Noninvasive Diagnosis of Cardiac Allograft Rejection
Another of Many Searches for the Grail

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Survival after cardiac transplantation has improved steadily over the past decade, with mortality at 1 year falling to as low as 10% in many centers. This improvement in survival is owed in part to better, more specific immunosuppressive agents such as cyclosporine and antilymphocyte antibodies.1–3 Also, to a large extent survival has improved because of the early diagnosis of rejection by surveillance endomyocardial biopsy, in most instances made before the development of allograft dysfunction.4,5 This early diagnosis is exceedingly important, as most clinicians experienced in transplantation are aware of the very high mortality in patients with acute rejection associated with consequent allograft dysfunction. Unfortunately, this early rejection diagnosis is purchased with up to 20 biopsy procedures for each patient within the first year after cardiac transplantation. Aside from the obvious costs associated with endomyocardial biopsy (approximately $1,300 at Oregon Health Sciences University, including hospital and professional charges), there is patient discomfort and potential risks from the multiple invasive procedures. Accordingly, the search for a noninvasive technique for the diagnosis of cardiac allograft rejection has continued to receive much interest in the field.

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What is required for a perfect screening test for cardiac allograft rejection is not dissimilar to any ideal screening test. It should be easy to administer repetitively and should be noninvasive, low cost, and most importantly, it should have a high sensitivity. The need for specificity varies, depending on implications for further diagnosis. In the diagnosis of cardiac allograft rejection, the screening test must be nearly 100% sensitive, given the potentially fatal implications of missing acute rejection. Even if specificity is only 50%, this would result in a decrement of one half of the biopsies performed on a routine basis.

Three general classes of studies have been investigated as possible screening tests for cardiac rejection: cardiac functional assessment, biochemical/immunologic assays, and myocardial imaging. It has been long appreciated that systolic function as assessed by ejection phase indexes is neither sensitive nor specific for allograft rejection.6,7 This has led to several studies investigating the use of diastolic indexes of left ventricular function as potentially more sensitive in this diagnosis. Paulsen and colleagues' first demonstrated that echocardiographic-derived indexes of diastolic function were abnormal in patients with acute allograft rejection. Subsequently, Valantine et al,8,9 Haverich et al,10 and Desruennes et al11 demonstrated that Doppler indexes of diastolic function were prolonged in rejecting patients. These groups reported sensitivities from 78% to 88%. Another study using radionuclide ventriculographic indexes of diastolic function reported similar results.12 Unfortunately, other studies13,14 have reported that diastolic dysfunction is common early after cardiac transplantation (probably related to ischemic injury) and may take as much as 3 months to completely resolve. In addition, Valantine et al15 and others16 report late "restrictive" physiology after cardiac transplantation. Additional factors such as donor-recipient size matching may play an important role in the diastolic properties of the allograft.14,17 Based on the available data, one must therefore conclude that functional indexes by themselves are unlikely to provide the sensitivity required for rejection screening of asymptomatic patients.

The second major area of investigation has attempted to directly assay for increases in alloimmune activity. The earliest studies reported by Copeland and colleagues18,19 involved the measurement of urinary polypeptides that reflected increased cellular proliferation or degeneration. This same group20 also evaluated changes in prolactin levels, a potential indication of immune modulation, as a noninvasive screen for rejection. Again, the sensitivity of these studies was approximately 80%. With the further understanding of immunologic activation, lymphocyte subsets, and activation markers, along...
with the development of flow cytometry techniques, several groups\textsuperscript{21-23} have investigated the possibility that the expression of activation antigens on circulating lymphocytes might predict rejection. Although one group\textsuperscript{23} reported a sensitivity of 94\% for the detection of rejection using cytotoxicologic monitoring, these striking results have not been confirmed by others.\textsuperscript{21,22} It is certainly possible that, given the appropriate immunoassay, an adequately sensitive screening test reflecting immunologic activation would be developed. One potential problem with this approach, however, is that what is occurring in the periphery may not reflect immunologic activity in the allograft.

Imaging and characterization of the myocardium to diagnose rejection have been attempted using a variety of techniques, including echocardiographic tissue characterization,\textsuperscript{24} backscatter analysis,\textsuperscript{25} indium-labeled white cells,\textsuperscript{26} and magnetic resonance imaging.\textsuperscript{27} Although preliminary results in animal models have been promising, the results in patients have yielded sensitivities that are once again inadequate.

The development of hybridoma technology by Kohler and Milstein\textsuperscript{28} in 1975 not only led to their receiving a Nobel prize but also to the development of a myriad of monoclonal antibodies currently in clinical use or in testing. One such is a monoclonal antibody to cardiac myosin developed by Khaw and colleagues.\textsuperscript{29} The antibody itself or its Fab fragment has been found to bind to myocytes in which the sarcolemma is no longer intact and when labeled with \textsuperscript{311}In has been demonstrated to be useful in the diagnosis of myocardial infarction\textsuperscript{30} and myocarditis.\textsuperscript{31} In this issue of \textit{Circulation}, Ballester and colleagues\textsuperscript{32} have extended their experience using indium-labeled antimyosin antibodies in the diagnosis of acute cellular cardiac allograft rejection. In their initial experience of 53 studies in 21 patients,\textsuperscript{33} an abnormal antimyosin uptake ratio (greater than 1.55) yielded a sensitivity of 95\% for the diagnosis of rejection requiring treatment (moderate or severe rejection). As might be anticipated, specificity was quite low (29\%), with a majority of patients with no rejection also exhibiting antimyosin uptake ratios out of the normal range. In a follow-up study of patients at least 1 year after cardiac transplantation,\textsuperscript{34} a negative antimyosin antibody scan assured the absence of rejection requiring treatment (sensitivity, 100\%), with four of the 11 patients with positive scans demonstrating clinically significant rejection (specificity, 33\%). Specificity, as anticipated, increased with an increasingly abnormal scan. Thus, both early and late after cardiac transplantation, scanning with indium-labeled antimyosin appears to be nearly 100\% sensitive but not specific. Based on these data, approximately one third of all biopsies on stable patients could therefore be avoided.

The current study incorporates patients from the previous two investigations but most importantly provides an additional 22 patients who are prospectively and serially studied at multiple time points in the first year after cardiac transplantation. The normal pattern of myocardial injury as assessed by allograft antimyosin antibody uptake over time can therefore be described. As reported, it appears that initially antimyosin uptake can be abnormal but should decrease steadily toward normal by approximately 3 months. By inference, myocardial injury sufficient to cause sarcolemmal disruption from initial ischemic injury, immunologic mechanisms, or other factors may persist for up to 3 months, with gradual resolution. These data would also suggest that if alloimmunologic injury is the mechanism, traditional cell-mediated rejection is an incomplete explanation, given the multiple negative biopsies during this period. In those patients who followed this normal pattern (15 of 33), their long-term follow-up was uneventful. In those 10 patients who exhibited persistent elevations in antimyosin antibody uptake, their course was less well defined, with three patients succumbing to acute cellular rejection.

The strength of this study is the definition of the normal pattern of diminishing antimyosin antibody uptake and by inference, myocardial injury over time after cardiac transplantation in a prospective, serially studied cohort. Any interpretation or therapeutic intervention based on an abnormal antimyosin antibody uptake pattern should at this time be considered cautious. Although the authors suggest that this abnormal pattern of antimyosin antibody uptake bodes poorly for the patient, the evidence for this is not strong. Of the 10 patients studied, three patients did well. Only three had clearly established immunologic consequences (cellular rejection). One additional patient had an abnormal ejection fraction potentially caused by any number of factors (donor factors, ischemic preservation injury, or rejection), and two patients had “vascular rejection,” a frequently used diagnosis but poorly defined entity (or nonentity). Finally, given the extremely low 1-month survival rate of their transplant experience during this prospective study, selection bias may play a role in overall outcome.

The confusion over both the mechanisms and the diagnosis of immune-mediated myocardial injury is not new. Most transplant physicians have experienced patients with multiple episodes of severe histological rejection who despite this maintain normal allograft function and conversely, patients presenting with cardiogenic shock who on biopsy or even autopsy have trivial interstitial infiltrates, minimal necrosis, and possibly some myocardial edema. Our current understanding of the rejection process is still quite primitive. Ongoing research such as the characterization of specific cell types invading the allograft, the role of cytokines in myocardial injury, and the probability of humoral injury to both the myocardium and the allograft vasculature will hopefully reduce this confusion.

The authors should be commended for this study. First, they have provided data, which to date best define the expected and presumably normal pattern
of antimyosin antibody uptake in the human cardiac allograft. Second, they have by inference identified this methodology as a tool of exquisite sensitivity for the detection of allograft myocardial injury (at a time when histology is normal). This may be extremely important in attempting to better delineate immune mechanisms of myocardial injury. Finally, assuming confirmation by other investigators, they have demonstrated that this methodology could result in a reduction in the number of surveillance endomyocardial biopsies after cardiac transplantation. Certainly this represents a "grail" for both transplant physicians and their patients.

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Noninvasive diagnosis of cardiac allograft rejection. Another of many searches for the grail.
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