by the Isotope Pharmacy, Copenhagen, Denmark. I. Rosenbaum is gratefully acknowledged for her advice concerning the manuscript.

This project was supported by grants from the Wolhardt-Warrer Foundation, The Foundation of Mr. & Mrs. Lauritz Peter Christensen, The Foundation of Mrs. Olga Bryde Nielsen, The medical research foundation for region 3 Denmark, The Foundation of Senior Lieutenant H. Jensen and his wife, and The Kathrine and Vigo Skovgaard Foundation.

REFERENCES

1. Knight LC. Radiopharmaceuticals for thrombus detection. *Semin Nucl Med* 1990;10:52–67.
2. Oster ZH, Srivastava SC, Som P et al. Thrombus radioimmunoscintigraphy: an approach using monoclonal antiplatelet antibody. *Proc Natl Acad Sci USA* 1985;82:3465–3468.
3. Rosebrough SF, Kudryk BJ, Grossman ZD et al. Radioimmunoimaging of venous thrombi using iodine-131 monoclonal antibody. *Radiology* 1985;156:515–517.
4. Knight LC, Maurer AH, Ammar IA, et al. Evaluation of indium-111-labeled anti-fibrin antibody for imaging vascular thrombi. *J Nucl Med* 1988;29:494–502.
5. Goldenberg DM, Sharkey RM, Ford E. Anti-antibody enhancement of iodine-131 anti-CEA radioimmunodetection in experimental and clinical studies. *J Nucl Med* 1987;28:1604–1610.
6. Andrew SM, Pimm MV, Perkins AC, Baldwin RW. Comparative imaging and biodistribution studies with an anti-CEA monoclonal antibody and its $F(ab)_2$ and Fab fragments in mice with colon carcinoma xenografts. *Eur J Nucl Med* 1986;12:168–175.
7. Tromholt N, Hesse B, Folkenborg O, Selmer J, Nielsen NT. Immunoscintigraphic detection of deep venous thrombophlebitis in the lower extremities with indium-111 labeled monoclonal antibody against tissue plasminogen activator. *Eur J Nucl Med* 1991;18:321–325.
8. Fuchs HE, Berger Jr H, Pizzo SV. Catabolism of human tissue plasminogen activator in mice. *Blood* 1985;65:539–544.
9. Rijken DC, Otter M, Kuiper J, Berkel TJC. Receptor-mediated endocytosis of tissue-type plasminogen activator (t-PA) by liver cells. *Thromb Res* 1990;10(suppl X):63–71.
10. Tanswell P, Seifried E, Su PCAF, Feuerer W, Rijken DC. Pharmacokinetics and systemic efforts of tissue-type plasminogen activator in normal subjects. *Clin Pharmacol Ther* 1989;46:155–162.
11. Korninger C, Stassen JM, Collen D. Turnover of human extrinsic (tissue-type) plasminogen activator in rabbits. *Thromb Haemostas* 1981;46:658–661.
12. Richardson AP, Mountford PJ, Heyderman E, Coakley AJ. Optimization and batch production of DTPA-labelled antibody kits for routine use in In-111 immunoscintigraphy. *Nucl Med Commun* 1987;8:347–356.
13. Kuiper J, Otter M, Rijken DC, van Berkel TJC. Characterization of the interaction in vivo of tissue-plasminogen activator with liver cells. *J Biol Chem* 1988;263:18220–18224.

EDITORIAL

Immunoscintigraphy of Thrombi

Noninvasive detection and localization of thrombi remains a major challenge in clinical diagnosis. To date, clinical investigations have mainly been carried out with radiolabeled monoclonal antibodies (Mabs), which are either specific for platelets or fibrin. In order to be clinically useful for immunoscintigraphy of thrombi, such Mabs should fulfill the following criteria:

1. They should bind with high affinity to an epitope which occurs at a high density at the thrombus surface.
2. The epitope should be highly or totally thrombus-specific.
3. Epitope exposure should be independent of thrombus age and composition.
4. Antibody binding should not be affected by anticoagulant or antiplatelet drugs.
5. The antibody should not be affected by radioisotope labeling procedures.

Received Sept. 3, 1991; accepted Sept. 3, 1991.
For reprints contact: P. Holvoet, Center for Thrombosis and Vascular Research, University of Leuven, Campus Gasthuisberg, O and N, Herestraat 49, B-3000 Leuven, Belgium.

The first report on successful thrombus radioimmunoimaging was published in 1985 (*1*). The 7E3 antibody enabled imaging of experimental venous and arterial thrombi 1.5–2.0 hr after injection and thus fulfilled the first criterion of a clinically useful antibody-based thrombus-specific agent (*2*). Binding of 7E3 to the platelet IIb/IIIa glycoprotein complex in the thrombus resulted in thrombus-to-blood ratios as high as 15 at 60–90 min after injection of the radiolabel, as a result of rapid accumulation of ^{99m}Tc-labeled 7E3 at the thrombus surface and a surprisingly rapid clearance of radiolabeled antibodies with an effective half-life of 20 min. However, because of its extensive cross-reactivity with circulating unactivated platelets, 7E3 does not fulfil the second criterion of an optimal antibody-based thrombus-specific agent. Furthermore, accumulation of 7E3 occurred only at the surface of growing thrombi, whereas maturation of thrombi resulted in the disappearance of the binding sites for 7E3. Consequently, only 1-day-old thrombi could be imaged. Finally, the binding of 7E3 to thrombi was greatly hampered in the presence of anticoagulant agents.

More recently, Mabs specific for activated platelets have been produced. Iodine-131-labeled KC_4, a Mab directed against an epitope in PAD-GEM (platelet activation-dependent granule-external membrane protein) (*3*) allowed detection of deep venous thrombi in femoral veins of baboons at 1 hr postinjection. Recently, ligand-induced binding site-specific antibodies (LIBS) have been described (*4*), which are specific for epitopes in the glycoprotein IIb/IIIa complex after its interaction with Arg-Gly-Asp-containing ligands such as fibrinogen or vitronectin. Such antibodies should discriminate between resting and activated platelets, but data on their use for thrombus imaging are presently not available.

The first Mab specific for fibrin, 59D8, was developed by Hui et al. (*5*), using a synthetic fibrin-like peptide representing the NH_2-terminal sequence of the beta-chain of fibrin exposed after removal of fibrinopeptide B by thrombin. A similar antibody, T_2G_{1s} was developed by Kudryk et al. (*6*). T_2G_{1s} enabled visualization of thrombi that were several days old, indicating that antibody binding sites were not affected by thrombus maturation (*7*). However, these antibodies are directed against an epitope that is removed early during fibrinolysis. Another fibrin-specific Mab, GC_4 (*8*), directed to an epitope on the D-fragment of fibrin that is exposed after digestion with plasmin, appears to be less sensitive to partial clot dissolution. Indeed, maturation of the thrombi for 24 hr resulted in an increased ratio, from 9 at 3 hr to 12 at 24 hr, with GC_4, but a decreased ratio, from 9 at 3 hr to 5 at 24 hr, with T_2G_{1s}. Surprisingly, heparin enhanced the uptake of GC4, resulting in a clot-to-blood ratio of 24 at 24 hr, but did not affect the binding of T_2G_{1s}.

In an experimental rabbit model, the binding of MA-15C5, a Mab specific for human fragment D-dimer of cross-linked fibrin, was not altered by maturation of human plasma clots for up to 72 hr before injection of radiolabeled antibody nor by heparin anticoagulation (*9*). All fibrin-specific antibodies allowed imaging of thrombi only 24 hr after injection of antibody as a result of the slow clearance of free antibody from the circulation. However, serial imaging at different time periods, between 10 min and up to 18 hr postinjection, allowed detection of deep venous thrombi in patients with an accuracy of 82% at 3 hr using ^{111}In-labeled anti-fibrin antibody AF. The accuracy increased to 92% at 18 hr postinjection. The sensitivity for diagnosis of deep venous thrombosis was not decreased by heparin therapy (*10*).

Several approaches to increase the clearance of labeled antibodies have been investigated. First, ^{99m}Tc-labeled Fab fragments with an effective half-life of 3 hr result in a 1.6-fold increased effective clearance rate and do not bind to Fc receptors in the liver, bone marrow, lymph nodes and spleen. Fab fragments, however, usually have a lower affinity than intact IgG and a higher diffusion rate resulting in higher background activity in tissues. A recombinant single-chain M_r 25,000 Fv fragment, comprising only the variable domains of the Mab MA-15C5 was found to bind to immobilized fragment D-dimer with an affinity constant similar to that of the intact antibody, but was cleared 40-fold faster (*11*). Provided the kinetics of binding to the thrombus of the Fv fragment and the intact antibody are comparable, the effective half-life of ^{99m}Tc-labeled Fv fragments should be less than 1 hr, which would allow more rapid visualization of a thrombus. However, Fv fragments suffer from similar disadvantages as mentioned above for Fab fragments.

The clearance of circulating radiolabeled antibodies could also be increased with the intravenous administration of human anti-murine antibodies (*12*). Thus, 15 min after the injection of 15–30 mg of human anti-murine antibodies, only 25% of the radiolabeled murine Mab persisted in blood.

In this issue of the *Journal*, Tromholt and Selmer (*13*) used yet another approach to thrombus imaging. The ^{111}In-labeled antibody, F_2, specific for tissue-type plasminogen activator (t-PA), was used to demonstrate focal fibrinolytic activity at the thrombus site. However, the high background activity, due to circulating antibody posed a major problem. Therefore, t-PA was injected shortly after the antibody administration with the aim to form antigen-antibody complexes, which would be rapidly cleared. More than 99% of the circulating antigen-antibody complexes were indeed eliminated by the liver within 10 min. Biologic background reduction caused by injection of t-PA thus allowed localization of a deep vein thrombus in the right crural region in one patient.

Although that result is most encouraging, a number of questions need to be resolved to establish the clinical applicability of this technique. First, the thrombus-specificity of the antibody specific for t-PA is questionable. Indeed, t-PA is not only present at the surface of a thrombus, but also at the surface of endothelial cells (*14*). Furthermore, t-PA-mediated fibrinolysis constitutes a physiologic mechanism which protects against thrombosis at the vessel wall-blood interface. Injection of 5 mg of t-PA following injection of radiolabeled antibody resulted in an increase of the thrombus-to-blood ratio from 1.2 to 1.6. This increase is surprisingly low, considering that 99% of the background radioactivity was cleared, which would have been anticipated to result in a 10-fold increase of the thrombus-to-blood ratio. Possibly, fibrin-bound t-PA/antibody complexes could have been removed from the thrombus by competing free t-PA.

Potential improvements of the imaging technique of Tromholt and Selmer might comprise the use of Fab fragments (with a higher effective clearance rate) and ^{99m}Tc instead of ^{111}In (with a 3-fold higher merit number, defined as the ratio of the signal versus the square root of the background, and a 5-fold shorter effective half-life). In conclusion, although the approach to lower the background radioactivity by injection of excess antigen might prove to be effective, its value as a useful diagnostic approach needs to be further evaluated.

P. Holvoet
D. Collen
Center for Thrombosis and Vascular Research,
University of Leuven
Leuven, Belgium

REFERENCES

1. Oster ZH, Srivastava SC, Som P, et al. Thrombus radioimmunoscintigraphy: an approach using monoclonal antiplatelet antibody. *Proc Natl Acad Sci USA* 1985;82:3465–3468.
2. Oster ZH, Som P. Of monoclonal antibodies and thrombus-specific binding. *J Nucl Med* 1990;31:1055–1057.
3. Palabrica TM, Furie BC, Konstam MA, et al. Thrombus imaging in a primate model with

antibodies specific for an external membrane protein of activated platelets. *Proc Natl Acad Sci USA* 1989;86:1036–1040.
4. Frelinger AL, Cohen I, Plow EF, et al. Selective inhibition of integrin function by antibodies specific for ligand-occupied receptor conformers. *J Biol Chem* 1990;265:6346–6352.
5. 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.
6. Kudryk BJ, Rohoza A, Ahadi M, et al. Specificity of a monoclonal antibody for the NH_2-terminal region of fibrin. *Mol Immunol* 1984;21:89–94.
7. Rosebrough SF, Grossman ZD, McAfee JG, et al. Aged venous thrombi: radioimmunoimaging with fibrin-specific monoclonal antibody. *Radiology* 1987;162:575–577.
8. Rosebrough SF, McAfee JG, Grossman ZD, et al. Thrombus imaging: a comparison of radiolabeled GC4 and T_2G_{1s} fibrin-specific monoclonal antibodies. *J Nucl Med* 1990;31:1048–1054.
9. Holvoet P, Stassen JM, Hashimoto Y, et al. Binding properties of monoclonal antibodies against human fragment D-dimer of cross-linked fibrin to human plasma clots in an in vivo model in rabbits. *Thromb Haemostas* 1989;61:307–313.
10. De Faucal P, Peltier P, Planchon B, et al. Evaluation of indium-111-labeled antifibrin monoclonal antibody for the diagnosis of venous thrombotic disease. *J Nucl Med* 1991;32:785–791.
11. Laroche Y, Demaeyer M, Stassen JM, et al. Characterization of a recombinant single-chain molecule comprising the variable domains of a monoclonal antibody specific for human fibrin fragment D-dimer. *J Biol Chem* 1991;266:16343–16349.
12. Stewart JSW, Sivolapenko GB, Hird V, et al. Clearance of ^{131}I-labeled murine monoclonal antibody from patients blood by intravenous human anti-murine immunoglobulin antibody. *Cancer Res* 1990;50:563–567.
13. Tromholt N, Selmer J. Biological background subtraction improves immunoscintography by subsequent injection of antigen. *J Nucl Med* 1991;32:2318–2321.
14. Russell ME, Quertermous T, Declerck P, et al. Binding of tissue-type plasminogen activator with human endothelial cell monolayers. *J Biol Chem* 1990;265:2569–2575.