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did not differ significantly from the uptake in 20 hr postinjection. The uptake in S. fMLFK-HYNIC rose to 36.8 ± 4.3 at 20 hr postinjection. E Radiopharmaceuticals Division, The Da Pont Merck Pharmaceutical Company Departments of Nuclear Medicine and Internal Medicinet University Hospital Nijmegen, postinjection onward. Abscess-to-background ratios increased to 99nrTc-HYNIC-MLF0Me were similar. With time, 99mTc-fMLFK-
tion.

Jeucyf-phenylalanyMysine-hydrazinonicotinamide (® 9nTC"fMLFK-
coli of specific binding to receptors on locally present leukocytes. To
tested.

agent 99nTc-HYNIC~MLFOMe was cleared from the abscess
HYNIO was retained in the abscess

infection and inflammation. Previous studies have shown high up-
potential to target to different infections and sterile inflammation was
molecular weight with low receptor binding affinity. In addition, the
infection in rats, rabbits and nonhuman primates can be clearly
rapid detection of inflammatory foci. The potential of several
immunopeptides and proteins has already been demonstrated
may make radiolabeled immunopeptides suitable agents for the


Methods: Twenty-four hours after induction of Escherichia coli, Staphylococcus aureus and zymosan abscesses, rabbits were i.v. injected with either 1 mCi of 99nrrTc>labeled formyl-methionyl-
nuclear medicine and internal medicine, university hospital nijmegen, nijmegen, the netherlands; and radiopharmaceuticals division, the du pont merck pharmaceutical company, north billerica, massachusetts

Chemotactic peptides have been proposed as vehicles to image infection and inflammation. Previous studies have shown high uptake at the site of infection soon after injection, most likely because of specific binding to receptors on locally present leukocytes. To investigate this hypothesis, the in vivo behavior of a synthetic chemotactic peptide was compared to a control peptide of similar molecular weight with low receptor binding affinity. In addition, the potential to target to different infections and sterile inflammation was tested. Methods: Twenty-four hours after induction of Escherichia coli, Staphylococcus aureus and zymosan abscesses, rabbits were i.v. injected with either 1 mCi of 99mTc-labeled formyl-methionyl-leucyl-phenylalanine (99mTc-fMLFK-HYNIC) or 99mTc-labeled hydrazinonicotinamide-methionyl-leucyl-phenylalanine-OME (99mTc-HYNIC-MLFOMe, control peptide). Gamma camera images were obtained at 5 min and 1, 4, 8 and 20 hr postinjection. Biodistribution was determined at 20 hr postinjection. Results: The blood clearances of 99mTc-fMLFK-HYNIC and 99mTc-HYNIC-MLFOMe were similar. With time, 99mTc-fMLFK-HYNIC was retained in the abscess (E. coli), whereas the control agent 99mTc-HYNIC-MLFOMe was cleared from the abscess (0.049 ± 0.011 versus 0.085 ± 0.003%/h) at 20 hr postinjection; p < 0.005). Abscess-to-contralateral muscle ratios of 99mTc-fMLFK-HYNIC rose to 36.8 ± 4.3 at 20 hr postinjection. E. coli, S. aureus and zymosan abscesses were clearly visualized from 4 hr postinjection onward. Abscess-to-background ratios increased to values varying from 4.4 ± 0.2 (zymosan) to 7.1 ± 0.6 (S. aureus) at 20 hr postinjection. The uptake in S. aureus and zymosan abscesses did not differ significantly from the uptake in E. coli abscesses.

Conclusion: fMLFK-HYNIC is retained in both acute infection and sterile inflammation by means of specific receptor binding if sufficient cellular infiltration is present. Key Words: chemotactic peptides; infection; inflammation; biodistribution; imaging

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During the past few years, there has been a growing interest in the development of radiolabeled immunopeptides and proteins as radiopharmaceuticals for the imaging of infection and inflammation. Their small size and receptor binding capacity may make radiolabeled immunopeptides suitable agents for the rapid detection of inflammatory foci. The potential of several immunopeptides and proteins has already been demonstrated (1-5). One promising approach is the use of chemotactic peptides (6).

Chemotactic peptides, released by bacteria, cause leukocytes to marginate to nearby endothelial surfaces, move extravascularly and accumulate at sites where a chemotactic agent has been generated, a process called chemotaxis (7). Migration of cells from the blood to sites of tissue damage in the extravascular tissue is crucial to the development of inflammation. Schiffman et al. (8) found that formyl peptides, synthetic analogs of natural bacterial products, were potent chemotactic factors for polymorphonuclear neutrophils (PMNs) and monocytes. These peptides initiate leukocyte chemotaxis by high-affinity binding to receptors present on both PMNs and mononuclear cells (9,10).

Fischman et al. (11) and Babich et al. (12) showed that acute infection in rats, rabbits and nonhuman primates can be clearly
visualized by $^{111}$In- and $^{99m}$Tc-labeled synthetic chemotactic peptides. Imaging of acute infection in rabbits with $^{99m}$Tc-labeled chemotactic peptides was superior to $^{111}$In-labeled white blood cells. High target-to-background ratios were obtained, probably as a result of accumulation of the chemotactic peptide at the site of infection.

Until now, increasing target-to-background ratios were assumed to be due to specific receptor binding of chemotactic peptides to leukocytes in inflammatory tissue, with concomitant clearance from the blood pool and other noninflamed tissues. These assumptions were based on correlations found between in vitro receptor binding and accumulation of the peptides at the site of inflammation in vivo. In this study, the specificity of focal uptake was studied directly in vivo. The in vivo behavior of the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine-l-lysine (fMLFK) was compared to the behavior of a size-matched control peptide with low receptor binding affinity. Furthermore, the potential of the chemotactic peptide to localize in different acute infections as well as in sterile inflammation was explored.

**MATERIALS AND METHODS**

**Peptide Synthesis**

fMLFK was synthesized and purified as described by Fischman et al. (1) and conjugated to hydrazinonicotinic acid (HYNIC) as described by Babich et al. (11).

The peptide methionyl-leucyl-phenylalanyl-O-Me (MLFOMe) was analogously synthesized and purified. HYNIC was conjugated to the N-terminal amine group of methionine. The peptide was used as control peptide in imaging and biodistribution studies.

**Technetium-99m Labeling of HYNIC-Conjugated fMLFK and MLFOMe**

In a 8-ml glass vial, 0.25 ml of 160 mg/ml tricine (in water), 100 µl of 0.05 mg/ml fMLFK-HYNIC or HYNIC-MLFOMe (in DMSO), 0.5 ml of 50–70 mCi $^{99m}$TcO$_4^-$ (in saline), 220 µl of ethanol and 30 µl of 1 mg/ml stannous chloride in 0.1 N HCl were mixed and incubated at room temperature for 0.5–1 hr. Subsequently, 400 µl of the reaction mixture was purified by reversed-phase chromatography using a 4.6 mm × 25 cm Zorbax Rx-C18 column. The following elution conditions were used: solvent A, 90% 25 mM sodium phosphate (pH 8) and 10% acetonitrile; solvent B, 50% 25 mM sodium phosphate (pH 8) and 50% acetonitrile; gradient, 0–100% solvent B over 25 min; flow rate, 1.0 ml/min; and UV absorption, 280 nm. The void fractions were collected in plastic tubes containing tricine (20–30 mg per tube) and transferred to a 25-ml round-bottomed flask. The solution was evaporated to dryness and then reconstituted with saline to proper concentrations for animal studies.

The radiochemical purity of the radiopharmaceuticals was determined by reversed-phase chromatography with the above described elution conditions and instant thin-layer chromatography on paper Whatman no. 1 strips with acetone and acetone: saline (3:1) as solvents.

**Receptor Binding Assays**

Human PMNs were isolated from heparinized venous blood obtained from healthy donors. Blood samples of 40 ml were mixed with an equal volume of Hank's balanced salt solution (HBSS), layered on a Ficoll–Hypaque gradient and centrifuged at 10°C and 1300 rpm for 30 min. The supernatant was discarded, and the PMN-rich pellet was resuspended in cold HBSS, to which cold 2% dextran solution (in saline) was added to remove the contaminating red blood cells. After 25 min of sedimentation at room temperature, the suspended cells were removed and centrifuged at 10°C and 1300 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 10 ml of cold hypotonic lysing buffer for a 5-min lysis of residual RBCs. After lysis, cold HBSS (40 ml) was added, and the cells were centrifuged at 10°C and 1300 rpm for 10 min. This final PMN pellet was washed once and resuspended to the desired cell concentration in phosphate buffered incubation buffer containing 140 mM NaCl, 1.0 mM KH$_2$PO$_4$, 5 mM Na$_2$HPO$_4$, 0.5 mM MgCl$_2$, 0.15 mM CaCl$_2$ and 0.5% BSA (pH 7.4) for fMLFK binding assays. For the cytochrome C reduction assays, cells were resuspended in HBSS.

**fMLF Binding Assay.** $^{[3]}$HfMLF (10 nM), fMLFK-HYNIC or HYNIC-MLFOMe and 100 µl of PMN solution (8 × 10$^5$ PMNs/ml) were added to a 96-well microplate with filters (0.65-µm pore size). The microplate was incubated for 60 min at room temperature with gentle agitation. The microplate was then placed on a filtration system. The wells were washed with incubation buffer three times and dried. The filters were removed from the microplate, placed into scintillation vials and agitated for 1 hr at room temperature in the presence of scintillation fluid. Percent inhibition of $^{[3]}$HfMLF binding to PMNs was calculated by dividing the specific binding (total binding – nonspecific binding) obtained in the presence of fMLFK-HYNIC or HYNIC-MLFOMe by that obtained in the absence of these peptides. EC$_{50}$ values were calculated by fitting the percent inhibition values to a regression line.

**Cytochrome c PMN Free Radical Release Assay.** The biologic activity of fMLFK-HYNIC was determined by measuring superoxide release of PMNs after they were exposed to fMLFK-HYNIC. Typically, PMN solution (1 ml, 1 × 10$^6$ PMNs/ml) was incubated with cytochalasin B (10 µM) and 100 µl of cytochrome c (40 µM) for 10 min at 37°C. Then, fMLFK-HYNIC was added, and tubes were incubated for 10 min at room temperature. After centrifugation (200 × g, 10°C for 10 min), the supernatants were transferred to cuvettes for spectrophotometric analysis to measure reduction of cytochrome c, reflecting the amount of superoxide release by PMNs. The percent of fMLFK-HYNIC response was determined. EC$_{50}$ values were derived from concentration-response curves.

**Animal Studies**

**Comparison of Technetium-99m-fMLFK-HYNIC and Technetium-99m-HYNIC-MLFOMe**. Abscesses were induced in the left thigh muscle of female New Zealand rabbits weighing 2.2–2.8 kg with 1.5 × 10$^9$ colony forming units of Escherichia coli in 0.5 ml. During the procedure, the rabbits were anesthetized with a subcutaneous injection of a 0.6 ml mixture of 0.315 mg/ml fentanyl and 10 mg/ml flumisonone. After 24 hr, when swelling of the muscle was apparent, groups of four rabbits were immobilized, placed prone on the gamma camera and injected with either 0.6 ml of 37 MBq $^{99m}$Tc-fMLFK-HYNIC or 0.6 ml of 37 MBq $^{99m}$Tc-HYNIC-MLFOMe in the lateral ear vein.

Images were recorded at 5 min and 1, 4, 8 and 20 hr postinjection with a single-head gamma camera equipped with a parallel-hole low-energy collimator. Images were obtained with a 75% symmetrical window over the 140-keV $^{99m}$Tc energy peak. After acquisition of 50,000–100,000 counts, the images were digitally stored in a 256 × 256 matrix.

The scintigraphic results were analyzed quantitatively by drawing regions of interest over the abscess, the uninfected contralateral thigh muscle (background) and the whole body. Abscess-to-background ratios and percentage residual activity in the abscess (abscess-to-whole body ratio × 100%) were calculated.

After completion of the final images, rabbits were killed with a lethal dose of sodium phenobarbital. Samples of blood, infected thigh muscle, uninfected contralateral thigh muscle, bone, bone marrow, lung, spleen, liver, kidneys and intestines were collected. The dissected tissues were weighed and counted in the gamma

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counter. To correct for radioactive decay, injection standards were counted simultaneously. The measured activity in samples was expressed as %ID/g. Abscess-to-contralateral muscle ratios and abscess-to-blood ratios were calculated.

The blood clearance of both labeled peptides was determined in groups of three rabbits with intramuscular E. coli infections per peptide. Blood samples were collected at 1, 5, 10, 20, 30, 60, 120 and 240 min after injection of the radiopharmaceuticals. The samples were weighed, measured and expressed as %ID/g.

In addition, white blood cell counts were measured in blood samples of healthy rabbits after injection of an equal amount of 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

as used in imaging and biodistribution studies. Blood samples were obtained at 1, 3, 5, 10, 30 and 60 min after injection.

**Technetium-99m-fMLFK-HYNIC in Staphylococcus aureus- and Zymosan-Induced Inflammations.** S. aureus abscesses were induced in the left thigh muscle of four rabbits as described above, with \( 5 \times 10^8 \) bacteria in 0.5 ml. Sterile inflammations were similarly induced in four rabbits by intramuscular injection of 1 ml of 7.5% zymosan in sterile saline. Twenty-four hours after induction of the abscesses, rabbits were i.v. injected with 0.6 ml of 37 MBq 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

The gamma camera imaging and determination of tissue biodistribution were performed as described above.

**Statistical Analysis**

All values are expressed as mean ± s.e.m. Statistical analysis was performed using a one-way ANOVA.

**RESULTS**

**Radiolabeling and Characterization of the Technetium-99m-Labeled Peptides**

Technetium-99m-fMLFK-HYNIC was eluted from the column in two overlapping peaks at 11-14 min. The control peptide, 

\[ ^{99m} \text{Tc-HYNIC-MLFOMe} \]

was eluted from the column with a similar pattern at 18-21 min. The elution pattern showed a small additional peak at 6 min, presumably representing TeO\(_4\)\(^{2-}\), which was typically less than 10%. Reversed-phase chromatography analysis of the injected preparations did not show degradation. The radiochemical purity of both radiopharmaceuticals was >96%.

The binding of [\(^3\)H]fMLF to receptors on PMNs could be completely inhibited by fMLFK-HYNIC. The IC\(_{50}\) value of fMLFK-HYNIC was 3 ± 2 nM. Nonspecific binding on average was less than 15%. In contrast, HYNIC-MLFOMe did not show any inhibition at 100 nM. Furthermore, fMLFK-HYNIC was an effective inducer of superoxide release of PMN, which was typically less than 10%. Reversed-phase chromatography analysis of the injected preparations did not show degradation. The radiochemical purity of both radiopharmaceuticals was >96%.

The % pre-injection value of 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

was determined in rabbits with intramuscular E. coli infections. Data are expressed as percentage of the injected dose. Error bars, s.e.m.

Although 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

rapidly cleared from circulation, the images clearly showed rapid accumulation in the abscess (Fig. 3). The abscesses could already be visualized at 4 hr postinjection. With time, increased abscess uptake was visible, resulting in clearly delineated abscesses at 20 hr postinjection. In contrast, the control peptide 

\[ ^{99m} \text{Tc-HYNIC-MLFOMe} \]

cleared from the abscess during the time course of the study. In addition, the images showed high uptake of 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

in spleen, liver, kidneys and bladder. Uptake in the lungs was high immediately after injection but rapidly decreased with time. The control peptide, 

\[ ^{99m} \text{Tc-HYNIC-MLFOMe} \]

showed marked accumulation in gallbladder and bowel.

**Animal Studies**

Comparison of Technetium-99m-fMLFK-HYNIC and Technetium-99m-HYNIC-MLFOMe in E. coli Infections. After injection of 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

in healthy rabbits, a minimal transient reduction in peripheral leukocyte levels of short duration was observed: immediately after injection, the white blood cell count decreased to 65% of the initial level and returned to 90%-95% within 3 min after injection (Fig. 1). The same pattern was observed in infected rabbits (data not shown).

Technetium-99m-fMLFK-HYNIC and the control peptide, 

\[ ^{99m} \text{Tc-HYNIC-MLFOMe} \]

rapidly cleared from the blood in a similar fashion (Fig. 2). After a fast initial biodistribution, 

\[ ^{99m} \text{Tc-fMLFK-HYNIC} \]

and 

\[ ^{99m} \text{Tc-HYNIC-MLFOMe} \]

cleared with t\(_{1/2}\) values of 16.4 ± 1.4 min and 13.4 ± 0.5 min, respectively, within the first hour and thereafter with t\(_{1/2}\) values of 203.2 ± 21.3 min and 301.4 ± 24.7 min, respectively.
The percentage residual activity (abscess-to-whole body ratio \( \times 100\% \)) in rabbits with an E. coli infection injected with either \( ^{99m}\)Tc-fMLFK-HYNIC or \( ^{99m}\)Tc-HYNIC-MLFOME Error bars, s.e.m.

Comparison of Technetium-99m-fMLFK-HYNIC in E. coli-, S. aureus- and Zymosan-Induced Inflammations. The images in Figure 3 demonstrate that E. coli- and S. aureus-induced infections and sterile inflammations induced by zymosan were all clearly visualized by \( ^{99m}\)Tc-fMLFK-HYNIC. Quantitative analysis of the scintigraphic data showed increasing abscess-to-background ratios in all three models to values varying from 4.4 ± 0.2 (zymosan) to 7.1 ± 0.6 (S. aureus) at 20 hr postinjection (Fig. 5).

Tissue biodistribution data, summarized in Table 1, were in agreement with the imaging data. Biodistributions of \( ^{99m}\)Tc-fMLFK-HYNIC in the three models were similar. There were no significant differences in uptake in S. aureus-, E. coli- or zymosan-induced inflammations. Both the abscess-to-contralateral muscle ratios and the abscess-to-blood ratios in the S. aureus and the zymosan models were as high as the ratios obtained in the E. coli model (Fig. 6). The sterility of the zymosan-induced inflammation was confirmed by microbiologic examination.
E. coli

DISCUSSION

Our results indicate that accumulation of fMLFK-HYNIC in acute infection and sterile inflammation takes place by virtue of binding to receptors on locally present PMNs and monocytes. The studied chemotactic peptide fMLFK-HYNIC was retained in infection, whereas a size-matched control peptide with low receptor binding affinity cleared from the infection with time.

To study the hypothesis of specific retention of fMLFK-HYNIC in infection and inflammation, we searched for a control agent with the most favorable characteristics for this purpose. HYNIC-MLFOMe met the requirements because it was a similar-sized peptide that highly resembled fMLFK-HYNIC and had a significantly lower affinity for receptors on PMNs and monocytes. Because it was more hydrophobic than fMLFK-HYNIC, a different route of clearance was observed. As shown by the scintigraphic data as well as by tissue biodistribution, fMLFK-HYNIC was cleared via both the kidneys and liver. Hepatobiliary clearance was the main route of clearance of HYNIC-MLFOMe. As a result, high activity uptake in liver, gallbladder and bowel was visible. Despite different routes of clearance, blood clearances of the two peptides was similar. Therefore, localization in infection could be compared. The different behavior of fMLFK-HYNIC compared to HYNIC-MLFOMe in terms of uptake of fMLFK-HYNIC in the abscess and clearance of HYNIC-MLFOMe from the abscess could be attributed to differences in receptor binding. The binding of fMLFK-HYNIC to receptors on leukocytes is furthermore reflected in high uptake of the agent in spleen and liver. In addition, the observation that ⁹⁹mTc-tricine did not accumulate in infection provided further evidence of specific receptor binding of fMLFK-HYNIC in inflammatory tissue (data not shown). These data are in line with data reported by Babich et al. (12). They found a correlation between the infection localization properties of chemotactic peptides in vivo and the fMLF receptor affinity, as determined in vitro. Nonspecific accumulation did not contribute significantly to infection localization.

Our data demonstrated that fMLFK-HYNIC localizes in both infection and sterile inflammation. These findings contradict results obtained by Fischman et al. They reported infection selective localization of chemotactic peptides, based on the observation that low levels of peptide were found in uninfected thermally injured tissues in rabbits (13,14) and sterile inflammations induced by intramuscular injection of anesthetic agents in nonhuman primates (15). However, assuming that the mechanism of localization of chemotactic peptides at sites of inflammation takes place by virtue of binding to receptors on PMNs and monocytes, it is to be expected that infiltration of significant numbers of these cells in tissue damaged by a sterile agent can be visualized with labeled chemotactic peptides. Therefore, most likely, accumulation of chemotactic peptide was not found in the described sterile lesions used by Fischman et al. because of a lack of cellular infiltration. This would be in agreement with low accumulation of peptide in turpentine induced inflammatory foci (data not shown), which are characterized by significant increased vascular permeability but minimal granulocyte infiltration (16,17). Apparently, increased vascular permeability only does not result in localization of the peptide at the site of the lesion. Other data support these suggestions: chemotactic peptides accumulate in chronic intestinal inflammation in rats and in lungs of rats with infiltration of neutrophils as a result of severe trauma (18,19). In accordance with these reports, our results demonstrate accumulation of chemotactic peptide in zymosan-induced sterile inflammation, an inflammation characterized by infiltration of PMNs and monocytes (20,21).

The slightly lower uptake of fMLFK-HYNIC in the zymosan-induced sterile inflammation compared to the uptake in E. coli- and S. aureus-induced infections could be explained by differences in severity of the inflammation. Injection of E. coli and S. aureus induced more severe inflammation than could be obtained by injection of zymosan. In fact, the severity of the infection can be increased by injection of more bacteria. Babich

<table>
<thead>
<tr>
<th>Organ</th>
<th>HYNIC-MLFOMe</th>
<th>fMLFK-HYNIC</th>
<th>S. aureus</th>
<th>Zymosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.005 ± 0.001</td>
<td>0.005 ± 0.0003</td>
<td>0.005 ± 0.001</td>
<td>0.004 ± 0.001</td>
</tr>
<tr>
<td>Abscess</td>
<td>0.005 ± 0.003</td>
<td>0.049 ± 0.011</td>
<td>0.056 ± 0.012</td>
<td>0.035 ± 0.007</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.001 ± 0.0004</td>
<td>0.001 ± 0.0003</td>
<td>0.001 ± 0.0003</td>
<td>0.001 ± 0.0002</td>
</tr>
<tr>
<td>Bone</td>
<td>0.002 ± 0.001</td>
<td>0.007 ± 0.005</td>
<td>0.01 ± 0.002</td>
<td>0.006 ± 0.005</td>
</tr>
<tr>
<td>Marrow</td>
<td>0.002 ± 0.001</td>
<td>0.036 ± 0.013</td>
<td>0.047 ± 0.009</td>
<td>0.050 ± 0.026</td>
</tr>
<tr>
<td>Lung</td>
<td>0.003 ± 0.0003</td>
<td>0.025 ± 0.010</td>
<td>0.048 ± 0.024</td>
<td>0.015 ± 0.004</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.003 ± 0.001</td>
<td>0.430 ± 0.111</td>
<td>0.501 ± 0.231</td>
<td>0.327 ± 0.066</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.020 ± 0.004</td>
<td>0.145 ± 0.014</td>
<td>0.138 ± 0.021</td>
<td>0.163 ± 0.030</td>
</tr>
<tr>
<td>Liver</td>
<td>0.013 ± 0.005</td>
<td>0.094 ± 0.017</td>
<td>0.114 ± 0.007</td>
<td>0.067 ± 0.011</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.073 ± 0.057</td>
<td>0.033 ± 0.014</td>
<td>0.052 ± 0.041</td>
<td>0.088 ± 0.050</td>
</tr>
<tr>
<td>Feces</td>
<td>0.196 ± 0.065</td>
<td>0.028 ± 0.011</td>
<td>0.052 ± 0.025</td>
<td>0.018 ± 0.013</td>
</tr>
</tbody>
</table>
et al. (2) obtained higher uptake values of fMLFK-HYNIC in E.
coli abscesses with $1 \times 10^{11}$ bacteria than we reported for
similar abscesses by injection of $1.5 \times 10^{10}$ bacteria. We could
not inject more E. coli because we used the maximum obtainable
E. coli concentration. S. aureus was more pathogenic; less
bacteria ($5 \times 10^{8}$) induced more severe infections compared to
E. coli, resulting in even higher uptake in S. aureus infections.
Concentrations of S. aureus of $5 \times 10^{7}$ bacteria/ml were lethal.
For induction of the sterile inflammations, we used the maxi-
num obtainable concentration of zymosan, which was as high as
7.5%.

The specific activity of the injected preparation could not be
determined because the diluted concentrations of fMLFK-
HYNIC used did not have appreciable UV absorbance at
280 nm. However, in vivo, the induced transient neutropenia
appeared to be minimal and of short duration, indicating that
excess of unlabeled fMLFK-HYNIC had been removed by
reversed-phase chromatography and that minimal amounts of
fMLFK-HYNIC were injected in the rabbits. A study in
nonhuman primates (22) showed that a similar minimal de-
crease in white blood cell count of short duration was observed
when a dose as low as 10 ng/kg of chemotactic peptide was
injected.

It has been shown that the nature of the coligand has effects
on the biodistribution of $^{99m}$Tc-labeled HYNIC-derivatized
chemotactic peptides (23). Most previous studies with these
peptides have used glucoheptonate as coligand for the radio-
labeling. Nevertheless, tricine was selected for our studies be-
cause, in the same animal model, higher abscess uptake as well
as higher abscess-to-background ratios were obtained when a dose as low as 10 ng/kg of chemotactic peptide was
injected.

**CONCLUSION**

Both acute infection and sterile inflammation can be specifi-
cally targeted with radiolabeled chemotactic peptides, provided
that these lesions are characterized by sufficient infiltration of
PMNs and monocytes. Therefore, in principal, distinction be-
 tween infection and inflammation cannot be made. The
lesions could be visualized as early as 4 h after injection. High
focal uptake was obtained. More studies are required to evaluate
whether chemotactic peptides are suitable agents for detection
of chronic inflammatory diseases because most tested models
to date are involved in acute infection or inflammation.

Several in vivo characteristics of chemotactic peptides need to
be optimized. Detection of lesions localized in liver, spleen,
kidneys or even bowels, especially in the case of less severe
lesions, will be difficult to detect with chemotactic peptides
because of high physiologic uptake in these organs. Because
this is a major limitation of almost every infection detection
agent, possible ways to modify the peptides to decrease back-
ground activity in the abdomen should be explored. In addition,
reduction of biologic activity of chemotactic peptides will be
necessary to apply chemotactic peptides clinically. The develop-
ment of a high specific activity $^{99m}$Tc-labeling has been a step
in the right direction but does not warrant safe administration
to humans. Therefore, further research in this area should
focus on the development of antagonists or biologically less
active agonists with similar binding characteristics.

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peptides, P. Mast for determination of the leukocyte counts and G.
Gruuters and H. Eijkholt (University of Nijmegen, Central Animal
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