Studies on Serum Anti-Heart Antibodies and Migration Inhibitory Factor against the Heart Tissue in Systemic Lupus Erythematosus*

NOBORU IIDA, M.D. AND YUICHI SHIOKAWA, M.D.

IN systemic lupus erythematosus cardiac lesions are found in the great majority of the autopsies, and affect the endocardium, myocardium and pericardium. Like other collagen diseases, autoantibodies against the various tissue constituents have been detected in the sera of the disease, and anti-nuclear anti-bodies which reacted with the homologous heart tissue have also been found. Still the role of the antibodies in the pathogenesis of cardiac injury has not been clarified. In this study the humoral antibodies and the cellular immunity against cardiac tissue were investigated in patients with systemic lupus erythematosus. As a cell-mediated immunity, the migration inhibition activity released from lymphocyte cell suspensions was examined and the results were discussed in relation to the clinical signs of cardiac lesions in the disease.

METHODS AND MATERIALS

Eighteen cases with systemic lupus erythematosus and accompanying cardiac lesions were studied. In each case the diagnosis of systemic lupus erythematosus was made according to ARA criteria. The diagnosis of carditis was based on the presence of a significant heart murmur, pericardial friction rub, radiologic evidence of cardiomegaly, a conduction abnormality on the electrocardiogram and clinical signs of congestive heart failure. The anti-streptolysin O titer remained within normal range in every patient.

The assay for the migration inhibition factor consisted of: 1) sensitive lymphocytes from affected patients and non-sensitive lymphocytes

as controls; 2) non-sensitive migrating macrophages, and 3) specific antigens. The factor was assayed as described by *Sørberg et al.*

In order to obtain a lymphocyte cell population, 5.0 ml of human peripheral venous blood was drawn into a disposal syringe containing 0.9% saline and 1000 units of phenol free Liquaemin Heparin. Ficoll-Conray 400 was added to the blood and it was then centrifuged at 1550 rpm for 30 minutes. The lymphocyte rich

Fig.1. Percentage migration in SLE.

Key Word:
MIF in SLE

Department of Internal Medicine, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, Japan

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Migration in Control

Migration in MIF

Percentage migration = 16.3 % (SLE)

Method

Fig.2. Migration inhibitory factor released from peripheral lymphocyte reacted to heart tissue.

fraction was removed and again centrifuged at 1550 rpm for 10 minute. The cell pellet was washed three times in TC-199. The lymphocyte suspension thus obtained was diluted in TC-199 containing 1000 units of penicillin and was adjusted to a concentration of $3-5 \times 10^6$ cell/ml.

To obtain the antigen, human heart tissue were taken immediately after death from a patient without an apparent immunologic dis-

order, the connective tissue was removed, minced with scissors and rinsed with cold saline solution in order to remove visible traces of blood. Extracts of the tissue were obtained by homogenization in the Vertis homogenizer, using twice its volume of 0.15 M phosphate buffer at pH 7.2 and centrifugation at room temperature at 3000 rpm. The supernatant was stored at -60°C until use.

Peritoneal exudate cells were obtained from DDY strain mice by lavaging the peritoneal cavity with chilled saline solution. The exudates were washed three times with TC-199 and resuspended at the concentration of 5 - 10 cell/ml in TC-199 containing 15% fetal calf serum and 1000 units penicillin. The isolated peritoneal sensitive and non-sensitive lymphocytes were adjusted to $3-5 \times 10^6$ cell/ml in TC-199 and added to the solution. The cell suspensions were transferred to capillary tubes which were sealed at one end, and were centrifuged at 800 rpm for 10 minutes. The tubes were broken at the cell medium interface, sealed and fastened with silicone grease in the bottom of a Mackness chamber. The chamber was filled with TC-199 containing the heart homogenate as an antigen whose protein concentration was adjusted to 100 $\mu\text{g}/\text{ml}$, was sealed and incubated at 37°C for 24 hrs in a 5% CO atmosphere. After 36 hrs of incubation, the chamber was photographed and the average area of the cell migration was measured by planimetry. The action of the antigen was expressed as a ratio of the average area of migration. The average area of migration with the antigen was divided by the average area of migration without the antigen, and the percentage of migration or migration index (MI) was calculated. When the index was more than 2 standard deviations from the average, it was considered evidence of migratory inhibition.

Serum anti-heart antibodies were determined by the tanned cell agglutination test (T.R.C.A.).

Tanned sheep red blood cells were incubated using a heart tissue homogenate as an antigen and with 0.15 M phosphate buffer saline for 10 minutes at 37°C , and then adjusted to 1.5% suspension of antigen coated sheep red blood cells. Using this suspension, the micro-titer method was performed.

RESULTS

Figure 1 illustrates the result of the migration inhibitory factor produced by the lymphocyte cell population from 10 cases of systemic lupus

TABLE I ANTI-HEART ANTIBODIES IN 40 CASES SLE (T.R.C.A.)

Cardiac lesion	No. of cases	Positive	P
Cardiomegaly	7	5 (71%)	> 0.05
Pericarditis	0	0 (0%)	
Endocarditis	2	1 (50%)	
Myocarditis	3	1 (33%)	> 0.05
ECG abnormality	9	6 (66%)	
Heart murmur	10	8 (80%)

Fig.3. Anti-DNA antibodies and % migration in SLE.

erythematosus with cardiac lesions, 4 cases of systemic lupus erythematosus without cardiac lesions and 4 cases of normal controls. When incubated with human heart homogenates as the antigen, the average migration index was 52.2% in patients with cardiac lesions and 87.3% in patients without cardiac lesions. The controls' index was 90.8% (Fig.1).

In addition, lymphocytes from 3 cases with pericarditis showed inhibition of macrophage migration. Figure 2 shows the marked inhibition seen in one case of systemic lupus erythematosus with pericarditis and panserositis (16.3%). Table I shows anti-heart antibodies with T.R.C.A. in 40 cases of systemic lupus erythematosus. Fifteen out of 40 cases were positive. There was a significant correlation between the high titer of anti-heart antibodies in the sera and the clinical signs

of cardiomegaly ($p < 0.05$). Figure 3 shows the relationship between anti-DNA antibodies and the percent of migration inhibition in systemic lupus erythematosus. There was no correlation between the degree of migration inhibition activities of lymphocytes and anti-DNA antibodies in the sera (Fig.3).

DISCUSSION

Cardiac lesions are frequently found in systemic lupus erythematosus. Libman Sachs' endocarditis, for example, was seen in 32 (Harvey) and 3 (Haserich) of the autopsied cases. In systemic lupus erythematosus, experimental and clinical data indicate that autoimmunity is responsible for the pathogenesis of the organ lesion of the disease. The disorder is characterized by the presence of a circulating serum factor capable of reacting with many different auto-logous tissue constituents. Among them, anti-nuclear antibodies have reacted with DNA and other nuclear substances have been widely investigated. The anti-heart antibodies, produced in rheumatic fever as a result of a cross reaction with the streptococcal antigen, have also been detected in the sera of systemic lupus erythematosus. The antibodies which reacted with cardiac tissue have been detected by precipitation, tanned red cell agglutination, complement fixation and immunofluorescence techniques. With the indirect immunofluorescence method anti-heart antibodies were reported in 20 of 32 patients with systemic lupus erythematosus. The pathogenic role of autoantibodies of the sera of cardiac lesions has not yet been clarified, but Das (1973) reported that antibodies were found with equal frequency in those with and those without clinical heart disease. Recently advances in immunology have progressed rapidly. In addition to humoral immunity, the role of cellular

immunity in the pathogenesis of organ lesions of collagen disorders has been vigorously pursued. A substance, named migration inhibition factor, is synthesized and released by the sensitive lymphocytes after contacting the antigen, while normal cells cannot release the substance after incubation with the antigen. In 1932, Rich and Lewis demonstrated that tuberculin induced inhibition of migration of macrophage whenever delayed hypersensitivity to tuberculin existed. An in vitro technique for the migration was introduced by Gorge and Vaughan in 1962 on the basis of capillary tube. David reported that migration of macrophage in normal animals was inhibited by the supernatant of culture of lymphocytes from sensitive animal when exposed to antigen. Recently macrophage inhibition tests have been widely used as in vitro correlates of delayed hypersensitivity and cellular immunity. According to our study the macrophage migration factor to cardiac antigen was generated by the lymphocytes of patient with systemic lupus erythematosus. The migration index was lower in patients with a cardiac lesion than those without it. A high titer of antiheart antibodies with T.R.C.A. was detected in the disease, more frequently in cases with the cardiac lesion than in cases without it. But there was no relationship between the presence of a high titer of autoantibodies in the sera and the migration inhibition factor released by the lymphocytes. The antigen used in this study was a crude extract of the human heart tissue which con-

tained every cellular component including the nuclear substance. High anti-nuclear activity is usually noted in this disorder being detected in both humoral and cellular immunity procedures, and therefore, in our study the autoantibodies in the sera, as well as the migration inhibition factor produced by the lymphocytes, could be attributed to the activity against nuclear components. Our examination showed, however, that the titer of the anti-DNA factor did not correlate well with the immunological reactivity against the heart tissue. In conclusion, the result suggested the possibility that the presence of cellular immunity against autologous heart tissue could participate in the pathogenesis of cardiac lesions in systemic lupus erythematosus, but further investigation is needed to clarify the problem.

REFERENCES

1. DAS, S. K. & CASSIDY J. T.: Anti-heart antibodies in patients with systemic lupus erythematosus. *Am. J. Med.* 265: 275-280, 1973.
2. GEORGE, M. & J. H. VAUGHAN: In vitro cell migration as a model for delayed hypersensitivity. *Proc. Soc. Exp. Biol. Med.* 111: 514, 1962.
3. DAVID, J. R., S. AL-ASKARI, H. S. LAWRENCE, & L. THOMAS: Delayed hypersensitivity in vitro. *J. Immunol.* 93: 264, 1964.
4. KAPLAN, M. H.: Autoimmunity to heart and its relation to heart disease. *A review. Progr Allergy* 13: 408-429, 1969.
5. KAPLAN, M. H.: Autoimmunity to heart, Textbook of Immunopathology. Vol. 2 Edited by PA Miescher, HJ Muller-Eberhard. New York, Grune and Stratton, 1969, pp.641-650