Experimental Autoimmune Myocarditis Produced by Adoptive Transfer of Splenocytes After Myocardial Infarction

Abstract—One possible mechanism for neurohumoral activation after myocardial infarction may be the generation of an immune response against cardiac self-antigens. We hypothesize that if there is a T cell–mediated reaction to self-antigens, the transfer of splenic lymphocytes from postinfarct rats into syngeneic rats with normal hearts should result in a T cell–mediated autoimmune myocarditis in the healthy syngeneic rats. Rats were killed 6 weeks after coronary ligation. Splenocytes from animals with large and small infarcts were purified from spleens, activated with concanavalin A, and injected in varying doses into normal syngeneic rats. These recipient rats were killed 6 weeks later, and histopathological studies were performed. Our results demonstrate in vivo evidence of lymphocyte-mediated myocardial injury by adoptive transfer of sensitized lymphocytes from rats who developed congestive heart failure after acute myocardial infarction. The amount of infiltrate and necrosis in the recipient rats appeared directly related to the size of the infarct from the donor rats. This suggests that larger infarcts lead to a greater inflammatory response as well as a greater propensity for alteration of cardiac surface antigens or the emergence of previously sequestered antigens. None of the other organs (kidney, liver, lung, or brain) had evidence of infiltrates. Two-dimensional echocardiography did not reveal systolic dysfunction. This study provides direct evidence of autoimmune myocardial injury produced by adoptive transfer of concanavalin A–activated splenocytes after myocardial infarction. We propose that neurohumoral activation early in the postinfarction period triggers a series of specific inflammatory and immunological events that lead to formation of specific clones of T cells. When these are activated and transferred into normal rats, cardiac-specific cellular infiltration occurs, occasionally accompanied by myocardial necrosis. This model should help to further explore the link between neurohumoral activation after myocardial infarction and the subsequent immune alterations that might be associated with the development and/or progression of congestive heart failure. Additionally, this might be a useful model in which to study other immune-mediated cardiomyopathies. (Circ Res. 1998;82:458–463.)

Key Words: myocardial infarction ♦ myocarditis ♦ concanavalin A

Evidence suggests that neurohumorally mediated mechanisms, mainly the sympathetic and renin-angiotensin nervous systems, are central to the development of heart failure after myocardial infarction.1–3 It is well known that adrenergic overactivity augments the inflammatory response, which affects cytokines such as interleukin-2, tumor necrosis factor, and gamma interferon (responsible for proliferation of both B and T lymphocytes), and may influence remodeling and/or progression of congestive heart failure.4–10 One possible mechanism for this neurohumoral activation may be the cogeneration of an immune response against cardiac self-antigens after myocardial infarction.7–11

In several models of autoimmune disease, including a rat model of myocarditis, transfer of splenic lymphocytes sensitized to an offending antigen into syngeneic rats led to adoptive transfer of the disease.12–18 This technique of “adoptive transfer” into syngeneic rats enables us to distinguish ischemia-related injury from the subsequent immune-mediated injury. Myocardial necrosis releases or exposes normally sequestered antigenic constituents that may cause proliferation of antigen-recognizing T cells that, if given the opportunity, might target the heart in an autoimmune response, assisting in the development and maintenance of congestive heart failure. We hypothesize that if these lymphoid cells (presumably T cells) are truly reacting to a self-antigen, when one transfers them from postinfarct rats with severe heart failure into syngenic rats with normal hearts, the memory cells would attack the heart, creating an autoimmune myocarditis. This investigation of such an inflammatory response in the recipient rats may significantly enhance our understanding of the role of the immune system in the development of heart failure after myocardial infarction.

Materials and Methods

Rats
Fisher 344, syngeneic inbred (Harlan Sprague Dawley, San Diego, Calif), age-matched male rats were kept two to a cage in a climate-
controlled environment with 12-hour light/dark schedules and unlimited water and Purina rat chow.

Infarction
Myocardial infarction was produced by suture ligation of the left coronary artery. After anesthesia with intramuscular injections of 100 mg/kg ketamine and 7 mg/kg xylazine, the rats were intubated and ventilated with a small animal respirator. A left parasternal thoracotomy was performed, and the heart was exteriorized by applying external pressure to the abdomen and bilateral thorax. The left coronary artery was visualized and ligated using a 4–0 silk suture, and the heart was returned to the thoracic cavity. The chest was quickly closed by a previously placed 2–0 purse-string suture, and the animal was removed from the ventilator. The perioperative mortality was ~50%.

Infarct Quantification
Animals were killed 6 weeks after coronary ligation. At that time, a clinical rating of cardiac size was assigned (1, normal; 2, slightly enlarged; and 3, markedly enlarged) on the basis of the relative size of the live heart before obtaining blood by cardiac puncture. The heart was then removed, and the atria and great vessels were dissected away. The ventricles were separated and weighed. The left ventricle was scribed in 10% formalin for histological determination of infarct size. In order to determine infarct size, the left ventricle was sliced in four transverse sections from apex to base, and 5-μm slices of each section were fixed and stained with Masson’s trichrome. Slides of each section were projected onto an 8×11 sheet of paper. The left ventricular epicardial and endocardial circumferences were traced, as were the epicardial and endocardial infarct lengths for each slice, by use of a digitizing tablet interfaced with an IBM PC-AT computer running Sigmascan software. The percentage of circumferential infarct was then calculated. A large infarct was classified as at least 40% involvement of the left ventricle. A small infarct was between 20% and 40%. A rat was classified as a control subject if there was less than a 5% infarct. An additional group of sham-operated control rats was analyzed.

Histopathology
Hearts were removed and fixed in 10% formalin, embedded, and sectioned as described.12,13 Sections were stained with hematoxylin and eosin. Microscopic findings were graded as follows by two observers in a blind study: 1, normal or the presence of a few small lesions, not exceeding 0.25 mm² in size, in a single section; 2, presence of multiple small lesions or a few moderate-sized lesions, not exceeding 5 mm²; and 3, presence of multiple moderate-sized lesions or larger, usually with accompanying myocardial necrosis. If observers differed in their readings, a third observer was brought in.

Additionally, the T-cell antigen receptor was stained with a mouse anti-rat α/β T-cell antigen receptor monoclonal antibody (clone R73, Biosource International), followed by the avidin-biotin complex immunoperoxidase technique using AEC as the chromogen. Tissue slices were also stained with a mouse anti-rat monocyte/macrophage monoclonal antibody (clone ED1, Biosource International), followed by the avidin-biotin complex immunoperoxidase technique using AEC as the chromogen. Finally, to detect B cells, some slides were stained with a mouse anti-rat IgG monoclonal antibody (Biosource International), again followed by the avidin-biotin complex immunoperoxidase technique using AEC as the chromogen.

Echocardiography
In a subset of recipient rats, two-dimensional echocardiography was performed with a phased-array echocardiographic machine, using a 3-MHz (short focus) transducer. Long-axis, short-axis, and subcostal views were obtained.
Data Analysis
Data shown are mean±SEM. The significance of differences between groups was determined by unpaired two-tailed t tests.

Results
Induction of Infarction
Rats that survived coronary ligation were killed at 6 weeks. The extent of infarction was estimated by visual inspection, surface electrocardiography, and trichrome staining (Fig 1). Rats with infarcts that involved 40% or more of the left ventricle by trichrome staining had hearts that were markedly enlarged, usually accompanied by a pericardial effusion. In most cases at least two of the three limb leads of the ECG showed Q waves, new from baseline.

Cellular Transfer and Infarct Size of Donor Rats
Rats were injected with varying numbers of activated splenocytes via the internal jugular vein and killed at 6 weeks. Fig 2 shows representative hearts stained with hematoxylin and eosin. Recipient animals that had received splenocytes from donor animals with large infarctions had evidence of focal areas of myocarditis (Fig 2a and 2b). This finding was more frequent and often more severe than in those animals whose splenocytes were transferred from rats with infarcts of <40% or from a group of sham-operated control rats (Fig 2c). The cardiac lesions in rats were composed of a mixed cellular infiltrate, predominantly lymphocytes and plasma cells. Some rats also had areas of fibrosis and frank myocardial necrosis (Fig 2d through 2f). Multiple sections of kidney, liver, lung, and brain failed to reveal any cellular infiltrate.

Sufficient Dose of Cells for Adoptive Transfer
None of the rats injected with concanavalin A–activated spleen cells at doses of under 1 million cells developed myocarditis. The Table relates the histological findings to the range of cells transferred and the infarct size of the donor animals. When

Figure 2. a, Low-power view of recipient heart 6 weeks after transfer of activated splenocytes from an animal with a large infarction. Note diffuse infiltration of lymphocytes and plasma cells as well as areas of fibrosis. b, High-power view of same area. Necrosis and fibrosis are visible. c, Low-power view of myocardial section from recipient animal who had a splenocyte transferred from a sham-operated rat. d, Low-power view of a recipient heart 6 weeks after transfer of activated splenocytes from a rat with a small infarction (<20%). Note spotty area of cell infiltration. e, High-power view of same area showing lymphocyte infiltration and spotty areas of necrosis. The lightly stained cells with open nuclei support the theory that there are antigen-activated cells. f, Another high-power view showing myocardial necrosis adjacent to cell infiltration.
Cardiac antigens have been widely recognized for some time in a host of cardiac diseases, including myocardial infarction, 12–18 recent evidence suggests that these antibodies may be functionally significant. 20–23 Nevertheless, there has been no direct proof that memory T or B cells produced against specific myocardial antigens may cause an autoimmune phenomenon that might be associated with the development and/or progression of congestive heart failure after myocardial infarction.

In several models of autoimmune disease, including experimental allergic encephalomyelitis, 24 and in a rat model of myocarditis, 13 transfer of splenic lymphocytes that have been sensitized to an offending antigen into syngeneic rats led to adoptive transfer of the disease, thus proving the autoimmune nature of the disease. The use of adoptive transfer allows one to address specific immune changes in the recipient animal, which are independent from the initial ischemic tissue injury. If the progression to congestive heart failure after myocardial infarction involves production of autoantibody or autoreactive T cells against the heart, then passively transferring immunocompetent T and B cells from...
rats with heart failure to normal rats might elicit subsequent cardiac injury, confirming a cause-effect relationship.

The time course of inflammation after myocardial infarction in mammals is well characterized. For the first few days, neutrophils infiltrate the necrotic muscle, phagocytosing and digesting it. Over the course of a few weeks, the infiltrate changes to macrophages and lymphocytes, cells capable of antigen presentation and processing. By 6 weeks, necrotic muscle has been removed, collagen has been synthesized by fibroblasts, and the local inflammation is subsiding. Anti-myocardial antibodies have previously been shown to appear during this and other processes, causing myocardial necrosis.

In the present study, we have shown for the first time in vivo evidence of lymphocyte-mediated myocardial injury by adoptive transfer of sensitized lymphocytes from rats that developed congestive heart failure after acute myocardial infarction 6 weeks earlier. The amount of infiltrate and necrosis appeared directly related to the size of the infarct from the donor animals, suggesting that larger infarcts lead to a greater inflammatory response, which produces greater immunogenicity of altered or previously sequestered antigens. An alternate interpretation is that hearts from donor rats with congestive heart failure and large infarctions have a greater number of tissue- and/or antigen-specific T cells that are represented proportionately in the splenocyte population. Therefore, when splenocytes are stimulated with concanavalin A, they may represent a greater number of cells capable of transferring disease. Increased sympathetic activity in postinfarct animals may enhance the infiltration of immune cells, generating larger numbers of memory cells, which, when activated and expanded by concanavalin A and injected into syngenic controls, reveal a cardiac-specific autoimmune re-

Figure 4. Additional histological markers. Top, Cardiac T-cell receptor stained with mouse anti-rat α/β T-cell antigen receptor monoclonal antibody. Middle, Cardiac monocytes/macrophages stained with a mouse anti-rat monocyte/macrophage monoclonal antibody. Bottom, Cardiac IgG stained with mouse anti-rat IgG monoclonal antibody. High-power views are on the right.
weeks) or with a greater number of injected splenocytes, a more impaired function in these animals, it is likely that with further time (weeks after transfer of T cells into recipient animals. Despite the focal areas of myocarditis, there was no diminution of systolic function. LV indicates left ventricle; AO, aorta; and PA, pulmonary artery. Left panels show systolic function; right panels, diastolic function.

Figure 5. Two-dimensional echocardiography performed 6 weeks after transfer of T cells into recipient animals. Despite the focal areas of myocarditis, there was no diminution of systolic function. LV indicates left ventricle; AO, aorta; and PA, pulmonary artery. Left panels show systolic function; right panels, diastolic function.

Conclusion

The present study provides direct evidence of autoimmune myocardial injury produced by adoptive transfer of concanavalin A–activated splenocytes after myocardial infarction. We propose that neurohumoral activation early in the postinfarction period triggers a series of specific inflammatory and immunological events that lead to formation of specific T cells, which may release damaging mediators (such as cytokines) or perhaps even attack structural antigens of the myocardium. When these cells are activated and then transferred to normal syngeneic rats, cardiac-specific cellular infiltration occurs, occasionally accompanied by myocardial necrosis. Thus, this simple model should help to further explore the link between neurohumoral activation after myocardial infarction and the subsequent immune alterations that might be associated with the development and/or progression of congestive heart failure. Additionally, this might be a useful model in which to study other immune-mediated cardiomyopathies and may provide insight for developing therapeutic strategies to reduce myocardial injury and development into heart failure by specific blockade of neurohumorally induced immune activation.

References

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