Experimental Myocarditis.

II. Cardiac Lesions in Rats Induced by Immunization with Heterologous Heart Extracts

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The production of myocardial lesions and circulating anti-heart antibodies has been studied in the present experiment. Twenty-one of Wistar rats were divided into 3 groups: 9 rats immunized with heterologous heart extracts, Freund's complete adjuvant and diphtheria-tetanus-pertussis (DTP) vaccine, 8 rats injected phosphate buffered saline adjuvant mixture with DTP vaccine and 4 rats untreated as control. Injection of heterologous heart extracts emulsified with Freund's complete adjuvant and DTP vaccine developed focal myocardial lesions in all of the subjected rats. The lesions were characterized by focal and diffuse interstitial cellular infiltration with myocardial necrosis. The circulating antibodies reacted to rat heart extracts were detected in 77.8% of the subjects. The antigen, reacted with the antibodies, located intracellular spaces in myocardium. The severity of cardiac lesions were not functional to the titer of circulating anti-heart antibodies. These data suggest that the circulating anti-heart antibodies may play little role in the pathogenesis of myocardial damage at least in this experimental myocarditis.

EXPERIMENTAL study of an immune myocarditis may provide a good model to assess the role of immune processes in the pathogenesis of myocardial damage. As reported previously, we found that homologous heart extracts injected with Freund's complete adjuvant and diphtheria-tetanus-pertussis (DTP) vaccine induced myocarditis in rats. There were no relationship between the prevalence of positive circulating anti-heart antibodies and myocardial lesions.

The purpose of this study is to investigate the pathogenesis of myocarditis of the rat immunized with heterologous heart extracts and to assess the role of circulating anti-heart antibodies in the experimental immune myocarditis.

MATERIALS AND METHODS

Experimental animals were 21 of Wistar rats, maintained on a standard balanced diet during experimental period. Animals were divided into 3 groups: 9 rats immunized with heterologous heart extracts, Freund's complete adjuvant and DTP vaccine, 8 rats injected phosphate buffered saline (PBS) adjuvant mixture with DTP vaccine and 4 rats untreated as the controls. Heterologous heart extracts were obtained from rabbit hearts.

Method of preparation of rabbit heart extracts, technique of histological examination and deter-
TABLE I  SUMMARY OF PATHOLOGICAL AND SEROLOGICAL FINDINGS

<table>
<thead>
<tr>
<th>No.</th>
<th>Heart Antigen</th>
<th>Complete Freund's adjuvant</th>
<th>DTP Vaccine</th>
<th>Rat with Myocarditis</th>
<th>Degree of severity</th>
<th>Positive anti-heart antibodies</th>
<th>Rat heart</th>
<th>Rabbit heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>PBS</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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* +: mild, ++: moderate, +++: severe

mination of circulating anti-heart antibodies were carried out as described in the previous report.

Immunodiffusion was carried out on glass slide covered with 1% Agarose in veronal buffer. The thickness of the gel was set at 1 mm. Wells of 5 mm of diameter were punched out at 5 mm distance. The plate was incubated in a moist chamber for 48 hours.

An indirect immunofluorescent technique was employed to detect the antigens reacting to circulating anti-heart antibodies using alcohol fixed paraffin embedded rat heart sections. Serum was diluted 1:5 with buffer, and then applied to the section. The section was incubated in a moist chamber for 30 min. After the section was washed out by PBS 3 times for 15 min, the section was incubated for 50 min with fluorescein isothiocyanate conjugated goat anti-rat gamma globulin. Again the section was washed out by PBS as described above. The stained section was mounted in glycerol buffer and were read with Leitze Dialux 20 fluorescent microscope.

RESULTS

The results of this experiment are summarized in Table 1. In all rats immunized by the emulsion of heterologous heart extracts and Freund's complete adjuvant and DTP vaccine, histological myocardial lesions were observed. The predominant changes in myocardium were focal and diffuse interstitial cellular infiltration with myocardial necrosis. The severity of myocardial lesions were arbitrarily graded as described in the previous report. In this experiment, the "mild"

Fig.1. Focus of cardiac lesion with diffuse interstitial cellular infiltration. H.E.  
×40

Fig. 2. Immunodiffusion reaction of the anti-rabbit heart rat serum with heart extracts. 1: heterologous anti-heart serum, 2: rat heart extracts, 3: rabbit heart extracts.

Fig. 3. Reaction of heterologous anti-heart serum with section of rat heart, showing intracellular staining pattern.

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lesion was seen in all animals, the "moderate" lesion in 5 and the "severe" lesion in 4.

All rats immunized with heterologous heart extracts had circulating anti-heart antibodies reacted to rabbit heart extracts in passive hemagglutination test. The circulating anti-heart antibodies were reacted to both rabbit and rat heart extracts in 7 of 9 rats.

Immunodiffusion analysis of the both heart extracts against heterologous anti-heart sera were carried out. As shown in Fig. 2, 2 precipidine lines developed between heterologous anti-heart sera and rabbit heart extracts, and one precipineline between heterologous anti-heart sera and rat heart extracts. A spar was formed between a precipidine line against rat heart extracts and one of 2 lines against rabbit heart extracts. These results showed that the circulating anti-heart antibodies detected in immunized animals might react to rat myocardium.

These anti-heart antiserum were tested for immunofluorescent staining properties using normal rat heart section. Intracellular substances were stained as shown in Fig. 3.

However, the titer of circulating anti-heart antibodies were not functional to severity of cardiac lesions.

DISCUSSION

In a previous report, we observed that 6 of 7 rats, injected with the emulsion of homologous heart extracts and Freund's complete adjuvant and with DTP vaccine, had histological lesions in myocardium. In the present study, it was demonstrated that myocardial lesions can be induced by heterologous heart extracts, Freund's complete adjuvant and DTP vaccine injection. These results support to Friedman's report demonstrating that pertussis vaccine potentiates the development of myocarditis.

Concerning the role of circulating anti-heart antibodies in producing tissue damage, there are many opinions. In the study of Laufer and Davies who used Freund's complete adjuvant, heterologous heart extracts highly produced the circulating antibodies and focal myocarditis.

While rats treated with homologous heart extracts showed myocarditis without antibody formation. Their in vivo and in vitro studies using passive immunization showed that specific anti-heart sera were not cytotoxic in vivo, but that heterologous sera were cytotoxic in vitro. Tompson and Halbert studied the cytotoxicity of heart antibodies for pulsating rabbit and rat heart cells in tissue culture, and showed that neither rabbit anti-rabbit-heart antibodies nor rabbit anti-rat-heart antibodies in the presence of rabbit complement were cytotoxic for beating rabbit heart cultures. On the other hand, in rabbit heart culture, cell damage was obtained by duck anti-rabbit-heart antibody in the presence of complement.

The present study indicated that 7 of 9 rats immunized with heterologous heart extracts had circulating antibodies against rat heart extracts, and antigens which react to the circulating antibodies located intracellular spaces in myocardium. However, there was no correlation between the severity of myocardial lesions and the titer of circulating anti-heart antibodies.

These data suggest that the circulating anti-heart antibodies may play little role in the pathogenesis of the present experimental myocarditis.

REFERENCES

5. TOMPSON A, HALBERT SP: The cardiac auto-immune system. III. Studies on the cytotoxicity of heart antibodies for pulsating rabbit and rat heart cells in tissue culture. Int Arch Allergy 40: 274, 1971