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EDITORIAL COMMENT

Are We Clear About the Mechanisms by Which Biopsy Evidence of Interstitial Fibrosis Following Cardiac Transplantation Helps Predict Late Post-Transplant Coronary Artery Disease?*

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Transplant-associated coronary artery disease (TxCAD), or coronary vasculopathy, is the leading cause of death after the first year of transplantation and, once diagnosed, is associated with a mortality rate of >40% at two years (1). Though coronary vasculopathy is widely believed to represent some form of chronic humoral and cellular rejection, some nonimmune risk factors such as older donor age, ischemia time, dyslipidemia, preexisting donor coronary artery disease (CAD) and post-transplantation cytomegalovirus infection have also been identified (2,3). These risk factors suggest that both immune and nonimmune causes probably interact to lead to coronary vasculopathy, although the precise mechanisms involved remain poorly understood.

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In this issue of the *Journal*, Yamani et al. (4) performed a prospective study involving 140 cardiac allograft transplant recipients and correlated the degree of post-transplant ischemia and interstitial fibrosis with the development of coronary vasculopathy progression, acute cellular rejection and long-term outcome. Coronary vasculopathy progression was determined by comparing baseline intravascular ultrasound (IVUS) measurements at one month post-transplant to end point measurements taken at one year post-transplant. Serial endomyocardial biopsies were evaluated for evidence of ischemia, fibrosis and vascular rejection, and patients were divided into four groups (ischemia, fibrosis, vascular and nonischemic) based on histologic and immunofluorescence criteria. To determine how each of these four biopsy categories were correlated with standard cellular rejection, a cellular rejection score was calculated for each patient based on the average International Society for Heart and Lung Transplantation (ISHLT) grade of rejection (5) during the first year post-transplantation. Thus, for every patient, in addition to routine assessments of cellular/humoral rejection episodes, the investigators determined whether patients developed biopsy evidence of peritransplant ischemia and which patients progressed to interstitial fibrosis and vascular rejection.

Of the 140 patients studied, a group of 32 (23%) patients did not demonstrate evidence of either peritransplant ischemia, fibrosis or vascular rejection. This group had the least coronary vasculopathy progression by IVUS, and the lowest proportion of patients developing significant late post-TxCAD. Interestingly, this "nonischemic" group did not have the lowest average cellular rejection scores, supporting the view that the number of acute rejection episodes does not by itself predict the development of post-transplant coronary vasculopathy. All the remaining 108 patients were found to have some evidence of either ischemia, fibrosis or vascular rejection—24 (17%) developed peritransplant ischemia alone while the remaining 84 patients developed either peritransplant ischemia followed by interstitial fibrosis (n = 62) or vascular rejection (n = 22). Of interest was the finding that 18 of these 22 patients (82%) in the vascular rejection group also had peritransplant ischemia followed by interstitial fibrosis. Therefore, of the 140 patients studied, 80 (57%) developed peritransplant ischemia followed by interstitial fibrosis.

The important finding in this study was that the 62 patients in the fibrosis group had the fewest average number of cellular rejection episodes and the lowest incidence of donor-specific human leukocyte antigen (HLA) sensitization, yet had the most severe coronary vasculopathy, the highest proportion of late TxCAD and the poorest >5-year event-free survival rates. Also, patients in the vascular (humoral) rejection group who had the highest average episodes of cellular rejection only rarely (6%) progressed to clinically significant late TxCAD. Thus, the investigators make a strong case for the presence of nonimmune mechanisms in mediating the pathogenesis of allograft vasculopathy.

Nonimmune mechanisms of coronary vascular injury or hidden immune mechanisms? Does a low incidence of acute cellular rejection and donor-specific HLA sensitization indicate that "nonimmune mechanisms" are mediating the coronary vascular injury? If the researchers (4) imply that classical *acquired* (antigen specific) humoral and cellular immune mechanisms are not associated with the development of early coronary vasculopathy and late TxCAD, then data from this study seem to lend support for this view. Patients within the fibrosis group had the lowest average episodes of cellular rejection, the lowest incidence of donor-specific HLA sensitization, yet experienced the highest incidence of coronary vasculopathy. However, we know that there are natural (nonantigen specific) *innate* immune

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mechanisms consisting of natural killer (NK) and macrophage cell lineages that have been implied to play a role in inducing coronary vasculopathy in murine organ transplant models (6). Specifically, a high frequency of organ transplant recipient mice incapable of mounting classical acquired immune responses (RAG-/- and SCID mice) have been shown to develop proximal coronary lesions, which were markedly reduced if recipients were treated with antibodies specifically directed at the NK cell lineages (6). A role for such potential innate nonantigen-specific immune-mediated mechanisms has not been appreciated so far; thus, such mechanisms are not included in the standard ISHLT cellular rejection scoring system (5).

Can immune mechanisms be involved in coronary vasculopathy in the absence of histologic evidence of acute cellular or humoral rejection? The answer to this question is yes. First, we know that in the ISHLT grading system for heart transplant biopsy specimens (5), the milder forms of rejection (grades 0 to 2) denote the presence of interstitial immune cell infiltration that do not usually require bolus immunosuppressive therapy. Thus, the low number of acute rejection episodes (ISHLT grade $\geq$3) in patients with post-transplant fibrosis is not synonymous with lack of immune cell trafficking within the myocardium. Second, donor alloantigens can be shed by the graft, picked up by roving macrophages/dendritic cells and taken to local lymph nodes, leading to alloimmune responses. This is termed the "indirect pathway," whereby soluble donor major histocompatibility complex (MHC) antigens are processed into peptides and these donor MHC peptides are presented by recipient MHC molecules to recipient effector T cells. Activation of recipient T cells could lead to the generation of interferon-gamma (IFN-γ) systemically at levels that could lead to coronary vasculopathy. The fact that indirect pathways have been shown to be involved in coronary vasculopathy (7) and that infusion of IFN-γ alone in the absence of leukocytes can induce arteriosclerosis (8) supports this view. Thus, one must be cautious in interpreting the data in the Yamani et al. (4) study, which suggest that the development of coronary vasculopathy in the absence of a high average cellular rejection score provides evidence for the role of nonimmune involvement in the form of ischemia and fibrosis.

Conversely, can coronary vasculopathy develop without histocompatibility mismatches and cellular rejection? Perhaps the strongest evidence that some form of innate, acquired and/or both immune mechanisms must be involved in the development of coronary vasculopathy has come from the fact that isogenic murine and rat cardiac transplants develop little detectable evidence of coronary vasculopathy. This observation has suggested that incompatibility (be it [MHC] class I or class II or non-MHC incompatibility) must contribute in some way to coronary vasculopathy. However, a recent study has suggested that under certain conditions, nonimmune mechanisms may be the major if not sole mediator of post-transplant vasculopathy. Using a genetically similar rat cardiac isograft model, Cantin et al. (9) have shown that significant coronary vasculopathy can develop without histocompatibility mismatches or cellular rejection.

Limitations. The limitations of the Yamani et al. (4) study are important. The 140 patients who completed the IVUS study at one year represented only one-third of the total of 422 patients who underwent heart transplantation at the Cleveland Clinic Foundation from 1992 to 1997. The basis by which patients would undergo serial IVUS studies may have led to unanticipated selection bias. In addition, all patients in the study were classified into nonischemic, ischemia, fibrosis and vascular rejection groups. The definition of the important fibrosis group was "patients who developed peritransplant ischemia followed by interstitial fibrosis in the absence of vascular rejection." Unfortunately, no specific criteria were provided on precisely how the histologic diagnosis of post-transplant fibrosis was made.

For example, is the presence of interstitial fibrosis on one biopsy sufficient or must multiple biopsies be affected? The bias associated with biopsy sampling is also an important issue in this regard. Thus, investigators in other centers will have to create their own criteria to try to reproduce these important findings.

Another major issue concerning the findings by Yamani et al. (4) is a lack of clarity as to the potential nonimmune-mediated mechanisms that perpetuate fibrosis for long periods of time following ischemia in such patients. Thus, fibrosis/smooth muscle cell proliferation and remodeling must require a continuous source of a number of growth factors/cytokines. What is the source of such growth factors and cytokines that are localized to the transplanted heart? If indeed ischemic events are initiators of this process, why is such a process perpetuated and why is such a process not regulated? Future studies are required to more definitively ascribe a role for such ischemic nonimmune-mediated events to induce post-TxCAD.

In addition, patients were evaluated by IVUS and serial endomyocardial biopsies for only one year post-transplant, and during that year both graft failure and clinically apparent coronary vasculopathy were very rare. It was over an average of 67 months of follow-up that the fibrosis group experienced poor long-term outcome and a higher proportion (32%) of moderate–severe vasculopathy. Yet, one year after transplantation, no pathology data were provided from autopsy and retransplantation specimens. Did the group with interstitial fibrosis in the first year after transplant continue to have lower cellular rejection scores past their first post-transplant year? Did the patients with the worst outcomes, namely graft failure and death, have evidence of advanced coronary vasculopathy without histologic evidence of interstitial and vascular inflammation? It is also important to note that clinical data that might have confounded the effect between the presence of fibrosis and coronary vasculopathy, such as lipid levels, were not provided.

In addition, two other points are worth mentioning. One

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is the specificity of the finding of interstitial fibrosis. It is difficult to understand how patients in the fibrosis group develop the most severe late TxCAD, whereas patients in the vascular rejection group, who also have fibrosis, only rarely develop coronary vasculopathy. The data in the study (4) suggest that when fibrosis is accompanied by vascular rejection it is not predictive of coronary vasculopathy progression.

Second, though the investigators (4) used immunofluorescence staining for IgM and complement to identify vascular rejection, they failed to look for other recognized markers associated with immune-mediated vascular injury. The expression of MHC class I and class II antigens and intercellular adhesion molecule (ICAM)-1 on arterial/arteriolar endothelium in transplant allografts during the first three months after transplant has been shown to predict the development of late TxCAD and graft failure (10). The finding of aberrant MHC and induced ICAM-1 expression on arterial endothelium would represent a more sensitive and specific marker of immune-mediated targeting than the average cellular rejection score.

Whatever the case, the study by Yamani et al. (4) demonstrates that early post-transplant fibrosis was associated with increased risk of coronary vasculopathy and poor overall long-term outcome. Is it possible that the simple histologic finding of interstitial fibrosis is a marker for a predisposition to develop late TxCAD? We believe that this may be possible. The presence of interstitial fibrosis on early post-transplant endomyocardial biopsies is secondary to remodeling due to postischemic reperfusion injury. Patients with a genetic predisposition to cardiac interstitial proliferation may be predisposed to intravascular proliferation in response to a myriad of stimuli, both immune and nonimmune mediated. The possibility that genetic predisposition may modulate immune responses following post-ischemic reperfusion is supported by recent studies documenting that specific interleukin-6 (IL-6) promoter polymorphisms influence the amount of IL-6 that individual patients produce after coronary artery bypass graft surgery (11). In the post-transplant setting, it is certainly possible that genetic influences on levels of growth factor(s) that may be implicated in both the development of interstitial fibrosis and coronary vasculopathy (i.e., fibroblast growth factor) are involved. If this is the case, then patients with a long-term risk for developing coronary vasculopathy may be simply identified by their predisposition to develop interstitial fibrosis in the year following cardiac transplantation.

Conclusions. Finally, though the data presented by Yamani et al. (4) are clearly interesting and provide impetus for continued studies on the mechanisms by which nonclassical immune-mediated mechanisms contribute to post-TxCAD, it is important to be wary of the terminology we utilize until such mechanisms are more fully defined. A role for the contribution of NK cells and indirect pathways of allorecognition in concert with the precise mechanisms that perpetuate long-term fibrosis and coronary vasculopathy needs to be a focus of future studies.

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