Localization of anti-mitochondrial antibody in experimental canine myocardial infarcts

(myocardial scintigraphy/cell membrane permeability/irreversible cellular damage)

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ABSTRACT Alterations in cell and subcellular membrane integrity occur during evolving ischemic myocardial injury. We tested the hypothesis that an antibody against human liver mitochondria [anti-mitochondrial antibody developing in a patient with primary biliary cirrhosis] could identify altered cell membrane integrity in experimental canine myocardial infarcts. The proximal left anterior descending coronary arteries of 12 dogs were ligated and 1 hr later ¹³¹I-labeled F(ab')₂ fragments from either a control human IgG (6 dogs) or anti-mitochondrial IgG (6 dogs) were injected. The ¹³¹I-labeled F(ab')₂ anti-mitochondrial fragments concentrated maximally in the central infarct subendocardium [infarct-to-normal ratio of 9.2 ± 3.5 (mean ± SD) vs. 4.6 ± 3.3 for control F(ab')₂ IgG, *P* < 0.05]. There was also 1½- to 2-fold greater anti-mitochondrial antibody F(ab')₂ accumulation in the central infarct epicardium and the peripheral infarct subendocardium and subepicardium. Thus, an anti-mitochondrial antibody obtained from a patient with primary biliary cirrhosis concentrates in irreversibly damaged myocardium after experimental canine myocardial infarction. Presumably this occurs because of altered cell membrane integrity, which allows exposure of mitochondria to the anti-mitochondrial antibody. The F(ab')₂ fragments of anti-mitochondrial antibodies labeled with suitable radionuclides should allow noninvasive scintigraphic detection of experimental acute myocardial infarcts.

Myocardial ischemia produces alterations in cell membrane integrity that develop early during the course of experimental ischemic myocardial injury (1-8). Such alterations in cell membrane permeability appear to precede the development of irreversible cellular injury (1, 2, 8); some investigators have even suggested that these alterations in membrane permeability play a causal role in the development of irreversible cellular injury (1, 2, 5, 6, 8).

Various radionuclide imaging tests have been developed for the detection of irreversible cellular injury (9-15), and recently Beller, Haber, and their colleagues (16-18) have described the use of radiolabeled specific antibodies against cardiac myosin as another scintigraphic method for detecting irreversibly damaged myocardial cells. In the present study, we have tested the hypothesis that an antibody developed against mitochondria in a patient with primary biliary cirrhosis might be used to detect altered cell membrane permeability.

MATERIALS AND METHODS

Experimental Animal Model. Thirty-three dogs with permanent occlusion of the proximal left anterior descending coronary artery were studied. Dogs were anesthetized with intravenous pentobarbital (15-25 mg/kg), intubated, and placed on a mechanical respirator. Their chests were opened

Table 1. Radiolabeled protein injected into dogs with experimental myocardial infarcts

Group	¹³¹ I-Labeled protein	Protein source	No. of dogs
1	IgG	Normal human control	11
2	IgG anti-mitochondrial antibody	Patient F.H. with primary biliary cirrhosis (Table 2)	10
3	F(ab') ₂	Same normal human IgG control used in group 1	6
4	F(ab') ₂	Same anti-mitochondrial antibody IgG used in group 2	6

and their proximal left anterior descending coronary arteries were ligated for 3-5 days. One to 2 mg of the control IgG, anti-mitochondrial IgG, or their F(ab')₂ fragments was injected intravenously into the respective animals 1 hr after coronary artery ligation (Table 1). After sacrifice of the animals, the hearts were removed and tissue was obtained from the various infarct and noninfarct left ventricular regions. Radioactivity in the tissue obtained was measured in a gamma scintillation counter (Autologic-Abbott); representative sections of these tissues were examined microscopically for the presence or absence of myocardial necrosis.

Selection of the Human Anti-Mitochondrial IgG Antibody. A patient (F.H.) with primary biliary cirrhosis was evaluated and sera demonstrating anti-mitochondrial antibody were identified (Table 2). This anti-mitochondrial antibody was selected by using routine fluorescence microscopy screening of blood samples obtained from patients with various types of liver disease.

Preparation of IgG and the F(ab')₂ Fragments from Control Sera and Sera Containing an Anti-Mitochondrial Antibody. IgG Preparation. Intact IgG without anti-mitochondrial or other cardiac intracellular antibody activity was obtained from a control individual and partially purified by ammonium sulfate precipitation (three times). It was dialyzed against 0.02 M sodium phosphate buffer, pH 8.0, and passed through a pre-equilibrated DEAE-cellulose column.

Preparation of F(ab')₂ Fragments. Purified IgG was digested with hog pepsin at 37°C for 20 hr according to the procedure described by Edelman and Marchalonis (19). The digested products were purified on a Sephadex G-150 (Pharma-

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Table 2. Results of immunofluorescence microscopy

Tissue	Control human IgG*	Human anti-mitochondrial IgG*
Rat kidney, normal	Negative	Positive
Canine myocardium, normal	Negative	Positive
Canine myocardium, infarct	Negative	Positive

Anti-mitochondrial human IgG exhibited a strong affinity for normal rat kidney, normal canine myocardium, and acutely infarcted canine myocardium. Positive, reacts at 1:100 dilution.

* Same results with unlabeled or ^{131}I -labeled antibodies.

cia) column equilibrated with phosphate-buffered saline, pH 7.7. Fractions containing F(ab')_2 fragments as proved by Ouchterlony double diffusion analysis were pooled.

Iodination of IgG and F(ab')_2 . Intact IgG and the F(ab')_2 control and anti-mitochondrial antibody fragments were iodinated by the iodine monochloride procedure of McFarlane (20) with some modifications. The radiolabeled products were separated from free iodine on an anion-exchange resin column (Dowex 1 X 8). The final labeled product had a specific activity of approximately 1 mCi (3.7×10^7 becquerels)/mg of protein.

Fluorescence Microscopy Studies. Immunofluorescence microscopy was performed by using the indirect fluorescent antibody technique (21). Fresh frozen cryostat sections were prepared from normal rat kidney, normal canine myocardium, and acutely infarcted canine myocardium. The sections were incubated with control human IgG and human anti-mito-

chondrial IgG (Table 2). Sections were then incubated with fluorescein-conjugated anti-human IgG. The sections were examined with an American Optical fluorescence microscope to determine the localization and intensity of antibody deposition (Table 2).

RESULTS

Control and Anti-Mitochondrial IgG. By immunofluorescence microscopy the anti-mitochondrial antibody obtained from patient F. H. exhibited intracellular localization in normal rat kidney, normal canine myocardium, and infarcted canine myocardium as shown in Table 2 and Fig. 1. Control human IgG did not show intracellular localization in these same organs or tissues (Table 2 and Fig. 1). However, ^{131}I -labeled normal human IgG and the ^{131}I -labeled anti-mitochondrial antibody accumulated to approximately the same extent in the center and periphery of experimental canine infarcts (Fig. 2).

Localization of Anti-Mitochondrial and Control F(ab')_2 Fragments. In contrast to the intact control and anti-mitochondrial IgG, ^{131}I -labeled F(ab')_2 fragments of the anti-mitochondrial antibody did concentrate in the center and periphery of the experimental canine infarcts in significantly greater amounts than did the control ^{131}I -labeled F(ab')_2 from normal human IgG (Fig. 3). The ^{131}I -labeled F(ab')_2 anti-mitochondrial antibody concentrated to the greatest extent in the subendocardium of the infarct center, but significant increases were also documented in the subepicardial region of the infarct center and in both the subendocardium and subepicardium of the infarct periphery (Fig. 3). In general, the ^{131}I -labeled F(ab')_2 anti-mitochondrial antibody concentrated

FIG. 1. (Left) Indirect immunofluorescence localization of human anti-mitochondrial IgG in a frozen section of normal canine myocardium. Note the intracellular accumulation of anti-mitochondrial IgG with a prominent granular pattern of localization. ($\times 640$.) (Right) Immunofluorescence microscopy shows no specific fluorescence in a frozen section of normal canine myocardium treated with control human IgG. ($\times 640$.)

FIG. 2. Uptake of ^{131}I -labeled anti-mitochondrial IgG (hatched bars, $n = 10$) and ^{131}I -labeled normal human IgG (stippled bars, $n = 11$) in control canine left ventricle (LV) tissue and experimental canine myocardial infarct regions. Uptake is measured as cpm/g. The bars represent the mean values; the cross bars represent SEM. Significance of the differences in the uptake ratios of anti-mitochondrial and normal IgG is given on the bottom line (NS, not significant). Note that the anti-mitochondrial and the normal human IgG concentrated to approximately the same extent within the experimental canine myocardial infarcts.

approximately $1\frac{1}{2}$ to 2 times more than did the control F(ab')_2 human IgG.

DISCUSSION

The data obtained demonstrate that the F(ab')_2 fragments of an antibody obtained from a patient with primary biliary cirrhosis concentrate in the center and periphery of experimental canine myocardial infarcts. In general, these antibody fragments concentrate to the greatest extent in the central subendocardial region of lowest flow and most severe cellular damage—i.e., the subendocardial portion of the center of the canine infarct (9, 22). This anti-mitochondrial antibody possesses species and tissue cross-reactivity so that it concentrates intracellularly in canine hearts with a localization pattern that suggests mitochondrial uptake (Fig. 1). The tissue uptake and concentration of the F(ab')_2 fragments of the anti-mitochondrial antibody in the setting of experimental canine myocardial infarction requires *in vivo* alterations in cell membrane integrity allowing intracellular penetration of the F(ab')_2 anti-mitochondrial antibody fragments; whether mitochondrial membrane alterations are also necessary is not yet known, but this antibody might combine with either membrane or intramitochondrial antigenic sites (23).

Under normal circumstances, control human IgG (150,000 daltons) does not concentrate in canine myocardial tissue but after occlusion of the proximal left anterior descending coronary artery there is an increased level of the intact control IgG in the infarct region. This presumably reflects nonspecific trapping of the intact IgG, because immunofluorescence microscopy showed that only the anti-mitochondrial antibody had an affinity for intracellular sites in cut sections of myocardium. However, smaller molecular fragments of the anti-mitochondrial antibody—i.e., the F(ab')_2 fragments (approximately 100,000 daltons)—do concentrate in the central and peripheral infarct tissue to a significantly greater extent than do control F(ab')_2 fragments. The F(ab')_2 fragments have a more rapid clearance and apparently less potential for nonspecific trapping (16, 18), although even the control F(ab')_2 fragments accumulated to a greater degree in infarcted myocardium than in control hearts (Fig. 3). This most likely represents some nonspecific trapping of even the lower molecular weight IgG fragments, but additional study of reactivity to isolated mitochondrial membranes will be necessary to be certain.

FIG. 3. Concentration of ^{131}I -labeled F(ab')_2 fragments of the anti-mitochondrial IgG (solid bars, $n = 6$) and of normal human IgG (stippled bars, $n = 6$) in experimental canine myocardial infarcts. The format of the figure is the same as in Fig. 2. Note that the ^{131}I -labeled F(ab')_2 fragments obtained from the anti-mitochondrial IgG concentrated in the experimental canine infarcts to a significantly greater extent than did the control ^{131}I -labeled F(ab')_2 fragments from normal human IgG.

Because ^{131}I does not provide good quality myocardial scintigraphic images, in this study we have determined that there is significant uptake of the F(ab')_2 anti-mitochondrial antibody in experimental canine infarcts only by scintillation counting of isolated tissues, but in future evaluations the F(ab')_2 anti-mitochondrial antibody may be labeled with ^{123}I , a positron emitter, or a single-photon-emitter to allow *in vivo* scintigraphic images to be obtained. The mean 9-fold increase in central infarct subendocardial uptake of the anti-mitochondrial F(ab')_2 fragments suggests that *in vivo* scintigraphic detection of experimental canine myocardial infarcts should be possible. Moreover, further purification of this antibody fragment or the use of antibodies with even greater antimitochondrial affinity may further increase these ratios. Finally, earlier identification of alterations in cell membrane permeability and localization on mitochondria after experimental myocardial infarcts have been established may be possible by using lower molecular weight fragments of the anti-mitochondrial antibodies (i.e., the Fab fragments, with molecular weight of 50,000). These possibilities need to be studied in future evaluations.

Thus, the use of antibodies directed against intracellular structures allows detection of cell membrane permeability alterations and may also allow one to identify specific intracellular organelles (or organellar injury) in a more direct manner (16–18). In addition, important concentration of these imaging agents does not occur in bone. Whether the anti-mitochondrial antibody will possess any particular advantages in comparison to the anti-myosin antibody (16–18) for scintigraphic detection of experimental (or potentially clinical) myocardial infarcts will have to be determined by future studies. However, it is of interest that the anti-mitochondrial antibody spontaneously develops in humans, as opposed to the requirement for injection of antigen into a heterologous species to elicit production. This antibody also provides an opportunity to identify a different cellular organelle—i.e., the mitochondrion—as opposed to the contractile protein myosin, after experimental cellular injury.

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