

Development of Infection and Inflammation Targeting Compounds

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Abstract: Nuclear medicine offers powerful noninvasive techniques for visualization of infectious and inflammatory disorders using whole body imaging enabling the determination of both localization and number of inflammatory foci. A wide variety of approaches depicting the different stages of the inflammatory response have been developed. Non-specific radiolabeled compounds, such as ⁶⁷Ga-citrate and radiolabeled polyclonal human immunoglobulin accumulate in inflammatory foci due to enhanced vascular permeability. Specific accumulation of radiolabeled compounds in inflammatory lesions results from binding to activated endothelium (e.g. radiolabeled anti-E-selectin), the enhanced influx of leukocytes (e.g. radiolabeled autologous leukocytes, anti-granulocyte antibodies or cytokines), the enhanced glucose-uptake by activated leukocytes (¹⁸F-fluorodeoxyglucose) or direct binding to micro-organisms (e.g. radiolabeled ciprofloxacin or antimicrobial peptides). Scintigraphy using autologous leukocytes, labeled with ¹¹¹In or ^{99m}Tc, is still considered the "gold standard" nuclear medicine technique for the imaging of infection and inflammation, but the range of radiolabeled compounds available for this indication is still expanding. Recently, positron emission tomography with ¹⁸F-fluorodeoxyglucose has been shown to delineate various infectious and inflammatory disorders with high sensitivity. New developments in peptide chemistry and in radiochemistry will result in specific agents with high specific activity. A gradual shift from non-specific, cumbersome or even hazardous approaches to more sophisticated, specific approaches is ongoing. In this review, the different approaches to scintigraphic imaging of infection and inflammation, already in use or under investigation, are discussed.

INTRODUCTION AND HISTORICAL PERSPECTIVE

Nuclear Medicine techniques have a lot to offer in visualization of infectious and inflammatory foci. Quite often these techniques do not lead immediately to a definitive diagnosis, i.e. a histological or a microbiological diagnosis. However, they point to parts in the body where a particular metabolic process is ongoing, leading to elevated uptake of a radiopharmaceutical. With the help of other techniques, such as puncture, biopsy and culture, a definitive diagnosis can be obtained.

There are several reasons why imaging of infection and inflammation becomes increasingly important in the next decade. The population is ageing; the application of implants and transplants is increasing. The number of immune compromised patients is growing, mainly because of frequent use of chemotherapeutic agents leading to neutropenia. Furthermore, the increased use of antibiotics leads to insensitivity for some of these pharmaceuticals.

At present inflammation is defined as the reaction of tissue to any injury, aiming at bringing serum molecules and cells of the immune system to the area where the injury takes place. Infection is defined as any injury caused by microorganisms. The injury leading to inflammation can vary from trauma, to ischemia, to neoplasm, and can be caused by infectious agents such as bacteria, viruses, fungi and parasites.

Injury induces the production of inflammatory mediators, being either vaso-active or chemotactic. Vaso-active mediators lead to increased vascular permeability, with edema and efflux of components from the intravascular to the extravascular space. Chemotactic mediators induce recruitment and stimulation of inflammatory cells, being mainly granulocytes in acute inflammation, while macrophages and lymphocytes are predominant in chronic types of inflammation. For the purpose of scintigraphic imaging of inflammation and infection various pathways can be used (Table 1). Non-specific radiolabeled compounds show increased extravasation at the site of inflammation due to the locally enhanced vascular permeability. Examples are ⁶⁷Ga-citrate and radiolabeled non-specific immunoglobulins. The second approach is based in the influx of

leukocytes, either by radiolabeling the patient's leukocytes *ex vivo* or by directly targeting leukocyte antigens or receptors *in vivo*. For the latter two methods, radiolabeled antigranulocyte monoclonal antibodies or receptor-binding ligands (chemotactic peptides, cytokines and complement factors) are administered. Most of these radiolabeled compounds preferentially bind to granulocytes and are thus most suitable in conditions with large influx of granulocytes such as in acute inflammatory and infectious processes. Radiolabeled interleukin-2 (IL-2) is suitable for imaging chronic inflammatory processes, because the IL-2 receptor is preferentially expressed on T-lymphocytes. On the other hand, imaging of activated endothelium is possible and involves targeting of activated endothelial adhesion molecules, for example anti-E-selectin. The mechanism of ¹⁸F-fluorodeoxyglucose (FDG)-uptake in inflammatory cells is related to leukocytes using glucose as an energy source only after activation during the metabolic burst. The accumulation of FDG in cells with increased glucose metabolism is specific. Increased glucose metabolism, however, is also present in malignant cells, so FDG-uptake is not specific for inflammatory processes as such. Finally, a new class of radiolabeled compounds consists of radiolabeled antibiotics, microbial peptides and enzyme substrates, which directly bind to or localize in microorganisms. The specific nature of uptake of these radiolabeled compounds in inflammatory processes is still a matter of debate, however.

In the context of the design of ideal radiopharmaceuticals for the imaging of infection and inflammation, it is desirable to formulate a wishlist of criteria. The radiopharmaceutical should be taken up rapidly and well retained at the site of inflammation/infection, together with a quick wash-out from the background, in order to achieve a good target to background ratio. Uptake in normal organs should be low. The preparation of the radiopharmaceutical should be quick and easy, preferably with technetium-99m as the radionuclide. For a diagnostic agent it is obvious that there should be no toxicity and also no immune response after administration of the radiopharmaceutical. It would be most helpful to have a radiopharmaceutical that allows discrimination between infection and sterile inflammation. Unfortunately we have to face the fact that none of the currently used agents for the scintigraphic detection and localization of infection/inflammation meets all of the criteria as listed above. So, there is the need to develop new and better agents. In order to be able to differentiate between normal and abnormal appearances on scintigraphic images it is important to understand the mechanisms of uptake of individual radiopharmaceuticals in normal

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Table 1. Overview of Characteristics of Radiopharmaceuticals Used for Imaging Inflammatory Processes

Physiological Characteristics	Targeting Mechanism	Tracer Class	Radiolabeled Compound
Enhanced vascular permeability	Non-specific uptake		⁶⁷ Ga-citrate Non-specific immunoglobulins
Endothelial activation	Antigen binding	Antibodies	F(ab') ₂ -anti-E-selectin
Enhanced influx of granulocytes	Granulocyte influx		Radiolabeled granulocytes
	Antigen binding	Antibodies	Anti-NCA-95 IgG, BW 250/183 (Scintimun®) Anti-SSEA-1 IgM (Neutrospec®) Anti-NCA-90 Fab', sulesomab (LeukoScan®)
	Receptor binding	Cytokines	IL-8
Enhanced influx of mononuclear cells	Receptor binding	Cytokines	IL-2
Presence of micro-organisms	Affinity for micro-organisms	Antimicrobial agents	Ciprofloxacin (Infecton®)
Increased metabolic requirements	Enhanced glucose uptake		FDG

and in diseased tissues and organs. Such understanding makes it possible to select the most appropriate radiopharmaceutical in a particular disease entity.

This review focuses on well-established and widely available radiopharmaceuticals as well as on "new" radiolabeled compounds in which human data are available.

SINGLE PHOTON RADIOPHARMACEUTICALS FOR IMAGING INFECTIOUS AND INFLAMMATORY PROCESSES

Non-Specific Radiolabeled Compounds

⁶⁷Ga-citrate

After injection, ⁶⁷Ga-citrate binds to circulating transferrin. Subsequently, this complex extravasates at the site of inflammation due to locally enhanced vascular permeability. In the inflamed tissue, ⁶⁷Ga is transferred from transferrin to lactoferrin that is locally excreted by leukocytes or to siderophores produced by microorganisms. Physiologically, 10 to 25% of the radionuclide is excreted *via* the kidneys during the first 24 hours. After 24 hours the principal route of excretion is hepatobiliary. After 48 hours about 75% of the injected dose remains in the body and is equally distributed among the liver, bone, bone marrow and soft tissues. ⁶⁷Ga-citrate has been used extensively in clinical practice in several pathological conditions demonstrating high sensitivity for both acute and chronic infection and non-infectious inflammation. There are several shortcomings that limit its clinical application, however. Specificity is poor due to the physiological bowel excretion and accumulation in malignant tissues and in areas of bone modeling. In addition, the radiopharmaceutical has unfavorable imaging characteristics such as a long physical half-life (78 h) and multiple high-energy photons (93-889 keV), causing high radiation absorbed doses. Moreover, optimal imaging often requires delayed imaging up to 72 hours after injection. These unfavorable characteristics and the development of newer radiopharmaceuticals have resulted in replacement of ⁶⁷Ga-citrate scintigraphy in the majority of inflammatory conditions by scintigraphy with labeled leukocytes. Leukocyte scanning, however, is of limited value in patients with suspected vertebral osteomyelitis. Sequential gallium imaging appears to be a better way to diagnose this condition. Also, in immunocompromized patients, ⁶⁷Ga-citrate imaging is the procedure of choice for detecting opportunistic respiratory tract infections. Finally, ⁶⁷Ga-citrate scintigraphy is still the gold standard for radionuclide imaging in patients with FUO, where it is able to detect both acute and chronic inflammatory conditions and neoplasms [1]. However, its limited specificity and the generally unfavorable characteristics when compared to FDG PET will in patients with FUO most probably result in the near future in replacement of this technique by FDG PET.

Non-Specific Immunoglobulins

Initially it was considered that human polyclonal immunoglobulin (HIG) was retained in inflammatory processes by interaction with Fc- γ -receptors expressed on infiltrating leukocytes. Later it was shown, however, that radiolabeled HIG accumulates primarily in inflammatory foci by non-specific extravasation due to locally enhanced vascular permeability. For clinical use, HIG has been labeled with both ¹¹¹In and ^{99m}Tc. Disadvantages of the use of ¹¹¹In are a relatively high radiation burden, suboptimal gamma radiation for *in vivo* imaging, often limited availability and high costs. ^{99m}Tc is a more attractive alternative in most cases because of its short half-life (6 h), availability and lower costs. Both agents have slow blood clearance and physiological uptake in the liver and spleen. A general limitation is the long time span of 24 h between injection and diagnosis. In a comparative study, it was shown that ^{99m}Tc-HIG labeled using hydrazinonicotinamide (HYNIC) as the chelator, has *in vivo* characteristics highly similar to those of ¹¹¹In-HIG [2]. In most cases it appeared to be suited to replace the ¹¹¹In-labeled compound. ^{99m}Tc-HIG imaging, however, has more limited sensitivity than ¹¹¹In-HIG scintigraphy in chest disease and in chronic inflammatory processes. Direct comparison of ¹¹¹In-HIG and ¹¹¹In-leukocytes patients with various subacute infections showed a slightly, but significantly better overall accuracy of ¹¹¹In-HIG scintigraphy [3]. The major indication for imaging with radiolabeled HIG seems to be localization of acute infection or inflammation of the musculoskeletal system [4]. Furthermore, ^{99m}Tc-HIG scintigraphy appears to be an effective method for monitoring of disease activity in patients with rheumatoid arthritis [5]. In addition, ¹¹¹In-HIG scintigraphy is clinically useful in pulmonary infection, particularly in immunocompromized patients [6]. In conclusion, ¹¹¹In- or ^{99m}Tc-HIG scintigraphy can be successfully used in various infectious and inflammatory diseases with a diagnostic accuracy comparable to that of radiolabeled leukocytes. When facilities for labeling leukocytes are not available or in severely granulocytic patients, HIG scintigraphy can be a good alternative for radiolabeled leukocytes. However, commercial kits are not available, impeding its general use in most hospitals.

Limitations of Non-Specific Radiolabeled Compounds in Infection/Inflammation Imaging

Although infectious and inflammatory processes can be visualized with radiolabeled compounds without a specific interaction between the agent and a tissue component in the inflammatory focus, this method has several limitations. Extravasation of molecules *via* diffusion is a slow process requiring prolonged high blood levels to allow for sufficient accumulation in the target tissue. High blood levels, however, entail relatively high background levels, especially in well-perfused tissues. Furthermore, in chronic inflammatory processes the vascular permeability tends to normalize. Finally, because non-specific radiolabeled compounds accumulate as a result

of a common feature of infection and inflammation, these agents cannot distinguish between infection and inflammation. It must be emphasized, however, that all radiolabeled compounds accumulate to some extent in this non-specific way in inflammatory foci. This mechanism is of particular importance in evaluating new radiolabeled compounds, because non-specific accumulation can be erroneously interpreted as being specific.

More Specific Radiolabeled Compounds

Imaging of Endothelial Cell Activation

Anti-E-Selectin Antibodies and Antibody Fragments

E-selectin is an endothelial adhesion molecule exclusively expressed on the luminal surface of activated endothelial cells and capable of binding to different populations of leukocytes [7]. The endothelial expression of E-selectin is stimulated by interleukin-1 (IL-1), tumor necrosis factor α , and bacterial lipopolysaccharide. A F(ab')₂ antibody fragment, derived from an anti-E-selectin monoclonal antibody, has been synthesized and labeled with ¹¹¹In. Uptake of ¹¹¹In-F(ab')₂-anti-E-selectin was demonstrated in the inflamed joints of patients with rheumatoid arthritis. Compared with ¹¹¹In-HIG, ¹¹¹In-F(ab')₂-anti-E-selectin provided superior images in these patients [8]. Imaging with ¹¹¹In-F(ab')₂-anti-E-selectin also identified areas of inflammation in Crohn's disease and ulcerative colitis, concordant with the results of ^{99m}Tc-leukocyte scanning [9]. More recently, diagnostic accuracy of ^{99m}Tc-F(ab')₂-anti-E-selectin proved to be comparable to ¹¹¹In-F(ab')₂-anti-E-selectin and higher than diagnostic accuracy of conventional bone scanning in patients with rheumatoid arthritis [10]. Another study from the same group showed that imaging with ¹¹¹In-anti-E-selectin-monoclonal antibody is also a sensitive method for assessment of disease activity in patients with rheumatoid arthritis and that targeting is more intense and specific than using ^{99m}Tc-HIG.

Imaging of Infiltrating Granulocytes

Radiolabeled Leukocytes

Imaging using *ex vivo* labeled autologous leukocytes was developed in the 1970s by McAfee and Thakur [11]. A blood sample of approximately 50 mL is collected and leukocytes are separated from red blood cells. These leukocytes are then labeled with radioactive isotopes (¹¹¹In or ^{99m}Tc) and reinjected. Using standard labeling procedures, only a few granulocytes are damaged by labeling, whereas most lymphocytes are mutilated. The damaged cells are rapidly cleared from the circulation after reinjection. After intravenous administration, the radiolabeled leukocytes initially sequester in the lungs with subsequent rapid clearance from the lungs. The radiolabel rapidly clears from the blood and in most cases uptake in granulocytic infiltrates is high while a substantial portion of the leukocytes accumulate in the spleen and the liver. Autologous leukocytes can be labeled with ¹¹¹In using ¹¹¹In-oxine. The use of hexamethylpropyleneamine oxime (HMPAO), a lipophilic ^{99m}Tc chelator, allows for efficient labeling of white blood cells with ^{99m}Tc. In contrast to ¹¹¹In-oxine, some of the ^{99m}Tc-HMPAO is released from the leukocytes after injection and subsequently excreted *via* the kidneys (within minutes) and the hepatobiliary system (after several hours). ^{99m}Tc-labeled leukocytes have replaced ¹¹¹In-labeled leukocytes for most indications, because of the more optimal radiation characteristics. As a result of the biodistribution of ^{99m}Tc-HMPAO-labeled leukocytes, the use of ¹¹¹In-labeled leukocytes is preferred for evaluation of the kidneys, bladder and gall bladder. ¹¹¹In-labeled leukocytes are also preferred if late images are needed as in chronic infection. Radiolabeled leukocytes do provide a good diagnostic accuracy. However, the preparation of this radiopharmaceutical is laborious. Isolating and labeling a patient's white blood cells takes a well-trained technician two to three hours. In addition, the need to handle potentially contaminated blood can result in transmission of blood-borne pathogens, such as hepatitis virus and

human immunodeficiency virus, to technicians or patients. The principal clinical indications for radiolabeled leukocytes include inflammatory bowel disease, osteomyelitis, follow-up of patients with infections of vascular or orthopedic prostheses and soft tissue infections [12]. There has always been concern that chronic infections could be missed using radiolabeled leukocytes, because these infections generate a smaller granulocyte response compared to acute infections. However, a study in 155 patients demonstrated that sensitivity of labeled leukocytes for detection of acute infections (90%) was not significantly different from sensitivity for detection of chronic infections (86%) [13].

Anti-Granulocyte Antibodies and Antibody Fragments

Ever since it became clear that infectious and inflammatory foci could be visualized by radiolabeled autologous leukocytes, investigators have tried to develop a method aiming to label white blood cells *in vivo*. The use of radiolabeled monoclonal antibodies against surface antigens as present on granulocytes has the advantage that labeling procedures are easier and do not require handling of potentially contaminated blood. Disadvantages of the use of monoclonal antibodies, however, are the high molecular weight, resulting in slow diffusion into sites of inflammation, a long plasma half-life and uptake in the liver due to clearance by the reticulo-endothelial system. A long interval is often required between administration of radiolabeled antibodies and acquisition of images in order to improve target-to-background ratios. Use of monoclonal antibodies of murine origin sometimes induces generation of human antimouse antibodies (HAMA), which can lead to allergic reactions and altered pharmacokinetics when repeated injections are given. This is, of course, a major limitation for follow-up studies. The use of antibody fragments (Fab' or F(ab')₂) or humanization of the antibodies could overcome most of these limitations. Theoretically, immunogenicity will be lower, blood clearance will be faster and accumulation in inflammatory foci will be higher. Moreover, since Fab' antibody fragments have an intrinsic lower affinity for the epitope, bone marrow uptake is lower, which is an advantage for imaging of infections of the central skeleton. Although radiolabeled anti-granulocyte antibodies and antibody fragments are developed as radiolabeled compounds for specific targeting of infiltrating granulocytes, recent studies have demonstrated that they localize in infectious processes to a large extent by non-specific extravasation due to locally enhanced vascular permeability. Binding of the antibodies to infiltrating leukocytes may contribute to the retention of the radiolabel in the inflammatory focus.

Anti-Non-Specific-Cross-Reacting Antigen-95

One of the most widely used anti-granulocyte antibodies is the commercially available murine anti-NCA-95 IgG (BW 250/183, Scintimun®), labeled with ^{99m}Tc. This antibody recognizes the non-specific cross-reacting antigen 95 (NCA-95, CD66) expressed on human granulocytes and (pro)myelocytes. It has been used successfully for imaging of various infectious and inflammatory processes including subacute infectious endocarditis [14], lung abscesses [15], septic loosening of hip and knee prostheses [16,17] and diabetic foot infections [18]. Peripheral bone infections were also adequately visualized [15], but sensitivity decreased in case the focus was located closer to the spine because of physiological bone marrow uptake, so imaging with ^{99m}Tc-anti-NCA-95 is less suitable for diagnosing vertebral osteomyelitis. Pulmonary infections other than abscesses were not visualized [15]. ^{99m}Tc-anti-NCA-95 scanning appeared to be a safe and reliable method for detecting infectious foci in neonates and infants with fever of unknown origin [19]. The preparation was also used in the evaluation of patients with inflammatory bowel disease, but it appeared to be less accurate than radiolabeled leukocytes partly due to non-specific bowel uptake [20,21]. Due to the relatively slow blood clearance, imaging 24 hours after injection is generally necessary for correct localization of the inflammatory process. The major drawback of radiolabeled

anti-NCA-95, however, is the production of HAMA after the first injection.

Anti-Stage Specific Embryonic Antigen-1.

Another Monoclonal antibody, anti-stage specific embryonic antigen-1 (anti-SSEA-1) IgM (Neutrospec[®]), recognizes CD15 antigens on granulocytes with high affinity ($K_d=10^{-11}$ mol/L). The *in vivo* binding exceeds 50%, suggesting involvement of more specific accumulation in inflammatory sites, such as *in vivo* migration of leukocytes from the circulation to the focus. ^{99m}Tc-anti-SSEA-1 IgM was successfully used in patients with various inflammatory and infectious diseases, such as osteomyelitis, diabetic foot ulcers and post-surgical infection [22] with similar diagnostic accuracy when compared to radiolabeled leukocytes. Imaging with ^{99m}Tc-anti-SSEA-1 IgM also proved to be a highly sensitive test for detection of appendicitis in equivocal cases [23]. ^{99m}Tc-anti-SSEA-1 IgM is a convenient radiolabeled compound (imaging after 1 hour, easy preparation) and no HAMA production has been found. Disadvantages are high liver uptake and transient mild neutropenia that has been observed after ^{99m}Tc-anti-SSEA-1 injection in several patients. This adverse effect can also be caused by other monoclonal antibodies as well as some cytokines. In most cases, however, this does not represent a clinical problem and does not impair image quality.

Anti-Non-Specific-Cross-Reacting Antigen-90 Fab

^{99m}Tc-labeled anti-NCA-90 Fab' (sulesomab, LeukoScan[®]), which binds to NCA-90 surface antigen on granulocytes, is a commercially available infection imaging agent. Promising results have been obtained in the scintigraphic detection of endocarditis [24] and nonclassic appendicitis [25,26]. ^{99m}Tc-anti-NCA-90 Fab' proved to be no alternative for radiolabeled leukocytes in patients with inflammatory bowel disease due to limited sensitivity [27]. Non-specific bowel activity is often present, especially in the delayed images. At first, scintigraphy using ^{99m}Tc-anti-NCA-90 Fab' appeared to provide rapid localization of bone and soft tissue infections with a negligible HAMA response rate and accuracy comparable to that of leukocyte scanning. In other studies, however, ^{99m}Tc-anti-NCA-90 Fab' scintigraphy was found to be less specific for the diagnosis of musculoskeletal infections than leukocyte scanning. In addition, false-negative results were found in several patients with chronic infections [28]. In diabetic foot infections, sensitivity (67%) and specificity (85%) of ^{99m}Tc-anti-NCA-90 Fab' scintigraphy was higher than sensitivity and specificity of ⁶⁷Ga scintigraphy, although sensitivity was not optimal [29]. These studies suggest that ^{99m}Tc-anti-NCA-90 Fab' scintigraphy could be used for imaging of acute orthopedic infections, with its greatest strength being a high negative predictive value. Positive studies may require further correlative imaging.

Cytokines

Cytokines are (glyco)proteins acting *via* interaction with specific cell surface receptors expressed mainly on leukocytes, but also on other cell types. Cytokine receptors are usually expressed at low levels on resting cells, but their expression is upregulated during activation. Cytokines potentially can be used to specifically target leukocytes *in vivo*, because they bind to specific receptors with high affinity in the nanomolar range, they have low molecular weights (<25 kD) and plasma clearance is rapid. Finally, many cytokines are of human origin and are therefore readily available and supposedly non-immunogenic.

Interleukin-8

Interleukin-8 (IL-8) is a small protein (8.5 kD) belonging to the CXC subfamily of chemokines or chemotactic cytokines, in which the first two cysteines are separated by one amino acid. IL-8 binds with high affinity ($0.3-4 \times 10^{-9}$ mol/L) to two different receptors (CXCR1 and CXCR2) expressed on granulocytes and promoters

chemotaxis of these cells. In a pilot study in eight patients, it was shown that ¹²³I-IL-8 could visualize osteomyelitis and cellulitis correctly [30]. Recently, a ^{99m}Tc-labeled IL-8 preparation was developed in our lab using HYNIC as a chelator resulting in a significantly higher specific activity [31]. Protein doses to be administered were lowered substantially due to much higher specific activity of this new ^{99m}Tc-IL-8 preparation diminishing concerns about the influence on leukocyte counts in patients. Studies in neutropenic and normal rabbits with turpentine-induced abscesses have shown that accumulation of ^{99m}Tc-IL-8 in the abscess is a highly specific, neutrophil-driven process and that the total fraction of ^{99m}Tc-IL-8 that accumulates in the inflamed tissue is extremely high (up to >15% of injected dose) [32]. Recently, the first clinical study using ^{99m}Tc-IL-8 scintigraphy in 20 patients suspected of different infectious diseases, was completed [33]. In our institution, injection of ^{99m}Tc-IL-8 was well tolerated. ^{99m}Tc-IL-8 rapidly cleared from the blood and most other organs. In 10 of 12 patients with an infection, ^{99m}Tc-IL-8 localized the infection 4 hours p.i. In the patients with non-infectious disorders, no focal accumulation of ^{99m}Tc-IL-8 was found. ^{99m}Tc-IL-8 scintigraphy could thus be a promising new tool for detection of infections in patients.

Platelet Factor 4 (PF4)

PF4 is like IL-8 a member of the CXC chemokines, but has no relevant affinity for either of the two CXC receptor types. In fact, the PF4-receptor has not been identified yet. PF4 is also called the "body's heparin neutralizing agent". At Diatide Research Laboratories, since 1999 part of the Berlex Laboratories (Seattle, WA), the 23 amino acid peptide P483 was synthesized. This peptide contains the heparin-binding region of PF4, a lysine-rich sequence to facilitate renal clearance and CGCG-sequence to allow labeling with ^{99m}Tc. When P483 was complexed with heparin its affinity for leukocytes increased and this complex (P483H) was studied in a rabbit model of infection. ^{99m}Tc-P483H clearly delineated the infectious foci as early as 4 hours after injection. Upon intravenous injection high pulmonary uptake is observed [34]. ^{99m}Tc-P483H has been studied in 30 patients to test its applicability as imaging agent for scintigraphic detection of infection and inflammation with good results (86% sensitivity, 81% specificity, 83% accuracy) [35]. Due to the high physiological uptake in the lungs, the agent is not suited for detection of pulmonary infections. In addition, in some patients excessive thyroid uptake was observed, which correlated with the peptide:heparin ratio.

Complement Factor 5a (C5a)

The complement factor 5a (C5a) and its natural metabolite C5a-des-Arg are involved in several stages of the inflammatory process. C5a-des-Arg only differs from C5a by the absence of the C-terminal Arg-residue of C5a. Both act on a common receptor on different cell types, including neutrophils and monocytes. The receptor binding affinity of C5a is one order of magnitude higher than the affinity of C5a-des-Arg. The biologic activity of C5a-des Arg is considerably lower than that of C5a. In rabbits with intramuscular *E. coli* infection, ^{99m}Tc-C5a rapidly visualized the infection with high uptake of the radiolabel in the affected muscle while uptake of ^{99m}Tc-C5a-des-Arg in the abscess was low [36]. Thus, none of the compounds in this study combined good imaging characteristics with reduced biologic activity.

Leukotriene B₄ (LTB₄)

Leukotriene B₄ (LTB₄) is a potent chemoattractant that activates granulocytes and macrophages and it is an important mediator in both acute and chronic inflammatory diseases. Two distinct types of leukotriene receptors have been identified (BLT1 and BLT2). LTB₄ has a high affinity for the BLT1 receptor ($K_d = 10^{-9}$ M) that is mainly expressed on human neutrophils, while the recently characterized BLT2 receptor is a low-affinity receptor ($K_a = 23 \times 10^{-9}$ M) expressed more ubiquitously. Binding of LTB₄ to BLT1 and BLT2

promotes chemotaxis and chemokinesis. In search for an effective infection-imaging agent, the LTB₄ receptor antagonist RP517 was synthesized. RP517 (MW 830 Da) has a quinolone function as a receptor binding moiety linked *via* a spacer arm to HYNIC to allow labeling with ^{99m}Tc (IC₅₀ = 2x10⁻⁹ M). In rabbits with *E. coli* infections, ^{99m}Tc-RP517 rapidly visualized the abscess with high abscess-to-background ratios [37]. ^{99m}Tc-RP517 could also visualize experimental endocarditis in dogs [38]. However, ^{99m}Tc-RP517 is a highly lipophilic compound that is cleared mainly *via* the hepatobiliary route, resulting in high uptake in the digestive tract relatively early after injection. The high physiologic uptake in the intestines limits the applicability of ^{99m}Tc-RP517 as an infection-imaging agent [37].

In order to produce a hydrophilic variant of RP517, a new compound designated as DPC11870-11, was synthesized. DPC11870-11 (MW = 3127 Da) consists of two quinolone moieties for receptor binding linked *via* a cysteic acid-based hydrophilic backbone and a DTPA moiety to allow labeling with ¹¹¹In. In rabbits with intramuscular *E. coli* infection, ¹¹¹In-DPC11870-11 rapidly delineated the infection with high abscess-to-background ratios. The agent cleared exclusively *via* the kidneys and no accumulation of radioactivity was observed in the gastrointestinal tract. Blocking experiments with an excess of the nonradiolabeled agent indicated that the localization of ¹¹¹In-DPC11870-11 was dependent on the specific interaction with LTB₄-receptors expressed in the infected tissue [39]. Recently, ¹¹¹In-DPC11870-11 was tested for its ability to visualize abdominal inflammation. Chemically induced colonic inflammation could clearly be delineated in rabbits. The ¹¹¹In-DPC11870-11 rapidly accumulated in the inflamed colon and in the images the affected colon-to-nonaffected colon uptake ratios exceeded 10. The images obtained with ¹¹¹In-DPC11870-11 were superior to those obtained with ¹¹¹In-labeled leukocytes and to the PET images obtained after injection with FDG [40].

Imaging of Infiltrating Mononuclear Cells

Interleukin-2

IL-2 is a glycoprotein with a molecular weight of 15.5 kD, which is synthesized and secreted by T-lymphocytes after specific antigen stimulation. During inflammation, activated lymphocytes express high-affinity IL-2 receptors and become a target for radiolabeled IL-2. IL-2 has been labeled with ¹²³I and ^{99m}Tc to enable imaging of chronic infection or inflammation. In several rat models of autoimmune diabetes and in rats with renal allografts, ¹²³I-IL-2 adequately detected areas of lymphocytic infiltration. Specific accumulation of ¹²³I-IL-2 has been confirmed by *ex vivo* autoradiography [41]. ¹²³I-IL-2 has also been used successfully in patients with type 1 diabetes. ^{99m}Tc-IL-2 was able to identify a subgroup of patients with type 1 diabetes with persistent inflammation at the time of diagnosis that might benefit from the use of immunomodulating drugs to preserve β cell function [42]. In patients with active Crohn's disease, ¹²³I-IL-2 allowed imaging of activated T-lymphocytes infiltrating the gut wall. The uptake of ¹²³I-IL-2 decreased after corticosteroid therapy, so this technique could be valuable in monitoring the effect of therapy [43]. Scintigraphic results using ¹²³I-IL-2 in patients with celiac disease was consistent with the histologically determined number of infiltrating IL-2 receptor-positive cells in the jejunal mucosa [44]. ^{99m}Tc-IL-2 also strongly accumulates in the thyroid glands of patients with Hashimoto's thyroiditis and Graves' disease [45]. No side effects were observed. These results suggest that radiolabeled IL-2 could be a suitable radiopharmaceutical for *in vivo* targeting of mononuclear cell infiltration as present in several autoimmune diseases.

Imaging of Microorganisms

Ciprofloxacin Ciprofloxacin is a fluoroquinolone that binds to bacterial DNA gyrase, which is present in all dividing bacteria. Since

it binds only to living bacteria, even to most bacteria that are resistant to this antibiotic, the use of ^{99m}Tc-labeled ciprofloxacin (Infecton[®]) theoretically allows distinction between sterile inflammation and infection. ^{99m}Tc-ciprofloxacin is mainly excreted *via* the kidneys, it has low liver metabolism and bowel uptake is usually very low. The lack of bone marrow uptake is particularly useful for the detection of bone infections. In patients with known or suspected sites of various bacterial infections, sensitivity of scintigraphy with ^{99m}Tc-ciprofloxacin varied from 70 to 85% and specificity was approximately 80 to 95% [46]. Comparison between ^{99m}Tc-ciprofloxacin and leukocyte imaging gave comparable sensitivities and specificity was 96% and 77%, respectively [47]. ^{99m}Tc-ciprofloxacin was shown to be a very sensitive and quite specific marker of bone and joint infections [48, 49]: sensitivity of ^{99m}Tc-ciprofloxacin imaging was higher when compared to scintigraphy with ^{99m}Tc-HMPAO-leukocytes [48] and three-phase bone scanning in combination with ⁶⁷Ga-citrate scintigraphy [49]. In patients suspected of postoperative spine infections, ^{99m}Tc-ciprofloxacin SPET showed a sensitivity of 100% and a specificity of 74%. An interval of at least 6 months after surgery decreased the likelihood of false positives [50]. ^{99m}Tc-ciprofloxacin has also been used successfully in patients with suspected infections of hip or knee prostheses [51]. Lately, the specificity of ^{99m}Tc-ciprofloxacin has been discussed extensively. In patients with suspected osteoarticular infections and patients with osteoarticular diseases without signs of infection, ^{99m}Tc-ciprofloxacin scintigraphy did not discriminate between infected and aseptic osteoarticular diseases and articular uptake was seen in many control patients [52].

Antimicrobial Peptides

Antimicrobial peptides (MW = 5-7 kDa) are produced by phagocytes, endothelial cells and many other cell types and are an important component of the innate immune system. The basis of their antimicrobial activity is their direct interaction with the bacterial plasma membrane by electrostatic and hydrophobic interaction. The preferential binding of various antimicrobial peptides to bacteria was demonstrated *in vitro* [53]. Due to this preferential binding these peptides potentially can be used to discriminate bacterial infections from nonbacterial infections and sterile inflammations. A fragment of the antimicrobial peptide ubiquicidin (UBI 29-41) labeled with ^{99m}Tc showed some uptake (1-2%ID) in the infected thigh muscle at 30 min p.i. This enhanced uptake was specific because coinjection of an excess of unlabeled UBI 29-41 peptide resulted in a significantly lower uptake of ^{99m}Tc-UBI 29-41. In addition, uptake in muscle tissue with inflammation induced by inoculation of dead bacteria was also significantly lower [54]. Similarly human neutrophil peptide-1 (HNP-1) labeled with ^{99m}Tc was characterized in mice with inflamed thigh muscles [55]. The abscess-to-background ratios obtained with this tracer were low (3-5) and decreased with time. In the peritoneal cavity of the infected mice, ^{99m}Tc-HNP-1 bound preferentially to bacteria rather than to leukocytes. The investigators propose that these peptides can be used to monitor the efficacy of antimicrobial therapy regimens and to distinguish between bacterial infection and sterile inflammation.

Radiolabeled FIAU

Recently, the radiolabeled thymidine kinase (TK) substrate 1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-iodouracil (FIAU) has been proposed as an agent to image bacterial infections, based on presence of endogenous TK in bacteria [56]. The authors showed that ¹²⁵I-FIAU accumulated in bacteria but not in TK-negative bacteria, indicating the uptake was TK-mediated. Recently, we investigated whether ¹²⁴I-labeled FIAU can discriminate between bacterial and sterile inflammation using PET [57]. Images clearly showed uptake in *Escherichia coli* -induced abscesses but no accumulation in turpentine-induced sterile abscesses. These data indicate that radiolabeled FIAU potentially can distinguish between bacterial infection and sterile inflammation.

POSITRON EMISSION TOMOGRAPHY (PET) FOR IMAGING OF INFECTIOUS AND INFLAMMATORY PROCESSES: IMAGING OF ENHANCED GLUCOSE UPTAKE USING FDG

FDG accumulates in tissues with a high rate of glycolysis, which not exclusively occurs in neoplastic cells. FDG-uptake is present in all activated leukocytes (granulocytes, monocytes as well as lymphocytes) enabling imaging of acute and chronic inflammatory processes. The mechanism of FDG-uptake in activated leukocytes is related to the fact that these cells use glucose as an energy source only after activation during the metabolic burst. FDG, like glucose, passes the cell membrane. Phosphorylated FDG is not further metabolized and remains trapped inside the cell in contrast to phosphorylated glucose that enters the glycolytic pathway. ^{18}F is a positron-emitting radionuclide with a physical half-life of 110 minutes. After annihilation of a positron with an electron, two 180°-opposed gamma rays are emitted simultaneously, which can subsequently be detected by a PET camera. Increased uptake and retention of FDG has been shown in lesions with a high concentration of inflammatory cells, such as granulocytes and activated macrophages. In an experimental rat model of turpentine-induced inflammation, FDG-uptake was elevated even more in chronic inflammation than in an acute inflammatory process [58]. In another rat model of *E. coli* infection, FDG-uptake in the infectious process was higher than uptake of ^{67}Ga -citrate, radiolabeled thymidine, methionine and human serum albumin [59].

FEVER OF UNKNOWN ORIGIN

The value of FDG PET has been studied in several studies in 292 patients with FUO [60-65] showing an overall helpfulness of FDG PET corrected for study population of 36%, which is very high compared to radiological techniques and ^{67}Ga -citrate scintigraphy. Although comparing these studies is difficult, because FDG PET was performed at different stages of the diagnostic process, no structured diagnostic protocol was used and the patient characteristics differed, FDG PET appears to be a valuable new imaging technique in these patients. The impossibility to differentiate between malignancy and infection or inflammation appears to be an advantage rather than a drawback in the investigation of patients with FUO. Another major advantage of FDG PET in the work-up of patients with FUO is the vascular FDG-uptake in patients with vasculitis. From two prospective studies comparing FDG PET with ^{67}Ga -citrate scintigraphy in a total of 58 patients with FUO, it was concluded that FDG PET was superior to ^{67}Ga -citrate scintigraphy because the diagnostic yield is at least comparable to that of ^{67}Ga -citrate scintigraphy and the results are available within hours instead of days [60,61]. Based on the results of these studies and resulting from the favorable characteristics of FDG PET, conventional scintigraphic techniques may be replaced by FDG PET in the investigation of patients with FUO in institutions where this technique is available.

PROSTHETIC JOINT INFECTION

Diagnosing prosthetic joint infection is very difficult, because radiographic methods and three-phase bone scanning cannot differentiate adequately between septic and aseptic loosening. FDG PET is very sensitive in detecting infected joint prostheses, but specificity varies from approximately 50 to 95% [66,67]. In a prospective study comparing FDG PET to $^{99\text{m}}\text{Tc}$ -labeled leukocytes in combination with bone scintigraphy in patients with a hip prosthesis suspected of infection and in controls with asymptomatic hip prostheses, the combined analysis of bone scintigraphy and leukocyte scintigraphy resulted in a comparable sensitivity, but a lower specificity for FDG PET [68]. It is concluded that FDG PET has a high sensitivity in diagnosing infected joint prostheses. Specificity, however, is lower than specificity of combined leukocyte scintigraphy and bone scanning. This limited specificity probably

results from persisting FDG-uptake around the prosthesis for many years after arthroplasty, even in uncomplicated cases [66]. Location of FDG-uptake is probably important since FDG-uptake along the interface between bone and prosthesis appears to be more specific for infection [69].

INFLAMMATORY BOWEL DISEASE

High FDG-uptake has been reported in areas with inflammation in patients with inflammatory bowel disease [70,71]. Specificity of FDG PET was comparable to MRI and antigranulocyte antibody scintigraphy in 59 patients with Crohn's disease, but sensitivity of FDG PET was significantly higher [71]. FDG PET also correctly detected histologically confirmed eosinophilic colitis, collagenous colitis and bacterial colitis in a small number of patients [72]. FDG PET could become a useful tool to detect disease activity in the terminal ileum and colon in patients with inflammatory bowel disease, but physiological, non-specific bowel uptake could be an important problem in clinical practice. More data are needed to justify routine application of PET in the management of inflammatory bowel disease.

CONCLUSIONS AND FUTURE DEVELOPMENTS

Scintigraphy using autologous leukocytes, labeled with ^{111}In or $^{99\text{m}}\text{Tc}$, is still considered the "gold standard" nuclear medicine technique for the imaging of infection and inflammation, but the range of radiolabeled compounds available for this indication is expanding rapidly. A gradual shift from basic, non-specific, cumbersome and even hazardous techniques to more intelligent approaches, based on small agents binding to their targets with high affinity, is ongoing. In general, the lower molecular weight should also lead to enhanced blood clearance reducing blood pool activity. New agents should also obviate the need to handle blood as this presents potential hazards of transmission of hepatitis virus or human immunodeficiency virus to both patients and medical personnel. Radiolabeled compounds are being designed enabling specific distinction between infection and non-infectious inflammatory disease and between acute and chronic processes. The ideal agent will thus be determined by the clinical situation. The advantages of $^{99\text{m}}\text{Tc}$ as a radionuclide will be fully explored. Labeling with high specific activity will reduce the doses used resulting in less undesirable agonistic activities. Undesirable agonistic activities will also be alleviated by chemical modification of the agonist. Furthermore, FDG PET may prove to be as useful in the rapid detection and management of infectious and inflammatory diseases as it is in the management of malignant diseases.

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