In Vivo Leukocyte Labeling: The Quest Continues

The development of in vitro methods to radiolabel and image leukocytes that migrate to sites of infection was a significant milestone in the history of nuclear medicine (1). Even today, in vitro labeled leukocyte imaging remains the radionuclide gold standard for the imaging of most infections in the immunocompetent population. However, there are significant limitations to the technique. In vitro labeling is labor-intensive, is not always available, and requires direct handling of blood products. The need to perform complementary marrow or bone imaging adds complexity and expense to the procedure and is inconvenient for patients (2).

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Considerable effort has been expended on developing in vivo methods of labeling leukocytes that would overcome the limitations of in vitro methods. Although limited investigations of leukocyte-avid peptides in humans have shown promise, most investigations have focused on antigranulocyte antibodies (3–19). Unfortunately, these techniques have met with only modest success. One of the first agents investigated was BW 250/183 (Granuloscint; CISBio International), a murine monoclonal IgG1 antibody that binds to the NCA-95 antigen on leukocytes. About 10% of the injected activity is neutrophil bound at 45 min after injection, with 20% of the activity circulating

freely in the blood (5). Although studies usually become positive by 6 h after injection, delayed imaging at 24 h increases sensitivity. Up to 40% of the injected dose accumulates in bone marrow, potentially obscuring small foci of infection. Sensitivity for osteomyelitis ranges from about 70% in the hips to 100% in the distal lower extremities, perhaps reflecting easier detection with decreasing amounts of marrow distally (5,7). A disadvantage of BW 250/183 is the incidence of a dose-dependent human antimurine antibody response, which ranges from less than 5% in patients receiving a single dose of the antibody to more than 30% in patients receiving repeated injections (8).

Sulesomab (Leukoscan; Immunomedics) is a 50-kDa-antigen–binding fragment (Fab′) of a murine monoclonal antibody of the IgG1 class that binds to the NCA-90 antigen on leukocytes (9). A significant advantage of this agent is the lack of a human antimurine antibody response. Uptake mechanisms include binding to circulating neutrophils and to leukocytes already present at the site of infection (6). Clinical results have been variable. In 1 investigation of suspected musculoskeletal infection, the sensitivity, specificity, and accuracy of the agent were 90%–93%, 85%–89%, and 88%–90%, respectively (12,13). In another series, however, the sensitivity, specificity, and accuracy were 76%, 84%, and 78%, respectively (11). In 1 series of people with diabetes and with suspected pedal osteomyelitis, the agent was 100% sensitive and specific, whereas another group of investigators reported a sensitivity of 80% and a specificity of 67% (13,14).

^{99m}Tc-Fanolesomab (NeutroSpec; Palatin Technologies) is a murine IgM antibody that binds to the CD15 antigen on human neutrophils, eosino-phils, and lymphocytes. The antibody binds at a higher frequency to neutrophils than to any other cell type. Binding increases proportionally with increasing numbers of circulating neutrophils and is upregulated with neutrophil activation. Fanolesomab binds to circulating neutrophils and to neutrophils and neutrophilic debris containing CD-15 receptors and already sequestered in the focus of infection (15,16).

In clinical trials, ^{99m}Tc-fanolesomab accurately diagnosed appendicitis and appendicular osteomyelitis and was approved for use in the United States in 2004 (17,18). In December 2005, it was withdrawn as a result of postmarketing reports of serious and life-threatening cardiopulmonary events, including 2 fatalities, which occurred shortly after administration. The explanation for these events is as yet unknown, and the future of ^{99m}Tc-fanolesomab is uncertain (19).

Eschewing antigranulocyte antibodies, Bleeker-Rovers et al. (20) chose to investigate a small protein, interleukin 8 (IL-8), and the results of that investigation are reported on pages 337–343 of this issue of *The Journal of Nuclear Medicine*. IL-8, which has a molecular mass of 8.5 kDa, binds with a high affinity to the CXC1 and CXC2 receptors present on neutrophils (21,22). Markedly reduced abscess uptake of ^{99m}Tc-IL-8 in neutropenic rabbits has been reported (23). The success of leukocyte imaging is also dependent on neutrophils, which are the predominant leukocytes labeled in vitro (2). Therefore, with reasonable confidence, researchers can predict a priori how IL-8 will perform in a given situation and design clinical investigations accordingly. For example, leukocyte imaging is not very useful for diagnosing opportunistic infections or spinal osteomyelitis (2). (Indeed, for the 1 patient with spinal infection in the

investigation of Bleeker-Rovers et al. (20), the IL-8 results were false-negative.) Designing clinical trials to study these entities with IL-8 would likely be neither cost-effective nor fruitful. Efforts would be better focused on appendicular osteomyelitis, prosthetic vascular graft infection, and inflammatory bowel disease, entities for which the utility of leukocyte imaging is well documented.

A significant disadvantage of leukocyte imaging is the labeling process itself. Safety concerns aside, the procedure is time-consuming, is laborious, and requires skilled personnel. Even when labeling is performed on site, the test is usually performed only during routine working hours. At sites that depend on outside radiopharmacies to perform labeling, availability may be even more restricted, especially in rural locations, where the distance between a medical center and a radiopharmacy can make performance of the test all but impossible. If commercially available cold IL-8 were available in kit form and if the radiolabeling were performed as described by Bleeker-Rovers et al. (20), the availability of the test could dramatically expand.

The results obtained with IL-8 in the investigation of Bleeker-Rovers et al. (20) were in agreement with the final diagnosis in 18 of 20 patients studied, a finding that is encouraging. Given the small number of patients studied and the preponderance of referrals for suspected musculoskeletal infection, the issue of efficacy really cannot be addressed. This investigation is best viewed as proof of principle; in the population studied, the agent was safe and accurate and warrants further investigation.

Safety concerns are, perhaps, the major impetus for developing in vivo leukocyte-labeling methods. An agent that can be safely injected directly into a patient, obviating direct contact with blood, would be a significant improvement over current methodology. Data on the safety of IL-8 in humans are scant. In 11 patients who received 50–100 μg of ^{131}I-IL-8, there was a transient decrease in the numbers of circulating leukocytes, with a return to baseline levels within about 1 h (24). In the study of Bleeker-Rovers et al. (20), a much smaller dose was used, and no significant changes in leukocyte counts were observed. Facial flushing in 1 patient was the only side effect noted. Although the drug is apparently safe, IL-8 has been studied in fewer than 50 patients. No significant safety issues arose during clinical trials of ^{99m}Tc-fanolesomab, in which more than 500 patients were studied. These issues became apparent only after the drug was in clinical use and several thousand patients had been injected!

In their preliminary investigation, Bleeker-Rovers at al (20) report on an agent that may be able to replace in vitro labeled leukocyte imaging for many indications. Much work remains, however, if IL-8 is to avoid the fate of many of its predecessors. The key to determining its true value is well-designed clinical trials. No single agent is equally useful for all situations; therefore, investigations in which entry criteria are broad, such as "suspected infection," are not ideal. Focused investigations of specific conditions, such as prosthetic joint infection, diabetic foot infection, and inflammatory bowel disease, although more costly, will provide far more meaningful information about the merits of IL-8.

Appropriate imaging protocols also need to be developed. From the article of Bleeker-Rovers et al. (20), one could conclude that a single set of images obtained within a few hours after injection are sufficient. Similar conclusions initially were drawn about fanolesomab; subsequent data, however, indicated that delayed imaging was sometimes required (25). Early and late imaging should be incorporated into future IL-8 investigations.

Equally important is the "standard of truth" against which IL-8 is judged. Regardless of their flaws or the difficulties encountered in obtaining them, histopathology or microbiology evaluations are the diagnostic gold standards for most diseases, and a requirement that they be obtained should be part of investigational protocols.

Because IL-8 imaging is a potential replacement for leukocyte imaging, intraindividual comparisons of IL-8 and leukocytes would further strengthen conclusions about the ultimate worth of IL-8.

Given the unfortunate events associated with ^{99m}Tc-fanolesomab, the importance of thoroughly investigating the safety of IL-8 cannot be over-emphasized. It will not be sufficient to demonstrate that IL-8 is safe for a 1-time use. It is not uncommon for patients to undergo radionuclide infection imaging procedures on multiple occasions, and it is important to confirm that repeated injections will compromise neither patient safety nor diagnostic accuracy.

The role of PET is increasing exponentially, and the importance of ^{18}F-FDG PET for the imaging of infection and inflammation is now well appreciated (26). ^{18}F-FDG, however, is a beginning, not an end. Investigators are already exploring the potential of labeling leukocytes in vitro with ^{18}F-FDG (27). The next logical step is the development of an in vivo method of labeling leukocytes with a positron emitter. Perhaps this goal can be accomplished with IL-8; at the very least, it merits consideration.

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