Evaluation of Indium-111-Labeled Anti-Fibrin Antibody for Imaging Vascular Thrombi

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Monoclonal antibody 59D8 developed by Hui et al., binds to fibrin but not fibrinogen. An ^{111}In-labeled Fab fragment of 59D8 was studied in vitro and in animal models to evaluate its potential for imaging thrombi and emboli in man. Rabbits and dogs were used as models for studying thrombus uptake in vivo. Thrombi and emboli up to 4 days old were successfully visualized at 4–24 hr postinjection in five of eight rabbits. In dogs, 0.5-hr-old and 24-hr-old thrombi were successfully imaged at 24 hr in six of eight animals, and 3/6 of these were positive at 3–4 hr postinjection. Thrombus-to-blood ratios in the dogs averaged 7.1 ± 1.3. The findings suggest this antibody may be useful for imaging thrombi in man.

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Radioisotopic methods for detecting deep venous thrombosis (DVT) still suffer from many limitations despite the continued search for a scintigraphic method for locating thrombi (*1*).

Radiolabeled antibodies have been tested as specific probes for thrombi in the past. Initial studies used polyclonal antibodies which reacted with fibrinogen as well as fibrin (*2–5*). These antibodies rapidly became bound to circulating fibrinogen, and thus traced the incorporation of fibrinogen into forming thrombi. Prolonged antibody residence time in the blood resulted in a delay of at least 48 hr before thrombi could be visualized. More recent studies have used both polyclonal (*6*) and monoclonal (*7*) radioiodinated antibodies which react with fibrin monomer but not with fibrinogen. Although fibrin-specific antibodies may have increased affinity for aged thrombi, they still suffer from a long residence time in the blood. This limits the rapidity with which thrombi can be detected in the presence of the blood background.

Previous studies with radiolabeled antibody for thrombus detection used iodine-131 (^{131}I) as the radiolabel. Although the technique of radioiodination of proteins has been well-studied and produces a moderately stable label (*8,9*) the physical characteristics of ^{131}I are not optimal for imaging and the use of radioiodine may result in a high radiation dose to the thyroid.

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In this study, we evaluated the ability of a monoclonal antibody reactive with human fibrin but not reactive with fibrinogen (*10*) to detect thrombi in animal models in order to predict its potential for imaging thrombi in man. A Fab fragment of the antibody was used to shorten the residence time of the labeled antibody in the blood circulation. In addition, the antibody was radiolabeled with indium-111 (^{111}In), which has improved physical characteristics for imaging.

METHODS

Source of the Antibody

The antibody used in these studies, 59D8, was previously described by Hui et al. (*10*). It was raised against a synthetic peptide comprising the first seven amino acids of the beta chain of human fibrin, a site which is not exposed in fibrinogen. Thus, it reacts with fibrin but not fibrinogen (*10*). The hybridoma cell line 59D8 was grown in cell culture and the antibody-containing cell supernatant purified by filtration. The 59D8 IgG was purified on Protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) as described by Ey et al. (*11*).

The immunoreactivity of 59D8 was determined using an ELISA method similar to the assay previously described (*10*). Fibrin monomer was prepared from human fibrinogen (AB Kabi, Stockholm, Sweden) essentially as described (*12*), except that iodoacetamide was not included. Fibrin-coated wells (50 μg/well, 50 μl per well) were blocked and washed, then five-fold serial dilutions of 59D8 assayed in duplicate. After washing, bound 59D8 was detected with 1:2,500 alkaline-phosphatase conjugated goat-anti-mouse IgG, using p-nitrophenyl phosphate as substrate. The average OD_{410} of duplicate wells

as plotted as log (mg/ml) 59D8 protein concentration versus OD_{410}. Species crossreactivity was studied by performing the above assay, using wells coated with fibrin from rabbit, dog, pig and sheep (Sigma, St. Louis, MO) in place of human fibrin.

Preparation of Fab fragment

The 59D8 IgG was digested with papain and the Fab fragments purified as described previously (*13*), with the following differences. The digest was carried out with 0.25 units of papain per mg of IgG for 6 hr in the presence of 0.1*M* cysteine, and stopped by adding 0.2*M* iodoacetamide. The 59D8 Fab was purified by anion exchange chromatography and diafiltered into 0.15*M* NaCl.

Derivatization with DTPA groups

The 59D8-Fab was derivatized with diethylenetriaminepentaacetic acid (DTPA) groups to facilitate stable binding of ^{111}In, according to the method of Hnatowich (*14*). Under these conditions, between 1.0 and 1.5 DTPA groups were coupled to each Fab. The immunoreactivity was re-tested and compared with previous results.

Radiolabeling with ^{111}In

Thirty-five microliters of 1.0*M* citrate, pH 5.0 was added to 0.5 mg DTPA-59D8-Fab in 325 μl of saline. Up to 1 mCi of ^{111}In as indium chloride (Amersham, Arlington Heights, IL) was then added. After 10 min, the incorporation of radioactivity was assessed by ascending thin layer chromatography (*15*) using ITLC-SG media developed in 0.1*M* citrate buffer, pH 5.0, or HPLC using a size-exclusion packing (Zorba X G-250, Dupont Chromatography Products, Wilmington, DE) eluted with 0.2*M* phosphate, pH 7.0. The two methods gave equivalent values for radiochemical purity. Prior to using the labeled antibody in any functional tests, 25 μl of 0.1*M* DTPA in 0.1*M* citrate, pH 5.7, was added to complex any unbound ^{111}In and prevent formation of radiocolloids which could give incorrect results (*16*). If the radiochemical purity was <90%, the labeled antibody was purified using ultrafiltration in a Centricon 30 unit.

Binding to Plasma Clots In Vitro

The ^{111}In-labeled antibody was tested for its ability to bind to human, canine and rabbit plasma clots in vitro, using a variation of the Ratnoff and Menzie assay (*17*). Clots were formed at room temperature from 0.5 ml ACD plasma, mixed with 0.5 ml 0.05*M* tris, pH 7.4, containing 0.15*M* NaCl (TBS), 12 μl of 1*M* $CaCl_2$ and 6 units of thrombin (Parke, Davis & Co., Detroit, MI), and were wound out on a wooden stick as they formed. In order to study the binding of antibody to actively forming clots, [^{111}In]DTPA-59D8-Fab was added to each clot before the addition of calcium and thrombin. The clots were wound out on a stick during 1 hr, then washed in saline and dissolved in 6*M* urea in 0.2N NaOH. The dissolved clot, wash and serum solutions were counted in a well counter to determine the fraction of total radioactivity associated with the clot.

In order to study the binding of labeled antibody to preformed clots, the clots were wound for 1 hr at room temperature without the addition of labeled antibody. The clots were then washed in saline and placed in a tube containing [^{111}In] DTPA-59D8-Fab in 1 ml of TBS, and incubated for 1 hr before washing with saline, dissolving and counting.

To estimate the amount of nonspecific uptake of radiolabeled antibodies by the plasma clots in vitro, an ^{111}In-control antibody, the DTPA-conjugated Fab fragment of R11D10 anti-human myosin monoclonal antibody (*13*, nonreactive with fibrin) was added to forming and preformed clots and tested for uptake as described above.

Thrombus Uptake Studies in Rabbits

Thrombi and emboli were induced in 21 rabbits by injection of a suspension of iron particles into each rabbit's left ear, while holding a magnet in place over the rabbit's left neck (*18*). The iron particles collected near the magnet and caused a thrombus to form in the left jugular vein. The location of the iron particles was confirmed by an x-ray without contrast. The thrombus was allowed to age for 15 min to 96 hr before injecting 250 μCi [^{111}In]DTPA-59D8-Fab into the rabbit's right ear. Immediately after injecting the antibody and flushing it in with saline, 50 μCi of ^{125}I-human fibrinogen prepared by the iodine monochloride method (*19*) was injected to serve as a control. An aliquot of each dose was saved for later counting to determine the amount of activity injected. Anterior and left lateral images of each rabbit were acquired using a gamma camera (Searle Pho-Gamma IV) interfaced to a computer (Medical Data Systems Med IV). The camera was fitted with a parallel hole collimator and was set to acquire the 247 keV photopeak of ^{111}In with a 20% window. Using a 64 × 64 matrix, 300k counts were acquired in each image. At 24 hr postinjection, a repeat x-ray was taken to confirm the location of the iron particles and the thrombus and a sample of blood were removed for counting in a well counter. For each thrombus, a thrombus-to-blood ratio was calculated:

$$\text{T:B ratio} = \frac{\text{counts/g thrombus}}{\text{counts/g whole blood}}$$

No corrections were made for an increase in the weight of the thrombus due to retained iron particles.

Two rabbits were injected with an ^{111}In-labeled control antibody, DTPA-conjugated Fab fragment of R11D10 anti-human myosin monoclonal antibody (*13*), nonspecific for fibrin. The thrombi were removed and counted relative to blood as described above.

Thrombus Uptake in Dogs

Thrombi were induced in the leg veins of eight anesthetized mongrel dogs by transcatheter placement of an embolization coil (*20*). A catheter was introduced percutaneously into a femoral vein as close as possible to the groin. The catheter was advanced distally as far as possible. An embolization coil, 3mm or 5mm in size (Cook Co., Bloomington, IN) was introduced into the catheter and advanced with a guidewire until it emerged from the distal end of the catheter, where it promptly expanded and lodged firmly in the vessel. The location of the coil was confirmed by an x-ray without contrast (Figs. 3C and 4B). At 30 min or 24 hr after placing the coil, 600 μCi of [^{111}In]DTPA-59D8-Fab was injected into a foreleg, followed by a saline flush and 75 μCi of ^{125}I-human fibrinogen. An aliquot of each dose was saved for later counting to determine the amount of activity injected.

Anterior images were obtained for up to 24 hr, using a gamma camera interfaced to a computer. The camera was fitted with a parallel hole collimator and was set to acquire the 247 keV photopeak of ^{111}In with a 20% window. Using a

64 × 64 matrix, 300k counts were acquired in each image. The thrombus and a blood sample were removed at 24 hr postinjection for weighing and counting.

In three additional dogs, the ability of a radiolabeled nonspecific control antibody to bind to thrombi was studied. The model was created in the same way as described above. Each dog received an injection of 600 μCi [^{111}In]DTPA-R11D10-Fab (*13*) followed by a saline flush and 75 μCi of [^{125}I] fibrinogen.

One additional dog received an intravenous injection of $^{111}InCl_3$. Images were acquired for 24 hr.

Blood Disappearance Rate

Samples of blood were taken at various intervals from six dogs following injection of [^{111}In]DTPA-59D8-Fab. The samples were anticoagulated with heparin, centrifuged to pellet cells, and 0.5 ml aliquots of plasma were counted. An aliquot of the dose was also counted to determine the amount of activity injected. The ^{111}In content (expressed as % of injected dose per ml of plasma) was plotted versus time. The data points after 30 min were fit to an exponential curve (straight line on a semilog graph) using a regression program on a computer (Cricket Graph, Cricket Software, Philadelphia, PA, used on a Macintosh Plus Computer, Apple Computer, Cupertino, CA), to give an equation of the form $y = a \cdot 10^{-bx}$ where a = y-intercept and b = slope. The half-life of the late component of the blood disappearance curve was calculated by dividing (−log 2) by the slope.

RESULTS

Retention of Immunoreactivity Following Fragmentation and Derivatization

Figure 1 shows the curves of immunoreactivity for 59D8 IgG and DTPA-Fab. There is no difference in the immunoreactivity between the two forms. The species crossreactivity studies were in agreement with the results of Matsueda et al. (*10*), indicating that dog fibrin had the best cross-reactivity, and rabbit fibrin the next best.

Labeling Efficiency

The efficiency of labeling DTPA-59D8-Fab with ^{111}In was 88.2 ± 10.9% (mean ± 1 s.d., based on 64 labelings).

Binding to Plasma Clots In Vitro

The binding of [^{111}In]DTPA-59D8-Fab to rabbit, canine and human plasma clots in vitro is given in Table 1. The binding of labeled antibody to pre-formed rabbit clots is ~¾ the magnitude of binding to human pre-formed clots. This indicated that the rabbit may be adequate to use as a model for studies in vivo of this antibody. The extent of uptake of nonspecific antibody was very low.

Thrombus Uptake in Rabbits

These studies were done in an attempt to determine the ability of [^{111}In]DTPA-59D8 Fab to bind to aged thrombi in an animal model. The early images (3 to 4 hr postinjection) showed primarily residual blood-pool activity and the 24-hr images revealed accumulation of radioactivity in the liver, kidneys and urinary bladder, and also in the shoulder joints. Many of the thrombi were visible on images acquired at 4 and 24 hr postinjection (Table 2). Typical images are shown in Figure 2. Our ability to visualize the uptake of labeled antibody in the thrombus depended largely on the location of the thrombus. Unfortunately, many of the thrombi embolized to the lungs, where it was very difficult to detect them so close to the high activity in the liver and heart. However, we were able to detect eight of ten of the thrombi which remained in the neck, even ones which were 96 hr old. Images of rabbits injected with control antibody showed primarily blood pool, liver,

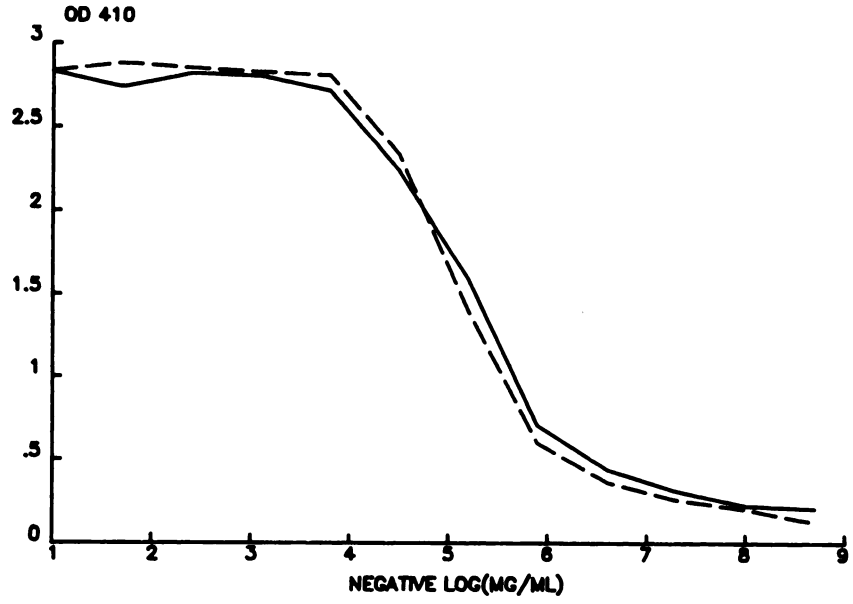

FIGURE 1
Immunoreactivity of 59D8 antifibrin antibody. ——— IgG; ---- DTPA-Fab fragment.

TABLE 1
Uptake of [^{111}In]Antifibrin Antibody by Plasma Clots In Vitro

Species	% Bound to clot* Forming clots	Pre-formed clots
Human	75 ± 4	12 ± 1
Rabbit	22 ± 3	9 ± 2
Canine	75 ± 0.4	10 ± 1
Human (control Ab)†	5.7 ± 0.1	1.3 ± 0.6

* Mean of 3 ± s.d.
† R11D10 antimyosin monoclonal antibody

and kidneys, with no discernable uptake in the region of the thrombus.

Table 3 lists the thrombus-to-blood ratios of [^{111}In] DTPA-59D8-Fab and [^{125}I]fibrinogen as a function of thrombus age. All of the ratios were rather low, despite the focal appearance of some of the thrombi in the neck; however, the ratios for [^{125}I]fibrinogen were also lower than expected. The uptake of antibody was comparable to that of fibrinogen at all times and was better than fibrinogen in thrombi 72 and 96 hrs old. In the control studies, administration of radiolabeled nonspecific antibody did not result in focal uptake at the site of the thrombus, and thrombus-to-blood ratios were found to be <1.0 (Table 2).

Thrombus Uptake Studies in Dogs

Because 59D8 antibody has excellent crossreactivity with canine fibrin, and because the information to be gained from the rabbit studies was limited owing to the poorer species cross-reactivity with rabbit fibrin, thrombus uptake studies were also carried out in dogs. Aged thrombi over 24 hr old were not attempted in dogs, however, because previous experience had shown that the dog's active fibrinolytic system made it difficult to study older thrombi (*21*).

The coils did not move from their initial placement positions because they were firmly wedged in place. The coils were completely enclosed in occlusive thrombus.

The thrombus-to-blood ratios obtained in the dogs are given in Table 4. The ratios, typically ~7 or 8:1, are all high enough to permit easy visualization in the extremities. However, accumulation of activity in the urinary bladder and surgical site made it difficult to distinguish thrombus uptake in those thrombi which were near the groin. Figures 3 and 4 illustrate examples of the appearance of images. Thrombi were clearly visible in six of eight animals at 24 hr postinjection. In Dogs 1 and 2, the coils were placed too close to the body and were obscured in the image by bladder activity; however, it is felt that these thrombi would have been visualized if they had been more distal. The uptake was also clearly apparent at 3–4 hr postinjection in Dogs 6, 7, and 8 (Fig. 3). The thrombi in the other dogs were visible at 24 hours but not at 3–4 hr; this is probably because they were located in areas with a great deal of blood background (Dogs 3 and 4), or very small thrombi (Dog 5).

TABLE 2
Ability to Visualize Uptake of [^{111}In]DTPA-59D8-Fab in Rabbit Thrombi and Emboli

	No. visualized/no. of lesions 4 hr	24 hr	at either time
Jugular vein thrombi	5/10	6/8	8/10 (two embolized)
Pulmonary emboli	0/10	2/12	3/12
In heart	0/1	0/1	0/1

As in the rabbit studies, the early images (3–4 hr) also showed blood-pool activity and uptake in the liver and kidneys. At 24 hr, the popliteal lymph nodes (*22*) and knee joints were also visible.

The values for the uptake of control antibody [^{111}In] DTPA-R11D10 in dogs are given in Table 4. The thrombus-to-blood ratios are low, indicating that the observed uptake of [^{111}In]DTPA-59D8 reflects specific binding of the antibody to fibrin. Images of the dog following injection of [^{111}In]chloride showed a pattern of bone and joint and lymph node uptake that was consistent with the appearance of the ^{111}In-labeled antibody images at 24 hr, suggesting that symmetrical knee and lymph node activity may be an indication of the formation of indium-labeled metabolites similar to the compounds formed after injection of indium chloride.

Absolute Uptake

The absolute uptake of each tracer in thrombi in rabbits and dogs are given in Table 5. Fibrinogen had a higher absolute uptake than 59D8-Fab in all cases.

Blood Disappearance Rate of [^{111}In]DTPA-59D8-Fab

The curves of disappearance of ^{111}In and ^{125}I activity from the plasma of one dog are plotted in Figure 5. It illustrates that the clearance of [^{111}In]DTPA-59D8-Fab from plasma is much more rapid than fibrinogen. The clearance curves were biphasic. When the half-times for the longer-lived component of the curves from all six dogs were averaged, the half-time (± 1 s.d.) was 8.46 ± 1.39 hr for [^{111}In]DTPA-59D8-Fab compared with 27.1 ± 4.0 hr for [^{125}I]fibrinogen. The average R for the fit was 0.99 for 59D8-Fab and 0.97 for Fbg. If one assumes that plasma volume is 35 ml/kg body weight, approximately 40% of the injected dose of 59D8 is still circulating at 3 hr postinjection, and ~8% is still circulating at 24 hr.

DISCUSSION

We have shown that ^{111}In-labeled DTPA-59D8-Fab has affinity for clots in vitro and for thrombi in two

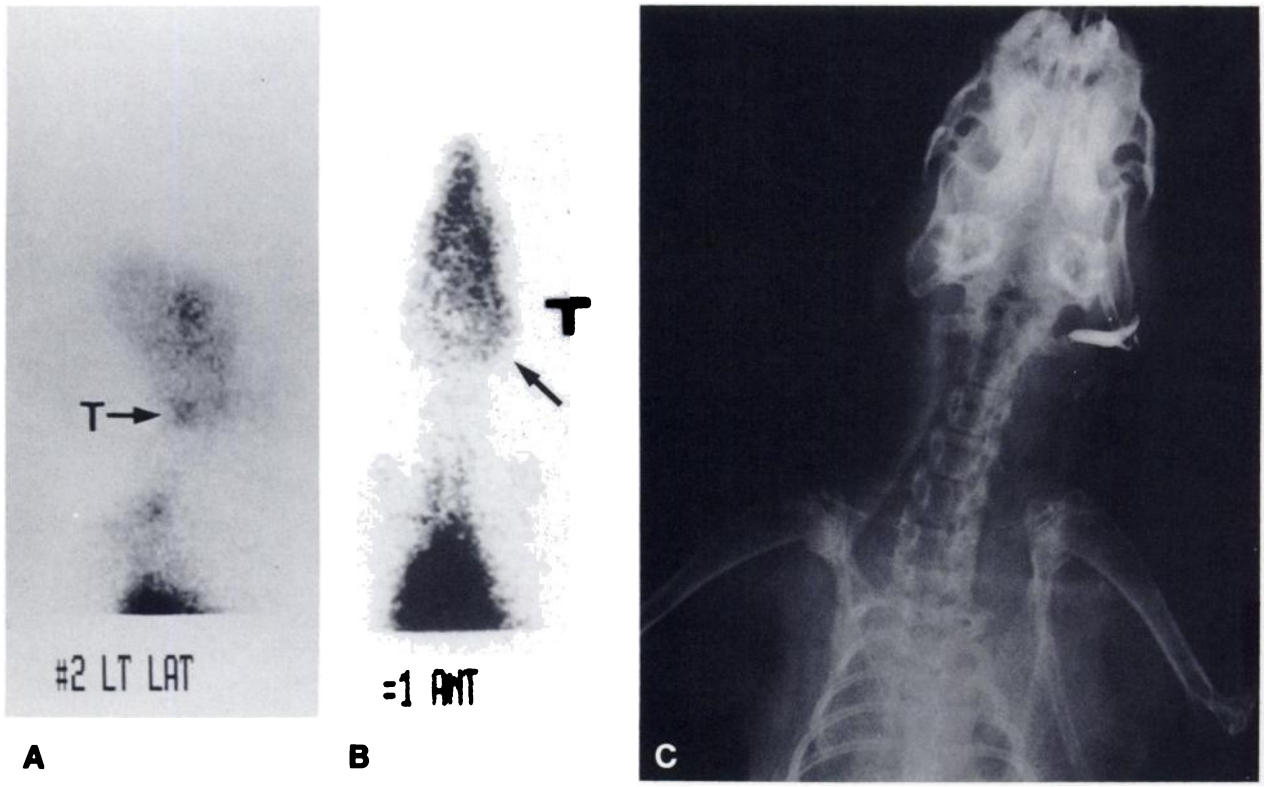

FIGURE 2
Images of a rabbit with a thrombus (T) in its jugular vein. The thrombus was aged for 96 hr before injection of the antibody. A and B: Left lateral and anterior gamma camera images taken at 24 hr postinjection demonstrate focal uptake at the site of the thrombus (arrow). C: An x-ray shows the location of the trapped iron particles.

animal models. The uptake in the thrombi in vivo was high enough to permit visualization by external imaging at 24 hr, and in some cases at 3–4 hr postinjection.

The antibody studied in this work has been shown to be highly specific for binding to fibrin in vitro and ex vivo (*10*). Even with highly specific binding, however, prolonged circulation of unbound radioactive species in the blood is a barrier to satisfactory imaging of thrombi. One early study of anti-fibrinogen antibody in animals (*2*) showed that the use of a goat-anti-rabbit antibody could quickly remove labeled rabbit-anti-human fibrin antibody from the circulation without affecting the thrombus-bound radioactivity, thus rapidly improving the target-to-background ratio. The Fab fragment used in these studies would be expected to have a faster rate of blood disappearance than intact IgG (*23*). Thus, the use of the radiolabeled Fab fragment makes earlier imaging possible without the need for the injection of a second antibody to reduce the blood background. In addition, Fab fragments should be less immunogenic than IgG (*23*).

TABLE 3
Uptake of [^{111}In]DTPA-59D8-Fab by Thrombi in Rabbits

Thrombus age	n*	Mean thrombus-to-blood ratio ± s.d. at 24 hr postinjection		
		[^{111}In]59D8-Fab	^{111}In-control†	[^{125}I]fibrinogen
1–5 hr	4	1.19 ± 0.42		0.94 ± 0.44
24 hr	15	2.17 ± 0.84		1.32 ± 1.09
48 hr	10	2.15 ± 1.02		0.93 ± 0.67
72 hr	11	1.23 ± 0.47		0.30 ± 0.11
96 hr	5	1.22 ± 0.46		0.53 ± 0.19
48 hr	3		0.94 ± 0.08	0.48 ± 0.16
96 hr	1		0.49	1.3

* Number of thrombi.
† R11D10 antimyosin monoclonal antibody, nonspecific for fibrin.

In these studies, the antibody fragment was labeled with ^{111}In, which has physical characteristics superior to the more common radiolabel for antibodies, ^{131}I: the physical half-life is shorter, there is no beta emission, the emitted gamma rays are of more suitable energies and are emitted in high yield. In addition, ^{111}In can be attached quantitatively to the DTPA-antibody without requiring additional purification.

Rosebrough et al. (*7*) studied the thrombus uptake of an ^{131}I-labeled monoclonal antibody (MAb/T2G1s, *24*) which may have the same epitope as 59D8. In a dog model similar to the one reported here, Rosebrough et al. found that the antibody achieved thrombus-to-blood ratios averaging 8.4 at 48 hr postinjection. We have observed comparable thrombus-to-blood ratios at

TABLE 4
Uptake of [^{111}In]DTPA-59D8-Fab by Thrombi in Dogs

Dog ID	Thrombus age	Thrombus-to-blood ratio* at 24 hr			Weight (mg)
		[^{111}In]59D8-Fab	Control	[^{125}I]fibrinogen	
D-1	30 min	8.7		13.7	110
D-2	30 min	7.3		10.5	141
D-3	30 min	7.6		7.4	86
D-4	30 min	4.8		5.4	282
D-5	24 hr	8.4		7.7	13
D-6	24 hr	5.6		5.3	102
D-7	24 hr	7.5		2.3	29
D-8	24 hr	6.7		n.d.	37
D-A	30 min		0.48†	7.3	201
D-B	30 min		0.73†	8.8	155
D-C	30 min		0.71‡	n.d.	153

* Counts per gram thrombus/counts per gram whole blood.
† ^{111}In-labeled antimyosin monoclonal antibody, nonspecific for fibrin.
‡ [^{111}in]chloride.

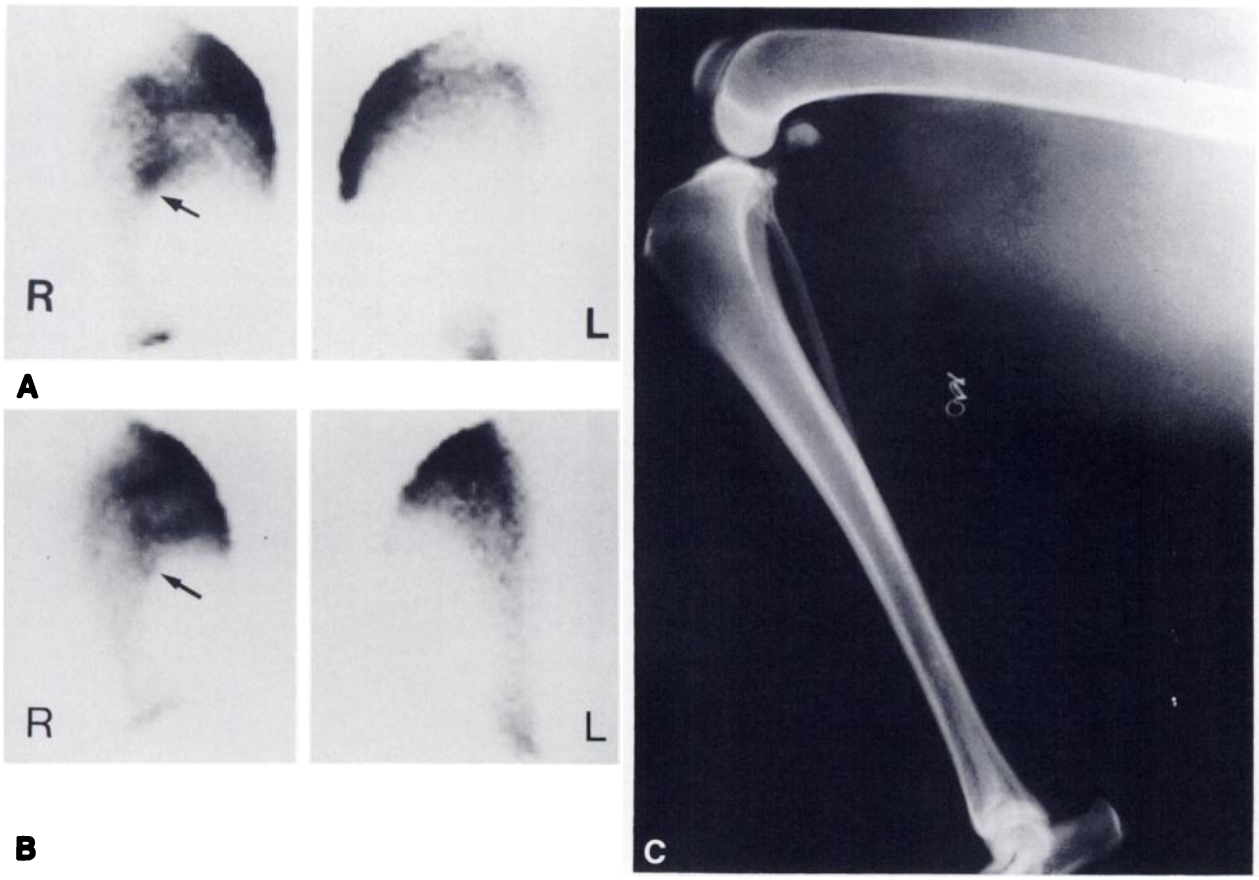

FIGURE 3
Gamma camera images of Dog 6 with a thrombus in the right leg demonstrate focal uptake in the site of the thrombus (indicated by arrow) but no apparent uptake in the same location on the control side (image at the same intensity settings). A: 24 hr postinjection. B: 4 hr postinjection. C: An x-ray shows the location of the coil.

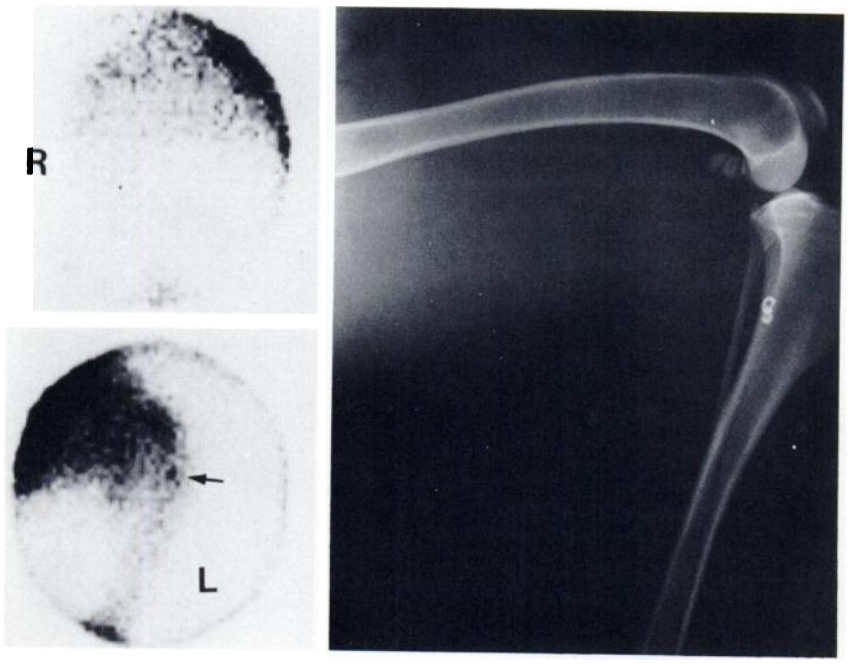

FIGURE 4
Images of Dog 5. A: Anterior gamma camera images taken 24 hr postinjection demonstrate focal uptake of [^{111}In]DTPA-59D8-Fab in the site of the coil (bottom, indicated by arrow) and absence of focal uptake in the same location on the control side (top). B: X-ray showing the position of the coil.

24 hr postinjection. This difference may be due to the longer residence time of their antibody in the blood: at 24 hr, 59% of the administered radioactivity was still circulating, compared with [^{111}In]DTPA-59D8-Fab (~8% still circulating).

Other radiolabeled antibodies have also recently been reported for use in thrombus defection. A radiolabeled antibody which binds specifically to platelets has been used to label platelets in vivo and trace their incorporation into thrombi in dogs (*25,26*). It is known that labeled platelets are incorporated primarily into fresh thrombi, however (*21*), so this technique still has the limitation of not being able to detect aged thrombi.

Other radiopharmaceuticals such as radiolabeled fragment E_1 (*27*), which is not based on monoclonal antibodies, are being evaluated as probes for both fresh and aged thrombi (*28,29*). In contrast to the fibrin-specific monoclonal antibodies, fragment E_1 has a more rapid rate of blood disappearance (faster than Fab fragments), recognizes fibrin dimers or strands but not fibrin monomer, and achieves a higher thrombus-to-blood ratio in the same length of time. Fragment E_1 has the disadvantages that it must be isolated from human blood products, and that it is susceptible to degradation in vivo so that the binding of radioactivity to a thrombus is of limited duration.

In this work, we have attempted to choose the best animal models suitable for the antibody and for the

TABLE 5
Absolute Thrombus Uptake by 24 hr Postinjection

Species	Thrombus age	% ID/gram (mean ± s.d.) [^{111}In]Fab	[^{125}I]Fbg
Dog	30 min	0.074 ± 0.061	0.430 ± 0.23
Dog	24 hr	0.081 ± 0.031	0.333 ± 0.176
Rabbit	3.5–4.5 hr	0.127 ± 0.015	0.371 ± 0.009
Rabbit	24 hr	0.115 ± 0.047	0.186 ± 0.073
Rabbit	48 hr	0.117 ± 0.064	0.239 ± 0.126
Rabbit	72 hr	0.053 ± 0.026	0.108 ± 0.031
Rabbit	96 hr	0.063 ± 0.006	0.204 ± 0.197
		Control antibody*	
Rabbit	48 hr	0.048 ± 0.005	0.171 ± 0.057
Dog	30 min	0.008	0.375

*Antimyosin monoclonal antibody.

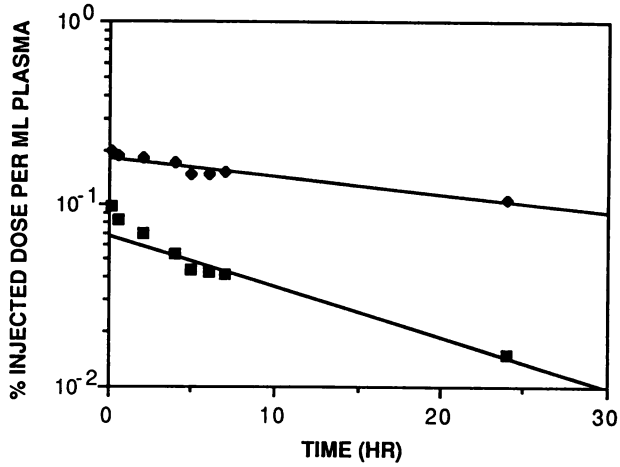

FIGURE 5
Blood disappearance curves in a dog. (■– – –■) [^{111}In] 59D8-Fab; (♦– – – –♦) [^{125}I]fibrinogen. Exponentials were fitted to the data after 30 min: [^{111}In]Fab ($T_{1/2}$ = 10.8 hr; R = 0.98) [^{125}I]Fbg ($T_{1/2}$ = 31.7 hr; R = 0.95).

measurements being made. The species crossreactivity studies showed that dog fibrin had the highest crossreactivity with the antibody (*10*), but dogs have an active fibrinolytic system, making it difficult to study aged thrombi. Other reports using the coil model in dogs claim to achieve thrombi several days old but expressed concern about whether the coil model was continuously thrombogenic (*30*). We judged rabbit fibrin to have adequate crossreactivity for studying the antibody in rabbits, based on species crossreactivity studies and clot uptake studies in vitro (Table 1). The iron particles/magnet method of inducing thrombi in rabbits was chosen for its lack of external surgical site, but we do not have conclusive evidence as to its suitability as a model of aged thrombi. The relatively high absolute uptake of fibrinogen in this model several days after thrombus induction (Table 5) suggest that this model may also be continuously thrombogenic. Further studies of the antibody in a different model of aged thrombi are needed.

The use of radiolabeled fibrinogen as a positive control not only permits evaluation of the continued fibrin deposition in the thrombus, but also permits correlation of the thrombus uptake of a new radiotracer with a standard tracer which has previously been used successfully in man for thrombus imaging (*31,32*). The data reported here suggest that [^{111}In]DTPA-59D8-Fab should be at least as good as iodinated fibrinogen for producing an adequate thrombus-to-blood ratio in man. The absolute uptake of fibrinogen was greater than antibody in all groups of thrombi tested, however, suggesting that the more rapid blood disappearance rate of the antibody is important in its ability to achieve comparable thrombus-to-blood ratios.

The focal uptake of [^{111}In]DTPA-59D8-Fab in areas of thrombi was clearly visible by 24 hr, when the thrombus was located well away from liver or bladder, which accumulated significant amounts of ^{111}In. Although uptake was occasionally visible at 3–4 hr, the image quality was hampered by residual blood pool.

The uptake of radioactivity observed in the knee joints is not unusual for ^{111}In (*33*) and may reflect the breakdown of the labeled antibody by the liver to release the radioactivity in some other form, perhaps as [^{111}In] transferrin, which has affinity for erythroid precursors in the bone marrow (*33*). It is known that small amounts of ^{111}In can exchange from DTPA-proteins while in the circulation, and the extent of this has been estimated to be 2% (*34*) to 9% (*35*) per day. No free ^{111}In was injected into the animals, because DTPA was added to each labeled antibody preparation to bind up any free ^{111}In before injection. Previous studies have observed the same pattern of ^{111}In uptake in knee joints in dogs, as well as the uptake by the popliteal lymph node following injection of [^{111}In]platelets (*21*). The symmetrical nature of the ^{111}In joint activity, however, was easily recognized and should therefore not be confused with vascular thrombi. Further, this uptake may be more pronounced in dogs than it is in humans.

In conclusion, we observed that the ^{111}In-labeled Fab fragment of 59D8 monoclonal antibody binds to thrombi in animal models with sufficiently high thrombus-to-blood ratios to permit imaging at 24 hr, and sometimes at 4 hours post injection. Therefore, this labeled antibody should bind to thrombi in man, permitting external imaging.

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REFERENCES

1. Knight LC. Thrombus-localizing radiopharmaceuticals. In: Fritzberg A, ed. *Radiopharmaceuticals: progress and clinical perspectives*. Boca Raton: CRC Press, 1986.
2. Spar IL, Goodland RL, Schwartz SI. Detection of preformed venous thrombi in dogs by means of ^{131}I-labeled antibodies to dog fibrinogen. *Circ Res* 1965; 17:322–329.
3. Reich T, Reynolds BM, Healy M, Wang MCH, Jacobson JH. Detection of venous thrombosis in the human by means of radioiodinated antifibrin-fibrinogen antibody. *Surgery* 1966; 60:1211–1215.
4. Kramer RS, Ashburn WL, Vasko JS, Morrow AG. Detection of intracardiac thrombi using radio-iodinated antifibrinogen (RIAF) and precordial scanning: experimental and clinical studies. *Ann Surg* 1967; 166:173–182.
5. Spar IL, Perry JM, Benz LL, et al. Detection of left atrial thrombi. Scintillation scanning after administration of ^{131}I rabbit antibodies to human fibrinogen. *Am Heart J* 1969; 78:731–739.
6. Bosnjakovic VB, Jankovic BD, Horvat J, and Cvoric J. Radiolabelled anti-human fibrin antibody: a new thrombus-detecting agent. *Lancet* 1977; 452–454.
7. Rosebrough SF, Kudryk B, Grossman ZD, et al. Radioimmunoimaging of venous thrombi using iodine-131 monoclonal antibody. *Radiology* 1985; 156:515–517.
8. Krohn KA, Knight LC, Harwig JF, Welch MJ. Differences in the sites of iodination of proteins following four methods of radioiodination. *Biochim Biophys Acta* 1977; 490:497–505.
9. Knight LC, Harwig SSL, Welch MJ. In vitro stability and in vivo clearance of fibrinogen or serum albumin labeled with ^{77}Br, ^{131}I, or ^{125}I by direct or indirect synthetic methods. *J Nucl Med* 1977; 18:282–288.
10. Hui KY, Haber E, Matsueda GR. Monoclonal antibodies to a synthetic fibrin-like peptide bind to human fibrin but not fibrinogen. *Science* 1983; 222:1129–1132.
11. Ey PL, Prowse SJ, Jenkins CR. Isolation of pure IgG, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. *Immunochem* 1978;

15:429–436.
12. Matsueda GR, Margolies MN. Structural basis for the species selectivity of a fibrin-specific monoclonal antibody. *Biochem* 1986; 25:1451–1455.
13. Khaw B-A, Mattis JA, Melincoff G, Strauss HW, Gold HK, Haber E. Monoclonal antibody to cardiac myosin: Imaging of experimental myocardial infarction. *Hybridoma* 1984; 3:11–23.
14. Hnatowich DJ, Layne WW, Childs RL, Lanteigne D, Davis MA. Radioactive labeling of antibody: A simple and efficient method. *Science* 1983; 220:613–615.
15. Shen V, Hetzel KR, Ice RD. Radiochemical purity of radiopharmaceuticals using Gelman Seprachrom ITLC chromatography. Technical bulletin 32, Gelman Instrument Co, Ann Arbor, MI, 1975.
16. Paik CH, Ebbert MA, Murphy PR, et al. Factors influencing DTPA conjugation with antibodies by cyclic DTPA anhydride. *J Nucl Med* 1983; 24:1158–1163.
17. Ratnoff OD, Menzie C. A new method for the determination of fibrinogen in small samples of plasma. *J Lab Clin Med* 1951; 37:316–320.
18. Grossman ZD, Wistow BW, McAfee JG, et al. Platelets labeled with oxine complexes of Tc-99m and In-111. Part 2. Localization of experimentally induced vascular lesions. *J Nucl Med* 1978; 19:488–491.
19. Knight LC, Budzynski AZ, Olexa SA. Radiolabeling of fibrinogen using the lodogen technique. *Thromb Haemostas (Stuttgart)* 1981; 46:593–596.
20. Chuang VP, Wallace S, Gianturco C. A new improved coil for tapered-tip catheter for arterial occlusion. *Radiology* 1980; 135:507–509.
21. Knight LC, Primeau JL, Siegel BA, Welch MJ. Comparison of In-111-labeled platelets and iodinated fibrinogen for the detection of deep vein thrombosis. *J Nucl Med* 1978; 19:891–894.
22. Evans HE, Christensen GC. Chapter 13: The lymphatic system. In: *Miller's anatomy of the dog.* Philadelphia: W.B. Saunders, 1979.
23. Haber E. Antibodies in cardiovascular disease and therapy. *Hospital Practice* 1986; 147172.
24. Kudryk B, Rohoza A, Ahadi M, Chin J, Wiebe ME. Specificity of a monoclonal antibody for the NH_2-terminal region of fibrin. *Mol Immunol* 1984; 21:89–94.
25. Som P, Oster ZH, Zamora PO, et al. Radioimmunoimaging of experimental thrombi in dogs using technetium-99m-labeled monoclonal antibody fragments reactive with human platelets. *J Nucl Med* 1986; 27:1315–1320.
26. Oster ZH, Srivastava SC, Som P, et al. Thrombus radioimmunoscintigraphy: An approach using monoclonal antiplatelet antibody. *Proc Natl Acad Sci* 1985; 82:3465–3468.
27. Olexa SA, Budzynski AZ. Binding phenomena of unique plasmic degradation products of human cross-linked fibrin. *J Biol Chem* 1979; 254:4925–4932.
28. Knight LC, Olexa SA, Malmud LS, Budzynski AZ. Specific uptake of radioiodinated fragment E_1 by venous thrombi in pigs. *J Clin Invest* 1983; 72:2007–2013.
29. Knight LC, Maurer AH, Robbins PS, Malmud LS, Budzynski AZ. Fragment E_1 labeled with 1-123 in the detection of venous thrombosis. *Radiology* 1985; 156:509–514.
30. Rosebrough SF, Grossman ZD, McAfee JG, et al. Aged venous thrombi: radioimmunoimaging with fibrin-specific monoclonal antibody. *Radiology* 1987; 162:575–577.
31. Charkes ND, Dugan MA, Maier WP, et al. Scintigraphic detection of deep-vein thrombosis with 1-131-fibrinogen. *J Nucl Med* 1974; 15:1163–1166.
32. DeNardo S, DeNardo GL. Iodine-123-fibrinogen scintigraphy. *Semin Nucl Med* 1977; 7:245–251.
33. Lilien DL, Berger HG, Anderson DP, Bennett LR. ^{111}In-chloride: a new agent for bone marrow imaging. *J Nucl Med* 1973; 14:184–186.
34. Yeh SM, Meares CF, Goodwin DA. Decomposition rates of radiopharmaceutical indium chelates in serum. *J Radioanal Chem* 1979; 53:327–336.
35. Hnatowich DJ, Griffin TW, Kosciuczyk C, et al. Pharmacokinetics of an indium-111-labeled monoclonal antibody in cancer patients. *J Nucl Med* 1985; 26:849–858.