Acute Myocardial Infarct Imaging with Indium-111-Labeled Monoclonal Antimyosin Fab

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Indium-111 monoclonal antimyosin Fab scintigraphy was used to detect myocardial necrosis in 52 of 54 patients (96.3%) with acute myocardial infarction. Infarcts were visualized when coronary arteries were persistently occluded (n = 10), became patent after thrombolysis (n = 33), or became patent after spontaneous reperfusion (n = 7). Posteroinferolateral visualizations were obtained in two patients with clinical and enzymatic evidence of infarction but normal electrocardiograms. Of the two patients in whom no infarcts were visualized, one had an anterior myocardial infarct. This patient underwent successful thrombolytic therapy, with attendant minimization of creatine kinase release. The other patient had a small, nonreperfused inferior myocardial infarct. Five patients with a history of remote infarction and acute necrosis showed antimyosin uptake only in regions concordant with the acute episodes of infarction, and radiolabeled antimyosin Fab localized in neither old infarcts nor normal, noninfarcted myocardium. Antimyosin Fab scintigraphy, thus, appears to be a highly specific means of delineating necrotic myocardium, at least in this limited and selected group of patients.

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Radioimmunoimaging with monoclonal antibodies has been advocated for in vivo visualization of specific markers in cells or tissues. Although radiolabeled monoclonal antibodies have been used primarily in the detection of tumors (*1–10*), studies in our laboratory demonstrate the feasibility of using radiolabeled monoclonal antibodies directed against cardiac myosin for the detection of zones of acute myocardial necrosis (*11–15*). Two concerns about this approach to imaging infarcts are the potential for leakage of intracellular antigen, which would result in a loss of antibody binding sites, and the possibility that antibodies would have insufficient access to the zone of necrosis because of a lack of blood flow. With respect to the first issue, cardiac myosin is a highly insoluble antigen, and remains within the cell at a high concentration long after the integrity of the membrane has been lost. Rupture of the cell membrane exposes ~ 2.4×10^{17} binding sites per gram of myocardium, a concentration two orders of magnitude greater than that expected for tumor antigens (*17*). With respect to the second issue, perfusion to the center of a zone of myocardial necrosis, albeit extremely low, is not zero (*18*).

Experimental studies demonstrated an inverse relationship between antibody localization and residual perfusion. Maximal antibody accumulation was observed in areas of lowest perfusion (*11,16*). To optimize photon flux, we modified the bifunctional chelation technique of Krejcarek and Tucker (*19*) to permit labeling with technetium-99m (^{99m}Tc) (*20*). Because animal studies demonstrated uptake of ^{99m}Tc-labeled antimyosin Fab within 6 hr of acute coronary occlusion, initial studies in patients were performed with ^{99m}Tc-labeled antibody (*21*). The early images in human subjects were of low contrast, and diagnostic quality images were typically recorded 12–18 hr after injection. Subsequently, this bifunctional chelation technique was

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adapted to permit transchelation labeling of DTPA-antibodies with indium-111 (^{111}In) in citrate at pH 5.5 (5–6) (*22*). The present study reports the findings from 54 patients who were injected with [^{111}In]antimyosin Fab during the early hours of acute myocardial infarction.

MATERIALS AND METHODS

Preparation of Monoclonal Antimyosin Fab (R11D10)

R11D10 was produced by the fusion of immune BALB/c spleen cells with SP2/0 myeloma cells according to the polyethylene glycol fusion method previously described (*16*). Monoclonality was ascertained by the process of limiting dilution (*16*) and the antibody was produced in the ascites form in pristane-primed mice. Sterile and apyrogenic preparation of the DTPA-labeled R11D10 Fab was performed by Centocor, Inc. (Malvern, PA). IgG was isolated from mouse ascites and Fab was then prepared by papain digestion (*16,23*). Undigested antibody was separated from the Fab fragments by protein A affinity chromatography and high pressure liquid chromatography (*16*). Sterile and apyrogenic R11D10 Fab was covalently linked with DTPA by the cyclic anhydride method of Eckelman et al. (*24*), as used by Hnatowich et al. (*25*). Aliquots (0.5 mg) were also tested for nucleic acids as well as for mouse antibody production (the latter to show that transfection of mammalian cells did not occur with myeloma-hybrid genome containing the immunoglobulin gene) as required by the FDA. Kits containing 0.5 mg DTPA-R11D10 Fab in sterile, apyrogenic physiologic saline or 0.1*M* sterile and apyrogenic citrate (2.5 ml; pH 5.0) were prepared by Centocor. Sterile and apyrogenic 10 mCi/ml ^{111}In chloride solution was purchased weekly from Amersham USA. When needed, 2-mCi aliquots of ^{111}In were used to label DTPA-R11D10 Fab.

Labeling Procedure and Patient Studies

An aliquot of 0.5 mg DTPA-R11D10 Fab was dissolved in 100 μl physiologic saline, and 1.8 ml of 0.1*M* citrate (pH 5.0) was added. To this mixture, 2 mCi of ^{111}In was added, and incubated at room temperature for 10–15 min. A small aliquot (~ 0.01 ml) was used to determine labeling efficiency by ascending thin layer chromatography on cellulose acetate sheets developed in 0.1*M* citrate, pH 5.0. The ratio of radioactive counts at the origin to that of the solvent front was used to compute labeling efficiency. Samples with > 85% incorporation of ^{111}In determined after 15–30 min of incubation at room temperature were considered usable. All ^{111}In-labeled R11D10 Fab was used at ~ 1 hr after the labeling procedure and quality control study.

Patient Selection

All protocols were reviewed and approved by the appropriate committees of the Massachusetts General Hospital (Isotopes, December 19, 1980; Human Studies, December 10, 1981) and the Food and Drug Administration (IND No. BB-IND-1858). Informed consent was obtained from all patients. A total of 54 patients with suspected acute myocardial infarction were studied (27 anterior, 27 inferior) (Table 1). Criteria for selection included: precordial chest pain typical of cardiac ischemia of at least 30-min duration, ST elevation of at least 0.1 mV in two or more leads of the electrocardiogram (with subsequent evolution of an electrocardiographic infarct pattern), and at least twice normal elevation of CK with associated elevation of the MB isoenzyme (*21*). Fifty of the patients studied were also subjected to early coronary angiography because they were part of a thrombolytic therapy protocol. Two patients did not show the electrocardiographic changes typical of acute myocardial infarction. These patients had precordial chest pain typical of cardiac ischemia of at least 30-min duration and twice normal elevation of total CK with associated elevation of the MB isoenzyme (Table 1: Patients 35 and 37). Two other patients with anterior myocardial infarcts (Patients 6 and 12) underwent reperfusion therapy by percutaneous transluminal coronary angioplasty.

Patients consenting to thrombolytic therapy underwent immediate catheterization and angiography. Those with angiographic evidence of spontaneous reperfusion did not receive thrombolytic therapy (n = 8). Forty-one patients received thrombolytic therapy with either recombinant tissue plasminogen activator or streptokinase. The location of the coronary artery lesion was determined by angiography. Classification was by the major artery involved: left anterior descending (LAD), right coronary (RCA), or left circumflex (LCX).

Serial blood samples were obtained from 14 patients to determine blood clearance of the ^{111}In-labeled antimyosin Fab. The blood sample drawn 5 min after intravenous administration was designated the 100% sample.

Reperfusion was successful in 31 of the 41 patients receiving thrombolytic therapy. Two additional patients showed initial reperfusion followed by reocclusion prior to antimyosin administration, and thrombolytic therapy was unsuccessful in the eight remaining patients. Of the 13 patients remaining from the group of 54, seven showed spontaneous reperfusion, three showed angiographically confirmed persistent occlusion, two underwent percutaneous transluminal coronary angioplasty only, and angiographic data are not available for one.

Prior to intravenous injection of antimyosin, ~ 0.01 ml of ^{111}In-labeled monoclonal antimyosin R11D10 Fab was administered intradermally and the injection site was observed for a wheal and flare response over 30 min. A 1.8-mCi aliquot of the ^{111}In-labeled R11D10 Fab in 0.18 ml citrate (diluted to 10–15 ml to minimize patient discomfort) was then administered by slow intravenous injection.

Gamma Scintigraphic Imaging

Patients were typically imaged ~ 24 hr, and occasionally as late as 48 hr, after intravenous administration of the ^{111}In-labeled R11D10 Fab. Although imaging as early as 6 hr and as late as 48 hr can be performed with this agent, analysis of data from the first 10 patients allowed us to select the 24-hr interval as the time when there was both minimal residual blood-pool activity and maximal photon flux from the zone of myocardial necrosis. Both the 178 and 247 keV photopeaks of the ^{111}In were used to record the planar scintigrams in the anterior and left anterior oblique 45° views using a large field-of-view scintillation camera equipped with a medium-energy collimator. Data were recorded in digital form in a dedicated nuclear medicine computer system. A 4-min acquisition time was used for each view.

TABLE 1
Patients with Suspected Myocardial Infarction

Patient no.	CK	CK-MB	AM scan	AM infarct location	RP	RP-time	Infarct location by ECG	Infarct location by CAG
1*	2,910	19	+	ANT	Y	5.3	ANT	LAD
2*	1,830	7	+	ANT	Y RO	PTCA	ANT	LAD
3*	810	13	+	ANT	N	—	ANT	LAD
4	543	17	+	ANT, SEP	SR		ANT	LAD
5*	2,360	14	+	ANT	Y	2.2	ANT	LAD
6	2,600	15	+	ANT	Y	PTCA	ANT	LAD (RCA)†
7	990	9	+	ANT	SR		ANT	LAD
8*	4,670	13	+	ANT	Y	5.1	ANT	LAD
9*	1,443	15	+	ANT	Y	5.8	ANT	LAD
10	840	21	+	ANT	SR		ANT	LAD
11*	2,720	12	+	ANT	Y	7.5	ANT	LAD
12	407	5	+	ANT	Y	PTCA	ANT	LAD
13*	1,820	11	+	ANT	N		ANT	LAD
14*	1,865	16	+	ANT	Y	4.3	ANT	LAD (RCA)†
15*	1,077	18	+	ANT	Y	4.5	ANT	LAD
16*	5,190	16	+	ANT	Y	5.2	ANT	LAD
17*	193	6	+	ANT	Y	3.3	ANT	LAD (RCA)†
18*	718	6	−	—	Y	6.2	ANT	LAD
19*	2,980	15	+	ANT	Y	6.2	ANT	LAD
20*	3,630	17	+	ANT	Y	5.0	ANT	LAD
21*	1,460	18	+	ANT	Y	5.6	ANT	LAD
22	326	20	+	ANT	SR		ANT	LAD
23	998	16	+	ANT	SR		ANT	LAD
24*	1,440	NA	+	ANT	Y	6.6	ANT	LAD
25*	2,260	12	+	ANT	Y	4	ANT	LAD
26*	724	22	+	ANT	Y	6.4	ANT	LAD
27*	578	20	+	ANT	N	—	ANT	LAD
28*	892	19	−	—	N		INF	RCA
29*	958	16	+	INF	Y	3.5	INF	RCA
30	845	18	+	INF	SR		INF	Normal
31	560	9	+	INF	SR		INF	RCA
32*	782	12	+	INF	Y		INF	RCA
33	850	24	+	INF	N		INF	RCA
34*	1,220	28	+	INF	RO		INF	RCA
35	378	14	+	POST	NA		NOR	NA
36*	1,220	15	+	INF	N	—	INF	RCA
37	722	20	+	POST	N		NOR	LCX
38*	1,660	14	+	INF	Y	6.3	INF	LCX
39*	388	26	+	INF	Y	5.5	INF	LCX
40*	3,100	13	+	INF	Y	3.0	INF	RCA
41*	581	6	+	INF	N	—	INF	RCA
42*	1,591	14	+	INF	Y	5.5	INF	RCA (LCX)†
43*	960	16	+	INF	RO		INF	RCA
44*	1,119	20	+	INF	Y	3.8	INF	RCA
45*	1,680	17	+	INF	Y	6.9	INF	RCA
46*	555	23	+	INF	Y	3.7	INF	RCA
47	540	15	+	INF	N	—	INF	RCA
48*	1,630	19	+	INF	N	—	INF	RCA
49*	2,120	8	+	INF	Y	3.5	INF	RCA (LCX)†
50*	350		+	INF	Y	4.0	INF	RCA
51*	100	5	+	INF	N	—	INF	RCA
52*	779	8	+	INF	Y	5.25	INF	RCA
53*	555	17	+	INF	Y	4.0	INF	RCA
54*	2,150	11	+	INF	Y	6.0	INF	RCA
					RO PTCA	7.0		

* Patients that received thrombolytic therapy.

† Location of old infarct (RCA involvement denotes inferior infarction, and LCX involvement denotes posterolateral infarction).

AM, antimyosin Fab; ANT, anterior; CAG, coronary angiogram; CK, total serum creatinine kinase activity in IU; CK-MB, MB isoenzyme; ECG, electrocardiogram; INF, inferior; LAD, left anterior descending coronary artery; LCX, left circumflex artery; N, no reflow; NA, not available; POST, posterior; PTCA, percutaneous transluminal coronary angioplasty; RCA, right coronary artery; RO, reocclusion; RP, reperfusion; SEP, septal; SR, spontaneous reflow; Y, yes reflow.

DATA ANALYSIS

The ^{111}In-DTPA-R11D10 Fab images were interpreted directly from the computer video display by two independent observers who were not aware of the patient's identity or of the other clinical studies performed. The data from the images were then related to the electrocardiogram, total serum creatine kinase, CK-MB levels, and angiographic findings. Location of infarction was determined from the video display and the zone of involvement was classified as anterior or inferior (posteroinferior was included as inferior).

STATISTICAL ANALYSIS

Blood clearance data obtained from 14 patients were analyzed with the RS/1 Analysis System Fit Function Routine.*

RESULTS

None of the patients showed positive wheal and flare reactions within 15–30 min of intradermal administration of Fab (~ 5–10 μg). Of the 54 patients, only two failed to show positive antimyosin localization in the region of the myocardium. Both patients were admitted for thrombolytic therapy. It was successful in the patient with an anterior myocardial infarct (Patient 18); however, recanalization did not occur in the patient with a very small inferior myocardial infarct (Patient 28), as evidenced by the small region of hypokinesis by acute angiography. The patients' total CK serum enzyme levels were 718 (6% CK-MB) and 892 (19% CK-MB) IU, respectively (Table 1). Examples from two patients with angiographically confirmed, nonreperfused coronary arteries are shown in Figure 1 (A and B). Both, Patients 13 and 48, showed unequivocal localization of ^{111}In-labeled monoclonal antimyosin Fab in the region of myocardial compromise as indicated by the admissions electrocardiograms and by the zone of abnormal wall motion identified on the contrast ventriculogram. Patients with delayed but successful thrombolysis all showed uptake of ^{111}In-labeled antimyosin Fab. Figure 2 shows the anterior and left anterior oblique 45° gamma images of a typical anterior myocardial infarct scan from a patient who underwent successful but delayed LAD reperfusion (Patient 9), and Figure 3 shows a typical inferior myocardial infarct scan from

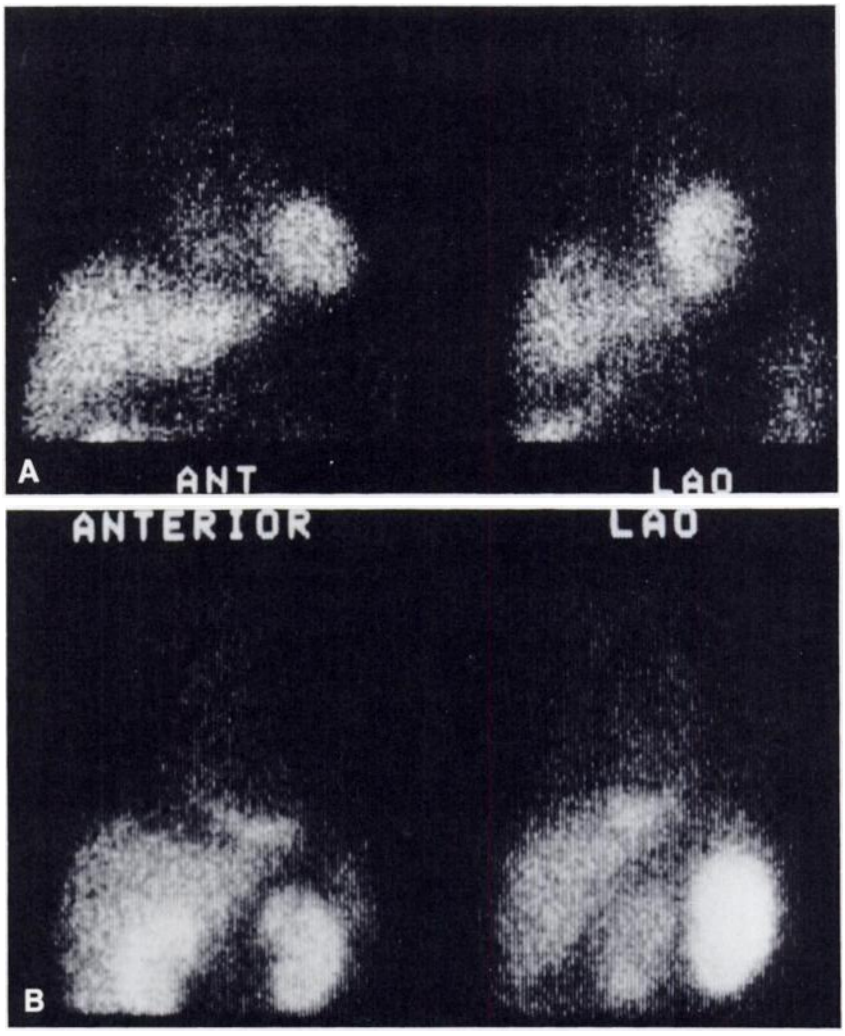

FIGURE 1
A: Anterior (left) and 45° LAO (right) gamma scintigrams ([^{111}In]antimyosin) 24 hr postinjection of a patient (13) with acute anterior myocardial infarction and angiographically confirmed, persistently occluded left anterior descending coronary artery. B: Anterior (left) and 45° LAO (right) gamma scintigrams 29 hr postinjection of a patient (43) with acute inferior myocardial infarction and angiographically confirmed, persistently occluded right coronary artery.

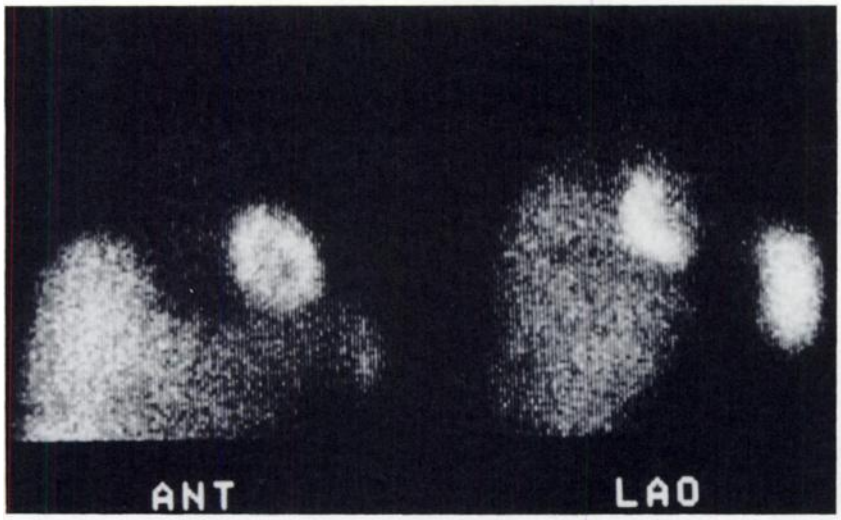

FIGURE 2
Anterior (left) and 45° LAO (right) gamma images ([^{111}In]antimyosin) 26 hr postinjection of a patient (9) with an acute anterior myocardial infarction and a left anterior descending coronary artery that had been successfully reperfused.

Patient 42, who was also successfully reperfused. Figure 4A shows the anterior and LAO 45° scintigrams of Patient 37, with a nondiagnostic electrocardiogram, demonstrating an inferoposterior localization of [^{111}In]-labeled antimyosin Fab. Although the electrocardiogram of Patient 37 (Fig. 4B) was not indicative of acute infarction, a peak CK of 722 IU was observed. An electrocardiogram taken 2 days later failed to show any evolutionary changes. Intense and homogeneous localization of [^{111}In]antimyosin within the infarct zone was not always observed. For example, Figure 5 shows the anterior and LAO images of Patient 7, who had anterior myocardial infarction and was studied 48 hr after i.v. administration of the radiolabeled antimyosin. It is apparent that the intensity of ^{111}In activity in the zone at risk is reduced and patchy. This patient's total peak CK was only 990 IU with 9% CK-MB, and the patient experienced angiographically confirmed spontaneous reperfusion.

Five patients in this study (Patients 6, 14, 17, 42, and 49) had previous myocardial infarcts. Antimyosin localization was observed only in the regions concordant with the acute episodes of infarction (Table 1). Previous infarct location is indicated in parenthesis. Uptake in old infarcts was not visualized with [^{111}In]antimyosin. Blood clearance of the ^{111}In-labeled antimyosin Fab in 14 patients was measured over 48 hr. Figure 6 demonstrates an exponential blood clearance, with a mean $T_{1/2}$ of ~ 5 hr. In this study, planar images were acquired from 18 to 28 hr after the onset of chest pain.

DISCUSSION

To date, monoclonal antibodies have shown promise as agents for the scintigraphic visualization of various target organs such as tumors (*5–10*) and infarcted myocardium (*12–14,16,22*). Although iodine-131 (^{131}I) has been the leading radionuclide for use in tumor imaging, its dosimetry precludes administration to patients for evaluations of non-neoplastic disorders and its use in many instances requires blood-pool subtraction to permit visualization of the tumors (*26*). The advent of metal-chelate radiolabeling techniques (*17,24,25*) pro-

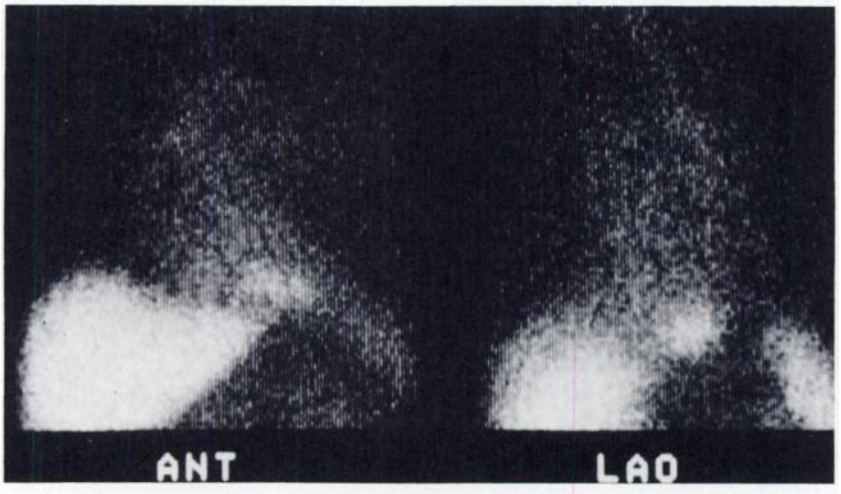

FIGURE 3
Anterior (left) and 45° LAO (right) gamma images ([^{111}In]antimyosin) 25 hr postinjection of a patient (42) with an inferior myocardial infarction and right and left circumflex coronary arteries that had been successfully reperfused.

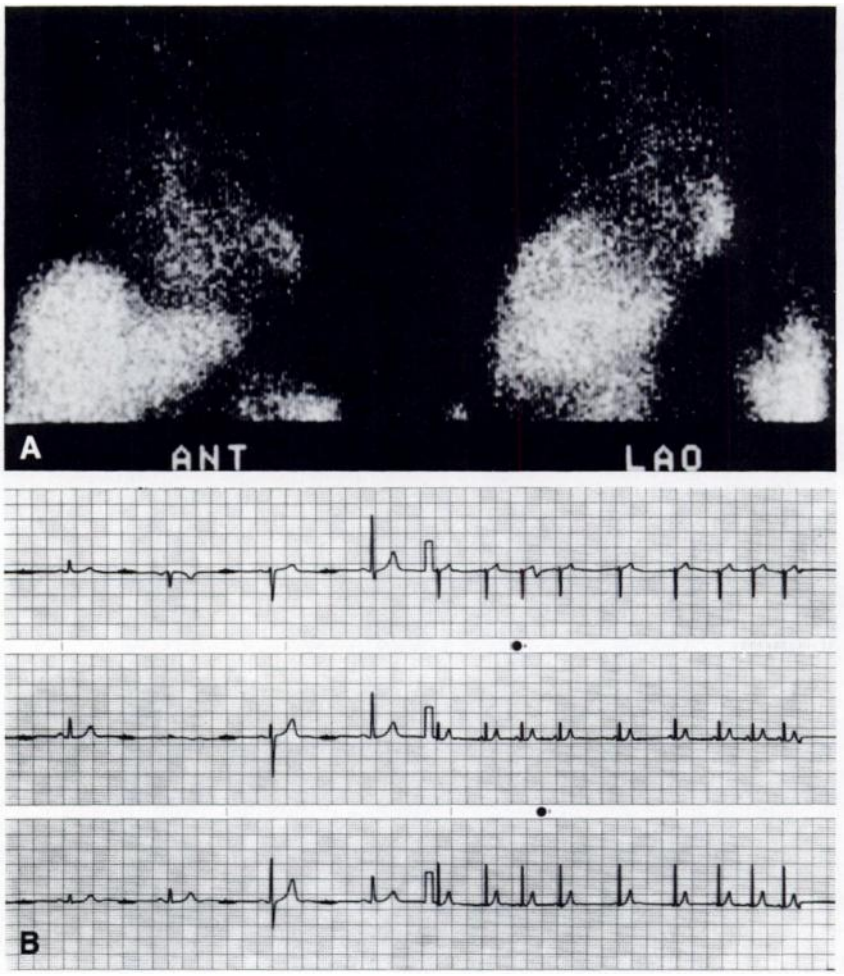

FIGURE 4
A: Anterior (left) and 45° LAO (right) gamma scintigrams ([^{111}In]antimyosin) 23 hr postinjection of a patient with an inferoposterior infarction whose acute electrocardiogram B was not diagnostic of acute myocardial infarction.

vides a means of preparing in kit form radioimmunoimaging agents that are suitable for use in an acute clinical care situation. The major objection to the routine use of ^{111}In as a radiolabel is the hepatic uptake of ^{111}In-labeled antibodies. To minimize localization in this site, we used citrate as a means of transchelating ^{111}In. The citrate serves two purposes: it prevents the indium from forming a colloid at a pH > than 4, and it permits the indium to transchelate to the DTPA-coupled antibody at a pH that minimizes damage to the protein. The minimization of hepatic localization allows the identification of even small infarcts that involve the inferior wall.

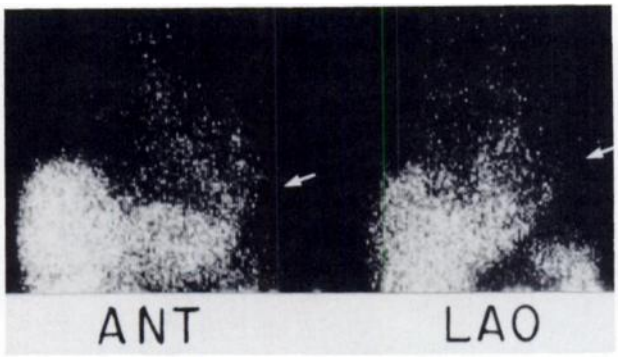

FIGURE 5
Anterior and 45° LAO gamma scintigrams of a patient (7) with an anterior myocardial infarction obtained 48 hr after antibody administration. The image shows minimal, diffuse and patchy uptake of ^{111}In activity in the anterior myocardium (arrows).

The minimal size of a lesion that can be visualized with ^{111}In-labeled antimyosin Fab has not yet been determined. However, we previously reported that ^{99m}Tc-labeled antimyosin Fab was capable of detecting inferior myocardial infarcts that showed a hypokinetic region by ventriculogram of ~ 3 cm in length. Because of the lower hepatic activity of ^{111}In-labeled antimyosin Fab in comparison with Fab labeled with ^{99m}Tc, it is likely that smaller infarcts might be detected with ^{111}In-labeled antibody. A sensitivity of 87% was observed for detection of acute myocardial infarction by planar imaging with ^{99m}Tc-labeled antimyosin (*21*). In the present study utilizing ^{111}In-labeled antimyosin Fab, sensitivity improved to 96.3%. Despite this improvement, and because diagnosis of infarction does not pose major clinical problems, the optimal value of antimyosin im-

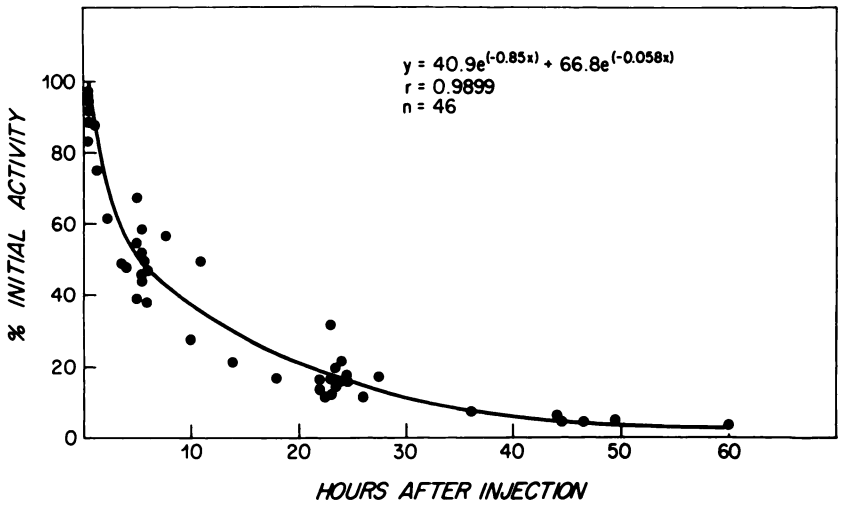

FIGURE 6
Blood clearance of intravenously administered ^{111}In-labeled monoclonal antimyosin Fab in 14 patients. The $T_{1/2}$ of the fast component was 0.8 hr ($p < 0.001$), that of the slow component was 12 hr ($p < 0.001$).

aging may well be in its application in the quantitative measurement of the extent of myocardial necrosis.

Two lines of evidence support the notion that antimyosin binds specifically in zones of acute myocardial necrosis. First, antimyosin did not localize in regions of prior infarction evidenced by ECG in five patients with both acute and remote infarction; and second, in all 54 patients studied, there was considerable normal, noninfarcted myocardium that did not show antimyosin accumulation. In this way each patient serves as his or her own control. It should be noted that the antibody does not concentrate in regions of previous infarction. This may be explained by the observation that necrotic myocytes were eventually replaced by fibrous tissue, and myosin is therefore no longer present.

Although 52 of 54 patients in this group showed positive antimyosin localization in the region of acute infarction, the intensity and distribution of the radioactivity is not the same in all cases. Images from some patients showed unequivocal but low intensity and diffuse localization of the radioactivity (Fig. 5), whereas, others showed intense homogeneous localization (Figs. 1–4). To date, we do not have sufficient postmortem pathologic evidence to determine whether low-intensity, diffuse ^{111}In antimyosin Fab localization in patients represents either subendocardial or patchy myocardial necrosis, nor can we ascertain whether homogeneous intense antimyosin uptake denotes homogeneous transmural infarction. However, in experimental studies in which canine hearts can be examined after [^{111}In]antimyosin Fab imaging and triphenyltetrazolium chloride histochemical staining, low-intensity, diffuse antimyosin localization corresponded with patchy or subendocardial infarction, and intense homogeneous antimyosin localization corresponded with homogeneous transmural infarction (unpublished data).

It is important to note that intense concentration of antimyosin was shown to occur in ten patients with persistent coronary artery occlusion. Examples shown in Figures 1A and B, 2, and 3 demonstrate concentration of antimyosin in two patients with successful but delayed coronary reperfusion.

It is also possible that a false-positive scan could result from residual blood-pool activity in patients with small or diffused infarction or in normal noninfarct patients if only the 24-hr images were to be analyzed. However, reimaging at 48 hr after antibody administration should minimize this uncertainty. By this time, there is only negligible blood-pool activity, which is not visualized (Figs. 5 and 6). Furthermore, patients with 10- to 14-day-old infarcts can still be imaged with ^{111}In antimyosin. Whether tracer uptake occurs throughout the area of acute infarction cannot be assessed without postmortem infarct comparison. It is also possible to administer multiple injections of antimyosin without eliciting human anti-murine antibody production. To date we have administered [^{111}In]antimyosin Fab two to four times in at least 15 patients with suspected recurrent or healing myocarditis. No sera from this limited number of patients showed presence of anti-murine Fab antibody activity.

In conclusion, ^{111}In labeling of antimyosin Fab improves the overall sensitivity for diagnosis of infarction to 96.3% (52 of 54 patients in this limited and selected group). It also reduces hepatic sequestration, allowing for the visualization of smaller infarcts.

NOTE

* BBN Research Systems, Cambridge, MA.

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