Viral Myocarditis: Immunopathogenesis and the Effect of Immunosuppressive Treatment in a Murine Model

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Serial changes in lymphocyte subset were investigated in an animal model of encephalomyocarditis (EMC) virus myocarditis in mice. The lymphocyte subset in the peripheral blood was measured by flow cytometry and, T cell subset in the myocardium was stained by labeled avidin-biotin technique. Helper-inducer T cells (Lyt 1 cells) decreased significantly on day 7, but there was no change in cytotoxic-suppressor T cells in the peripheral blood. During the acute stage a significant number of T cells (Thy 1.2 cells) were seen in the heart with helper-inducer T cells predominating.

The effect of prednisolone and cyclosporine on viral myocarditis was studied in our experimental model. Prednisolone or cyclosporine given in the early stage aggravated the course, and they did not have beneficial effects during the early recovery period.

CLINICALLY, viral myocarditis may present in a wide variety of ways, ranging from a total lack of clinical manifestations to sudden, unexpected death, which is not uncommon and may be caused by a fatal arrhythmia, high-degree atrioventricular block, or circulatory collapse. In addition to the broad spectrum of clinical presentations of acute viral myocarditis, possible complications and late sequelae are of concern. Myocarditis may be subacute or even chronic, leading to progressive myocardial failure and death. The relative infrequency of cases of acute viral myocarditis, and the difficulty of recognizing the lesions, make clinical investigation difficult. We, therefore, looked towards experimental models of viral myocarditis for systemic investigations.

Advances in immunologic approaches have

expanded our knowledge of the course of myocarditis, experimentally as well as clinically. We have investigated lymphocyte subset in the course of viral myocarditis and assessed immunosuppressive therapy in an animal model.

Lymphocyte Subset in Viral Myocarditis

Four-week-old Balb/c mice were inoculated intraperitoneally with EMC virus as described previously¹. Mice were sacrificed 7, 14 and 28 days after virus inoculation. Lymphocyte subset in the peripheral blood was measured by flow cytometry using monoclonal antibodies to T cells (Thy 1.2), helper-inducer T cells (Lyt 1), cytotoxic-suppressor T cells (Lyt 2) and B cells (goat anti-mouse IgG). Six-week-old Balb/c mice without virus inoculation served as controls.

For immunocytochemical analysis, frozen sections of the myocardium were stained to determine T cell subset by labeled avidin-biotin technique. T cell subset was expressed as a percentage of the inflammatory cells enumerated

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Fig.1. Lymphocyte subset in the peripheral blood. Thy 1.2 (T cells) increased 2 and 4 weeks after EMC virus inoculation, and Lyt 1 (helper-inducer) cells decreased significantly 1 week after infection.

by methyl green counter staining.

The percentage of Thy 1.2 cells increased on days 14 and 28 (Fig. 1). Lyt 1 cells decreased significantly on day 7. However, there was no change in Lyt 2 or B cells.

Immunocytochemical study of the heart showed that a significant number of infiltrating cells were Thy 1.2 positive cells (10%, 11%, 10% on days 7, 14 and 28 respectively), that Lyt 1 cells increased on day 14 (8%), but that Lyt 2 cells did not show a significant change and were in low frequency (Fig. 2). This study demonstrated that helper-inducer cells showed significant changes during the course of EMC virus myocarditis and suggests that these cells may be involved in the immunopathogenesis of this disease.

Immunosuppressive Therapy of Viral Myocarditis

1. Effect of Prednisolone

The effect of prednisolone on viral myocarditis was studied in Balb/c mice with encephalomyocarditis virus myocarditis. Prednisolone was injected intramuscularly, 10 mg/kg once a day, on days 4 to 13 (experiment 1) and on days 8 to 17 (experiment 2). The control mice in each experiment received injections of distilled water.

In experiment 1, myocardial virus titers were maximal but neutralizing antibodies rarely

Fig.2. Lymphocyte subset in the heart in situ. Frozen section was stained by labeled avidin-biotin technique using monoclonal antibody against Thy 1.2 (T cells, left), Lyt 1 (helper-inducer T cells, middle) and Lyt 2 (cytotoxic-suppressor T cells, right). A significant number of infiltrating cells were Thy 1.2 positive cells and Lyt 1 cells predominate.

present on day 4, and viral titers were still elevated and antibody titers were high on day 8. The survival rate of the prednisolone group was significantly lower than that of the control group. On day 10, the antibody titers of the prednisolone group were significantly lower than those of the control group, while the viral titers remained significantly elevated, whereas viruses were rarely isolated in the control group. In experiment 2, the survival rate and antibody titers were not significantly different in the prednisolone and control groups. In both experiments, no viruses were isolated on day 14.²

The study suggests that prednisolone given in the early stage aggravates the course of acute viral myocarditis. However, they may not have detrimental effects if given when the neutralizing antibody titer levels are high, although they are not expected to have a beneficial effect.

2. The Effect of Cyclosporine

Eight-week-old DBA/2 mice were infected with EMC virus. Cyclosporine (25 mg/kg/day) was administered subcutaneously for 3 weeks, starting (1) at 1 week after infection, and (2) at 3 weeks after infection. In the first experiment there was a significantly higher mortality but no evident reduction in myocardial pathology. In the second experiment, there was no improvement in mortality or any reduction in myocardial histopathologic lesions. Furthermore, treated

mice had significantly greater heart weight/body weight ratios, and liver weight/body weight ratios, suggesting more severe heart failure.³

Thus, the use of immunosuppressive therapy with prednisolone or cyclosporine in this murine preparation of acute viral myocarditis was associated with greater mortality when administered early in the illness, and no beneficial effect was seen when administered during the early recovery period.

The implication of the present study with respect to inflammatory myocarditis in man is that therapy with prednisolone or cyclosporine, although still potentially useful for myocarditis secondary to primary autoimmune disorders, may be deleterious when the disease results from an acute viral infection, at least in the early period of viral replication.

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