Antibodies to ADP-ATP Carrier—an Autoantigen in Myocarditis and Dilated Cardiomyopathy—Impair Cardiac Function

Karsten Schulze, MD, Bernhard F. Becker, MD, Rolf Schauer, and Heinz P. Schultheiss, MD

The adenosine diphosphate (ADP)—adenosine triphosphate (ATP) carrier of the inner mitochondrial membrane is identified as an autoantigen in myocarditis and dilated cardiomyopathy. Sera of patients with these diseases contain autoantibodies to the ADP-ATP carrier capable of inhibiting nucleotide transport in vitro. Recently, an antibody-related infringement of energy metabolism was shown in intact perfused hearts isolated from guinea pigs immunized with the ADP-ATP carrier. A decreased cytosolic-mitochondrial difference of the phosphorylation potential of ATP was measured that originated from a reduction in mitochondrial-cytosolic nucleotide transport. Nonimmunized animals did not show these changes in energy metabolism, despite being in a comparable metabolic state and performing equal external heart work. To establish whether antibodies to the ADP-ATP carrier can also alter cardiac function, hemodynamic parameters of isolated hearts of guinea pigs that were preimmunized with the carrier protein were measured. Cardiac metabolism was stimulated by exposing the hearts to a high calcium concentration in conjunction with a maximum elevation of the afterload. Mean aortic pressure, stroke volume, stroke work, and external heart work were found to be lowered significantly \((p<0.005)\). The external heart work of the immunized hearts reached only about 20\% of the level performed by control hearts. Myocardial oxygen consumption was lowered 2.5-fold, whereas the extent of lactate production was found to be more than doubled. These results show a diminished cardiac performance of hearts from animals immunized with the ADP-ATP carrier. Our findings demonstrate that autoimmunity to the ADP-ATP carrier may contribute to the pathophysiology of dilated cardiomyopathy as a subsequent stage of myocarditis by causing an autoantibody-mediated reduction in cardiac function on the basis of an imbalance between energy delivery and demand. (Circulation 1990;81:959–969)

Currently, most clinical cases of myocarditis in humans are suspected to be of viral etiology. Although the pathogenetic mechanisms are only vaguely understood, many observations indicate that certain patients with acute viral myocarditis suffer from dilated cardiomyopathy later.\(^1,2\) An autoimmunologic process, initiated by the viral infection, may aggravate and perpetuate the myocarditis and result in dilated cardiomyopathy. Virus persistence has been observed in hearts of these patients.\(^3\)

In previous studies, we have been able to identify the adenosine diphosphate (ADP)-adenosine triphosphate (ATP) carrier, the nucleotide-transporting protein of the inner mitochondrial membrane, as an autoantigen in patients with myocarditis and dilated cardiomyopathy. The autoantibodies were characterized as organ and conformation specific, and they inhibited the nucleotide exchange in vitro by blocking the substrate-binding site of the carrier protein.\(^4–6\)

In experimental studies, these antibodies were able to influence the carrier function, even in vivo.\(^7\) Guinea pigs immunized with the isolated ADP-ATP carrier showed a decreased cytosolic-mitochondrial difference of the phosphorylation potential of ATP. This decisive parameter in myocardial energy metabolism depends on the function of the ADP-ATP carrier. To detect changes in the phosphorylation potential, cytosolic and mitochondrial nucleotide concentrations in the isolated, perfused hearts...
of the animals were measured. While mitochondrial ATP and cytosolic ADP levels were raised, mitochondrial ADP and cytosolic ATP levels declined. As a consequence, the mitochondrial value of the phosphorylation potential of ATP was markedly higher and the cytosolic value was lower than in the hearts of nonimmunized control animals.7

These data indicate that the autoantibodies found in patients with myocarditis and dilated cardiomyopathy may not be merely an epiphenomenon. Furthermore, our results provide first evidence for a possible novel mechanism of an immunologically mediated dysfunction of the myocardial cell, namely, due to energy deficiency. The present study was designed to consider whether this observed disturbance in myocardial energy metabolism also leads to an alteration of myocardial function.

Methods

Isolation of the ADP-ATP Carrier

Beef heart mitochondria were isolated as described by Smith.4 The solubilization and isolation of the ADP-ATP carrier followed the procedure previously described.9–12 The bovine and the guinea pig antigen are equally suited for immunization because the ADP-ATP carrier has no species specificity.

In brief, mitochondria were first loaded with the tightly binding and specific carrier inhibitor carboxyatractylate (CAT), the suspension of mitochondria (10 mg protein/ml in 250 mM sucrose, 10 mM Tris-HCl, 50 mM ADP; pH 7.2) being supplemented with [3H]CAT (4 mmol/g protein; specific activity, 4,000 counts/min-mmol), left for 30 minutes at 4°C, and centrifuged for 30 minutes at 10,000g. The protein is, thus, fixed in the c-conformation (i.e., facing the cytosolic side of the membrane) and is protected against detergent-induced denaturation. The [3H]CAT binding also serves as a marker during the isolation procedure. The pellet was subsequently treated with the detergent Triton X-100 (Sigma, Munich; concentration, 6% wt/vol).

After solubilization and centrifugation (30 minutes, 140,000g), the supernatant was applied to a hydroxyapatite column (4 mg protein/ml column vol) that had been equilibrated with a solution of 0.1 M NaCl, 0.5% Triton X-100, and 10 mM 2-(N-morpholino)-propane sulfonic acid (MOPS) at pH 7.2. The protein was eluted at 4°C with the same buffer. The enriched carrier protein (assessed from the CAT-to-protein ratio) was pooled and applied to a gel chromatography column (6×100 cm, Ultrogel ACA 34, LKB, Sweden). The protein was eluted with a buffer consisting of 175 mM Na2SO4, 10 mM MOPS, and 0.5% Triton X-100 (pH 7.2). To remove minor contaminants, excess Triton X-100, and phospholipids from the protein, the eluant was finally subjected to sucrose density gradient centrifugation with a linear gradient of 5–20%.13 The total enrichment was about 10-fold.

Immunization Procedure

Blood was taken 1 week before immunization from guinea pigs to obtain preimmune sera. Fifteen female guinea pigs of the same age (weight, 1,000 g; Pirbright, Ivanovas, Munich, FRG) were immunized with the ADP-ATP carrier isolated from beef heart mitochondria. For immunization, a total of 50 μg of carrier protein was injected (30 μg s.c. and 20 μg i.p.) every second week for 4 months. The CAT protein complex (0.1 mg protein suspended in 100 mM NaCl, 10 mM MOPS, and 0.5% Triton X-100) was emulsified with an equal volume of Freund's complete adjuvant for the first injection, and all subcutaneous booster injections were administered in Freund's incomplete adjuvant. The antigen injected intraperitoneally was always given without Freund's adjuvant.

Isolation of Immunoglobulins

Immunoglobulin (Ig) was fractionated using protein-A sepharose in 10 mM phosphate-buffered saline (Pi/NaCl; pH 7.4) containing 0.1% sodium azide. The column (0.9×15 cm) was stored at 4°C. After adding the serum, the column was washed with phosphate-buffered saline until the absorbance of light of the eluate at 280 nm was at background level. Immunoglobulin was then eluted with 0.58% (wt/vol) glacial acetic acid in 0.15 M NaCl (pH 2.8). After neutralization of the eluate with 0.2 M Tris, the IgGs were dialyzed against the phosphate-buffered saline.11

Indirect Micro–Solid-Phase Radioimmunoassay

A modified solid-phase radioimmunoassay (SPRIA).5,11 was performed on flexible, u-shaped polyvinyl microtiter plates (Dynatech). The ADP-ATP carrier was diluted to the optimal concentration (0.5 mg/ml) in a solution of 100 mM Na2SO4, 10 mM MOPS, and 0.5% Triton X-100 (pH 7.2), and a 100-μl aliquot was incubated at 4°C for 4 hours. After washing three times with 3% fetal calf serum in phosphate-buffered saline, the precoated plates were incubated with 100 μl of a filler (3% fetal calf serum) for 1 hour at 4°C to block any remaining active binding sites of the polyvinyl plate. After further washing, the antigen-coated wells were incubated overnight at 4°C with 90 μl of the serum to be tested. Subsequent to washing, specifically bound antibodies were detected with iodinated protein A (100,000 counts/min-well, New England Nuclear). This involved incubation for 4 hours at 4°C in which the wells were washed three times, allowed to dry, and counted in a gamma spectrometer. All assays were performed in duplicate. The separate controls for nonspecific binding, performed parallel to each test with “no antigen” (filler only), a “no serum,” and “control serum” limitations, routinely yielded values 2–6% that of the total activity.
In Vitro Measurement of the Adenine Nucleotide Transport

The ability of the individually generated antibodies to inhibit the nucleotide transport was tested in vitro by measuring the exchange of $^{14}$C-labeled ADP of isolated mitochondria with the inhibitor-stop method. Isolation of mitochondria from guinea pig hearts was performed according to Smith. Batches of isolated mitochondria (20 mg) were next loaded with 0.8 $\mu$Ci $[^{14}]$ADP each. The ADP exchange was started by adding 10 $\mu$l unlabeled ADP (10 mM) to 200 $\mu$l of suspended mitochondria. After 40 seconds, the ADP transport was stopped by adding carboxyatractylate. The value for background radioactivity (cpm control) was obtained by adding carboxyatractylate only to the suspension. The adenine nucleotide translocation, being a 1:1 exchange between intramitochondrial and extramitochondrial nucleotides, may be calculated as percentage exchange of the total intramitochondrial content (cpm total) according to the equation:

$$\text{Percentage exchange} = \left( \frac{\text{cpm (assay)} - \text{cpm (control)}}{\text{cpm (total)} - \text{cpm (control)}} \right) \times 100$$

Western Blot

The isolated ADP-ATP carrier, total protein from guinea pig heart mitochondria, and marker proteins were separated on sodium dodecyl sulfate (SDS)–polyacrylamide slab gels. One section of the gel was stained with coomassie blue, and the other section was electrophoretically blotted on a wet nitrocellulose sheet (0.45 mm, Bio Rad, Munich, FRG), which was sandwiched between filter paper and Scotchbrite pads that were supported on a porous plastic grid. The running buffer (pH 8.3) contained 25 mM Tris, 200 mM glycine, and 30% methanol. The electrophoretic transfer was run overnight at 4°C with 0.2 A. The nitrocellulose was then placed for 2 hours in a buffer of 50 mM Tris/HCl (pH 7.4) and 150 mM NaCl (TBS) supplemented with 2.5% bovine serum albumin. Afterward, it was incubated for 2 hours with guinea pig antiserum in TBS with 2.5% bovine serum albumin. To remove unbound antibodies, six washings of 5 minutes each were performed with TBS not containing bovine serum albumin. Those antibodies that remained bound to the nitrocellulose were stained by horseradish peroxidase–conjugated anti-IgG.

Adsorption of Antibodies on the Isolated Carrier Protein

For immunoadsorption studies, the staphylococcal protein A antibody adsorbent was used. After preadsorption of the ADP-ATP carrier with 50 $\mu$l of 10% (wt/vol) suspension of a protein A–bearing strain of the bacterium Staphylococcus aureus (Cowan I) to remove any spontaneously reactive material, the isolated ADP-ATP carrier from heart was incubated with the antisera from the immunized guinea pigs at 4°C for 4 hours. Then, 150 $\mu$l of a 10% (wt/vol) suspension of protein A–bearing staphylococci was added to bind the antigen-antibody complexes. After incubation for 15 minutes at 4°C, the bacterial adsorbent was washed three times by centrifugation (2,000g, 6 minutes, 4°C). The supernatants containing the free antibodies were collected, and the residual antibody activity against the ADP-ATP carrier from heart was determined with the solid-phase radioimmunoassay. Control adsorption studies performed with the antisera and the Staphylococcus cells alone did not change the antibody activity in the supernatant after centrifugation of the bacterial cells.

The Working Heart Preparation

The hearts of the 11 guinea pigs whose sera contained the highest antibody titers were isolated and perfused as working heart preparations, as described by Becker et al and Bünge et al. The nonrecirculating perfusion medium used was a modified Krebs-Henseleit buffer containing (mM) 127 NaCl, 4.7 KCl, 24.9 NaHCO$_3$, 1.25 CaCl$_2$, 0.6 MgSO$_4$, and 1.2 K$_2$HPO$_4$, which was enriched with 0.3 mM pyruvate, 5.5 mM glucose, and 5 units/l insulin. The buffer was equilibrated at 37°C with 94.4% O$_2$-5.6% CO$_2$ (pH 7.4).

Perfusate was applied via a cannula tied into the left atrium, and all other atrial openings were ligated. The preload of 12 cm H$_2$O and the mean developed afterload determined the pressure, volume, and work of the left ventricle. No external work was performed by the right ventricle. The right atrial veins were ligated, and the coronary venous effluent was drained through a cannula inserted in the pulmonary artery.

Atrial filling and aortic pressures were monitored by Statham P23BB and P23Db strain gauges (Gould, Cleveland, Ohio), respectively. Heart rate was derived from the phasic pressure signals with a Beckman Cardiotachometer (Fullerton, California). All parameters were recorded on a Beckman Dynograph R411. External heart work was calculated as the sum of pressure, volume, and work (cardiac output×pressure gradient across the left ventricle) and acceleration work (0.5×ejected volume×mean velocity of flow$^2$) during ejection.

The myocardial oxygen consumption was derived from the difference of O$_2$ tension between aortic perfusate and coronary effluent, which was measured with two Clarke-type electrodes (Bachofer, Reutlingen, FRG), and the coronary flow rate. The lactate released into the coronary effluent was measured enzymatically.

To enhance metabolic demand, all hearts (control and immunized groups) were stimulated by applying calcium at a high concentration (perfusion concentration, 4.0 mM). In addition, after stabilization of the working heart preparations (approximately 30 minutes), the aorta was ligated.
to induce a maximal pressure load. Hemodynamic parameters were measured against the occluded aorta after 40 minutes of work.

Statistical Analysis
The Student's t test for unpaired samples was used. Values are given as mean±SD.

Peroxidase Staining
Cryostat sections of the myocardium of the immunized guinea pigs were air-dried for 60 minutes and fixed in acetone for 10 minutes. The unlabeled antibody–enzyme method was performed in a modified way. First, incubation with excess sheep anti-guinea pig immunoglobulin serum or with normal sheep globulin for negative control was performed. An anti-guinea pig immunoglobulin serum was induced in sheep by subcutaneously injecting 5 mg guinea pig IgG with complete Freund's adjuvant. Seven days after the last of three booster injections in 14-day intervals, the animals were exsanguinated. Second, incubation with peroxidase–anti-peroxidase complex was performed. Each incubation lasted 60 minutes at room temperature and was terminated with amino-ethyl-carbazol. The sections were counterstained with hemalum. The absence of nonspecific reactions was demonstrated in heart sections from normal rabbits.

Results
Of the 15 guinea pigs immunized with the isolated ADP-ATP carrier, 11 animals showed a marked increase of antibodies against the carrier protein (Figure 1). All preimmune sera were nonreactive for the ADP-ATP carrier. As assessed by measuring the back-exchange of ADP on isolated heart mitochondria in five cases, a significant inhibition of nucleotide transport indicated an antibody-mediated inactivation of the carrier function in vitro (Figure 2). No inhibition of the nucleotide exchange rate was observed by measuring nucleotide transport from kidney or liver mitochondria. The organ-specificity of the immunoinactivating antibodies was also proven by Western blot technique (Figure 3) and by immunoabsorption of the antibodies on the isolated carrier protein from heart, whereas no further inhibition of the function of the ADP-ATP carrier was observed.

Immunohistochemical studies by immunoperoxidase staining of the hearts of the immunized animals revealed an extracellular and an intracellular deposition of immunoglobulins (Figure 4). In contrast, the control hearts showed no staining of the myocardium.

The hearts of five control animals and 11 immunized animals that exhibited a significant rate of antibody production against the ADP-ATP carrier were isolated and perfused as working heart preparations. The energetic demand of all hearts was stimulated by imposing a maximal pressure load for 40 minutes. This was achieved by applying calcium at a concentration of 4.0 mM to the perfusate concomitant with aortic occlusion.

Under these conditions, the hearts of the immunized animals showed a marked decrease in cardiac performance (Figure 5). The hemodynamic data for each single experiment are listed in Table 1. Group 1 contains the five control hearts (nonimmunized animals), and group 2 includes the 11 hearts of

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Scatterplot of the binding of antibodies from sera of guinea pigs immunized with the ADP-ATP carrier to the isolated ADP-ATP carrier in solid-phase radioimmunoassay. Values for radioactivity after incubation with 125I-labeled protein A are experimental results minus background. Dotted line represents mean±2 SDs of the control group. ○, Controls (n=5, left) and immunized animals not exceeding the mean±2 SD of the controls (n=4, right); Δ, immunized animals without inhibition of 14C-ADP exchange (n=6); ▲, immunized animals with inhibition of 14C-ADP exchange (n=5).

![Figure 2](http://circ.ahajournals.org/)

**FIGURE 2.** Scatterplot of the in vitro assessment of the inhibition of nucleotide transport by sera of guinea pigs immunized with the ADP-ATP carrier. The exchange of 14C and ADP by isolated mitochondria was measured after 40 seconds. Dotted line represents mean±2 SDs of the control group. ○, Controls (n=5); Δ, immunized animals without inhibition of 14C-ADP exchange (n=6); ▲, immunized animals with inhibition of 14C-ADP exchange (n=5).
immunized animals with specific "anti-carrier" antibodies. Group 2 is divided into two subgroups (2A and 2B) comprising data from the hearts of those immunized animals, the antibodies of which had proven not to be carrier-inactivating in vitro (group 2A, six animals) or to be carrier-inactivating in vitro (group 2B, five animals). Heart rate did not differ among groups. All other parameters measured were lowered either significantly \((p<0.05\), group 1 vs. group 2) or highly significantly \((p<0.005\), group 1 vs. group 2B). Compared with controls, mean aortic pressure development decreased by 24% in group 2 and by 42% in subgroup 2B. Coronary flow declined by 36% in group 2 and by 63% in subgroup 2B. Stroke volume decreased to a similar degree. External heart work fell even more markedly—by 50% in group 2 and by 83% in subgroup 2B. Comparing group 2A with group 1 (controls) revealed only a slight depression of these hemodynamic parameters. This depression was not significant in any parameters mentioned above except in the mean aortic pressure, which was reduced by 12% \((p<0.05)\).

It is obvious from these results that the hearts of those five animals that had developed carrier-inactivating antibodies in vitro showed a far greater reduction in heart function (Figure 5). Indeed, two of these hearts were not even able to cope with the high calcium stimulation: They were incapable of developing sufficient pressure to support coronary perfusion and, thus, progressively failed. No external heart work was performed by these two hearts, and perfusion had to be continued according to the Langendorff technique.

Values for the rate-pressure product, the myocardial oxygen consumption, oxygen extraction, and lactate release are given in Table 2. With the exception of myocardial oxygen extraction, which showed no significant changes, all other parameters differed either significantly \((p<0.05\), group 1 vs. group 2) or highly significantly \((p<0.005\), group 1 vs. subgroup 2B) when immunized and nonimmunized hearts were compared. No significant changes were observed in comparison of subgroup 2A with group 1 (controls). In comparison with controls, the rate-pressure product decreased by 28% in group 2 and 48% in subgroup 2B (Figure 6). The corresponding fall in myocardial oxygen consumption was 31% and 60%, respectively. Myocardial oxygen extraction was found slightly enhanced in group 2 as a whole and in subgroup 2B. The release of lactate in the coronary effluent rose by 59% in group 2, mainly because of a dramatic rise of 137% in subgroup 2B. However, a linear correlation between the rate-pressure product and the coronary flow was observed in all groups of control and immunized animals’ hearts (Figure 7).

Discussion

Immunization of guinea pigs with the isolated, purified ADP-ATP carrier from heart results in an appearance of organ-specific antibodies against the carrier protein. Eleven of 15 immunized animals contained carrier-specific antibodies. The specificity of these antibodies has been demonstrated by Western blot technique, by radioimmunoassay, by immunoadsorption studies, and by measurement of nucleotide transport rate on isolated mitochondria. In the Western blot, the antibodies reacted with the transport protein when SDS gels of mitochondria or the isolated carrier protein had been blotted on the nitrocellulose paper.

The antibodies of the five animals of subgroup 2B proved to inhibit the nucleotide exchange of isolated heart mitochondria but not of mitochondria from kidney or liver. This inactivating ability seems to be due to a binding of the antibodies at the substrate-binding site of the nucleotide transport protein. Organ-specificity was further demonstrated by immunoadsorption studies.

According to the results presented here, working heart preparations from guinea pigs that had been immunized with the ADP-ATP carrier protein show a marked decrease in cardiac function compared...
with hearts from nonimmunized animals. The functional parameters were measured after 40 minutes of metabolic stimulation by calcium addition and aortic occlusion. Rates (min⁻¹) of mean developed aortic pressure, coronary flow, stroke volume, stroke work, and external heart work performed by the left ventricle were obtained. These hemodynamic parameters were found to be diminished in the immunized animals' hearts compared with control hearts by about 30–50% (p<0.05). When the performance of the control hearts was compared with only hearts isolated from animals with sera inhibiting the nucleotide exchange of isolated mitochondria, the respective data were found to have been lowered to a much greater degree. For example, external heart work diminished from 375 to 62 mJ/g/min (−81%). Hearts of immunized animals of subgroup 2A (no carrier inactivation in vitro) showed a much smaller depression of hemodynamic parameters that, with the exception of mean aortic pressure, was not significant (p<0.05). This tendency of a slight reduction of cardiac performance in subgroup 2A, too, can be explained by two reasons. At first, the in vivo testing of cardiac performance is more sensitive than the measurement of carrier activity in vitro on isolated mitochondria. Second, immunization with the carrier protein induces the forming of a variety of polyclonal antibodies with concentration-dependent effects.³⁸ Therefore, in the in vivo testing with a
FIGURE 5. Bar charts of parameters of cardiac function of isolated perfused hearts from guinea pigs after stimulation with 4.0 mM Ca in conjunction with 40 minutes of aortic ligature. Values are given as mean±SD. □, Controls (n=5); □, immunized animals without inhibition of nucleotide transport in vitro (n=6); □, immunized animals with inhibition of nucleotide transport in vitro (n=5). HR, heart rate; MAP, mean developed aortic pressure; CF, coronary flow; SV, stroke volume; and EHW, external heart work of the left ventricle (min⁻¹).

### TABLE 1. Data From Isolated, Perfused Hearts of Normal Guinea Pigs and Guinea Pigs Immunized With the ADP-ATP Carrier

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (l/min)</th>
<th>MAP (mm Hg)</th>
<th>CF (ml/min)</th>
<th>SV (μl/g)</th>
<th>EHW (mJ/g/min)</th>
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<td>19.6±9.6</td>
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(group 2A+2B)

HR, heart rate; MAP, mean aortic pressure; CF, coronary flow; SV, stroke volume; EHW, external heart work of isolated perfused hearts of normal guinea pigs (group 1, n=5) and guinea pigs immunized with the ADP-ATP carrier (group 2, n=11).

Subgroup 2A comprises hearts of animals the sera of which were not capable of inactivating carrier transport in vitro (n=6), whereas the sera of subgroup 2B were capable of inactivating carrier transport in vitro (n=5).

Data were obtained after stimulation with 4.0 mM Ca in conjunction with 40 minutes of aortic ligature.

*Hearts perfused in the Langendorff mode (retrograde coronary perfusion at 60 mm Hg; no external pressure-volume work).

Values are given as mean±SD for each group.
lower antibody concentration, other antibody-mediated effects on carrier function might be more relevant than in the in vitro testing.

The concern of whether the reduction of cardiac function is due to an inactivation of the mitochondrial nucleotide translocator or whether other factors, such as ischemia or impairment of the oxidative phosphorylation, lead to the pronounced failing of the hearts from animals immunized with the ADP-ATP carrier must be addressed.

Myocardial oxygen consumption fell from 17.2 (controls) to 11.8 μmol/g/min for all immunized animals and to 6.8 μmol/g/min in the hearts of animals with sera inactivating the carrier in vitro. However, this is no hint for ischemia in the immunized animals’ hearts but, apparently, is an adaption of oxygen consumption to the lowered cardiac performance. This could be demonstrated by an unchanged oxygen extraction in the control and the immunized animals’ hearts. Furthermore, Figure 7 shows an adequate coronary flow in relation to the cardiac oxygen demand, as indicated by the rate-pressure product.

However, the normal oxygen extraction of the hearts from subgroup 2B and the heightened rate of glycolysis, as indicated by the increase in myocardial lactate production, evidences an impairment of energy metabolism in the hearts of immunized animals. In previous experiments, oxidative phosphorylation of ATP seems to not be disturbed.7 This was shown by the elevated mitochondrial phosphorylation potential of ATP in hearts of guinea pigs previously having undergone the same immunization procedure as described above. In brief, these animals were subjected to a nonlimiting cardiac work load equal to that of control hearts. Under this condition, the oxygen consumption of the immunized hearts did not differ from the control value, but the immunized hearts showed a very high mitochondrial phosphorylation potential of ATP, as

### Table 2. Additional Data From Isolated, Perfused Hearts of Normal Guinea Pigs and Guinea Pigs Immunized With the ADP-ATP Carrier

<table>
<thead>
<tr>
<th>Group</th>
<th>HR×MAP (mm Hg/min)</th>
<th>O₂-EXT (%)</th>
<th>MVo₂ (μmol/g/min)</th>
<th>LAC (μmol/g/min)</th>
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| 2A    | 17,316             | 79.9       | 13.0              | 2.0              |
|       | 15,768             | 87.2       | 14.6              | 1.6              |
|       | 18,426             | 80.5       | 17.0              | 1.3              |
|       | 19,929             | 68.8       | 17.9              | 1.6              |
|       | 20,520             | 53.6       | 14.8              | 1.9              |
|       | 17,556             | 88.3       | 18.9              | 2.0              |
| Mean±SD | 18,252±1,762       | 76.4±13.2 | 16.0±2.2          | 1.73±0.28        |

| 2B    | 11,200             | 59.9       | 5.5               | 3.9              |
|       | 16,188             | 61.8       | 10.1              | 2.9              |
|       | 5,160              | 53.6       | 1.7               | 2.5              |
|       | 9,576              | 91.6       | 6.0               | 5.8              |
|       | 11,448             | 91.9       | 10.5              | 6.8              |
| Mean±SD | 10,714±3,965       | 71.8±18.5 | 6.8±3.6           | 4.36±1.86        |

| Mean±SD | 14,826±4,831       | 74.3±15.1 | 11.8±5.6          | 2.93±1.82        |

(groups 2A+2B)

HR×MAP, rate-pressure product; O₂-EXT, myocardial oxygen extraction given as percentage of oxygen supplied with the perfusate; MVo₂, myocardial oxygen consumption; LAC, lactate release of isolated perfused hearts of normal guinea pigs (group 1, n=5) and guinea pigs immunized with the ADP-ATP carrier (group 2, n=11).

Subgroup 2A includes hearts of animals the sera of which showed no inactivation of carrier transport activity in vitro (n=6), whereas the sera of animals from subgroup 2B showed inactivation of carrier transport activity in vitro (n=5). Mean±SD are given for each group.
calculated from the subcellular nucleotide and phosphate distributions. In these particular experiments, the increase of the mitochondrial phosphorylation potential of ATP in immunized hearts was paralleled by a decrease of the cytosolic phosphorylation potential and an activation of glycolysis. These data indicate an inadequate delivery of high-energy phosphates via the mitochondrial-cytosolic ATP transporter to the cytosol.

In conclusion, the biochemical and functional data clearly suggest the hypothesis that antibodies against the ADP-ATP carrier cause a dysfunction of the heart by an antibody-mediated disturbance of cellular energy metabolism.

The antibody-mediated alteration of the carrier function can be realized by several ways. First, the antibodies might inhibit carrier function as a direct result of antibody binding to the carrier protein. Second, as the carrier is synthesized in the cytosol and imported posttranslationally into the mitochondria,22,23 the antibodies might react with the primary translation product, which has the same apparent molecular weight as the mature protein, hindering the complete and functional active incorporation of the carrier protein into the mitochondrial inner membrane. Third, the anti-carrier antibodies might cause antigenic modulation of the protein, increasing carrier degradation. Fourth, the antibody binding to the cell surface might influence carrier function indirectly by activating a messenger system.

The first three proposed possible inhibitory ways of action of antibodies against the function of the ADP-ATP carrier are confirmed by immunoperoxidase staining. By this method, a deposition of immunoglobulins in the myocardium of animals immunized with the ADP-ATP carrier could be demonstrated.7 Several authors report on the intracellular occurrence of antibodies and their ability to elicit functional effects.24-26 An intracellular uptake of antibodies could be realized by receptor-mediated endocytosis.27 Indeed, after incubating isolated myocytes with gold-labeled antibodies against the ADP-ATP carrier, the adherence of the gold particles to the cytoplasmatic membrane and their appearance in vesicles inside the cell could be revealed by electronmicroscopy.28 The cell surface receptor protein corresponding to the carrier with which the antibodies cross-react could be identified as a 47-kDa subunit of the connexon.29 Furthermore, a deposition of antibodies at the mitochondrial membranes could be shown in electronmicroscopic studies by the peroxidase–anti-peroxidase

![Figure 6](http://circ.ahajournals.org/)

**FIGURE 6.** Bar charts of rate-pressure product (HR\(\times\)MAP), myocardial oxygen extraction (\(O_2\)-EXT), myocardial oxygen consumption (\(MVO_2\)), and lactate release (LAC) of isolated perfused hearts from guinea pigs that had been immunized with the ADP-ATP carrier. Data were recorded after stimulation by 4.0 mM Ca in conjunction with 40 minutes of aortic ligation. Values are given as mean \(\pm\)SD. □, Controls (n=5); □, immunized animals without inhibition of nucleotide transport in vitro (n=6); □, immunized animals with inhibition of nucleotide transport in vitro (n=5).

![Figure 7](http://circ.ahajournals.org/)

**FIGURE 7.** Relation between the rate-pressure product and the coronary flow of isolated perfused hearts of guinea pigs that had been immunized with the ADP-ATP carrier. Data after stimulation by 4.0 mM Ca in conjunction with 40 minutes of aortic ligation. ○, Controls (n=5); □, immunized animals without inhibition of nucleotide transport in vitro (n=6); □, immunized animals with inhibition of nucleotide transport in vitro (n=5).
technique in the hearts of previously immunized animals.7

The fourth possibility of how antibodies can inhibit carrier function is supported by the observation of a cross-reactivity between the ADP-ATP carrier and the calcium channel, shown by immunological, electrophysiological, and functional methods.30,31 It could be demonstrated that carrier-specific antibodies bind specifically on the calcium channel of the cell surface and enhance the calcium influx into the cell.30,31 Thus, in vivo, these antibodies could possibly induce a chronic calcium overload that also could influence indirectly the carrier function because an increased intracellular calcium might decrease the phosphorylation potential difference over the inner mitochondrial membrane.

The consequences of the data presented here are 1) the proof that antibodies against intracellular antigens can, in principle, exert effects on organ function. The influence of these antibodies against the ADP-ATP carrier on cardiac function is 2) of clinical importance because autoantibodies against the cardiac ADP-ATP carrier were found in the sera of patients with myocarditis and dilated cardiomyopathy.6,32–35 This is similar to the experimental data showing that these autoantibodies inhibit the nucleotide transport in vitro. This immunoinactivating capacity of the autoantibodies also is organ-specific and is caused by an antibody binding to the substrate-binding site of the carrier protein. Furthermore, recent data indicate that the nucleotide exchange rate of mitochondria isolated from explanted hearts from patients with dilated cardiomyopathy also is diminished.36 The clinical and experimental data can be taken as an indication for the central role that the autoaggression against the ADP-ATP carrier might play in the pathogenesis of myocarditis and dilated cardiomyopathy.

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