When Is Too Much Too Much and Yet Not Enough? Alas, a Plethora of Opportunities but Where's the Beef?

Inflammation is common to many diseases and should be regarded as a nonspecific common pathway. There are major roles for imaging in both noninfectious and infectious inflammation. In the former, detection and localization of disease are important, and there is an opportunity to monitor interventions during the development of a new therapy and during the use of an established therapy in the management of an individual patient. In these instances, sensitivity is essential and can be achieved at the expense of specificity. On the other hand, 2 general indications for infection imaging exist: detection and localization of infection in patients with fever of unknown origin, and diagnosis of infection in patients with preexisting localization. In these instances, sensitivity and, importantly, specificity for infection are required. Indeed, in some instances specificity at the level of the infectious agent could be the ultimate goal, as, for example, in the case of the patient with an abscess in whom culture material is not readily available. Among many, Becker (1), and Weiner and Thakur (2) have provided excellent reviews on the topic of radiopharmaceuticals for inflammatory and infectious diseases.

The article by van der Laken et al. (3) in this issue of The Journal of Nuclear Medicine represents another effort to develop an ideal radiopharmaceutical for imaging inflammation. Major categories of radiopharmaceuticals

for inflammation and infection imaging are as follows: ^{67}Ga-citrate transferrin receptor or lactoferritin; labeled white blood cells (WBCs); labeled human polyclonal immunoglobulin G (IgG); labeled antigranulocyte monoclonal antibodies (MAbs); labeled nanocolloids; labeled chemotactic peptide analogs (fMet-Leu-Phe); labeled cytokines (interleukin-1 [IL-1], interleukin-2 [IL-2], and interleukin-8 [IL-8]); labeled receptor ligands (somatostatin); and labeled antibiotics against infection (ciprofloxacin).

Despite enthusiasm for the opportunities that exist to use tracer methodology to understand, and then apply, cytokines in medicine, I believe there are more critical needs than yet another radiopharmaceutical, if scintigraphy is to fulfill its potential in inflammation and infection. The potential of labeled IL-8 for imaging infection and inflammation was investigated by van der Laken in a rabbit model of infection. High accumulation in the infection within a few hours after injection and rapid clearance from the background characterized the biodistribution of IL-8. Accumulation of ^{123}I-IL-8 in the abscess was most likely related to binding of IL-8 to its receptor on neutrophils in the infectious tissue or, alternatively, in the circulation followed by migration of the neutrophils to the site of infection. These characteristics approximate the profile of a radiopharmaceutical that is interesting for application in inflammation. Immediately after injection, IL-8 induced transient leukopenia that was followed by leukocytosis; the leukopenia and leukocytosis were caused mainly by changes in granulocyte counts. The neutropenia has been shown to be caused by cell

stiffening that results in transient trapping of activated neutrophils in capillaries (4), in particular in the microvessels of the lungs (5). A major drawback of IL-8 in the context of diagnosis is its biologic activity. IL-8, and other cytokines, must be labeled at very high specific activities to reduce the likelihood of side effects from these potent "hormones." IL-8 is a peptide with chemotactic and activating effects on neutrophils. It is recognized today as the prototype of a class of tissue-derived, inflammatory, chemotactic cytokine mediators. IL-8 is a member of a family of proinflammatory cytokines that were identified on the basis of their stimulation of neutrophil chemotactic activity. Intracutaneous application of IL-8 induced local exudation and a massive, long-lasting accumulation of neutrophils (6). High concentrations of IL-8 have been observed and correlated with tissue neutrophils in diseases such as rheumatoid arthritis (7), adult respiratory distress syndrome (8), and ulcerative colitis (9). IL-8 targets inflammatory tissue, where massive infiltration of neutrophils is present, by specific receptor binding to these cells. IL-8 and related chemotactic cytokines share properties with C5a, fMet-Leu-Phe, platelet-activating factor (PAF), and leukotriene B$_4$ (LTB$_4$), indicating that they qualify as classical chemotactic agonists.

Labeled cytokines are an interesting class of peptide radiopharmaceuticals. Cytokines act through an interaction with specific cell-surface receptors expressed on known cell populations. Cytokine receptors, usually of high affinity, are generally expressed at low levels on resting cells but their expression can be upregulated during activa-

tion. Cytokines have low molecular weight, rapid half-life and blood clearance, and high-affinity binding to a specific receptor. Only a few have been studied so far for the diagnosis of inflammation and tumors. Biologic activities of potent cytokines may lead to toxicities, even when the cytokine is labeled at high specific activities.

Recently, labeled IL-1α has been shown to accumulate in sterile inflammations and focal infections in mice at high target-to-background ratios through interaction with its receptor (10). Systemic side effects of IL-1 limited its clinical application (10). As a consequence of activation, infiltrating cells express the receptor for IL-2 on their surface membrane (11); labeled IL-2 accumulates at sites of WBC infiltration after binding to IL-2 receptor-expressing cells, particularly activated T-lymphocytes. Studies in humans have shown that ^{123}I-IL-2 can be used for the in vivo detection of activated lymphocytes. Labeled IL-2 might have a role in the management of patients with autoimmune diseases and other chronic diseases characterized by mononuclear cell infiltration. Lymphocytic infiltration has been visualized with labeled IL-2 in patients with thyroiditis (12). In general, cytokines mediate specific biologic activities, most frequently at very low amounts, so that their use for diagnostic purposes in patients is often not possible. In addition, some cytokine receptors are practically ubiquitous.

When tissue damage occurs or a microorganism invades the body, mechanisms are activated to remove the noxious stimulus. Soluble mediators are released and amplify the local response by recruitment from the blood of cells, often granulocytes, and plasma components. Vasodilatation and increased endothelial permeability are induced, which facilitate further extravasation of cells and proteins. Moreover, the expression of adhesion molecules on endothelial cells and leukocytes is stimulated. The process starts within minutes of the injury and usually resolves in hours or days. If the inflammatory agent persists, the infil-

trate becomes predominantly mononuclear, consisting of lymphocytes and cells of the monocyte–macrophage series. When microorganisms infect a tissue, they are sensed by neutrophils through chemoattractants released by the microorganisms or arising from the inflammatory reaction of the infected tissue. Several chemotactic agonists have been identified, including C5a (13) and N-formylmethionyl oligopeptides of bacterial origin, such as fMet-Leu-Phe (14), PAF, and LTB$_4$ (15). Despite major differences in structure and origin, these agonists have similar biologic activities. They stimulate neutrophils through distinct receptors and elicit the functions necessary for antimicrobial defense. In 1991, Fischman et al. (16) first reported the potential of ^{111}In-labeled chemotactic peptide analogs of fMet-Leu-Phe (437 Da) for imaging infection. Subsequently, Babich et al. (17) reported that ^{99m}Tc-labeled chemotactic peptides also localized at the site of infection in the same rat model. Although these reports documented the uptake of labeled peptides at the infection site, early imaging was not optimal. Their specificity for infection and the mechanisms of localization have not been thoroughly documented. fMet-Leu-Phe is produced by bacteria and binds to a specific receptor on the surface membrane of granulocytes; it is rather toxic. Although promising, further studies on their specificity for infection and their toxicity are needed.

Twenty-five years ago, ^{67}Ga-citrate was first introduced to image tumor and inflammatory or infectious lesions. Many mechanisms of localization have been documented. Leukocytes at the site of inflammation or infection excrete some of their intracellular lactoferrin, which remains localized and bound to macrophages. ^{67}Ga-citrate may leak through the vascular epithelium at the site of infection, where it is then bound to the lactoferrin (18). Despite a lack of specificity, ^{67}Ga-citrate is still widely used clinically for the detection of infections (19,20). ^{67}Ga-citrate is characterized by good sensitivity but poor specificity; it accumulates in tumors

and acute, chronic, septic, and sterile inflammatory lesions (21). [67]Ga delivers a high absorbed radiation dose and has unfavorable characteristics for imaging.

WBCs, labeled with ^{111}In or ^{99m}Tc, remain the reference method for inflammation and infection imaging, in spite of disadvantages. After injection, the labeled WBCs specifically migrate into inflamed tissues. The diagnostic accuracy of labeled WBCs has been established in patients with chronic inflammatory bowel disease, osteomyelitis, infected vascular prostheses, kidney diseases, lung infection, and fever of unknown origin (1). The technique shows high diagnostic accuracy for the detection of acute inflammation (1). Although labeled WBCs have had undisputed success in detecting infections and inflammations, there are significant limitations. WBC labeling is a time-consuming process that requires special facilities and technical expertise. Because of these limitations, attempts to develop new radiopharmaceuticals continue.

More recently, the use of labeled nonspecific human immunoglobulins (IgG) has been introduced for inflammation and infection imaging; their use is safe and simple. The mechanism of action is associated in part with increased vascular permeability and in part with binding to the Fc receptor expressed by infiltrating cells (22). They have been labeled with ^{99m}Tc and [111]In. Higher target-to-background ratios have been observed with ^{111}In-IgG than with ^{99m}Tc-IgG; for this reason, ^{111}In-IgG is preferred for the study of chronic inflammation (23). ^{111}In-IgG can be used for many diseases with a diagnostic accuracy comparable with that of WBCs. ^{111}In-IgG appears to be useful in the detection of Pneumocystis carinii pneumonia and other pulmonary infections (24).

Labeled MAbs directed against surface leukocyte antigens or endothelial adhesion molecules have been used for the detection of inflammatory processes. Four MAbs—CEA-47, BW250/183, IMMU-NN3, and MCA-480—have been examined extensively for

abscess or infection detection in patients. The most widely used labeled MAb is the commercially available BW250/183, an IgG1, provided as a ready-to-label kit for ^{99m}Tc labeling. Localization in infection is thought to be mediated in part by migration of antibody-labeled circulating labeled granulocytes and in part by nonspecific extravasation. Noncellular inflammation is also visualized. A novel approach to the detection of inflammation is based on the use of labeled MAbs directed against adhesion molecules. Adhesion molecules, such as intercellular adhesion molecule 1, vascular cell adhesion molecule, and E-selectin (endothelial leukocyte adhesion molecule), are hyperexpressed and promote the migration of WBCs into inflamed tissue. The use of MAbs has several advantages. They are easy to use. They also show good specificity and sensitivity. MAb imaging directed against a specific microorganism has been described for *P. carinii*, with a reported sensitivity of 86% and a specificity of 87% (25). Although impressive, this MAb approach can be used only in a patient population with a high likelihood of having the specific infectious agent. ^{67}Ga-citrate or radiolabeled WBCs, IgG, and whole antibodies require 12–24 h before a diagnosis can be established (26). Radiopharmaceuticals providing a same-day answer are desirable. The advantage of MAb fragments, compared with the whole MAb, is their smaller molecular weight. They penetrate better into tissue, are less immunogenic, clear from the blood more rapidly, and permit earlier diagnosis (27).

Other radiopharmaceuticals provide new possibilities for earlier diagnosis. Nanocolloids—small particles, 30 nm in diameter, produced from albumin—are taken up by the cells of the reticuloendothelial system and are rapidly cleared from the circulation. They leak into inflamed tissues because of increased vascular permeability and accumulate after phagocytosis by macrophages (28). Somatostatin receptors (SSRs) exist on human lymphoid tissue; SSR ligands, such as commer-

cially available Octreoscan (Mallinckrodt Inc., St. Louis, MO), hold promise for autoimmune and chronic inflammatory diseases.

Many diseases are characterized by neutrophil infiltrates without infection. Labeled autologous WBCs migrate into the site of inflammation by diapedesis; in contrast, most radiopharmaceuticals, such as nanocolloids and nonspecific human immunoglobulin, accumulate in the inflammation after nonspecific extravasation. The diagnosis of acute inflammation can be achieved with most currently available radiopharmaceuticals. With most radiopharmaceuticals, it is not possible to distinguish between sterile and infected acute inflammation. Bacterial infection can pose a substantial diagnostic dilemma. Most radiopharmaceuticals pinpoint the site of inflammation but do not distinguish between infection and inflammation. Criteria for radiopharmaceuticals for infection imaging include the following: specific for infection; differentiate infection from inflammation, tumor, and so forth; early localization in and detection of infection; provide high-contrast images; and inexpensive, readily and widely available, and safe.

A new approach for the specific detection of infection is the use of labeled broad-spectrum antibiotics, which accumulate in the target microorganism where they are metabolized. It should not be assumed that an antibiotic must preferentially target infection to be therapeutic! Interesting results have been obtained using a ^{99m}Tc-labeled quinolone, Infecton, which is based on the antibiotic ciprofloxacin. The site of infection could be detected, even before migration of labeled WBCs was evident (29). When the results were compared with those from a labeled WBC study, the concordance rate was 68%. Of 18 discordant results, ^{99m}Tc-Infecton was correctly positive in 8 of 9 positive studies and correctly negative in 4 of 9 negative studies. Four of 5 falsely negative studies were in patients who had taken antibiotics. ^{99m}Tc-Infecton gave better imaging results than did labeled WBCs. Comparison between ^{99m}Tc-Infecton and WBC

imaging gave sensitivities of 84% and 81% and specificities of 96% and 77%, respectively.

Recent advances in our understanding of the pathophysiology of inflammatory and infectious processes at the molecular level, combined with progress in radiopharmaceutical sciences, have provided a plethora of radiopharmaceuticals for the diagnosis of inflammation and infection. Labeled WBCs remain the reference method, but, in the future, alternative approaches may improve the specificity and the ease of using these techniques. A radiopharmaceutical that distinguishes between sterile and septic inflammation is clearly desirable and feasible.

Why are not radiopharmaceuticals more basic to the management of patients with infection and inflammation? Very simply, it is a failure to conduct focused, prospective, outcome-oriented, multicenter trials. This is a failure shared by individuals and organizations and, hopefully, one soon to be addressed. Until such time, an alternative is equally urgent. As small, single-institution published trials continue, systematic syntheses of the results are needed. Meta-analysis is a technique for combining study results to strengthen conclusions about the individual studies when taken as a whole (30). The approach allows determination of the diagnostic efficacy of the modality under study, including overall estimates of accuracy, and the proportion of patient management changes. Meta-analyses of published data for specific radiopharmaceuticals and specific inflammation or infection indications are urgently needed to provide data for decision models. As additional studies become available, these meta-analyses can be updated.

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REFERENCES

1. Becker W. The contribution of nuclear medicine to the patient with infection. *Eur J Nucl Med.* 1995;22:1195–1211.

2. Weiner RE, Thakur ML. Imaging infection/inflammations: pathophysiologic basis and radiopharmaceuticals. *Q J Nucl Med.* 1999;43:2–8.

3. van der Laken CJ, Boerman OC, Oyen WJG, van de Ven MTP, van der Meer JWM, Corstens FHM. Radiolabeled interleukin-8: specific scintigraphic detection of infection within a few hours. *J Nucl Med.* 1999;41:463–469.

4. Worthen GS, Schwab B, Elson EL, Downey GP. Mechanisms of stimulated neutrophils: cell stiffening induces retention in capillaries. *Science.* 1989; 245:183–186.

5. Laterveer L, Lindley IJD, Heemskerk DPM, et al. Rapid mobilization of hematopoietic progenitor cells in Rhesus monkeys by a single intravenous injection of interleukin-8. *Blood.* 1996;87:781–788.

6. Colditz I, Zwahlen R, Baggiolini M. Neutrophil accumulation and plasma leakage induced in vivo by neutrophil-activating peptide-1. *J Leukoc Biol.* 1990;48:129–137.

7. Endo H, Akahoshi T, Takagishi K, Kashiwazaki S, Matsushima K. Elevation of interleukin-8 (IL-8) levels in joint fluids of patients with rheumatoid arthritis and the induction by IL-8 of leukocyte infiltration and synovitis in rabbit joints. *Lymphokine Cytokine Res.* 1991;10:245–252.

8. Miller EJ, Cohen AB, Nagao S, et al. Elevated levels of NAP-1/interleukin-8 are present in the airspaces of patients with the adult respiratory distress syndrome and are associated with increased mortality. *Am Rev Respir Dis.* 1992;146:427–432.

9. Izzo RS, Witkon K, Chen AI, Hadjiyane C, Weinstein MI, Pellechia C. Interleukin-8 and neutrophil markers in colonic mucosa from patients with ulcerative colitis. *Am J Gastroenterol.* 1992;87: 1447–1452.

10. van der Laken CJ, Boerman OC, Oyen WJG, et al. Specific targeting of infectious foci with radioiodinated human recombinant interleukin-1 in an experimental model. *Eur J Nucl Med.* 1995;22:1249–1255.

11. Semenzato G, Zambello R, Pizzolo G. Interleukin-2 receptor expression in health and disease. In: Waxman J, Balkwill F, eds. *Interleukin-2.* Oxford, UK: Blackwell Scientific; 1992:78–105.

12. Chianelli M, Mather SJ, Procaccini EC, et al. In vivo detection of lymphocytic infiltration in thyroid autoimmune diseases by 99mTc-IL-2 [abstract]. *Eur J Nucl Med.* 1996;23:1055P.

13. Fernandez HN, Henson PM, Otani A, Hugli TE. Chemotactic response to human C3a and C5a anaphylatoxins. I. Evaluation of C3a and C5a leukotaxis in vitro and under stimulated in vivo conditions. *J Immunol.* 1978;120:109–115.

14. Schiffmann E, Corcoran BA, Wahl SM. N-formyl-methionyl peptides as chemoattractants for leukocytes. *Proc Natl Acad Sci USA.* 1975;72:1059–1062.

15. Baggiolini M, Dewald B, Thelen M. Effects of PAF on neutrophils and mononuclear phagocytes. *Prog Biochem Pharmacol.* 1988;22:90–105.

16. Fischman AJ, Pike MC, Kroon D, et al. Imaging focal sites of bacterial infection in rats with indium-111-labeled chemotactic peptide analogs. *J Nucl Med.* 1991;32:483–491.

17. Babich JW, Graham W, Barrow SA, et al. Technetium-99m-labeled hydrozino derivatized chemotactic peptide analogs for imaging focal sites of bacterial infection. *J Nucl Med.* 1993;34:1964–1974.

18. Sohn M, Jones B, Whitting JJ, et al. Distribution of gallium-67 in normal and hypotransferrinemic tumor-bearing mice. *J Nucl Med.* 1993;34:2135–2143.

19. Halpern S, Hagan P. Gallium-67-citrate imaging in neoplastic and inflammatory diseases. In: Freeman LM, Weissman H, eds. *Nuclear Medicine Annual.* New York, NY: Raven Press; 1980:219–265.

20. Lavender JP, Lowe J, Barker JR, Burn JI, Chaudri MA. Gallium-67 citrate scanning in neoplastic and inflammatory lesions. *Br J Radiol.* 1971;44:361–366.

21. Borman T, Johnson R, Sherman F. Gallium scintigraphy for the diagnosis of septic arthritis and osteomyelitis in children. *J Pediatr Orthop.* 1986;6: 317–325.

22. Fischman A, Rubin R, White J, et al. Localization of Fc and Fab fragments of nonspecific polyclonal IgG at focal sites of inflammation. *J Nucl Med.* 1990;31:1199–1203.

23. Oyen WJG, Classens RAMJ, van der Meer JWM, Corstens FHM. Biodistribution and kinetics of radiolabeled proteins in rats with focal infection. *J Nucl Med.* 1992;33:388–394.

24. Khalkhali I, Mena I, Rauh DA, Diggles LE, Pham HL, Mason GR. 111-Indium-DTPA-IgG lung imaging in patients with pulmonary and HIV infection. *Chest.* 1995;107:1336–1341.

25. Goldenberg DM, Sharkey RM, Udem S, et al. Immunoscintigraphy of *Pneumocystis carinii* pneumonia in AIDS patients. *J Nucl Med.* 1994;35:1028–1034.

26. McAfee JG, Gagne G, Subramanian G, Schneider RF. The localization of indium-111-leukocytes, gallium-67 polyclonal IgG and other radioactive agents in acute focal inflammatory lesions. *J Nucl Med.* 1991;32:2126–2131.

27. Becker W, Goldenberg G, Wolf F. The use of monoclonal antibodies and antibody fragments in the imaging of infectious lesions. *Semin Nucl Med.* 1994;24:1–13.

28. Schrijver MD, Streule K, Senekowitsch R, Fridrich R. Scintigraphy of inflammation with nanometer-sized colloidal tracers. *Nucl Med Commun.* 1987;8: 895–908.

29. Vinjamuri S, Hall AV, Solanki KK, et al. Comparison of 99mTc Infecton imaging with radiolabeled white-cell imaging in the evaluation of bacterial infection. *Lancet.* 1996;347:233–235.

30. Gambhir SS. Decision analysis in nuclear medicine. *J Nucl Med.* 1999;40:1570–1581.