Causal Association between Major Histocompatibility Complex and Myocardial Infarction in NZW × BXSB F1 Mice

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The NZW × BXSB F1 male mice that a model for systemic lupus erythematosus (SLE) develop myocardial infarction. To determine whether the gene(s) linked to the major histocompatibility complex (MHC) of NZW mice are involved in myocardial infarction, we developed an H-2-congenic NZW.H-2d strain and compared the incidence of myocardial infarction in NZW × BXSB F1 (H-2^eb) male mice to that in NZW. H-2^d × BXSB F1 male mice (H-2^eb). H-2^eb heterozygous F1 male mice showed a higher incidence of myocardial infarction than H-2^eb F1 male mice. This observation suggests that the myocardial infarction seen in SLE may be related to MHC. (Jpn Circ J 1995; 59: 98-102)

SYSTEMIC lupus erythematosus (SLE) is an immune disorder that involves the heart. Several studies have demonstrated cardiovascular complications, such as Libman-Sacks endocarditis, pericarditis, myocarditis and atrophicventricular block\(^1\)-\(^5\) in this disorder. Myocardial infarction has also been reported in SLE, and has been suggested to be caused by atherosclerosis? arteritis? or thrombosis? Myocardial infarction in SLE is also related to antcardiolipin antibody?

NZW × BXSB F1 male mice, an animal model of SLE, often develop myocardial infarction\(^10\)-\(^14\) associated with thrombocytopenia\(^15\),\(^16\). These mice may also provide a model for antiphospholipid syndrome\(^17\). We previously reported that H-2 was related to SLE in this animal model\(^18\).

To determine the relationship between the major histocompatibility complex (MHC) H-2 and myocardial infarction, we studied the incidence of myocardial infarction and the serum concentration of antcardiolipin antibodies in NZW × BXSB F1 male mice.

MATERIALS AND METHODS

Mice

BXSB and NZW mice were originally obtained from Japan SLC, Inc. (Shizuoka, Japan) and maintained in our laboratory. The H-2 congenic NZW.H-2d strain was established by selective backcrossing of the NZB × NZW F1 hybrid to NZW strains for 12 generations. Female NZW and NZW.H-2d mice were crossed with male BXSB mice to produce NZW × BXSB F1 and NZW.H-2d × BXSB F1 mice, respectively. A total of 13 (NZW × BXSB) F1 male mice, 8 (NZW. H-2^d × BXSB) F1 male mice, and 8 BXSB male mice were used.

Pathological Examination

These mice were sacrificed at the age of 3.5 to 5.5 months. The hearts from all three groups of mice were excised and fixed with

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10% formaline solution. Hearts were cut in the middle of the ventricle and divided into three transverse pieces. These pieces were embedded in paraffin, cut into 4 μm-thick sections, and stained with hematoxylin and eosin, azan, PAS, and PTAH. The specimens were then examined microscopically.

Measurement of Anti-cardiolipin Antibody

To detect the antibody to cardiolipin, each well in a polystyrene microtiter plate (Dynatec Laboratories, Alexandria, VA, USA) was coated with cardiolipin by the following procedure. Fifty microliters of 50 μg/ml cardiolipin in ethanol was evaporated under air at room temperature. Two hundred microliters of 10% fetal calf serum (FCS) in phosphate buffered saline (PBS) was added to each well which had been coated with cardiolipin and the preparation was incubated for 1 h at room temperature to block nonspecific binding of Ig to the well surface. After the preparation was washed three times with PBS containing 0.05% Tween 20 (PBS-Tween), 50 μl of serum obtained from each of the three groups of mice, diluted 1:200 with 1% BSA in PBS, was added to each well and the preparation was again incubated for 1 h at room temperature. After the preparation was washed five times with PBS-Tween, 50 μl of appropriately diluted peroxidase-conjugated goat anti-mouse γ antibody was added. The preparation was incubated for 60 min at room temperature and washed. Fifty microliters of 0.4 mg/ml substrate (o-phenylenediamine dihydrochloride) diluted in 0.1 mol/L citrate phosphate buffer, pH 5.0, with 0.5 μg/ml H₂O₂ was then added. Absorbance at 492 nm was read with an automated spectrophotometer (BIO-RAD, Richmond, CA, USA; Model 2550, EIA reader).

Statistical Analysis

Statistical analysis was performed using Student's t test. A P level of <0.05 was considered to indicate statistical significance.

RESULTS

To determine whether the gene(s) linked to the H-2 complex is involved in myocardial infarction, we compared the incidence of myocardial infarction and serum anticardiolipin antibody level between NZW × BXSB F1 (H-2e/b) and NZW.H-2d × BXSB F1 (H-2d/b) male mice.

Among the pathological findings, myocardial necrosis, cell infiltration, replacement fibrosis, and eosinophilic degeneration of the myocytes were seen in the myocardium of these mice (Fig. 1). Myocardial infarction was sometimes associated with hemorrhage caused by thrombocytopenia. The area of myocardial infarction was not as large as that produced by stenosis of the epicardial seg-
ment of a coronary artery. These myocardial changes appeared to be due to the ischemia caused by microthrombi in the intramural small coronary arteries (Fig. 2).

The incidence of myocardial infarction in NZW×BXSB was significantly higher than that in NZW. H-2d×BXSB F1 (11/13 [84%] vs 2/8 [25%], \(p<0.01\)) or in BXSB male mice (11/13 [84%] vs 3/9 [33%], \(p<0.05\)) (Fig. 3).

Serum anticardiolipin antibody levels in H-2\(d\) male mice were significantly higher than those in H-2\(d\) male mice (28.2 ± 5.3 IU vs 8.0 ± 4.1 IU, \(p<0.01\)) or in BXSB male mice (10.6 ± 3.0 IU, \(p<0.05\)), respectively (Fig. 4).

**DISCUSSION**

In this study, we found that the incidence of myocardial infarction was altered when H-2 was changed in a specific strain of mice.

Atherosclerosis is a major cause of myocardial infarction in humans. The relationship between coronary atherosclerosis and familial hyperlipidemia has been studied genetically. Although thrombosis is also a cause of myocardial infarction, the genetic relationship between thrombosis and myocardial infarction has not been evaluated.

Anticardiolipin antibody has been linked to myocardial infarction due to thrombosis in SLE. NZW×BXSB F1 male mice produce autoantibodies against cardiolipin. The cause of myocardial infarction in these mice is reported to be thrombosis in the small coronary arteries. We also observed thromboses in intramural small coronaries and demonstrated high titers of anticardiolipin antibodies. Although the precise relationship with H-2 has not been determined, our results suggest that H-2 is related to the incidence of myocardial infarction, and that such infarction is due to thrombosis and anticardiolipin antibodies.

MHC antigens play a crucial role in the activation and regulation of the immune system. The regulation of MHC antigens by cytotoxic T lymphocytes and helper T cells is believed to be established by MHC class.

A relationship between autoimmune disease in various organs and MHC has been suggested as has a relationship between MHC and heart disease. Dilated cardiomyopathy is reportedly related to HLA DR and DQ. In viral myocarditis, the genetic susceptibility to virus is related to the pathologic spectrum of myocarditis. Myocarditis induced by cardiac myosin is influenced by the major histocompatibility complex SSA autoantibody, which is linked to atrioventricular block, is reportedly correlated with HLA-DR/DQ.

Antiphospholipid antibodies have been

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found in patients without underlying immune disorders, as well as in those with SLE.\(^{31}\) The presence of an increase in anticiardiolipin antibodies was strongly associated with cardiac pathology.\(^{32}\) In addition, anticiardiolipin antibody may be linked with the progression of preexisting atherosclerotic vascular disease.\(^{33}\) Therefore, myocardial infarction in some patients may be controlled by a MHC that induces high titers of anticiardiolipin antibody.

While the causes of myocardial infarction are multifactorial, that associated with thrombosis may be related to MHC. Further studies of H-2 and myocardial infarction are required to elucidate this relationship.

REFERENCES


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