24. Rodwell JD. Engineering monoclonal antibodies. *Nature* 1989;342:99–100.
25. Holvoet P, Collen D. Immunoscintigraphy of thrombi. *J Nucl Med* 1991; 32:2321–2323.
26. Nedelman MA, Shealy DJ, Boulin R, et al. Rapid infarct imaging with a technetium-99m-labeled antimyosin recombinant single-chain Fv: evaluation in a canine model of acute myocardial infarction. *J Nucl Med* 1993;34:234–241.
27. Cerqueira MD, Stratton JR, Vracko R, Schaible TF, Ritchie JL. Noninvasive arterial thrombus imaging with ^{99m}Tc monoclonal antifibrin antibody. *Circulation* 1992;85:298–304.
28. Knight LC. Do we finally have a radiopharmaceutical for rapid, specific imaging of venous thrombosis? *J Nucl Med* 1991;32:791–794.
29. Francis CW, Marder VJ. A molecular model of plasmic degradation of crosslinked fibrin. *Semin Thromb Hemost* 1982;8:25–35.
30. Loscalzo J, Rocco TP. Imaging arterial thrombi. *Circulation* 1992;85:382–385.
31. Boniface GR, Lee FT, Milner LJ, et al. Pharmacokinetics of radiolabeled anti-fibrin Mab DD-3B6/22 and factors effecting its localisation to thrombi. In: Maddalena DM, Snowdon GM, Boniface GR, eds. *Advances in radiopharmacology*. Wollongong: University of Wollongong Press; 1989:26–36.
32. Whitaker AN, Elms MJ, Masci PP, et al. Measurement of cross-linked fibrin derivatives in plasma: an immunoassay using monoclonal antibodies. *J Clin Pathol* 1984;37:882–887.
33. Perkins AC, Lonsdale RJ. Monoclonal antibodies for cell labelling with particular reference to thrombus imaging. In: Baum RP, Cox PH, Hör G, Buraggi GL, eds. *Clinical use of antibodies*. London: Kluwer Academic Publishers; 1991:111–120.
34. Dewerchin M, Lijnen HR, Van Hoef B, De Cock F, Collen D. Biochemical properties of conjugates of urokinase-type plasminogen activator with a monoclonal antibody specific for cross-linked fibrin. *Eur J Biochem* 1989; 185:141–149.
35. Knight LC. Radiopharmaceuticals for thrombus detection. *Semin Nucl Med* 1990;20:52–67.
36. 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.

EDITORIAL

Thrombus-Specific Imaging: Approaching the Elusive Goal

The importance of developing a thrombus-specific imaging method is a goal of undisputed clinical importance. Whereas thrombus formation after vascular injury is cardinal in hemostasis, it is also the cause of unwanted effects due to occlusions and emboli in the arterial and venous circulation. Early detection and treatment of pathological intravascular thrombosis is effective. The high incidence of side effects from thrombolytic therapy, however, necessitates accurate identification of patients who will not benefit from such treatment.

A noninvasive, rapid and specific method for thrombus detection which will also enable monitoring thrombus dissolution is therefore highly desirable. Such a method should preferably allow imaging of arterial and venous thrombi of various ages and help determine whether a thrombus is still amenable to thrombolytic therapy. This topic has been reviewed intensively, including mesenteric ischemia and thrombolysis (*1–6*).

The search for thrombus-specific imaging agents began two decades ago when radioiodinated fibrinogen was first evaluated (*7*). Since then, a plethora of thrombus imaging agents have been described, including agents that are incorporated into thrombi and agents that bind to components of previously formed thrombi (*7–29*).

Radiolabeled platelets (*8*) and antiplatelet antibodies will bind to forming thrombi (*14,15,19*), antifibrin antibodies (*16,20–23*), anti-activated platelet antibodies (*24,25*) and active or inactivated tissue type plasminogen activator (t-PA) (*26–28*) will bind to formed, older thrombi.

Theoretically, agents targeted to forming thrombi should be suitable for imaging fresh propagating thrombi. In addition, an agent may show preferential binding to a thrombus type based on the abundance of that specific factor in either arterial or venous thrombi. Thus, radiolabeled platelets or antiplatelet antibodies will display higher affinity for arterial thrombi that are rich in platelets, while venous preformed thrombi will probably be better imaged with agents that bind to fibrin, antifibrin antibodies or by native or inactivated t-PA.

Although venous thrombosis and embolism occur frequently, resulting in a high incidence of morbidity and mortality, arterial thrombosis is of greater concern. Thrombus formation on atherosclerotic plaques may result in complete occlusion of coronary arteries, leading to critical and often fatal infarction. Although imaging arterial thrombosis is of great importance, little success has been achieved to date (*14,15,29–31*). This disappointing situation may have resulted from the use of compounds with inadequate thrombus-to-blood ratios and small-size arterial thrombi, particularly in the coronary circulation.

The greatest success has been achieved in imaging venous thrombosis with monoclonal antifibrin antibodies. The reports in the literature include antibodies that react with the NH_2 group on the alpha (*32*) or beta terminus (*16,33–34*), on the D-domain of the noncrosslinked fibrin (*35*), on the fibrin D-domain after plasmin digestion (*36*) or on cross-linked DD-dimer regions (*37*). In the current issue of the *Journal*, Bautovich et al. report human studies using an antifibrin antibody directed against cross-linked human fibrin dimer (*38*). Bautovich et al. offer convincing evidence of the safety, feasibility and efficacy of the monoclonal antifibrin antibody method. By using a Fab' fragment preparation labeled with ^{99m}Tc, thrombus imaging was accomplished 2–6 hr after injection, a significant improvement over previously reported compounds that required 6–24 hr of preparation before successful imaging. Moreover, this method demonstrated pulmonary emboli. False-negative

Received Nov. 2, 1993; revision accepted Nov. 18, 1993.
For correspondence or reprints contact: Prantika Som, DVM, Medical Department, Brookhaven National Laboratory, Bldg. 490, Upton, NY 11973.

scans might have resulted from epithelialization of thrombi due to the long interval between the thrombophlebitis-confirming studies (IPG, contrast venography) and scintigraphy. Nevertheless, the antifibrin antibody method deserves large scale clinical trials.

Other preliminary studies indicate that an alternative for the use of antibodies may soon become practical. Small peptides with platelet affinity labeled with ^{99m}Tc become incorporated into thrombi (*39,40*). These small peptides with specific amino acid sequences are more advantageous than complex, large molecular weight antibodies or fragments since they can be produced synthetically and are therefore less expensive. They are minimally antigenic and there is little danger of viral contamination, resulting in less stringent procedural requirements. Finally, blood clearance of peptides is fast when compared to antibodies. In the future, these preparations may replace many of the antibody methods. Correlative studies with antibodies and peptides will determine the outcome of this prediction.

The results of the studies of Bautovich et al. clearly indicate that similar efforts towards developing methods for arterial thrombus imaging should be made (*31,41*).

Prantika Som
Zvi H. Oster
Brookhaven National Laboratory
Upton, New York
Suny at Stony Brook
Stony Brook, New York

REFERENCES

1. Oster ZH, Som P. New perspective in thrombus-specific imaging: Radiolabeled monoclonal antibodies. *AJR* 1989;152:253–260.
2. Grossman ZD. Radionuclide thrombus imaging: an idea whose time has come? *Radiology* 1989; 173:22.
3. Oster ZH, Som P. Of monoclonal antibodies and thrombus-specific imaging [Editorial]. *J Nucl Med* 1990;31:1055–1058.
4. Knight LC. Scintigraphic methods for detecting vascular thrombus. *J Nucl Med* 1993;34:554–561.
5. Oster ZH, Som P, Zamora PO. Mesenteric vascular occlusion: a new diagnostic method using a radiolabeled monoclonal antibody reactive with platelets. *Radiology* 1989;171:636–656.
6. Wang G-J, Oster ZH, Som P, et al. A monoclonal antibody reacting with platelets for monitoring thrombolysis. *Nucl Med Biol* 1991;18:275–280.
7. Kakkar VV, Nicolaides AN, Renny JT, et al. Iodine-125-labelled fibrinogen test adapted for routine screening for deep-vein thrombosis. *Lancet* 1970;1:540–542.
8. Thakur ML, Welch MJ, Joist JH, et al. Indium-111-labeled platelets: studies on preparation and evaluation of in vitro and in vivo function. *Thromb Res* 1976;9:345–357.
9. Knight LC, Maurer AH, Robins PS. Fragment E labeled with I-123 in the detection of venous thrombus. *Radiology* 1985;156:509–514.
10. Harwig SL, Harwig JF, Sherman LA, et al. Radioiodinated plasminogen: an imaging agent for pre-existing thrombi. *J Nucl Med* 1977;18: 42–45.
11. Persson BRR, Darte L. Labeling plasmin with technetium-99m for scintigraphic localization of thrombus. *Int J Rad Appl Instrum[A]* 1977;28: 97–104.
12. Som P, Rhodes BA, Bell WR. Radiolabeled streptokinase and urokinase and their comparative biodistribution. *Thromb Res* 1975;6:247–253.
13. Holvoet P, Collen D. Immunoscintigraphy of thrombi [Editorial]. *J Nucl Med* 1991;32:2321–2322.
14. 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.
15. 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.
16. Rosebrough SF, Kudrik B, Grossman ZD, et al. Radioimmunoimaging of venous thrombi using iodine-131 monoclonal antibody. *Radiology* 1985;156:515–517.
17. Alavi A, Palevsky HI, Gupta N, et al. Radiolabeled antifibrin antibody in the detection of venous thrombosis: preliminary results. *Radiology* 1990;175:79–85.
18. Schaible TF, Alavi A. Antifibrin scintigraphy in the diagnostic evaluation of acute deep venous thrombosis. *Semin Nucl Med* 1991;21:313–324.
19. Peters AM, Lavender JP, Needham SG, et al. Imaging thrombus with radiolabeled monoclonal antibody to platelets. *Br Med J* 1986;293: 1525–1527.
20. Jung M, Kletter K, Dudczak R, et al. Deep vein thrombosis: scintigraphic diagnosis with In-111-labeled monoclonal antifibrin antibodies. *Radiology* 1989;173:469–475.
21. Walker KZ, Milner LG, Bautovich GJ, et al. Detection of experimental thrombi in rabbits with an ^{131}I-labeled fibrin-specific monoclonal antibody. *Eur J Nucl Med* 1990;16:787–794.
22. Rosebrough SF, McAfee JG, Grossman ZD, et al. Thrombus imaging: a comparison of radiolabeled GC4 and T2Gls fibrin-specific monoclonal antibodies. *J Nucl Med* 1990;31:1048–1054.
23. Scopinaro F, Di Loreto M, Banci M, et al. Anti-D dimer monoclonal antibodies: a possible scintigraphic agent for immunodetection of thrombi. *Nucl Med Commun* 1992;13:723–729.
24. 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.
25. Miller DD, Boulet AJ, Fermin OT, et al. In vivo technetium-99m S12 antibody imaging of platelet alpha-granules in rabbit endothelial neointimal proliferation after angioplasty. *Circulation* 1991;83:224–236.
26. Fry ETA, Mack DL, Monge JC, et al. Labelling of human clots in vitro with an active mutant of t-PA. *J Nucl Med* 1990;30:187–191.
27. Tromholt N, Selmer J. Biological background subtraction improves immunoscintigraphy by subsequent injection of antigen. *J Nucl Med* 1991;32:2318–2321.
28. Ord JM, Hasapes J, Dugherty A, et al. Imaging of thrombi with tissue-type plasminogen activator rendered enzymatically inactive and conjugated to a residualizing label. *Circulation* 1992; 85:288–297.
29. Chouraqui P, Maddahi J, Fung P, et al. Rapid in vivo imaging of arterial thrombi by Tc-99m labeled anti-activated platelet monoclonal antibody [Abstract]. *J Nucl Med* 1991;32:1005.
30. Berridge DC, Perkins AC, Frier M, et al. Detection and characterization of arterial thrombus using a platelet-specific monoclonal antibody (P256Fab'). *Br J Surg* 1991;78:1130–1133.
31. Cerqueira MD, Stratton JR, Vracko R, et al. Noninvasive arterial thrombus imaging with ^{99m}Tc monoclonal antifibrin antibody. *Circulation* 1992;85:298–304.
32. Scheefers-Borchel U, Muller-Berghaus G, Fuhge P, et al. Discrimination between fibrin and fibrinogen by a monoclonal antibody against a synthetic peptide. *Proc Natl Acad Sci USA* 1985;82:7091–7095.
33. Hui KY, Haber E, Matsueda GR. Monoclonal antibodies to a synthetic fibrin-like peptide bind to human fibrin but not to fibrinogen. *Science* 1983;222:1129–1132.
34. Kudryk B, 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.
35. Gargan PE, Ploplis VA, Scheu JD. A fibrin specific monoclonal antibody which interferes with the fibrinolytic effect of the tissue plasminogen activator. *Thromb Haemost* 1988;59:426–431.
36. Rosebrugh SF, McAfee JG, Grossman ZD, et al. Immunoreactivity of ^{111}In and ^{131}I fibrin-specific monoclonal antibody used for thrombus imaging. *J Immunol Methods* 1989;169:123–129.
37. Rylatt DB, Blake AS, Scottis LE, et al. An immunoassay for human D dimer using monoclonal antibodies. *Thromb Res* 1983;31:767–778.
38. Bautovich G, Angelides S, Lee F-T, et al. Detection of deep venous thrombi and pulmonary embolus with ^{99m}Tc-DD-3B6/22 anti-fibrin monoclonal antibody Fab' fragment. *J Nucl Med* 1994;35:195–202.
39. Knight LC, Lister-James J, Dean RT, et al. Evaluation of Tc-99m labeled cyclic peptides for thrombus imaging [Abstract]. *J Nucl Med* 1993;34(Suppl):17P.
40. Muto P, Morgano G, Lastoria S, et al. Initial clinical experience with Tc-99m P280, a synthetic peptide useful for imaging thrombi and pulmonary emboli. *Radiology* 1993;189(suppl): 303.
41. Loscalzo J. Imaging arterial thrombi—an elusive goal [Editorial]. *Circulation* 1992;85:382–385.