5. Hand PM, Nuti M, Colcher D, Schlom J. Definition of antigenic heterogeneity and modulation among human mammary carcinoma cell populations using monoclonal antibodies to tumor associated antigens. *Cancer Res* 1983; 43:728–735.
6. Colcher D, Esteban JM, Carrasquillo JA, et al. Quantitative analyses of selected radiolabeled monoclonal antibody localization in metastatic lesions of colorectal cancer patients. *Cancer Res* 1987; 47:1185–1189.
7. DeNardo SJ, DeNardo GL, O'Grady LF, et al. Treatment of a patient with B cell lymphoma by I-131 Lym-1 monoclonal antibodies. *Intl J Biol Markers* 1987; 2:49–53.
8. DeNardo SJ, DeNardo GL, O'Grady LF, et al. Pilot studies of radioimmunotherapy of B-cell lymphoma and leukemia using I-131-Lym-1 monoclonal antibody. *Antibody, Immunoconjugates, and Radiopharmaceuticals* 1988; 1:17–33.
9. Mach J-P, Carrel S, Forni M, Ritschard J, Donath A, Alberto P. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. *N Engl J Med* 1980; 303:5–10.
10. Epstein AL, Chen FM, Taylor CR. A novel method for the detection of necrotic lesions in human cancers. *Cancer Res* 1988; 48:5842–5848.
11. Chen FM, Taylor CR, Epstein AL. Tumor necrosis treatment of ME-180 human cervical carcinoma model with ^{131}I labeled TNT-1 monoclonal antibody. *Cancer Res* 1989; 49:4578–4585.
12. Jones PL, Gallagher BM, Sands H. Autoradiographic analysis of monoclonal antibody distribution in human colon and breast tumor Xenografts. *Cancer Immunol Immunother* 1986; 22:139–143.
13. Pervez S, Eppenetos AA, Mooi WJ, et al. Localization of monoclonal antibody AUA1 and its $F(ab')_2$ fragments in human tumour xenografts: An autoradiographic and immunohistochemical study. *Int J Cancer* 1988; (suppl)3:23–29.
14. Ong GL, Mattes MJ. Penetration and binding of antibodies in experimental human solid tumors grown in mice. *Cancer Res* 1989; 49:4264–4273.
15. Epstein AL, Marder RJ, Winter JN, et al. Two new monoclonal antibodies, Lym-1 and Lym-2, reactive with human B lymphocytes and derived tumors, with immunodiagnostic and immunotherapeutic potential. *Cancer Res* 1987; 47:830–840.
16. McFarlene AS. Labelling of plasma proteins with radioactive iodine. *Biochem J* 1956; 62:135–143.
17. Gaffar S, Epstein AL. Cell based radioimmunoassays to quantitate the immunoreactivity of TNT monoclonal antibodies directed against intracellular antigens. *Hybridoma* 1990: in press.
18. Dvorak HF, Nagy JA, Dvorak JT, Dvorak AM. Identification and characterization of the blood vessels of solid tumors that are leaky to circulating macromolecules. *Am J Path* 1988; 133:95–109.
19. Thomlinson RK, Gray LM. The histological structure of some human lung cancers and possible implications for radiotherapy. *Br J Cancer* 1955; 9:539–549.
20. Jain RK. Delivery of novel therapeutic agents in tumors: physiological barriers and strategies. *J Natl Cancer Inst* 1989; 81:570–576.
21. Majno G, Palade GE, Schoefl GI. Studies on inflammation II. The site of action of histamine and serotonin along the vascular tree: a topographic study. *J Biophys Biochem Cytol* 1961; 11:607–626.
22. Fischman AJ, Rubin RH, Khaw BA, et al. Detection of acute inflammation with ^{111}In-labeled nonspecific polyclonal IgG. *Semin Nucl Med* 1988; XVIII:335–344.
23. Morrel EM, Tompkins RG, Fischman AJ, et al. Autoradiographic method for quantitation of radiolabeled proteins in tissues using indium-111. *J Nucl Med* 1989; 30:1538–1545.
24. Warren BA. The vascular morphology of tumors. In: Peterson HI, ed. *Tumor blood circulation*, Orlando, FL: CRC Press: 1979:77–85.

Editorial: One Step Forward with Nonspecifically-Specific Monoclonal Antibodies

Although the monoclonal antibody technology introduced by Kohler and Milstein (*1*) provided initial "great expectations" in the search for the proverbial "magic bullet," it has proven very difficult to develop clinically useful monoclonal antibodies against human tumors for radioimmunoimaging and radioimmunotherapy. The present state-of-the-art requires different antibodies for different tumor types. Recent clinical trials have offered some encouraging results, but inevitably poor tumor localization significantly limits the efficacy of each new monoclonal antibody.

The problems associated with the use of monoclonal antibodies in tumor detection and therapy are well known. Heterogeneity in the distribution of antibodies may result from nonuniform expression of target tumor markers, irregular tumor vasculature, and aberrant microdiffusion dynamics (*2–5*). Nonuniform disposition of malignant cells within the tumor mass, as well as the variable expression of tumor markers on the tumor cells, and the continuous release of tumor-associated antigens restrict the homogeneous accessibility of specific antibodies (*2,6*). Furthermore, tumor vascular architecture is highly unorthodox and may be comprised of variably perfused areas (*4,7*). Necrotic areas in the tumor mass have sparse vasculature, making them relatively avascular (*4*). Ultrastructural breaches in the vascular mural integrity (*8*) coupled with more hydrophilic and enlarged interstitium, should theoretically, be more conducive to extravasation and diffusion of the macromolecules (*5*). Yet the exaggerated interstitial pressures resulting from the compression of the ever-

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growing tumor-interior (*4*) and the lack of lymphatic drainage (*6*) severely restrict the inward diffusion of macromolecules. Necrotic interstitium, large intervascular distances, and high interstitial pressures result in an unusual delay for diffusion of monoclonal antibodies (*5*). On the other hand, the role of vascular leakiness has been credited as the predominant factor in the nonspecific mechanism of localization of polyclonal human immunoglobulin G (IgG) in certain tumors (*9*), as well as in inflammatory (*10*) and atherosclerotic lesions (*11*). This nonspecific accumulation of human IgG is higher for intact IgG and its Fc fraction than for Fab (*11*). Thus, Fab may have the least nonspecific diffusion properties.

The study in this issue of *The Journal of Nuclear Medicine* by Chen et al. (*12*) is based on the hypothesis that rapidly proliferating tumors contain a sizable population of degenerating or dead cells. The vascular inadequacy and impaired phagocytic response in such tumors should allow accumulation of these degenerating cells. The presence of degenerating cells in large areas of necrosis may offer a means to distinguish rapidly growing malignant tumors (with high cell death rate and inappropriate removal of dead tissue) from normal tissue (with low cell death rate and continuous removal of debris) if an antibody to an insoluble intracellular antigen were used (*13–16*). Accumulation of the radiolabeled antibody at the target necrotic tumor sites should generate a snow-ball effect, whereby more tumor cells are killed. This should lead to exposure of more intracellular antigens which in turn should lead to increased accumulation of the radiolabeled antibody (*17*). In the study of Chen et al., TNT-1 monoclonal antibodies directed against nuclear histone antigens were used to localize tumors hosted in nude mice, which contained necrotic centers (*12*). This concept, using an antibody specific for an insoluble intracellular antigen has been amply documented with myosin-specific antibodies (*18–19*). The intracellular myosin must necessarily be exposed to the extracellularly introduced antimyosin only after myocyte necrosis (*20*). Since myocyte necrosis is an obligatory component of myocardial infarction and myocarditis, the noninvasive scintigraphic diagnostic application of radiolabeled antimyosin Fab can be demonstrated in localization and quantification of myocardial infarction (*21–22*) and in recognition of myocarditis associated with acute dilated cardiomyopathy (*23*) and cardiac transplant rejection (*24*). Radioimmunoimaging of malignant melanoma with an antibody to an intracellular antigen is also based on same principle (*25*).

The study of Chen et al. (*12*) provides three important leads to the understanding of the concept of a generalized tumor localizing agent.

1. Since TNT-1 antibodies are specific for normal insoluble nuclear antigen, which is present in all cells but becomes available to react with the antibody only after tissue necrosis, this antibody has potential as a generalized tumor localizing and therapeutic monoclonal antibody. TNT-1 may be the elusive magic bullet which no tumor can escape, irrespective of malignancy, antigenic-heterogeneity, or modulation. It circumvents the requirements for specific antibodies to tumor-associate antigens for therapy, and promotes the concept of localization of antibodies specific for a common antigen in the tumor mass. However, it is limited by its absolute necessity for the presence of necrotic centers in the tumors.
2. The study contradicts the concept of nonspecific localization of antibodies or other macromolecules in tumor mass. Two antibodies of IgG 2a subclass (LYM-1, TNT-1), used in the present study have shown a differential and characteristic distribution. LYM-1 antibody directed against a surface antigen of Raji cell lymphoma, did not penetrate into the core of ME-180 carcinoma. The lack of nonspecific diffusion of gamma globulins into the tumors is contrary to reported observations (*9*).
3. Significant localization of TNT-1 antibodies occurred in the necrotic tumor zones, an area usually inaccessible to radiolabeled tumor-antigen specific antibodies due to the relative avascularity of the tumor centers (*4*). Tumor-specific antibodies, however, have been demonstrated to react with the target-tumor antigens, predominantly in the better perfused areas of the tumor. Thus, in the necrotic core of the tumors, while restricting nonspecific accumulation of other antibodies due to its avascularity, localization of TNT-1 antibodies is facilitated by antibodies' specificity for exposed antigens of degenerated tumors cells.

Clearly, the hypothesis of Chen et al. has potential. There are also, not unexpectedly, possible drawbacks to their methods which pertain to a broad spectrum of nonspecifically specific TNT-1 antibodies. The major obstacle towards the palatability of the proposed approach is the very concept which makes it so attractive—the self-perpetuating effect of the binding of radiolabeled TNT-1 to histones in necrotic tumors (which should lead to more tumor cell death). According to this effect, localization of TNT-1 even in Raji tumors which contain small and diffuse necrotic foci, should increase with time. The report by Chen et al. (*12*) indicated only that there was "patchy labeling of the deeper parts of the tumors at 2, 3, and 5 days" without indication of whether an increase was seen from Day 2 to 5. Similarly, well-differentiated and slow growing tumors such as the intraductal tumors of the breast, are traditionally accepted to be free of, or to possess only minimal necrotic centers. The use of antibodies such as

TNT-1 in the localization and therapy of these tumors would appear to be limited. Furthermore, it might be assumed from this and other papers from this group, that these antibodies would be able to localize in tiny metastatic foci with tiny necrotic centers. If so, a potential danger exists, since a nonmalignant, normal reticuloendothelial system contains degenerating foci (this would lead to undesirable normal cell death which would be compounded with the passage of time). The ubiquitous presence of lysosomal enzymes in the necrotic zone of tumors may also adversely affect the persistence of the proteinacious vehicles (antibodies) of radioactivity.

Although many questions are left unanswered, the study offers an improved modality for radioimmunoimaging and therapy, and provides a definite step forward.

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REFERENCES

1. Kohler G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256:495–497.
2. Edwards PAW. Heterogeneous expression of cell surface antigens in normal epithelia and their tumors, revealed by monoclonal antibodies. *Br J Cancer* 1985; 51:149–160.
3. Enblad P, Glimelius B, Busch C, et al. Antigenic heterogeneity in adenocarcinomas of rectum and their secondaries. *Br J Cancer* 1987; 55:53–55.
4. Jain RK. Determinants of tumor blood flow. *Cancer Res* 1988; 48:2641–2658.
5. Jain RK. Delivery of novel therapeutic agents in tumors: physiological barriers and strategies. *JNCI* 1989; 81:570–576.
6. Humm JL, Cobb LM. Nonuniformity of tumor dose in radioimmunotherapy. *J Nucl Med* 1990; 31:75–83.
7. Gullino PM. Extracellular compartments of solid tumors. In: Becker FF, ed. *Cancer.* New York: Plenum Press; 1975:327–354.
8. Ludatsher RM, Gellei B, Barzilai D. Ultrastructural observations on the capillaries of human thyroid tumors. *J Pathol* 1979; 128:57–62.
9. Kairemo KJA, Wiklund TA, Liewendahl K, et al. Imaging of soft-tissue sarcomas with indium-111-labeled monoclonal antimyosin F_{ab} fragments. *J Nucl Med* 1990; 31:23–31.
10. Rubin RH, Fischman AJ, Callahan RJ, et al. ^{111}In-labeled nonspecific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989; 321:935–940.
11. Fischman AJ, Rubin RH, Khaw BA, et al. Radionuclide imaging of experimental atherosclerosis with nonspecific polyclonal immunoglobulin G. *J Nucl Med* 1989; 30:1095–1100.
12. Chen FM, Taylor CR, Epstein AL. A comparative autoradiographic study demonstrating differential intratumor localization monoclonal antibodies to cell surface (LYM-1) and intracellular (TNT-1) antigens. *J Nucl Med* 1990; 31:1059–1066.
13. Steel GG. Cell loss as a factor in the growth rate of human tumors. *Eur J Cancer* 1967; 3:381–387.
14. Cooper EH. The biology of cell death in tumors. *Cell Tissue Kinet* 1973; 6:87–95.
15. Cooper EH, Bedford AJ, Kenny TE. Cell death in normal and malignant tissues. *Adv Cancer Res* 1975; 21:59–120.
16. Epstein AL, Chen FM, Taylor CR. A novel method for detection of necrotic lesions in human cancers. *Cancer Res* 1988; 48:5842–5848.
17. Chen FM, Taylor CR, Epstein AL. Tumor necrosis treatment of ME-180 human cervical carcinoma model with ^{131}I-labeled TNT-1 monoclonal antibody. *Cancer Res* 1989; 49:4578–4585.
18. Khaw BA, Beller GA, Haber E, Smith TW. Localization of cardiac myosin-specific antibodies in myocardial infarction. *J Clin Invest* 1976; 58:439–446.
19. Khaw BA, Fallon JT, Beller GA, Haber E. Specificity of localization of myosin specific antibody fragments in experimental myocardial infarction: histologic, histochemical, autoradiographic and scintigraphic studies. *Circulation* 1979; 60:1527–1531.
20. Khaw BA, Scott J, Fallon JT, Cahill SL, Haber E, Homcy C. Myocardial injury: quantitation by cell sorting initiated with antimyosin fluorescent spheres. *Science* 1982; 217:1050–1053.
21. Khaw BA, Gold HK, Yasuda T, et al. Scintigraphic quantification of myocardial necrosis in patients after intravenous injection of myosin-specific antibody. *Circulation* 1986; 74:501–508.
22. Khaw BA, Yasuda T, Gold HK, et al. Acute myocardial infarct imaging with ^{111}Indium-labeled monoclonal antimyosin F_{ab}. *J Nucl Med* 1987; 28:1671–1678.
23. Yasuda T, Palacios IF, Dec GW, et al. Indium-111 monoclonal antimyosin antibody imaging in the diagnosis of acute myocarditis. *Circulation* 1987; 75:306–311.
24. Frist W, Yasuda T, Segall G, et al. Noninvasive detection of human cardiac transplant rejection with ^{111}Indium labeled monoclonal antimyosin (F_{ab}) imaging. *Circulation* 1987; 76:81–85.
25. Welt S, Mattes MJ, Grando R, et al. Monoclonal antibody to an intracellular antigen images human melanoma transplants in nu/nu mice. *Proc Natl Acad Sci USA* 1987; 84:4200–4204.