Editorial Comment

Of Molecules and Myocardium
PCR Diagnosis of Viral Myocarditis in Cardiac Biopsies

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The development of endomyocardial biopsy as a standard procedure has enabled cardiologists to obtain a direct histological diagnosis in a subset of cardiac disease states. In particular, right ventricular biopsy has become invaluable in the assessment of rejection after heart transplantation, in the tissue diagnosis of cardiac amyloidosis, and in the documentation of adriamycin cardiomyopathy. In each of these disease states, the diagnosis rests on the finding of pathognomonic histological features in small biopsies of ventricular tissue. Because many cardiac diseases do not produce specific changes, the usefulness of right ventricular biopsies has been rather restricted.1

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The limitations of cardiac biopsies have been especially problematic in the assessment of viral myocarditis and idiopathic dilated cardiomyopathy. The histological changes of a cellular infiltrate within the myocardial sample are not necessarily specific evidence for the diagnosis of viral myocarditis. Culturing the virus from the biopsy sample is a rare occurrence, and the small amount of available tissue usually precludes standard biochemical approaches for the detection of viral determinants. Because the biopsy is often performed outside of the window of acute infection, biopsy results are often inconclusive. Furthermore, it can be difficult to discern which type of virus is the infectious culprit, whereas a negative biopsy does not exclude a viral etiology for the cardiomyopathy. Because end-stage cardiomyopathy leads to similar histological changes of fibrosis and scarring, independent of the etiology, it is often impossible to determine whether the onset of cardiomyopathy is a consequence of an earlier episode of viral myocarditis.

During the past few years, the genomes of enteroviruses and other myocarditic viruses have been cloned and the DNA sequences elucidated.* Based on the availability of specific viral gene probes, several investigators recently demonstrated that hybridization techniques can be used to detect traces of the viral nucleic acids in cardiac tissue, leading to a more sensitive means to assess myocarditis and to identify the specific virus that is involved.2 The present report by Jin et al.,3 in this issue of Circulation, extends this concept by describing the first use of the polymerase chain reaction (PCR) for this purpose. PCR can amplify a single copy of DNA to several million copies. Using PCR and enteroviral specific probes, Jin et al demonstrate the ability to detect traces of the viral nucleic acid from five of 48 patients. This incidence is somewhat lower than previous reports with conventional slot-blot hybridization and may either reflect differences between the patient populations or a higher number of false-positive results in previous studies, arising from cross-hybridization between human genomic and viral sequences. In certain instances, patients with clinical cardiomyopathy, negative pathology, and histories suggestive of myocarditis displayed detectable enteroviral sequences, thus demonstrating the potential sensitivity of the method.

This study, as well as hundreds of other experimental and clinical investigations, is based on the landmark discovery of PCR by researchers at the Cetus Corporation, who were the first to amplify DNA in a completely in vitro reaction through repeated thermal cycles.4 The usefulness of PCR was revolutionized by modifying the original technique that used a heat-labile DNA polymerase to that which was based on a Taq DNA polymerase isolated from microorganisms that survive in natural hot springs. Recognition that the bacteria Thermus aquaticus proliferates at elevated temperatures led to the discovery of the Taq DNA polymerase that synthesizes DNA under conditions that would quickly render other DNA polymerases useless. The


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beautiful simplicity of the PCR reaction depends on automated thermal cycles of sequential melting of the DNA template, annealing of specific short primer segments of DNA that are complementary to the template ends, and subsequent synthesis of the new DNA strands by the Taq polymerase molecule in the presence of an abundance of the primers and deoxynucleotides. After the completion of one round of synthesis, numerous thermal cycles are repeated, theoretically resulting in the geometric synthesis of several million copies of DNA from a single initial template in a typical 30-cycle reaction. In short, the PCR reaction represents a powerful way to amplify DNA copies millions of times in an in vitro system that is independent of molecular cloning and the insertion of the DNA into bacteria or other surrogate systems. This power can also be a detriment, because any single copy gene product that contaminates the PCR sample can serve as a template and lead to false-positive results. Thus, one must carefully scrutinize all PCR results.

In the present study, the short DNA primers have been designed to be complementary to sequences within the enteroviral genome. By using specific primers that will bind only to viral sequences, one can amplify and thus identify a single molecule of DNA present in the few myocardial cells obtained from a myocardial biopsy specimen. The power of the PCR procedure has led to its widespread application in molecular biology to efficiently find rare genes within a cDNA or genomic DNA library, to identify complex related gene families, and to obviate the necessity of molecular cloning in certain experimental circumstances. In addition, the ability to amplify DNA from small tissue samples such as a hair follicle or blood has led to its use in forensic medicine. Most recently, PCR has also had a major impact in clinical medicine, including the development of 1) prenatal screening for sickle cell anemia, thalassemias, and sex determination; 2) diagnostic tests for gene products of diverse infectious disease agents; 3) detection of oncogenes within tumor tissues; and 4) assays of point mutations linked to various genetic disorders.

Although the present study represents one of the first applications of PCR to cardiovascular diagnosis, the ability to amplify the genetic material from small numbers of myocardial cells may lead to a new horizon in clinical cardiovascular research. The molecular study of genetic and acquired cardiac diseases has traditionally been hampered by the inability to clone and characterize the genes that are aberrantly expressed or structurally altered in these pathological states. PCR should allow the construction of libraries from cardiac biopsies and subsequent opportunities for the molecular characterization of human cardiac disorders that was not previously possible. The research journal Science has named the Taq DNA polymerase molecule and its use in the polymerase chain reaction as the first “Molecule of the Year.” As the application of the PCR becomes more fully developed within the field of cardiovascular medicine and biology, perhaps Taq polymerase may eventually reach a similar status with the readership of Circulation.

References

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