have been revealing to compare immune responses in early versus advanced lesions. For example, IFN-γ responses as assessed by IFN-γ-inducible chemokine production diminish over time in human coronary artery grafts in immunodeficient mice reconstituted with human allogeneic T cells despite the progressive increase in IFN-γ production.16 This may be due to injury of graft vascular cells with attenuated cytokine responses or to IFN-γ–dependent induction of inhibitors of JAK-STAT signaling, eg, SOCS1. The common limitation of all descriptive studies is the absence of experimental proof with regard to mechanisms. The findings of Loosdregt et al and other investigators represent convincing evidence on which to base investigational trials of specific therapeutic agents to inhibit IFN-γ production or IFN-γ responses in cardiac transplantation patients.

Besides their findings on the polarization of T-cell immune responses, Loosdregt et al7 also reinforce certain previous interesting observations in graft arteriosclerosis. First, the intima consists of a leukocyte- and matrix-rich inner layer and a smooth muscle cell—dominant outer layer, as noted by Atkinson et al.17 This may represent infiltrating host T cells and preexisting donor intima, respectively. Second, there are extravascular accumulations of leukocytes with organized T- and B-cell areas reminiscent of lymphoid neogenesis, as described by Baddoura et al,18 that may serve as a site of extra-anatomic antigen presentation. Because these extravascular accumulations have a low expression of memory or activated T-cell markers and chemokines/chemokine receptors used by effector T cells,7 it will be interesting to test for their expression of naïve T-cell markers, as well as addressins and chemokines/chemokine receptors used by lymphocytes for lymph node homing. Third, the media represents a site of immunoprivilege and is relatively spared of infiltrating leukocytes, as observed by Burns et al.16 The mechanism for preventing T-cell trafficking in this compartment is presumed to be mechanical barriers formed by medial elastic laminae.

In contrast to the pathological role ascribed to Th1-type cells by Loosdregt et al, another article in this issue of Circulation by Tu et al19 describes beneficial effects to cardiac allografts accruing from a different population of IFN-γ–producing CD4+ T cells. The latter are circulating T cells with an anti-cytomegalovirus (CMV) specificity, whereas the former are graft-infiltrating T cells, most of which are presumably alloresponsive. Cardiac recipients with detectable CMV-specific CD4+ T cells within the first month of transplantation (early responders) had greater freedom from acute rejection and less loss of lumen area during the first posttransplantation year compared with patients who developed anti-CMV responses after 1 month of transplantation (late responders). Because the 2 groups of patients also differed in the detection of CMV-specific CD4+ T cells at the time of transplantation, the early responders may represent patients with preexisting CMV immunity, although immune monitoring was not performed before transplantation. The beneficial allograft effects were associated with lower systemic viral loads 2 to 4 months after transplantation in early responders compared with late responders. Notably, all the recipients received prophylactic therapy against CMV with ganciclovir for 1 month after transplantation, and there were no episodes of symptomatic CMV infection.

How may immune responses against CMV be related to reduced cardiac allograft disease? A negative correlation of CMV infection with patient survival and graft outcomes has been recognized for many years. The deleterious effects of CMV disease have persisted into the modern era of transplantation despite improved immunosuppressive management, viral monitoring, and prophylactic and therapeutic antiviral agents. The effects of CMV infection on transplanted organs may be divided into direct infectious diseases and indirect manifestations on immunity and allograft injury.20 The direct cause of infectious disease syndromes by CMV is usually clinically apparent; however, even asymptomatic viral replication is associated with an increased incidence of graft arteriosclerosis.21 There are multiple mechanisms whereby CMV infection may injure the allograft, including intragraft viral infection, systemic inflammation, bystander activation of alloreactive T cells, and intragraft activation or recruitment of CMV-specific T cells.22

The findings of Tu et al19 partially clarify the mechanisms of CMV-mediated allograft injury. They demonstrate that detectable circulating CMV-specific CD4+ and CD8+ T cells do not positively correlate with parenchymal rejection or arterial remodeling of cardiac allografts. Therefore, it is unlikely that anti-CMV adaptive immune responses directly injure the allograft through cytokine secretion or bystander activation of alloresponsive T cells. In fact, the presence of CMV-specific CD4+ T cells early after transplantation is associated with improved graft outcome. The allograft was not examined in this study; however, CMV is rarely isolated from transplanted organs.22 Thus, it is unlikely that Th1 immune responses against CMV have a direct beneficial effect on the allograft, eg, by clearing donor cells of CMV. CMV-specific T cells may be nonspecifically recruited by the activated endothelium of transplanted organs and have been demonstrated within allografts.23 Tu et al19 found that early responders of CMV immunity did not acquire alterations in general T-cell immune responses as assessed by IFN-γ production by CD4+ T cells to polyclonal stimulation with bacterial enterotoxin. This argues against the possibility that the beneficial graft outcomes associated with early anti-CMV immunity are due to systemic or intragraft suppression of recipient alloresponsive T cells. A possible explanation for the reduced allograft disease in early responders of Th1 immune responses to CMV may be the inhibition of subclinical viral replication as demonstrated by measures of systemic viral load. CMV infection is related to systemic inflammation resulting from activation of innate immune responses; eg, CMV antigenemia correlates with elevated plasma levels of tumor necrosis factor in transplant patients.24 We have recently found that circulating inducible IL-12 and constitutive IL-18 correlate with elevated plasma levels of IFN-γ and IFN-γ–inducible chemokines and worse outcomes in patients with coronary atherosclerosis and that IL-12 and IL-18 induce IFN-γ responses in artery-infiltrating T cells in an antigen-independent fashion (unpublished observations). Tu et al may extend their work by determining the mechanisms...
relating CMV-specific Th1 cells and acute and chronic graft injury.

Tu et al.19 use 1-year arterial remodeling changes as measured by IVUS as an assessment of graft outcome. Coronary artery intimal thickening \( \geq 5 \) mm in the first year after transplantation by IVUS is a validated predictor of the subsequent development of graft arteriosclerosis;20 however, many of the early responders of CMV immunity appear to have lesser degrees of intimal expansion, and longer follow-up is required to determine the significance of the IVUS findings with regard to inward remodeling and lumen loss. Depicting the arterial remodeling data as a mean value obscures the heterogeneity of changes in vessel size. Studies that show individual patient data reveal a complex process of outward or inward remodeling of allograft coronary arteries.26 The mechanisms underlying outward and inward arterial remodeling may differ. It is unclear whether the early detection of CMV-specific CD4+ T cells is specifically associated with increased inward remodeling or decreased outward remodeling because both may result in smaller mean vessel size and greater lumen loss. Using a different study design, Potena et al.27 found that cardiac allograft recipients who received preemptive ganciclovir treatment for CMV seropositivity had less outward remodeling and greater lumen loss as assessed by IVUS at 1 year after transplantation compared with patients who did not require anti-CMV treatment. However, similar qualifications about illustrating results as mean values also apply to the latter study. It may be revealing to separately analyze the effects of CMV infection and CMV-specific immune responses on inward versus outward vascular remodeling to determine whether one or both processes are affected.

**Conclusions**

Adaptive immune responses after transplantation may have different effects on allografts. Losodregt et al.7 show that intra-arterial Th1-type immune responses are associated with graft arteriosclerosis, whereas Tu et al.19 find that early detection of systemic CMV-specific Th1 cells is associated with less acute rejection and vascular lumen loss. The specificity and temporal and spatial properties of adaptive immune responses in transplantation patients determine whether the effects on the allograft are deleterious or beneficial.

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None.

**References**


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