Leukocyte Receptor-Binding Radiopharmaceuticals for Infection and Inflammation Scintigraphy

On pages 786–793 of this issue of *The Journal of Nuclear Medicine,* van Eerd et al. (*1*) present the results of their investigations into the localization mechanisms of ^{111}In-labeled leukotriene B4 antagonist (DPC11870), a recently developed radiopharmaceutical (*2*) for infection and inflammation scintigraphy. Belonging to the growing group of receptor-binding radiopharmaceuticals, designed to label leukocytes in vivo, DPC11870 is distinguished by high affinity to the leukotriene B4 receptor that is abundantly expressed on the granulocyte cell surface. The rationale for in vivo labeling is to alleviate the labor intensity and the hazard of cross infection during the handling of autologous blood in the course of ^{111}In and ^{99m}Tc ex vivo white blood cell (WBC) labeling. The genesis of this group can be traced to the development (about 2 decades ago) of monoclonal antibodies raised against an antigen present on leukocytes. That initial effort (*3*) eventually culminated in the recent clinical introduction of ^{99m}Tc-fanolesomab in the United States. It soon became clear that, all other factors being equal, using smaller carrier molecules should be better, for they are generally easier to manufacture, radiolabel, and deliver to the target, and they clear from the nontarget tissues by faster excretion. The initial success in that pursuit was marked by the development of an antibody fragment with good binding to antigen-90 on the granulocyte surface (*4*). At about the same time, small peptide radiopharmaceuticals began to stimulate interest (*5*). An improved understanding of small peptide interactions at sites of inflammation and infection has created a fertile knowledge base of such radiopharmaceutical candidates, whereas advances in peptide synthesis and radiolabeling chemistry made this area of research into a fruitful endeavor (*6*). Even smaller nonpeptide leukocyte-binding molecules, such as the frontrunner DPC11870, soon received attention, holding yet greater promise for infection and inflammation scintigraphy (*7*). The ultimate catalyst for progress in this field, however, is the clinical demand for accurate and easy-to-use radiopharmaceuticals specifically designed for particular clinical presentations of suspected infection or inflammation.

An understanding of granulocyte kinetics is an essential prerequisite for the proper interpretation of the work done by van Eerd et al. (*1*) or, for that matter, any investigation that concerns WBC scintigraphy. Three physiologic compartments are involved in such kinetics: first, the pool in the marrow undergoing development and release with average granulocyte residence of 10 d; second, the total blood pool, which comprises the circulating and so-called marginating granulocyte subpools; and third, the graveyards within which the blood granulocytes are physiologically destroyed at a rate that leads to their entire replacement within the circulation every 10 h. The inflammatory lesion itself constitutes the fourth compartment into which granulocytes from the second compartment migrate. Because the bone marrow is an important component of both the second and the third compartments (pooling granulocytes to the same extent as the spleen and destroying them with an efficiency comparable to that of the spleen and liver), it has a more complex kinetic role in the setting of myeloid-targeting agents, such as ^{111}In-DPC11870, compared with cells conventionally labeled ex vivo. Therefore, it is not surprising that ^{111}In-DPC11870 shows prominent bone marrow uptake, reflecting the location of the great majority (>80%) of the myeloid cell mass and indicating that the LTB4 receptor is expressed early in granulocyte maturation. For the same reason, the monoclonal antibodies to granulocyte receptors have to some extent all given prominent images of bone marrow and indeed at one stage were exploited for imaging cancer metastases in the marrow that predate bone secondaries. Circulating granulocytes would be preferentially targeted if the radiopharmaceutical were directed toward a receptor that is expressed only on mature cells. Indeed, the ideal granulocyte-seeking agent would avidly target only cells that had migrated across the vascular endothelium and express a unique postmigration receptor. This seems to be an element in the repertoire of ^{99m}Tc-sulesomab (*8*) and effectively is the mechanism underlying ^{18}F-FDG uptake in inflammation. The kinetics of ^{111}In-DPC11870 are not, therefore, straightforward, and 2 important considerations emerge. First, one would not expect similar kinetics for cells labeled in vivo and for those labeled with the compound ex vivo because the role of the first compartment is eliminated with respect to the latter. Second, proof of the concept of labeled cells trafficking from compartment 1 to the lesion,

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via compartment 2, critically depends on sequential blood sampling and demonstration that the activity is firmly cell bound rather than free to move to a higher-avidity receptor. The alternative hypothesis, that the changing organ radioactivity is the result of ^{111}In-DPC11870 redistribution between compartments, remains a possibility. On the other hand, if we were to have had evidence for internalization of the LTB4 receptor-ligand complex after binding to ^{111}In-DPC11870, thus promoting stable binding of the ^{111}In, it would have supported the concept of cell-bound radionuclide trafficking from marrow to lesion. Another factor is the fate of ^{111}In-DPC11870 when a granulocyte carrying it is phagocytosed in the reticuloendothelial system or by macrophages in the inflammatory lesion or undergoes disintegration to become incorporated in pus.

As more information becomes available concerning ^{111}In-DPC11870, it can be measured against the requirements of an ideal inflammation- or infection-seeking radiopharmaceutical. These include, first, high and rapid uptake in the target; second, long enough residence in the target to allow for optimal imaging; third, low affinity for nontarget organs and tissues (especially within regions of clinical interest); fourth, rapid blood clearance to improve the target-to-nontarget ratio; fifth, a lesional uptake proportional to the inflammatory activity or to the concentration of live infectious microorganisms; sixth, suitability for a ^{99m}Tc (or ^{18}F) kit formulation; seventh, uncomplicated labeling that allows low-hazard handling and administration; eighth, long shelf life of the cold kit; and ninth, an acceptable cost. It should, of course, adhere to basic radiopharmaceutical principles, including absence of pharmacologic effects at diagnostic administered activities, lack of immunologic responses, and acceptable radiation dosimetry.

Another backdrop for evaluating a new radiopharmaceutical is the realization that infection and inflammation do not have a homogeneous clinical presentation, nor do they have a single arrangement of molecular and cellular interaction at their active focus. Hence, different clinical patterns would be best studied by a particular kind of radiopharmaceutical with a mechanism of action based on a corresponding pathophysiology and circumstances created by the background tissues. For example, a patient with granulocytopenia and spiking fever would not be a good candidate for an ex vivo labeled WBC scan. In this clinical presentation it would be better to use a radiopharmaceutical with uptake based on increased vascular permeability at the focus of infection, such as ^{67}Ga-citrate (*9*), the oldest nonspecific inflammation- and infection-seeking radiopharmaceutical. Although clearly violating the first, third, fourth, and fifth requirements of the perfect radiopharmaceutical, ^{67}Ga-citrate nevertheless remains useful after nearly 3 decades, especially in the diagnostic evaluation of suspected spinal osteomyelitis, immunocompromised host, and fever of unknown origin (*9*). For most indications, however, ^{111}In and ^{99m}Tc ex vivo–labeled WBCs have replaced ^{67}Ga-citrate, especially in the areas of abdominal and diabetic foot infection. Interference by physiologic uptake of a radiopharmaceutical in the bone marrow is another complication that either requires compensation by concomitant bone marrow imaging or selection of a radiopharmaceutical that has insignificant bone marrow localization. The latter option is exemplified by the use of ^{99m}Tc-ciprofloxacin in suspected infection of orthopedic prostheses (*10*) and spinal osteomyelitis (*11*). As our knowledge of mechanisms involved in infection and inflammation continues to grow, so will the sophistication of the design and selection of newer radiopharmaceuticals.

The first 2 requirements for an ideal radiopharmaceutical are most relevant to sensitivity and are fulfilled to varying degrees by all currently available agents. ^{111}In-DPC11870 accumulates in the abscess gradually, allowing good visualization by 8 h in the animal model (*1*). However, the image contrast and radiopharmaceutical concentration in the abscess were significantly better at 24 h, well suited to the choice of ^{111}In as the label. It is well known that in patients imaged with ^{111}In-chelate–labeled WBCs, early imaging has a somewhat lower detection rate and must be combined with imaging at 24 h. Sensitivity of early imaging was better with more acute and intense infections (*12,13*), as would be predicted for ^{111}In-DPC11870. When this new agent is tested in clinical trials, it will be important to establish the sensitivity for detecting infectious foci at different times after administration and the effect of comparing images acquired early and late after administration and in different types of infections (e.g., acute vs. chronic).

The third requirement addresses radiopharmaceutical specificity, of which 2 types need to be recognized. The first is the ability of a radiopharmaceutical to distinguish a focus of inflammation, caused by either a sterile or an infectious process, from normal tissue uptake or accumulation in other pathologic sites (such as recent fracture or activated bone marrow). The second is the ability of a radiopharmaceutical to accumulate on the basis of avidity to a viable infectious microorganism, rather than in reactive inflammation. All clinically available radiopharmaceuticals, including ^{111}In-chelate–labeled WBCs, lack adequate infection specificity. ^{111}In-DPC11870 is similar in this respect, as it has previously been shown to accumulate in experimental inflammatory (noninfectious) colitis (*14*). On the other hand, an ability to specifically distinguish infection from sterile inflammation would be useful in clinical practice. The earlier hope that ^{99m}Tc-ciprofloxacin may possess infection specificity (*15*) did not materialize in clinical trials (*16*). The closest to this goal are analogs of a natural mammalian antimicrobial agent—a 59-amino-acid peptide ubiquicidin (UBI 1–59). It binds significantly more to bacteria than to WBCs, with even stronger affinity exhibited by its shorter analogs UBI 18–35, UBI 22–35, UBI 29–41, and UBI 31–38. Prob-

ably the most studied analog, UBI 29–41, rendered some promising results in an animal model (*17–21*) that led to its recent entry into clinical trials. Other naturally occurring antibacterials with a potential for successful creation of infection-specific radiopharmaceuticals are the "clip-out" small peptide analogs of lactoferrin (*22*), as well as labeled alafosfalin (*23*) and human neutrophil peptide-1 (*20,24*).

When the goal is to assess disease severity and response to treatment at the site of inflammation or infection, radiopharmaceutical accretion rate or absolute uptake at the optimal imaging time should be proportional to the degree of inflammation or concentration of viable offending microorganisms, respectively. Such a feature would be important in determining effective therapy for inflammation and infection in selected patients, and in assessing new therapeutic agents. Although some evidence exists to support the use of radiolabeled WBCs to monitor activity of inflammatory bowel disease (*25*), none of the clinically used radiopharmaceuticals have been shown to reliably monitor the effectiveness of antimicrobial therapy. The latter goal may be within the reach of some novel small peptide infection-specific radiopharmaceuticals, such as UBI 29–41, which in animal models accumulates at the infected focus in proportion to the concentration of viable bacteria (*26*).

Fanolesomab kit for in vivo WBC labeling with ^{99m}Tc was recently approved in the United States for the diagnosis of atypical appendicitis, underscoring the clinical-indication specificity established early in studying newer inflammation-seeking radiopharmaceuticals. As with ^{111}In-DPC11870, the main advantage of this agent over WBCs labeled in vitro is avoidance of the risk to the patient and medical personnel associated with ex vivo blood handling. However, significant liver and renal uptake, with some hepatobiliary elimination, is likely to limit the utility of fanolesomab. Although early results suggest that its performance in diabetic feet and orthopedic infections may be on a par with ^{111}In ex vivo–labeled WBCs, it is highly unlikely to do well in the assessment of renal and gallbladder infection. Moreover, no matter how one labels WBCs, such agents are likely to exhibit significant activity in the bone marrow, complicating interpretation of suspected infection around orthopedic hardware. If used for this indication, ^{111}In-DPC11870 would likely require the assistance of conjoint ^{99m}Tc-sulfur colloid imaging. Another WBC-imaging shortcoming, which ^{111}In-DPC11870 is likely to share, is interference from uptake in the spleen and moderate activity in the liver.

Recently, ^{18}F-FDG received attention as a potential inflammation- and infection-seeking radiopharmaceutical. Like ^{67}Ga-citrate, it first became established in oncology and later found application in infection and inflammation. Although ^{67}Ga-citrate made a rapid extension into everyday inflammation and infection imaging, ^{18}F-FDG is still not commonly used despite excellent supportive evidence. The difference is explained by the contemporary practice in the United States of requiring acceptable evidence that a test is cost effective before it can be reimbursed. Clinical use of a diagnostically effective test is very much driven by demonstration of cost-effectiveness, as exemplified by ^{18}F-FDG PET in oncology. A similar litmus test will be applied to ^{111}In-DPC11870 or any other novel radiopharmaceutical.

Rapid progress in the field of small peptide, and even smaller nonpeptide, leukocyte-binding radiopharmaceuticals is likely to continue, if not accelerate, as investigators strive toward an ideal agent tailored to answer specific clinical questions. Which one will cross the finish line of widely accepted clinical use first is hard to predict, because not only will it have to be better than available radiopharmaceuticals, but it will also have to successfully compete with a plethora of other modalities. But of even more importance is that any candidate must stand the rigor of testing in a specific clinical setting to fulfill the demands of evidence-based, cost-effective contemporary medical practice.

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