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Chronic Myocarditis — What's Going on ? —

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Myocarditis is characterized histologically by the infiltration of inflammatory cells in the myocardium accompanied by myocardial cell damage in a pattern differing from that of ischemic heart disease and most cases of acute myocarditis in patients living in developed countries are thought to be due to viral infection[1]. Although an etiologic link between viral myocarditis and dilated cardiomyopathy (DCM) has long been recognized, the nature of this relationship is controversial. The progression from viral myocarditis to DCM is thought to involve autoimmunity and the presence of persistent viral infection[2]. When viral genome was detected in the myocardium of heart transplant patients with DCM, the persistent viral infection theory gains prominence. However, recent data demonstrated that the number of transplant cases with such viral infection is less than expected[3].

We previously demonstrated the abnormal immunologic responses in patients with DCM[4]. We also developed a murine model of chronic viral myocarditis in A/J mice that were inoculated with Coxsackievirus B3. This model demonstrated that during the chronic myocarditis, CD 4 + T cells were found to be the main infiltrates in this model. The intercellular adhesion molecule-1 (ICAM -1) was expressed on the endothelial cells of vessels in and around the infiltrated lesions, and, in contrast, major histocompatibility complex (MHC) class I antigen, and MHC class II antigen were expressed on infected myocardial cells. More interestingly, the Coxsackievirus B3 genome was not detected in the myocardia of animals with chronic myocarditis[5].

In this study, to investigate an autoimmune link to the pathogenesis of chronic myocarditis, we heterotopically transplanted normal A/J mouse hearts into A/J mice with chronic myocarditis and analyzed immunologic factors such as IL1-α and TNF-α, and the expression of ICAM-1, MHC class I and II in transplanted hearts. Heterotopic cardiac transplantation was performed by a modification of the method of Corry et al[6]. Normal A/J mouse hearts were transplanted into the same strain of nonmyocarditis mice (group A), as well as into mice with chronic myocarditis (group B). Two weeks postoperatively, the grafts were excised, and compared immunologically with the hearts grafted into normal A/J mice. Conventional histological examination of infiltrated T cells and macrophages was performed, and the expression of intercellular adhesion molecule-1 (ICAM-1), major histocompatibility complex (MHC) class I antigen, and MHC class II antigen was evaluated by immunoenzymatic staining. The concentrations of interleukin 1-α (IL1-α) and tumor necrosis factor (TNF-α) in the grafts were measured with an enzyme-linked immunosorbent assay. The viral RNA genomes were not detected in the mice with chronic myocarditis, but their transplanted hearts did show myocarditis. In the hearts with induced myocarditis, infiltrated mononuclear cells consisted of CD 4 +T cells, CD 8 +T cells (CD 4 +cell number>

CD 8 +cell number), and macrophages. ICAM-1, MHC class I antigen, and MHC class II antigen were expressed in the vascular endothelial cells and myocardial cells in and around the infiltrated lesions. The concentrations of IL1-α and TNF-α in group B was significantly higher than in group A[7].

In our recent study, we also demonstrated that 1) the viral RNA genomes were not detected in the mice with chronic myocarditis, but the transplanted hearts obtained from chronic ongoing myocarditic mice did show myocarditis, 2) CD 4 +T cells were mainly found in inflammatory lesions, with ICAM-1, MHC class I antigen, and MHC class II antigen being expressed in the myocardium of transplanted hearts in the chronic ongoing myocarditic mice, and 3) the concentration of both IL1-α and TNF-α significantly increased in the tranplanted hearts into cronic ongoing myocarditic mice.

Witebsky et al[8]. proposed a rationale for the autoimmune basis of clinical disease. They were consciously modeled on Koch's postulates, and required that an autoimmune response be recognized, either as an autoantibody or a cell-mediated immunity, that the corresponding antigen be identified, and that an analogous autoimmune response be induced in an experimental animal. In addition, the immunized animal must also develop a similar disease. Recently, Rose et al[9]. proposed novel criteria for autoimmune disease based on new information gained from the use of molecular biology and hybridoma techniques. These include direct evidence of the transfer of a pathogenetic antibody or pathogenetic T cells ; indirect evidence based on the reproduction of the autoimmune disease in experimental animals ; and circumstantial evidence from clinical clues. Using an animal model, we first demonstrated that myocarditis was transferred to a normal heart that was transplanted into a mouse with chronic myocarditis. This finding suggested the possibility that circulating pathogenetic T cells in the A/J mouse with chronic myocarditis responded to the myocardium of the transplanted normal heart.

Our results showed that the infiltrated mononuclear cells consisted of CD 4 +T cells, CD 8 +T cells, and macrophages. Cell number of CD 4 +T cells infiltrated into the transplanted hearts obtained from chronic ongoing myocarditic mice was twenty times higher, on the other hand, that of CD 8 +T cells was ten times higher compared with that of noninfected mice in this experiment. These CD 4 $^+$ T cells are expected to react primarily with adhesion molecules and MHC class II antigens, and may induce graft injury by secretion of cytokines and recruitment of nonspecific macrophages and monocytes, which can also cause graft injury by a delayed type hypersensitivity reaction. On the other hand, CD 8 +T cells, which interact primarily with monocyte, expressing MHC class I antigens, can induce cell injury directly, by either a calcium-dependent or independent mechanism[10]. Importantly, a particular subset of CD 4 +T cells, the T helper 1 cells has been shown to contribute to the development of organ-specific autoimmune disease[11]. Results of experimental T cell-mediated organ-specific autoimmune diseases, such as experimental autoimmune encephalitis[12] and insulin-dependent diabetes mellitus[13], strongly support an immunological role in disease progression. Nevertheless, there remain some questions about the source of mononuclear cell infiltration from the host cell, which are 1) whether it is triggered by the transplanted heart which is immunologically slightly different from the host, 2) whether it is activated by cross -reacting autoantibodies or 3) whether this is a reaction due to cross-reacting antigens, or to molecular mimicry. We previously confirmed that 12 week-old male A/J (H-2a) mice, certified virus-free, accepted skin grafts from the same strain of mice. We therefore speculated that the mononuclear cell infiltration was not due to both cross -reacting antigens and molecular mimicry, but also to an autoimmune response which was occurred in the recipient animal.

With respect to rejection of the cardiac graft, there are two possible pathways proposed ; the direct and indirect pathways. First, the direct pathway involves peptides in the groove of allogeneic MHC molecules that are expressed on donor antigen-presenting cells, and reflects the recognition of these

peptide differences within the a-helices in contact with T cell receptors. Second, the indirect pathway involves the T cell receptor recognition of donor MHC peptides in the groove of self-MHC class II molecules presented by recipient antigen-presenting cells[14]. In our experimental model, we used the same inbred strain of mice, so that, it is unlikely that the myocarditis which occured in the infected mice was due to the rejection. It is possible that the induced myocarditis in the donor hearts was caused by a circulating autoreactive T cells to the myocardium of the recipient.

The concentration of IL1-α and TNF-α was elevated in the hearts which were transplanted into the infected mice compared to the normals. Huber et al[15]. showed that Coxsackievirus B3-induced myocarditis may depend upon the release of specific cytokines, IL-1 or IL-2, during infection. These authors also demonstrated that the activation of Th1 cells may be important in disease pathogenesis in Balb/c mice with the myocarditic H3 virus variant and the nonmyocarditic H310A1 virus variant. These cytokines are also known to regulate the expression of ICAM-1 on the vascular endothelial cells[16,17]. Furthermore, in this experiments, we showed that, in the grafts which were transplanted into the infected animals, ICAM-1, MHC class I antigen, and MHC class II antigen were not only expressed in the vascular endothelial cells but also in myocardial cells. These findings implicate an involvement of the immune system in the induction of myocarditis in these animals.

The relationship between chronic myocarditis and DCM is controversial. If these two diseases are related, our model may suggest the possibility of the post-transplantation immune response to the myocarditis-related DCM patient who underwent cardiac transplantation. It is likely that many patients with myocarditis that have undergone cardiac transplantation, and have recieved long-term immunosuppressive agents that would have concealed the post-transplantation immune response in myocarditis-related patients.

There are several reports, indicating that candidates for autoantigens, β-adrenergic receptors[18], laminin[19], mitochondorial adenine nucleated translocator[20], branched chain α-keto acid dehydrogenase protein[21], and cardiac myosin[22]. We also proposed that α-gal antigen and three kinds of myocardial cell membrane proteins are possible antigens for autoreactive T cells to the myocardium as well[4].

Finally, there has been the adoptive transfer experiments, showing that the virus can surreptitiously persist in immunocytes at low levels, and then re-infect the heart in the new host[23]. We could not exclude such possility by the PCR method used in this experiments.

In conclusion, in this study we demonstrated that the viral RNA genomes were not detected in the mice with chronic myocarditis, but their transplanted hearts did show myocarditis, suggesting that an autoimmune response may play a key role in the progression of chronic myocarditis. Further experiments are required to verify the autoimmune mechanism of chronic myocarditis and the development of subsequent DCM, and the autoantigen that is responsible for the transmission of myocarditis in cardiac transplant models.

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