Specificity of Localization of Myosin-specific Antibody Fragments in Experimental Myocardial Infarction

Histologic, Histochemical, Autoradiographic and Scintigraphic Studies

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SUMMARY **The concentration of radioiodinated $(Fab')_2$ fragments of cardiac specific antimyosin antibody in myocardial infarcts has been shown previously to be inversely proportional to regional myocardial blood flow. The myocardial localization of antibody fragments has also been visualized by gamma camera scintigraphy. We now correlate the site of ^{125}I-antimyosin $(Fab')_2$ uptake with histochemical and histologic evidence of myocardial infarction. One millicurie of ^{125}I-antimyosin $(Fab')_2$ was administered intravenously 4 hours after left anterior descending coronary artery ligation to anesthetized dogs. The dogs were allowed to recover for 48 hours. The hearts were then excised, perfused with 1% triphenyl tetrazolium chloride (TTC) and formalin-fixed. One-centimeter-thick transverse slices were cut; gamma scintigrams for ^{125}I-antimyosin $(Fab')_2$ localization, macro- and microautoradiograms and histologic studies were performed. Samples of the remaining tissues were assayed for ^{125}I activity by gamma scintillation counting. Comparison of areas of ^{125}I-antimyosin $(Fab')_2$ uptake on scintigrams and macroautoradiograms with TTC infarct location and size showed close correlation. Maximal ratios of ^{125}I-antimyosin $(Fab')_2$ between infarcted and normal tissue (48 ± 13 [SEM]) were in the subendocardium at the infarct center in areas of most severe cellular necrosis. Microautoradiographic studies showed maximal grain density at the region of maximal myocyte necrosis. This study demonstrates that ^{125}I-antimyosin $(Fab')_2$ localization is highly specific for necrotic myocardial cells.**

ANTIBODIES SPECIFIC for cardiac myosin (Ab) concentrated in experimental canine myocardial infarcts to a markedly greater degree than nonimmune IgG.[1-3] Ab uptake was inversely related to regional myocardial blood flow, the greatest uptake being in regions of most severe blood flow reduction.[1, 2]

To determine the specificity and site of Ab localization in infarcted myocardium we report histochemical, histologic, scintigraphic and autoradiographic observations that confirm that Ab binds only to cardiac myosin in necrotic myocytes.

Methods

Preparation of ^{125}I-antimyosin $(Fab')_2$ Fragments

Canine cardiac myosin purified by the method of Katz et al.[4] was emulsified in complete Freund's adjuvant and injected into New Zealand white rabbits as previously described.[1, 5] Purification of antimyosin antibody from immune rabbit serum was effected by myosin-Sepharose affinity chromatography.[1] The immunoadsorbent was prepared by coupling cardiac myosin to cyanogen bromide activated Sepharose.[1, 6] Ab was then subjected to pepsin digestion for 20 hours at 37°C for preparation of $(Fab')_2$ fragments,[7] the bivalent antigen binding fragments with two-thirds the molecular weight of whole Ab. $Ab(Fab')_2$ fragments were separated from undigested Ab by Sephadex G-100 (90 × 2.5 cm) column chromatography.[1] The $Ab(Fab')_2$ fragments thus obtained were labeled with iodine-125 by the lactoperoxidase procedure of Marchalonis.[8]

Experimental Protocol

Ten mongrel dogs were anesthetized by intravenous pentobarbital (30 mg/kg) before left thoracotomy was performed under sterile conditions. Serial ligation of the confluent branches of the left anterior descending coronary artery was performed at 2–3-minute intervals until approximately 40% of the anterior left ventricular wall appeared cyanotic.[9] The thoracotomy was then closed and the dogs were allowed to recover. All dogs appeared active after recovery from anesthesia. Sham operation was carried out in two additional dogs. Four hours after ligation of the left anterior descending coronary artery (LAD) each dog received 1 mCi of ^{125}I-antimyosin $(Fab')_2$ intravenously. The dogs were allowed to recover for 48 hours and then were sacrificed by pentobarbital overdose. The hearts were excised and stained with triphenyltetrazolium chloride (TTC) by aortic perfusion as previously described.[3, 10] The TTC-stained heart was fixed in 10% buffered formalin and the ventricle then sliced at 1-cm intervals parallel to the atrioventricular groove. The slices were photographed and imaged with a portable gamma camera equipped with a low-energy collimator. Scintigraphic data were computer-analyzed to produce enhanced scintigraphic images.

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One-millimeter subslices were taken for macroautoradiography, histologic examination and microautoradiography. The remainder of the infarcted and normal myocardial tissues was cut into 1–2-cm specimens according to the TTC staining pattern and counted in a gamma scintillation counter. Ratios of ^{125}I-antimyosin $(Fab')_2$ in infarcted-to-normal myocardium were computed and compared with regions of infarction determined by TTC staining.

Macroautoradiographs were prepared from 1-mm subslices by wrapping the tissue in cellophane and placing the slices in direct contact with a sheet of Kodak no-screen x-ray film. Exposure times varied from 5–14 days.

Microautoradiographs were prepared from routinely processed histologic sections. After cutting and deparaffinizing, the unstained sections were dipped into a 50% solution of Kodal NTB2 nuclear track emulsion. After 2 weeks of exposure the emulsion-covered slides were developed in Kodak D-19, the slices were stained with hematoxylin and eosin and examined with oil immersion objective for evaluation of grain distribution.

In four other dogs, the LAD was occluded for 5 hours by selectively placing a specially designed Sones catheter under fluoroscopy as previously described.[3] After 5 hours, the occlusive catheter was removed and 0.5 mCi of ^{131}I-antimyosin $(Fab')_2$ was injected directly into the left main coronary artery. The dogs were sacrificed 24 hours later and the excised hearts were subjected to TTC staining and macroautoradiography. Ratios of antibody in the test tissues to normal posterior myocardium were also determined by gamma scintillation counting.

Results

Regions of myocardial infarction were identified by the absence of TTC staining. This stain identified areas containing dehydrogenase enzymatic activity, which has been shown to be depleted from infarcted tissue. Figure 1 shows a representative ventricular slice stained with TTC and its corresponding macroautoradiograph. Regions of infarction seen as lighter areas did not stain with TTC. These areas correspond to the areas of infarction as determined by macroautoradiography, which appears in the figure as a darkly shaded region. Figure 2 shows the same ventricular slice, and the outline on the right shows ratios of ^{125}I-antimyosin $(Fab')_2$ in indicated regions to normal posterior left ventricular myocardium. Highest ratios of antimyosin $(Fab')_2$ uptake corresponded to regions of minimal TTC staining. Ratios of antimyosin uptake as high as 46:1 were noted in central regions of myocardial necrosis. In figure 3, gamma scintigrams and the corresponding macroautoradiographs with the outlines of the ventricular slices are shown. The area of increased radioactivity on the scintigraphic images corresponds to the zone of infarction as seen on macroautoradiographic images, again demonstrating identity of delineated areas. Of the 10 dogs in this study, three were shown to have normal myocardium by histochemical and histologic criteria. In these animals there was lack of ^{125}I-Ab localization. Figure 4 shows TTC-stained ventricular slices of a heart from a dog subjected to catheter coronary occlusion followed by reperfusion and antibody administration. The slices show a hemorrhagic infarct (dark areas). The bottom row is the corresponding macroautoradiograph, again demonstrating close correspon-

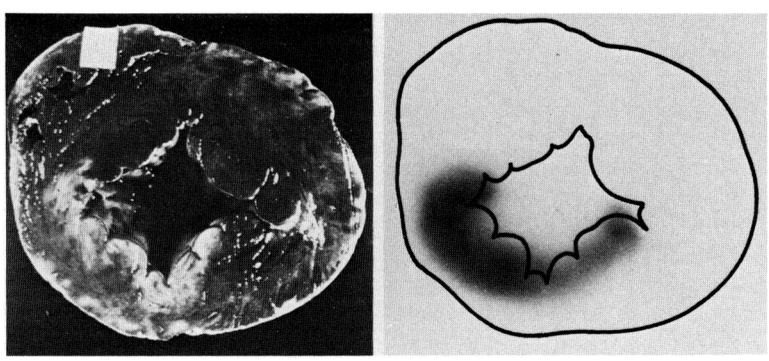

FIGURE 1. *Histochemically delineated myocardial infarction in a ventricular slice (left) seen as white or lighter colored regions, and normal myocardium seen as darker regions. The corresponding macroautoradiograph is shown on the right with the outline of the ventricular slice.*

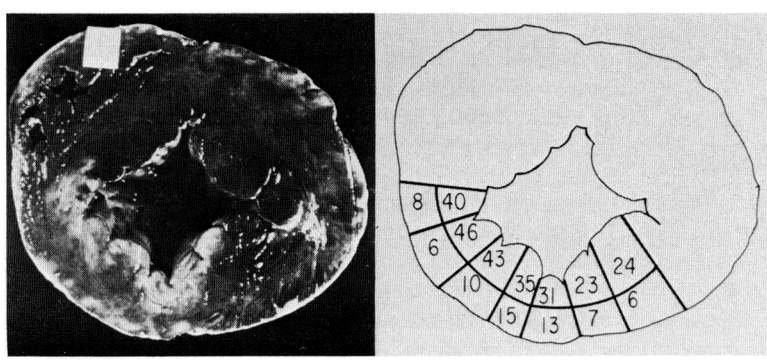

FIGURE 2. *The same ventricular slice stained with triphenyl tetrazolium chloride as in figure 1 (left) and the corresponding ratio of antibody uptake demonstrated in the indicated areas. Ratios of antibody uptake were determined as antibody activity in test tissue/antibody in normal posterior ventricular myocardium.*

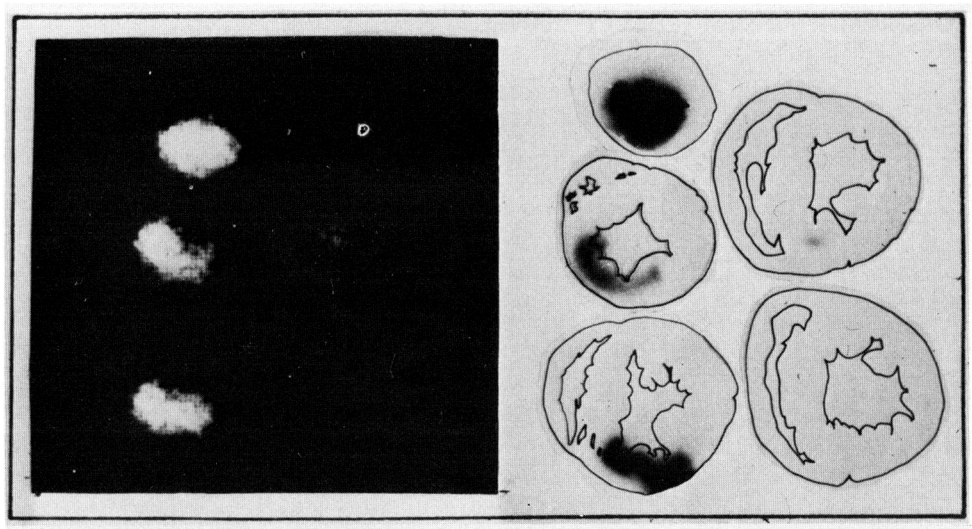

FIGURE 3. *Gamma scintigraphic images of the ventricular slices (left) from a dog with experimental myocardial infarction and the corresponding macroautoradiographs with outlines of the ventricular slices (right).*

dence between histochemically delineated infarction and autoradiographic antimyosin $(Fab')_2$ delineated infarction.

Localization of ^{125}I-antimyosin $(Fab')_2$ was also examined microscopically. Figure 5 shows that ^{125}I-antimyosin $(Fab')_2$ concentrates only in regions of myocytes identified as necrotic by hematoxylin and eosin staining. Regions of adjacent noninfarcted myocardium contain only a few grains in the interstitial and vascular spaces. However, a high grain density was present over necrotic myocytes characterized by absent or pyknotic nuclei and hyperiosinophilic cytoplasm. Figure 6 shows another microautoradiograph of the necrotic myocardium from a different dog showing an intense accumulation of grains over necrotic myocytes.

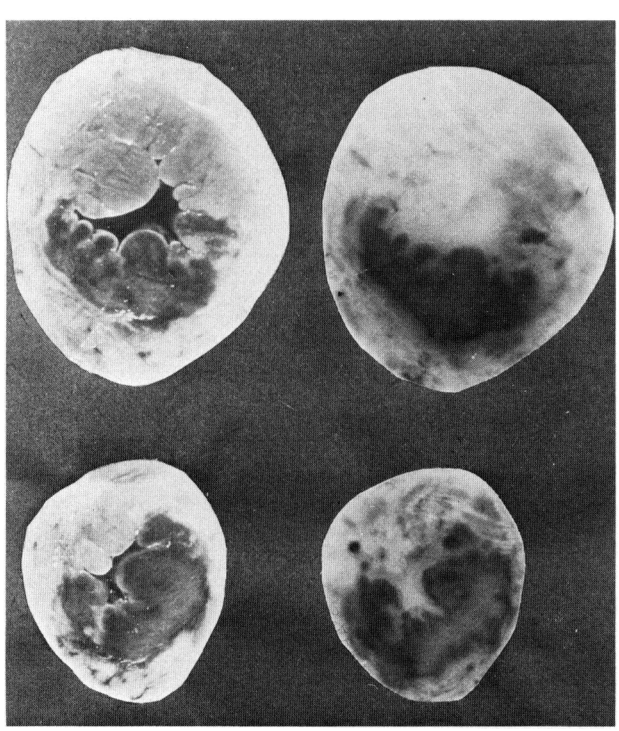

FIGURE 4. *Ventricular slices stained with triphenyl tetrazolium chloride (top row) with hemorrhagic infarct (dark) and the corresponding macroautoradiograph (bottom row) of a dog with catheter occlusion of the left anterior descending coronary artery followed by reperfusion.*

Hearts from the two sham-operated dogs did not show localization of ^{125}I-antimyosin $(Fab')_2$ by scintigraphy; nor were there regions of increased Ab uptake. Ratios of ^{125}I-antimyosin $(Fab')_2$ in test region to normal posterior myocardium were between 1 and 2, which is within the normal range of background variation. TTC staining was uniform, and histologic examination confirmed absence of infarction.

Discussion

We have previously shown that antimyosin $(Fab')_2$ labeled with radioactive isotopes of iodine localizes in regions of myocardial infarction after intravenous injection. In this study we compared the exact site of antimyosin antibody localization with other criteria for infarction to determine if antibody localization reflects myocardial cell death. It appeared that regions of antimyosin $(Fab')_2$ localization as determined by scintigraphy, macroautoradiography and scintillation counting of tissue fragments corresponded to areas of infarction as demonstrated by the absence of TTC staining as well as conventional histologic techniques of infarct identification. At a microscopic level antibody is bound only by necrotic myocytes, where cytoplasmic and nuclear details have been lost. The mechanism of antimyosin $(Fab')_2$ localization in the infarcted myocardium is probably related to enhanced access of macromolecules to intracellular sites after loss of cell membrane integrity. Since the macro-

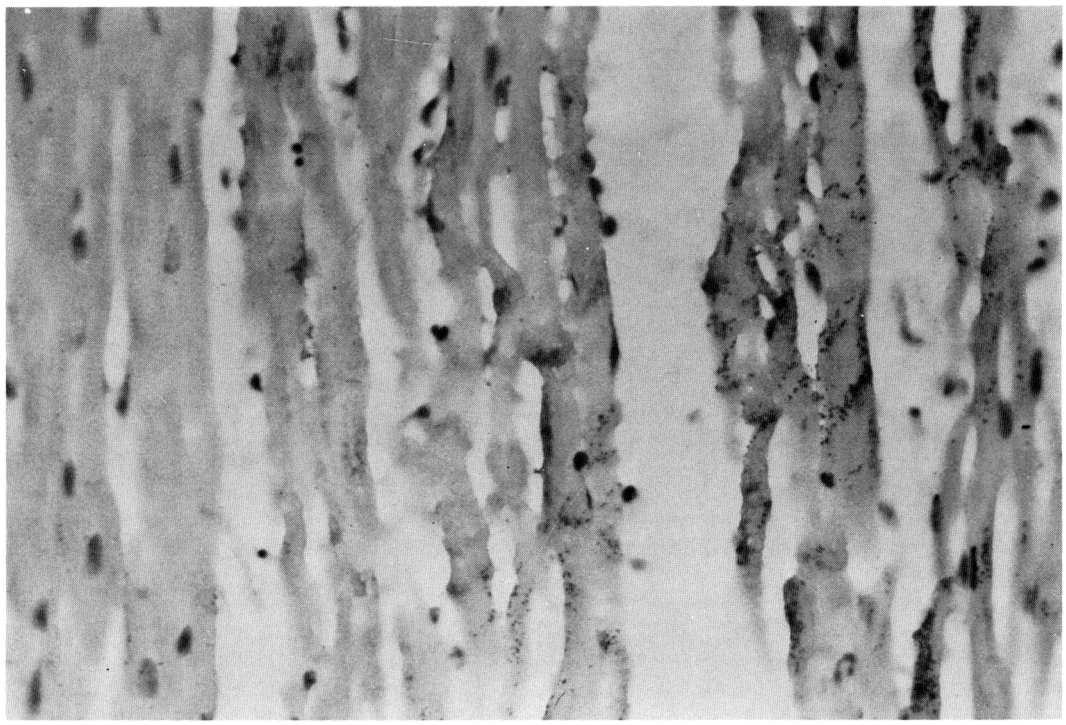

FIGURE 5. *Microautoradiograph showing localization of ^{125}I-Ab(Fab')$_2$ (grains) in the necrotic myocytes (center and right) and lack of Ab localization in normal myocytes (left). Histology was studied after hematoxylin and eosin staining of the sections (magnification × 300).*

FIGURE 6. *Microautoradiograph showing necrotic myocytes and localization of ^{125}I-Ab(Fab')$_2$ (grains) in the infarcted myocardial section from another dog (magnification × 500).*

molecule in this case is an antibody specific for an intracellular protein, myosin, binding to intracellular sites occurs. Normal myocytes by histologic criteria, which presumably have an intact cell membrane, do not show evidence of uptake of $(Fab')_2$.

The mechanism of localization of antimyosin $(Fab')_2$ is unlike that of technetium-99m pyrophosphate or ^{3}H-diphosphonate, which had been reported to depend on binding to intracellular calcium deposits.[11] Buja and co-workers[12] showed extensive deposition of ^{3}H-diphosphonate, a model for agents that bind to calcium, not only in the center of an infarct but also in cells at the periphery, whereas myosin antibody seems to be found only within or on necrotic myocytes. Diphosphonate was also seen in the interstitial spaces.

The specificity of myosin-specific antibody for necrotic myocytes, and the correspondence of the area of infarction with antibody uptake, suggest that this method has promise in the precise localization of infarcts in vivo.

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