Characterization of Anti-Heart Mitochondria Autoantibodies Produced in Dogs following Myocardial Infarction

By Robert E. Kelley, Merle S. Olson, and R. Neal Pinckard

ABSTRACT

A complement-fixing, heart-reactive serum component that develops in dogs following experimental myocardial infarction was characterized with respect to its immunoglobulin nature and its subcellular membrane and organ specificities. The immunoglobulin nature of the heart-reactive serum factor was established by the following evidence: (1) the factor was not removed from the serum following absorption of up to 90% of the first component of dog complement with bovine serum albumin (BSA)-anti-BSA immune precipitates, (2) the serum factor sedimented in the 19S region of sucrose density gradients, and (3) affinity chromatography using a Sepharose column coupled with rabbit anti-dog κ-chain-specific antibody resulted in the removal of nearly all of the heart-reactive substance. The major subcellular autoantigenic locus of the dog heart was determined to be the outer mitochondrial membrane. Serum containing the autoantibody demonstrated a six-fold greater autoantibody titer when outer mitochondrial membrane was used as the test antigen than it did when inner mitochondrial membrane was used. Absorption experiments suggested that the autoantigenic determinants residing on inner and outer mitochondrial membranes were unique to the individual membranes. Significant cross-reaction of the anti-heart autoantibody between heart and skeletal muscle mitochondria was observed, but no cross-reaction was seen with dog liver mitochondrial membranes.

KEY WORDS inner and outer mitochondrial membranes IgM heart autoantigens complement-fixing anti-heart autoantibody affinity chromatography complement

The development of anti-heart autoantibodies following myocardial infarction or during other cardiac disease has been reported by many investigators (1-5). In these studies, the incidence of anti-heart autoantibody production in patients following myocardial infarction varied from 3% to 80%. The inconsistency in the demonstration of anti-heart autoantibodies probably reflects differences in both the immunological tests used and the nature of the test antigen preparations derived from cardiac tissue. Among the various techniques used to demonstrate the presence of anti-heart autoantibodies, the complement-fixation assay appears to be the most sensitive method. In most of the aforementioned studies, the production of anti-heart autoantibodies was thought to be involved in the pathogenesis of cardiac diseases; little consideration has been given to the concept that autoantibody formation to tissue constituents represents a normal physiological response to tissue necrosis (6).

A previous communication from our laboratory (7) has indicated that a complement-fixing, anti-heart mitochondria autoantibody is produced in dogs following experimentally induced myocardial infarction. The evidence that this heat-stable (56°C for 30 minutes), heart-reactive substance is indeed an autoantibody is based on the fact that it fixes guinea pig complement in the presence of dog heart mitochondrial membranes and has a sedimentation coefficient of approximately 19S. Because we have recently demonstrated the existence of an antibody-independent activation of human complement by human heart mitochondrial membranes (8) and because the serum of many of the dogs used in our studies possesses a "naturally occurring" autoantibody reactive with heart subcellular membranes, further characterization of the immunoglobulin nature of the heart-reactive serum factor produced in dogs following myocardial infarction was necessary. In addition, the exact subcellular location of the autoantigen(s) in the mitochondrial fraction of cardiac tissue and the organ specificity of the anti-heart autoantibody...
Methods

Mitochondria were prepared from dog cardiac tissue obtained from mongrel dogs. Immediately following the administration of a lethal dose of 50% (w/v) sodium pentobarbital (0.5 ml/kg body weight), the heart was removed and washed in an ice-cold solution containing 0.25M sucrose and 0.01M Tris-chloride, pH 7.4. The tissue was trimmed of connective tissue and fat and passed through an electric meat grinder (2-mm holes) into seven volumes of a solution of 0.25M sucrose and 0.01M Tris-chloride, pH 7.4. Complete homogenization was accomplished using a Polytron tissue disintegrator (setting 5, 45 seconds). The crude homogenate was centrifuged at 600 g for 10 minutes. The 600-g supernatant fraction was centrifuged at 5,500 g for 10 minutes, and the pellet containing the mitochondria was resuspended and centrifuged at 5,500 g for 10 minutes. The mitochondrial pellets were resuspended in the homogenization buffer, aliquoted into small serum vials, and frozen quickly at -20°C. The 5,500-g supernatant fractions were pooled and centrifuged at 17,000 g for 20 minutes, and the resulting supernatant fractions were centrifuged at 105,000 g for 60 minutes. The 105,000-g pellet contained the sarcoplasmic reticulum, and the supernatant fraction consisted of the cytosol.

Dog liver mitochondria were isolated using the procedure described for rat liver by Parsons et al. (9). Dogs were anesthetized with sodium pentobarbital (30 mg/kg body weight). Prior to removal from the anesthetized dog, the liver was perfused for 5 minutes with ice-cold 0.25M sucrose by cannulating the portal vein. Mitochondria from dog skeletal muscle were isolated according to the procedure described by Swanson (17). Inorganic phosphate, and 2 mM MgCl₂, mixed by gentle homogenization in a glass-

an ice bath for 5 minutes. The mitochondrial suspension was diluted with one-third volume of a solution of 1.8M sucrose, 2 mM adenosine triphosphate, and 2 mM MgCl₂, mixed by gentle homogenization, and allowed to incubate for 5 minutes at 4°C. Aliquots of the suspension (3 ml) were sonicated three times for 5-second intervals using a Bronson Sonifer (microtip, 75 w). The sonicated mitochondria were layered onto a discontinuous sucrose gradient consisting of 7 ml of a solution of 1.18M sucrose and 0.01M Tris-chloride, pH 7.4, and 5 ml of a solution of 0.76M sucrose and 0.01M Tris-chloride, pH 7.4. The gradients were centrifuged at 62,000 g for 4 hours in a Beckman SW 25.1 rotor. Two crude outer mitochondrial membrane fractions were separated at the interfaces of the discontinuous gradient; the inner mitochondrial fraction sedimented in a pellet at the bottom of the tube. The outer membrane fractions were removed from the interfaces using a syringe, and these membrane fractions were mixed and diluted to a total volume of 18 ml by the addition of an appropriate amount of a solution of 0.25M sucrose and 0.01M Tris-chloride, pH 7.4. The inner membrane pellet was resuspended in a total of 18 ml of the sucrose-Tris solution. The inner and outer membranes each were layered onto discontinuous sucrose gradients and centrifuged as described earlier. The outer membrane fractions were removed from the gradients, frozen, and stored at -20°C. The inner membrane pellet was resuspended in sucrose-Tris buffer, frozen, and stored at -20°C.

The relative purity of the various subcellular fractions was assessed by comparing the specific activities of several marker enzymes in each preparation. The following enzymes were used: mono-

amine oxidase (14), rotenone-insensitive NADH-

cytochrome c reductase (15), and cytochrome oxidase (16). Glucose-6-phosphatase was used as a sarcoplasmic reticulum marker and was assayed as described by Swanson (17). Inorganic phos-

phate was estimated by the method of Gomori (18).

Acute myocardial infarction was produced in mongrel dogs by the injection of sized microspheres into the left coronary artery (19). The dogs were monitored following infarction by electrocardiography and also by changes in the levels of serum creatine phosphokinase (20). All of the dogs infarcted in this study had both serum en-

zyme elevations and electrocardiographic altera-

tions diagnostic for acute myocardial infarction. Serum samples were assayed for the presence of anti-heart mitochondria autoantibody by performing complement-fixation tests as described by Pinckard et al. (7). When the peak anti-heart autoantibody titer had developed, the dogs were anesthetized with sodium pentobarbital and bled by jugular vein puncture. The serum obtained was divided into 2-5-ml samples and stored frozen at -20°C.

To localize the autoantigenic determinants on the various subcellular fractions, spectrophotomet-

ric complement-fixation tests were performed. The appropriate dilutions of serum were made in 0.15M sodium barbital-buffered saline, pH 7.3, contain-

ing 1% for serum albumin. To 100 uliter of
of guinea pig complement containing 1.50 CH<sub>100</sub> units of complement followed by the addition of 100 μl of the subcellular membrane fraction being tested. The mixture was incubated for 30 minutes in a 37°C water bath, sensitized sheep erythrocytes (100 μl of 1 × 10<sup>6</sup> cells/ml) were added, and the mixture was incubated for 1 hour at 37°C with shaking at 15-minute intervals. The reaction was stopped by the addition of cold 0.15M NaCl and 0.01M ethylenediaminetetraacetic acid (EDTA). The test tubes were centrifuged at 9,000 g for 5 minutes, and the supernatant fractions were assayed spectrophotometrically at 415 nm for the presence of hemoglobin. The percent lysis was determined by comparison of the experimental optical density with that of a 100% lysis control (100 μl each of buffer, complement, antigen, and sheep red blood cells); 50% hemolytic end points were determined by graphing techniques (21) according to the procedure described by Kabat and Mayer (22). Under the experimental conditions of the complement-fixation test just described, none of the concentrations of subcellular membranes exhibited any pro- or anticomplementary activity.

Serum samples were absorbed with various subcellular membrane preparations at 0°C. The serum samples were added to the appropriate membranes and incubated for 2 hours. The suspensions were centrifuged at 15,000 g for 90 minutes at 4°C. The supernatant fraction was removed and tested for the presence of autoantibody activity using the spectrophotometric complement-fixation test. The control for this absorption experiment was serum that had not been treated with mitochondrial membranes but had been subjected to all other manipulations.

To remove the first component of dog complement (C1) from the serum, dog serum (1.0 ml) was absorbed with bovine serum albumin (BSA)-anti-BSA complexes (5 mg/ml). BSA-anti-BSA complexes at equivalence were prepared using heat-inactivated rabbit antiserum and crystalline BSA. Serum samples were incubated with BSA-anti-BSA immune precipitates at 1 hour at 0°C in the presence of 0.01M EDTA. The complexes were removed by centrifugation at 1,000 g for 30 minutes. The procedure was repeated once, and the serum was frozen at -20°C. This absorption procedure removed more than 90% of the initial C1 activity as determined by standard hemolytic C1 assays.

Serum which had been absorbed with BSA-anti-BSA complexes was subjected to sucrose density gradient ultracentrifugation to isolate the 19S immunoglobulin fraction as described previously (7). Continuous sucrose gradients (12-38%) were immunoglobulin fraction as described previously (7). 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Results

The first objective of the present study was to determine whether the heart-reactive serum factor present in untreated dogs and in dogs following myocardial infarction was an autoantibody or whether it was related to C1. An experiment was performed to determine whether the removal of C1 by absorption of dog serum with BSA-anti-BSA immune precipitates would also remove the heart-reactive serum factor. Two different types of serum were used in this experiment: (1) serum obtained 15 days after experimental myocardial infarction from a dog that did not possess preexisting heart-reactive serum factor prior to infarction and (2) sera obtained prior to any experimental treatment.
from two dogs that had a preexisting heart-reactive serum factor. Prior to the experiment described in Figure 1, it was demonstrated that treatment of the dog sera with BSA-anti-BSA immune precipitates led to a reduction of more than 90% in total hemolytic C1 and total complement activity. Absorption with immune precipitates yielded the three types of results illustrated in Figure 1: (1) the preexisting heart-reactive serum factor of some dogs was completely removed on absorption (dog A), (2) about 50% of the preexisting activity was removed from the serum of some dogs on absorption (dog B), and (3) the induced heart-reactive serum factor present in infarcted dogs was not affected by the absorption procedure (dog C). The results of the BSA-anti-BSA complex absorption experiments indicated that the heart-reactive factor seen in dog serum following myocardial infarction (dog C) probably was not C1. However, the preexisting anti-heart activity of dog sera possibly could be ascribed to either dog C1 or to a preexisting anti-heart autoantibody.

To substantiate that the induced heart-reactive serum factor in the serum from dog C and the preexisting heart-reactive serum factor in the serum from dog B were autoantibodies, experiments utilizing affinity chromatography were performed. Sera from dogs B and C were absorbed with BSA-anti-BSA complexes as described in Figure 1, and the absorbed sera were placed on sucrose density gradients. Following centrifugation, gradient fractions 6-9 containing the 19S serum proteins were removed, pooled, and subjected to affinity chromatography as described in Methods. The preexisting 19S heart-reactive factor that remained in the serum of dog B following absorption with immune precipitates was removed completely by the Sepharose 4B column coupled with anti-dog α-chain-specific rabbit antisera (Fig 2A). The induced heart-reactive factor present in the serum of dog C which developed following experimental myocardial infarction was reduced markedly on passage through a Sepharose column coupled with the anti-dog α-chain-specific antisera as compared with that in the same serum passed through a column coupled with normal rabbit sera (Fig. 2B).

Having demonstrated that the induced heart-reactive factor was a 19S immunoglobulin M(IgM) anti-heart autoantibody, the next objective was to define the location(s) of utilizing these autoantibodies. Prior to initiating this characterization, it was essential to obtain preparations of membranous subcellular constituents derived from cardiac tissue. Most of the procedures established for fractionating mitochondria into their inner

<table>
<thead>
<tr>
<th>Marker enzyme</th>
<th>Mitochondria</th>
<th>Inner mitochondrial membrane</th>
<th>Outer mitochondrial membrane</th>
<th>Sarcoplasmic reticulum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoamine oxidase</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase</td>
<td>818</td>
<td>992</td>
<td>344</td>
<td>70</td>
</tr>
<tr>
<td>(rotenone insensitive)</td>
<td>2.10</td>
<td>1.76</td>
<td>29.40</td>
<td>71.7</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>2.50</td>
<td>1.56</td>
<td>2.50</td>
<td>3.0</td>
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All enzyme activities are expressed in nanomoles of substrate oxidized per mg of protein.
and outer membrane components are suitable for liver tissue but do not result in an efficient separation of the outer mitochondrial membrane from mitochondria derived from tissues such as heart. To accomplish this separation, a modification of the procedure of Whereat et al. (13) was used. The relative purities of each membrane fraction were established; the results shown in Table 1 indicate the enrichment of various marker enzyme activities in the appropriate subcellular fractions. Although monoamine oxidase was not detected in heart mitochondria, analysis for another outer mitochondrial membrane marker, rotenone-insensitive NADH-cytochrome c reductase, indicated that there was four times more activity of this enzymatic marker in the outer mitochondrial membrane fraction of heart than there was in the inner mitochondrial membrane fraction. The inner mitochondrial membrane fraction was enriched with the enzyme cytochrome oxidase compared with the outer mitochondrial membrane fraction. The analyses for glucose-6-phosphatase, a sarcoplasmic reticulum membrane marker, indicated that significant amounts of this enzyme in a mitochondrial fraction occurred only in the outer membrane fraction. The results of the analyses for the various marker enzymes indicated that individual dog heart subcellular fractions obtained by the fractionation procedures described were enriched for the appropriate marker enzymes and were adequate for use in the present studies.

Experiments were performed to assess the autoantigenicity of various subcellular fractions (Table 2). A protein concentration of 250 µg/ml was used for each of the test autoantigens. Autoantigenic activity was observed in the mitochondrial and sarcoplasmic reticulum fractions but not in the cytosolic fraction of dog cardiac tissue. Fractionation of the cardiac mitochondria into their component membranes indicated that both the outer mitochondrial membrane and the inner mitochondrial membrane had autoantigenic activity (Fig. 3). Evidence indicating that the outer mitochondrial membrane was significantly more autoantigenic than the inner mitochondrial membrane on a milligram of protein basis was obtained using the spectrophotometric complement-fixation assay and checkerboard titrations of two membranous autoantigens. When inner mitochondrial membrane was used as the test antigen in the complement-fixation test, the end points of the titrations increased with increasing inner membrane protein until the concentration exceeded 3.0 mg inner membrane protein/ml. Above this concentration of membrane there was no increase in the complement-fixation test end points with a further increase in antigen concentration. In contrast, the outer mitochondrial membrane gave increasing end points for all concentrations tested. Although the maximum complement-fixation end point was not determined for outer mitochondrial membrane at the highest membrane protein concentration tested, there was approximately a sixfold greater autoantibody titer when the outer mitochondrial membrane autoantigen was used than there was when the inner mitochondrial membrane autoantigen was

![Graph](image-url)
used at the identical protein concentration of 2.0 mg/ml.

Although the outer mitochondrial membrane fraction of dog cardiac tissue appeared to be the major autoantigenic subcellular locus, it should be pointed out that this fraction contained some contaminating inner mitochondrial membrane (Table 1). The same statement also can be made with respect to the inner mitochondrial membrane fraction. To determine whether there are individual autoantigenic sites specific for inner and outer mitochondrial membrane fractions, absorption experiments were performed. Because the small size of the outer mitochondrial membrane vesicles precluded their rapid removal from the absorbed serum by centrifugation, experiments using outer mitochondrial membrane as the absorbing species were not performed. Hence, in an attempt to absorb autoantibody directed toward autoantigenic determinants located on the outer mitochondrial membrane, freshly prepared intact mitochondria were used as the absorbing species. Dog serum containing absorbed with varying concentrations of either intact dog heart mitochondria or inner mitochondrial membrane derived from these same mitochondria. Following the absorption procedure, each serum was tested for remaining autoantibody using the complement-fixation test with either intact mitochondria or inner mitochondrial membrane as the test antigen. When serum was absorbed with increasing concentrations of mitochondria (0.01–1.0 mg/ml mitochondrial protein) and tested with intact mitochondria as the antigen, the complement-fixation test titers were decreased to zero as the amount of absorbing membrane was increased (Fig. 4A). When the same absorbed serum was tested with inner mitochondrial membrane as the antigen, the complement-fixation titer was reduced two log₂ dilutions but was not further reduced as the absorbing membrane concentration was increased. The results of a similar experiment using dog heart inner mitochondrial membrane as the absorbing antigen are shown in Figure 4B. The autoantibody titer of the absorbed serum decreased to zero when it was tested against inner mitochondrial membrane, but the titer to whole mitochondria was not reduced significantly during the absorption. Since absorption with intact mitochondria should expose only the outer mitochondrial membrane to

**FIGURE 4**

Effect of absorption with intact heart mitochondria and inner mitochondrial membrane on the induced anti-heart mitochondria autoantibody present in dog serum. The concentration of mitochondria and inner mitochondrial membranes used as the test antigen was 1 mg/ml in all cases.

**FIGURE 5**

Effect of absorption with dog heart inner mitochondrial membrane on the induced anti-heart mitochondria autoantibody of dog serum using isolated inner (open circles) and outer (solid circles) mitochondrial membranes.
the autoantibody in the serum, the results of this absorption experiment indicated the possibility that the autoantigenic activities of the inner and outer mitochondrial membranes were different.

To confirm the apparent dissimilarity of the inner and outer mitochondrial membrane autoantigenic determinants, additional absorption studies were performed using the more accurate spectrophotometric complement-fixation test. An experiment was carried out using various concentrations of inner mitochondrial membrane as the absorbing species and both inner and outer mitochondrial membranes as the test antigens. As can be seen in Figure 5, the reactivity of the absorbed serum with dog heart inner mitochondrial membrane decreased steadily as the concentration of the absorbing membrane (inner mitochondrial membrane) increased. In contrast, when the same absorbed serum was tested with outer mitochondrial membrane, no effect on the complement-fixation test end point was observed. Again, these data support the existence of separate antigenic determinants on dog heart inner and outer mitochondrial membranes.

The organ specificity of the anti-heart mitochondria autoantibodies was assessed to determine possible cross-reaction between mitochondrial membranes derived from heart, skeletal muscle, and liver. Preliminary experiments indicated that when a serum containing anti-heart autoantibody was tested with a crude homogenate of skeletal muscle, a positive complement-fixation test was observed. Serial blood samples obtained from a dog that had undergone experimental myocardial infarction by microsphere injection into the coronary artery were assayed in the complement-fixation test using either heart or skeletal muscle mitochondria as the test antigen. The results are shown in Figure 6; they indicate that the autoantibody titers that developed following myocardial infarction were identical whether heart or skeletal muscle mitochondria were used as the test antigen. Since the procedure used for microsphere injection resulted in very little skeletal muscle damage, the results were interpreted as indicating a strong cross-reaction between skeletal muscle and heart mitochondria.

Dog serum obtained from the same dog used in the experiment described in Figure 6 was absorbed with BSA-anti-BSA immune existing anti-liver mitochondria autoantibody. When serum from this dog obtained prior to experimental myocardial infarction was tested with liver mitochondria as the antigen, a titer of 1:128 was obtained. However, this serum sample did not react with either heart or skeletal muscle mitochondria (Fig. 6). A serum sample from this same dog collected 10 days following myocardial infarction was tested with liver mitochondria and exhibited a titer unchanged from the preinfarction level of 1:128; this serum did contain significant levels of autoantibodies which reacted with both heart and skeletal muscle mitochondria.

Absorption experiments were performed using the serum from the same dog considered in Figure 6 containing induced anti-heart mitochondria autoantibody and intact mitochondria from heart and skeletal muscle as the absorbing species. The absorbed serum was tested for complement-fixing activity using heart, skeletal muscle and liver mitochondria as the test antigens. The results shown in Table 3 indicate a strong cross-reaction between skeletal muscle and heart mitochondria; however, there was little if any cross-reaction of the heart-reactive autoantibody with liver mitochondria. These absorption experiments were repeated using heart and skeletal muscle inner mitochondrial membranes as both absorbing species and test antigens with nearly identical results, e.g., cross-reaction was observed between heart and skeletal muscle mitochondrial membranes but not with liver membranes.

![Temporal development of anti-heart mitochondria autoantibody in a dog following myocardial infarction. Serial serum samples were tested with both heart mitochondria (closed circles) and skeletal muscle mitochondria (open circles) as the test antigen at a concentration of 1](http://circres.ahajournals.org/content/65/11/868/F1.large.jpg)
TABLE 3

<table>
<thead>
<tr>
<th>Test antigen</th>
<th>Absorbing material</th>
<th>Heart mitochondria</th>
<th>Skeletal muscle mitochondria</th>
<th>Liver mitochondria</th>
<th>Control (unabsorbed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Heart</td>
<td>38</td>
<td>39</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>Skeletal</td>
<td>Muscle</td>
<td>46</td>
<td>35</td>
<td>135</td>
<td></td>
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<tr>
<td>Mitochondria</td>
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Serum was absorbed with 2 mg/ml of the mitochondrial preparation.

Discussion

Recent studies in our laboratory (8) have indicated that a complement-fixing, heart-reactive serum factor is produced in dogs following experimental myocardial infarction. This factor has been tentatively described as an IgM on the basis of its heat stability at 56°C for 30 minutes and its sedimentation coefficient of approximately 19S. Other studies in our laboratory (7) have demonstrated that human Cl, which sediments in the 19S region of sucrose density gradients, is capable of binding to human heart mitochondrial membranes with the subsequent fixation of complement. Hence, it was considered essential to develop more direct evidence that the heart-reactive serum factor that we have been calling an autoantibody is indeed an immunoglobulin. Specific removal of Cl, by absorption with rabbit anti-BSA-BSA immune precipitates did not remove the induced heart-reactive factor that was not removed by absorption with immune precipitates. It is tempting to speculate that recent heart damage of unknown etiology resulted in the production of the preexisting anti-heart autoantibodies seen in our studies (8).

To characterize the location and the specificities of the heart subcellular membrane autoantigens, cardiac tissue was fractionated into its component subcellular membranes. One of the major autoantigenic loci was determined to be the mitochondrial fraction. Further subfractionation of the mitochondrial fraction into its inner and outer membranes indicated that the outer mitochondrial membrane was more reactive than the inner mitochondrial membrane on a milligram of protein basis (Fig. 3). The dramatic differences in the autoantibody titers toward outer and inner mitochondrial membranes suggested individual autoantigenic specificities for the two membranes. This suggestion was documented by the subsequent cross-absorption experiments shown in Figures 4 and 5. Other studies (25) from our laboratory have similarly demonstrated that the autoantigenic determinants on rat liver subcellular membranes including inner and outer mitochondrial membranes are unique with little or no cross-reaction.

The data shown in Figure 6 and Table 3 indicate that the anti-heart mitochondria autoantibody cross-reacts with heart and skeletal muscle mitochondria. This cross-reaction probably occurs both on the inner and outer mitochondrial membranes. Although the induced anti-heart mitochondria autoantibody seems to react with both heart and skeletal muscle mitochondria, our previous studies have demonstrated that skeletal muscle damage occurring in dogs undergoing thoracotomy without cardiac damage does not lead to the development of an autoantibody reactive with heart mitochondria (7) or with skeletal muscle mitochondria (unpublished observations). An indication that heart and liver mitochondria do not share autoantigenic determinants was provided by the fact that absorption of serum containing both preexisting anti-liver mitochondria autoantibody and induced anti-heart mitochondria autoantibody and induced anti-heart mitochondria autoantibody with isolated heart mitochondria resulted in removal of only the induced anti-heart mitochondria autoantibody.

In conclusion, these studies demonstrate that the induction of myocardial infarction in dogs results in the production of anti-heart mitochondrial autoantibodies.
ies in our laboratory similarly indicate that human anti-heart mitochondria autoantibodies produced following acute myocardial infarction react with both inner and outer mitochondrial membranes, the outer membrane being most reactive on a milligram of protein basis. It is proposed that the development of anti-heart mitochondria autoantibodies in experimental animals and in man occurs as the result of an apparently normal autoimmune response to membranous subcellular organelles to which the host is not immunologically tolerant (26). Hence, the development of anti-heart autoantibodies with the described specificities probably could be utilized as an indicator of recent myocardial cell necrosis. It would be interesting in future studies to compare the subcellular membrane autoantigenic specificities of the anti-heart autoantibodies induced by uncomplicated myocardial infarction with those associated with the postinfarction and commissurotomy syndromes or possibly with anti-heart autoantibodies seen in other cardiac diseases. Such studies may demonstrate whether these latter cardiac disease states result from an abnormal autoimmune response.

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