The Localization of Indium-111-Leukocytes, Gallium-67-Polyclonal IgG and Other Radioactive Agents in Acute Focal Inflammatory Lesions

John G. McAfee, George Gagne, Gopal Subramanian, and Richard F. Schneider

Divisions of Nuclear Medicine and Radiological Sciences, Department of Radiology, SUNY Health Sciences Center, Syracuse, New York

A variety of radioactive agents, injected directly intravenously have demonstrated foci of inflammation by gamma camera imaging, avoiding the in vitro preparation of labeled leukocytes. This study sought to find out if any of these agents mimicked the biodistribution in abscesses and non-target organs of labeled mixed leukocyte suspensions. Eight different agents were compared with ^{111}In-oxine labeled leukocytes in an acute soft tissue *E. coli* abscess and an acute arthritic lesion in 24 dogs one day after intravenous administration. These included ^{67}Ga-citrate, human and canine polyclonal immunoglobulin (IgG), rabbit anti-dog polyclonal IgG, serum albumin, monoclonal antibody TNT-1 F(ab')$_2$ against nuclear antigens, ^{57}Co-porphyrin and serum albumin nanocolloid. None of these agents achieved abscess concentrations approaching those obtained with labeled leukocytes, and their abscess/blood and abscess/muscle concentration ratios were considerably lower. No statistically significant differences were found between the different radiolabeled proteins evaluated. The abscess concentration of ^{99m}Tc-nanocolloid was much lower than that of other agents, and the results with the oldest agent, ^{67}Ga-citrate, were disappointing in these acute experiments.

J Nucl Med 1991; 32:2126–2131

A large number of radioactive agents have successfully demonstrated focal inflammation by gamma camera imaging (*1*). The nuclear medicine physician has to select which approach to use in different clinical situations. Many centers use the combination of ^{67}Ga and autologous mixed leukocyte suspensions labeled with ^{111}In-lipophilic chelates [oxine or tropolone]. Probably the best ^{99m}Tc agent for leukocyte labeling in vitro is HMPAO [hexamethylpropyleneamine oxime]. However, the cell labeling yield with this agent varies from batch to batch (*2*, *3*). In soft-tissue lesions in dogs, the abscess concentrations and abscess/muscle ratios with ^{99m}Tc-HMPAO cells are only about one-third as high as the corresponding values with ^{111}In-oxine cells (*2*) at 24 hr. In induced canine osteomyelitis in the tibia (*3*), the lesion concentrations are about 30%–40% lower with ^{99m}Tc-HM-PAO cells than with ^{111}In-oxine cells. The diffuse activities seen in the abdomen after several hours and the normal urinary activity are major disadvantages of ^{99m}Tc-HMPAO. In vitro phagocytic labeling of leukocytes with ^{99m}Tc-albumin colloid has been advocated from time to time. In one technique (*4*), 50% of the label is released from the cells in a few hours, suggesting considerable cell-surface adherence rather than irreversible phagocytosis.

In recent years, other agents have been tried which can be directly injected intravenously, avoiding the time-consuming process of harvesting and labeling leukocytes in vitro. A nanometer-sized albumin colloid [nanocolloid, Nanocoll TN, Solco Basle Ltd, Birsfelden, Switzerland] has been advocated particularly for osteomyelitis or arthritis of the extremities (*5*, *6*). The tissue uptake is thought to reflect increased capillary permeability in inflammatory lesions. The plasma clearance of the nanocolloid is much more rapid than macromolecules such as plasma albumin or globulins, so that images with ^{99m}Tc-labeled nanocolloid can be obtained in 30 min or 2 hr. Experimental lesions are demonstrated better with nanocolloid [mean particle size 30 nm] than with albumin microcolloid. In one clinical study (*7*), nanocolloid was superior to ^{67}Ga in osteomyelitis and septic arthritis, but could not differentiate sterile from septic lesions or from surgical skeletal trauma. Anionic phospholipid liposomes also have been proposed for focal inflammatory lesions, including arthritis (*8*).

Originally developed against carcinoembryonic antigen (*9*), an antigranulocyte IgG$_1$ monoclonal antibody designated BW250/183 by Behringwerke is available in Germany for in vivo granulocyte labeling with Tc-99m. After intravenous injection, about 20% becomes bound to granulocytes in vivo (*10*); thereafter, the activity localizes in abscesses perhaps by both granulocyte migration and direct

Received Mar. 8, 1991; revision accepted Jun. 4, 1991

For reprints contact: John G. McAfee, MD, Division of Nuclear Medicine, Department of Radiology, The George Washington University Hospital, 901 23rd St., N.W., Washington, DC 20037.

binding to exposed antigen of damaged cells at the inflammatory site. Because of the high degree of species-specificity of cell-surface antigens, this approach is not amenable to animal experiments. Nonspecific polyclonal human IgG labeled with ^{111}In after DTPA conjugation and with ^{99m}Tc has successfully localized inflammatory foci in many clinical reports (*11–14*) following the original work in experimental animals (*15*). Its mechanism of localization is not fully understood and is controversial. Binding to leukocyte Fc receptors has been postulated (*16*) and rejected (*17*).

Years ago, radiolabeled porphyrins were tried for tumor localization. Most of these, such as ^{57}Co-tetraphenylporphinesulfonate (TPPS), become tightly bound to plasma proteins resulting in slow blood clearance (*18*). More recently (*19*), TPPS accumulated in mice at the site of injections of histamine, a well-known mediator of the inflammatory response. In a clinical trial of ^{99m}Tc-TPPS in 16 patients with inflammatory lesions, images were all positive at 2 hr, similar to the findings at 24 hr with ^{111}In-oxine leukocytes administered simultaneously (*18*).

In this paper, we have compared the biodistribution of several proteins labeled with ^{131}I or ^{125}I, ^{99m}Tc-nanocolloid, ^{57}Co-porphyrin and ^{67}Ga with ^{111}In-oxine-labeled leukocytes, in a canine abscess model reported previously (*20*). The essence of this study was to find out if any of these agents administered directly intravenously approached the abscess concentration and lesion contrast of labeled leukocytes.

MATERIALS AND METHODS

All proteins were labeled with either ^{131}I or ^{125}I by the Iodobead technique, as previously described (*21*). These included human polyclonal IgG [Sandoglobulin, Sandoz] and human serum albumin for comparison. To exclude the possibility of species differences, radioiodinated canine polyclonal IgG also was used. According to Bach (*22*), the binding of most IgG antibodies to Fc receptors of leukocytes is more efficient when complexed to antigen, or when aggregates of IgG are formed. Accordingly, radioiodinated rabbit polyclonal antibody against canine IgG was also radioiodinated, in the hope that antigen-antibody complexes would form in vivo and become bound to leukocytes.

Epstein (*23*) developed a monoclonal antibody designated TNT-1, an IgG2a F(ab')$_2$ fragment against abundant insoluble nondiffusible human nuclear antigens which become accessible in abnormally permeable degenerating or necrotic tumor cells. This MAb reached relatively high tumor/non-target ratios in human tumor xenografts in mice. This agent was screened for abscess localization after radioiodination because of the possibility that purulent material might contain abundant nuclear antigen from necrotic neutrophils or other cells.

Cobalt-57-TPPS was prepared as previously described (*18*). This was used instead of the ^{99m}Tc-complex, because the latter was very unstable. Technetium-99m nanocolloid was prepared from Nanocoll Kits [Solco Basle Ltd, Birsfelden, Switzerland], ^{67}Ga-citrate and ^{111}In-oxine were obtained commercially. The purification methods and labeling yields for the various agents are given in the Appendix.

In 24 mongrel dogs, bacterial abscesses were induced by the intramuscular injection of a concentrated culture of *E. coli* [$>10^{10}$ organisms in 0.5 ml] into the lateral head of the right triceps muscle, as described previously (*20*). The following day, under pentobarbital anesthesia, 30 mg of sodium urate crystals suspended in sterile water were injected into the left stifle (knee) joint to induce a neutrophilic exudation maximal in about 4 hours, simulating the acute inflammatory response of gout (*24*). In this lesion, most of the cells in the exudate remain intact, so that cell-bound radioactivity can be quantified and separated from extracellular activity by simple centrifugation. In contrast, purulent material contains so much cellular debris that intracellular and extracellular components cannot be separated.

In this group of 24 dogs, autologous mixed leukocytes were harvested, labeled with ^{111}In-oxine and reinjected intravenously as previously reported (*20*), 24 hr after the induction of the *E. coli* abscess, simulating the routine clinical method of most laboratories. This method was the standard for comparison for other agents administered simultaneously. Fourteen dogs received ^{67}Ga-citrate also. Four dogs were given human polyclonal IgG, 4 canine IgG, 4 rabbit anti-dog IgG, and 5 were given monoclonal antibody TNT. Three dogs were given labeled serum albumin, three ^{57}Co-TPPS and three ^{99m}Tc-nanocolloid. Only three radionuclides were administered in each dog, facilitating the Compton corrections for the gamma emissions of different energies in each of the three channels when tissue samples were counted in an LKB automatic well gamma counter. The approximate average activities administered were as follows: 700 μCi ^{111}In-oxine leukocytes; 90 μCi ^{67}Ga-citrate, 110–170 μCi ^{131}I-labeled human or dog IgG, 230 μCi ^{125}I-albumin, 130 μCi ^{125}I-anti-dog rabbit IgG, 300 μCi ^{131}I-TNT-1 Mab F(ab')$_2$, 50 μCi ^{57}Co-porphyrin, and 10 mCi ^{99m}Tc-nanocolloid. The activity administered varied somewhat in each experiment, but the results were always normalized with a dilute standard of the quantity administered. Appropriate corrections were made for the Compton contributions of the hgher energy gamma emitters such as ^{131}I in the photopeak windows of the lower energy radionuclides such as ^{125}I.

Following the previously reported protocol (*20*), gamma camera images of the abscess area and knees were obtained of the ^{111}In-labeled leukocytes at 4 and 24 hr. Blood and plasma samples were obtained at 2 min, 1, 2, 3, 4 and 24 hr after the radioactive injections. The left stifle joint was aspirated at 4 and 24 hr. After killing at 24 hr, tissue samples were weighed and radioassayed by well counting of lung, liver, spleen, muscle, marrow, abscess contents and whole blood and plasma samples. Cell-bound radioactivity was estimated from the whole blood and plasma counts and the microhematocrit. The 4- and 24-hr stifle joint aspirates were counted and centrifuged. Both the cell button and supernatant were then counted. The data were expressed as percent administered activity per gram and as percent administered activity per 1% animal weight. The tissue and organ concentrations of different agents were compared by analysis of variance and differences between means were examined by Tukey's procedure (*25*).

RESULTS

The images of the abscess and joint lesions obtained with ^{111}In-oxine leukocytes were positive in all of the dogs. The *E. coli* abscess concentrations at 24 hr of the nine radioactive agents under study, expressed as the percent administered activity per gram, are compared in Figure

1A. The absolute concentration of ^{111}In-leukocytes is almost ten times higher than most of the other agents, and the concentration of nanocolloid about 100 times lower than the majority. After omitting the highest and lowest values, ^{111}In-leukocytes and ^{99m}Tc-nanocolloid, analysis of variance was performed on the remaining concentrations. The only difference between means ($F = 2.78$, $p < 0.05 > 0.025$) was the relatively high value of canine IgG compared with the low value for ^{67}Ga. This was of marginal significance. Human and canine IgG were not significantly different than serum albumin. The concentrations of the various proteins and protein-bound porphyrin are shown in Figure 1B on an expanded scale.

The 24-hr abscess/blood concentration ratios are shown in Figures 2A–2B. The values for ^{111}In-leukocytes and gallium were higher than those of the labeled proteins, Co-porphyrin and nanocolloid. The 24-hr abscess/muscle ratio for ^{111}In-leukocytes is more than ten times higher than those of all other agents, and gallium and nanocolloid had the lowest ratios (Figs. 3A–B). By analysis of variance of all agents except ^{111}In-leukocytes, ($F = 6.50$, $p < 0.005$),

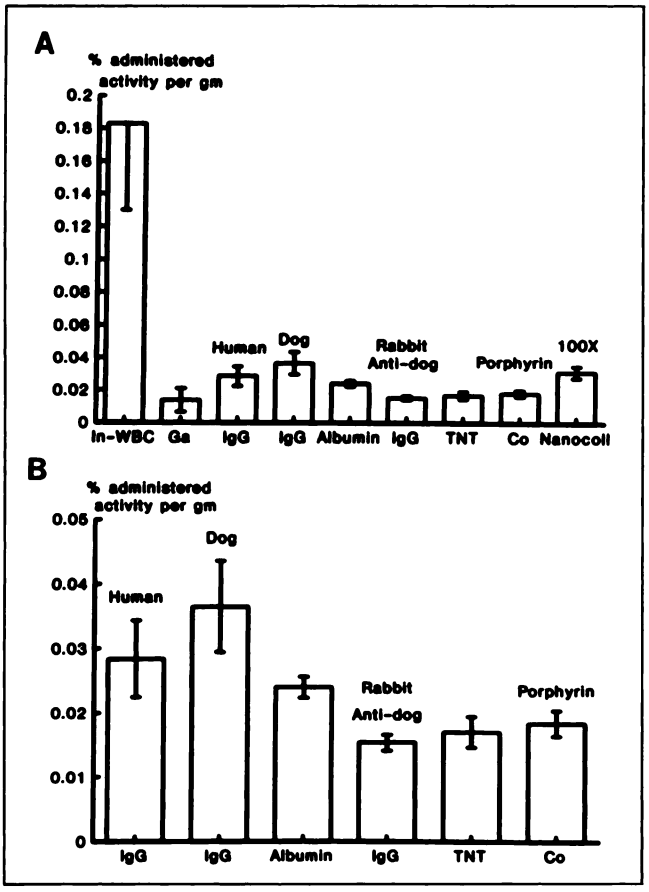

FIGURE 1. Abscess concentrations at 24 hr after radioactive administrations. Mean values ± s.e.m. (A) The concentration of In-leukocytes is much higher, and that of Tc-nanocolloid much (100× scale) lower than the other agents. (B) Same data as in A, on a different scale, omitting In-leukocytes, gallium and Tc-nanocolloid, comparing the various proteins and protein-bound porphyrin.

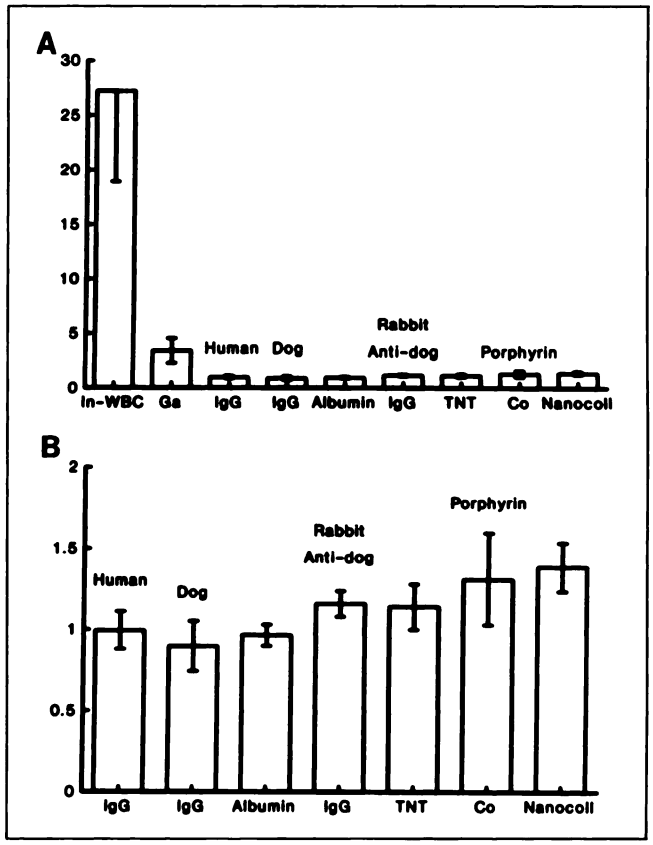

FIGURE 2. Abscess/blood ratios. (A) ^{111}In-leukocytes have the highest ratio. (B) Same data as in A, omitting ^{111}In-leukocytes and gallium.

the ratios for nanocolloid and particularly gallium were significantly lower than either canine or human polyclonal IgG.

After the aspirates of the left stifle joint were centrifuged to separate the cell-bound from supernatant radioactivity, a mean of 91% of the ^{111}In-oxine aspirate activity was in the cellular button at 4 hours and 89% at 24 hours. With rabbit anti-dog IgG, 13% was found in the cell button at 24 hr. For TNT-1, 13–15% was recovered in the cell button at 4 and 24 hr, perhaps because the $F(ab')_2$ fragments diffuse more readily than intact immunoglobulins. The amount of nanocolloid in the joint aspirates was negligible or about 100 times less than the labeled proteins. In contrast, for most of the other agents, including albumin, only 5%–6% of the activity was in the cell button, and even this amount may have been due to soluble activity trapped during cell precipitation.

The distribution of the nine radioactive agents in the major organs at 24 hr is summarized in Table 1. Of the labeled proteins, human and canine IgG and serum albumin had the highest blood concentrations at 24 hr. Somewhat lower concentrations were found with rabbit anti-dog polyclonal IgG, TNT-1 $F(ab')_2$ and ^{57}Co-porphyrin. The organ concentrations of nanocolloid were extremely low, except for the liver and marrow. The lung concentra-

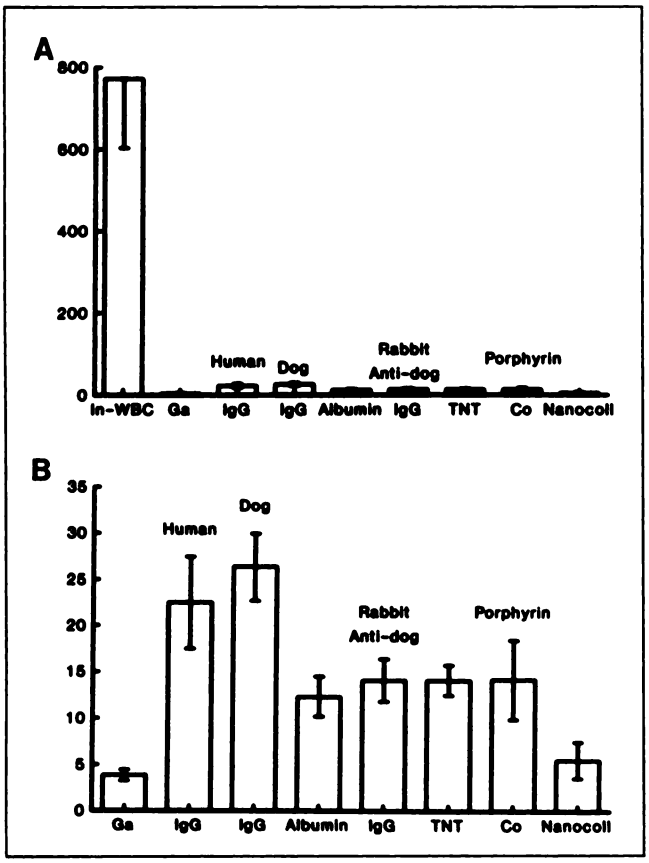

FIGURE 3. Abscess/muscle ratios. (A) The ^{111}In-leukocyte ratios dwarf the values of the other agents. (B) Same data as in A, on a different scale, omitting ^{111}In-leukocytes.

tions were highest for ^{111}In-leukocytes, followed by canine IgG albumin and ^{57}Co-porphyrin. Indium-111-leukocytes had the highest concentrations in the liver and spleen. The hepatic and splenic concentrations of gallium were similar to one another. Anti-dog rabbit IgG had an unanticipated high uptake in the spleen. Besides nanocolloid, the muscle concentration of ^{111}In-leukocytes was relatively low, probably reflecting the low blood level by 24 hr. The marrow concentration of ^{111}In-leukocytes was the highest, followed by nanocolloid and gallium.

DISCUSSION

Multiple comparisons of the various agents proposed for imaging focal inflammatory lesions in patients are practically difficult. Hence, we compared eight different radioactive agents in a canine abscess and joint inflammatory lesion to labeled leukocytes as a "gold standard." Canine granulocytes labeled with ^{111}In-oxine have a normal maximal recovery and survival in the circulating blood (*20*) close to that of humans, (*26*) (32%–36% and 6–7 hrs, respectively) unlike the behavior of rodent granulocytes. This study was limited only to acute lesions of soft tissues, emphasizing lesion concentrations measured by direct in vitro radioassay and contrast ratios with blood and muscle at 24 hr. These results may be different at various time intervals after injection, in subacute and chronic inflammations, in osteomyelitis and in patients with immunodeficiency, particularly when donor leukocytes are not used. Within the experimental limitations of this comparison, none of the agents injected directly intravenously approached the high lesion concentrations or contrast ratios of the leukocytes labeled in vitro. Our results with ^{67}Ga-citrate in these acute lesions were particularly unimpressive. Likewise, with ^{99m}Tc-nanocolloid, we found very low concentrations in the soft-tissue lesions, despite some preliminary good results in osteomyelitis of the extremities (*27*).

Modern preparations of human immunoglobulin are now so safe that they are being used in massive doses as anti-infective agents, in prophylaxis of infection in immunodeficient patients, and special preparations with high titers of antibody for specific target pathogens (*28*). Polyclonal IgG labeled with ^{111}In or ^{99m}Tc has undergone numerous successful clinical trials for inflammatory foci, whereas other proteins rarely have been used. Comparisons of different radiolabeled proteins for detection of inflammatory foci have been limited and the published

TABLE 1
Organ Concentrations in Dogs at 24 Hours (%Administered Activity/1% Animal Weight Mean Values; s.e.m. in parentheses)

	^{111}In-WBC	Ga	Human IgG	Dog IgG	Albumin	Rabbit anti-dog IgG	TNT	^{57}Co-porphyrin	Nanocolloid
Blood	1.68	0.576	5.65	6.96	4.82	2.46	2.93	2.79	0.0397
	(0.231)	(0.166)	(0.279)	(0.660)	(0.115)	(0.840)	(0.291)	(0.455)	(0.00352)
Lung	3.50	1.45	2.06	3.53	2.53	1.28	1.94	2.51	0.0712
	(0.520)	(0.346)	(0.399)	(0.880)	(0.328)	(0.193)	(0.330)	(0.676)	(0.00704)
Liver	14.3	6.67	1.81	2.09	1.73	2.77	1.40	3.96	11.8
	(1.10)	(1.89)	(0.383)	(0.705)	(0.296)	(0.114)	(0.236)	(1.30)	(0.918)
Spleen	22.6	5.08	2.19	1.21	1.71	11.7	0.739	2.05	0.915
	(3.15)	(1.62)	(0.361)	(0.448)	(0.860)	(0.182)	(0.131)	(0.147)	(0.298)
Muscle	0.0480	0.349	0.249	0.236	0.392	0.214	0.237	0.269	0.0121
	(0.0074)	(0.0863)	(0.0384)	(0.0315)	(0.0409)	(0.0278)	(0.236)	(0.0331)	(0.00313)
Marrow	4.30	2.26	1.17	1.39	1.13	1.45	0.670	1.95	2.95
	(0.759)	(0.444)	(0.153)	(0.352)	(0.143)	(0.122)	(0.151)	(0.509)	(0.531)

results conflicting. In *E. coli* thigh abscesses in rats, ^{111}In-IgG showed greater accumulation at 3 and 24 hr than either ^{67}Ga- or ^{99m}Tc-serum albumin (*15*). However, most preparations of ^{99m}Tc-albumin are notoriously unstable, especially by 24 hr. In another study of sterile inflammatory lesions in rabbit muscle (*29*), inflamed-to-normal muscle ratios were only slightly better with ^{111}In-IgG than with ^{131}I-albumin, and inferior to those of ^{99m}Tc-leukocytes. In *E. coli* abscesses in mice, abscess concentrations measured by direct radioassay were about the same for ^{67}Ga, ^{99m}Tc-IgG and ^{99m}Tc-albumin at 4 and 24 hr (*30*). In one clinical comparison (*31*), five osteomyelitic lesions were demonstrated with both ^{99m}Tc-IgG and ^{111}In-leukocytes, with one false-positive IgG study. Five of six positive leukocyte studies also were positive on IgG images. In another small series of 12 patients with sites of infection (*32*), the target/non-target ratios of ^{99m}Tc-polyclonal IgG were not as high as those of ^{99m}Tc-antigranulocyte mAb. The present study indicated only minimal differences between various radiolabeled proteins in their concentrations in inflammatory lesions and in their contrast ratios, and none of these differences were statistically significant.

Experimental evidence to date does not support the notion that exogenous radiolabeled polyclonal IgG binds in vivo to Fc receptors of leukocytes, successfully displacing endogenous IgG. For all three types of Fc surface receptors of polymorphs, the binding of monomeric IgG is poor, and the plasma contains one thousand times molar excess over receptors of circulatory cells (*33*). Polymeric IgG has a low affinity for one type of Fc receptor and some affinity for a second present in limited numbers per cell. Fc and Fabc fragments bind with high affinity to a third receptor, but the number of binding sites per cell is very small (*33*). In another in vitro study (*34*), binding of ^{99m}Tc-polyclonal IgG to granulocytes was only 2.7%, but to monocytes, 20%. Moreover, this binding was blocked by small amounts of carrier IgG. In contrast, the binding of ^{99m}Tc-antigranulocyte Mab to granulocytes was higher, and not blocked by carrier IgG. In the present study, only about 5% of the labeled proteins, including IgG, were found in the precipitated leukocyte pellet of the joint inflammatory lesions, compared with the binding of about 90% for the ^{111}In-labeled leukocytes. These findings suggest that polyclonal IgG, like other macromolecular proteins, detects inflammatory lesions by passive diffusion in areas of increased permeability of the microvasculature.

In conclusion, the results of this study suggest that the continued use of in vitro labeled fresh leukocyte suspensions still should be considered for optimal delineation of inflammatory foci, despite the inconvenience and time consuming steps of cell harvesting and labeling. However, meticulous cell preparation and prompt reinjection are essential to preserve cell viability and migration. If these cannot be achieved, the other options should be considered.

The search for better agents injected directly intravenously to detect and localize foci of infection is important, and should be continued until the lesion concentration and contrast are comparable to those of labeled leukocytes. From a clinical standpoint, ^{99m}Tc-agents have inherent advantages over ^{67}Ga, because this radionuclide is always on hand in emergency situations and images obtained in a few hours may provide useful answers.

APPENDIX

Purification of the Radiolabeled Agents and Labeling Yields

The radioiodinated proteins in 500–700 ml were passed through a BIORAD PIODG gel filtration column (molecular weight cut off 10 kD) pretreated with eluting solution 0.05 *M*, pH 7 Tris in 0.15 *M* NaCl. After draining, 2.5 ml eluting solution were added and discarded. Next, 4 ml of eluting solution were added and collected. The percent labeling yield was calculated from the activity in this 4 ml eluate compared to the total added activity.

Quality control of the labeled products was performed using an ITLC method. Whatman 31 ET paper strips 1 cm × 6 cm and 0.1 *M* ammonium sulfate with 2.5% triethylamine was used as solvent. In this system, unbound radioiodine moved with the solvent front. The percent purity was calculated as 100 minus percent activity in the solvent front.

Cobalt-57-prophyrin was purified by loading on an alumina column, washing with water to remove the cobalt chloride and eluting the complex with 1:1 NH_4OH and water. Quality control of this product was performed with reverse phase TLC using Whatman medium C-18 glass plate, and solvent 0.05 *M* sodium phosphate pH 5.0, methanol and tetrahydrofuran in a 32:48:19 ratio. The cobalt chloride remained at the origin.

The ^{99m}Tc-nanocolloid labeling yield was determined by ITLC.

Mean %Labeling Yields

Radioiodinated Proteins	
Human serum albumin	94.7
Human polyclonal IgG	93.8
Canine polyclonal IgG	96.2
Rabbit anti-dog IgG	96.3
TNT F(ab')$_2$	97.6
Cobalt-57-porphyrin	88
Technetium-99m-nanocolloid	98
Mean leukocyte labeling efficiency of ^{111}In-oxine	90.7

ACKNOWLEDGMENTS

The authors wish to thank Dr. Alan L. Epstein, Department of Pathology, University of Southern California for generously supplying his monoclonal antibody TNT-1 F(ab')$_2$ fragment for this comparative study.

This research was supported by grant CA-32853 from the National Cancer Institute/DHHS, its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

REFERENCES

1. McAfee JG. What is the best method for imaging focal infections? [Editorial]. *J Nucl Med* 1989;30:413–416.
2. McAfee JG, Subramanian G, Gagne G, Schneider RF, Zapf-Longo C.

^{99m}Tc-HM-PAO for leukocyte labeling: experimental comparison with ^{111}In oxine in dogs. *Eur J Nucl Med* 1987;13:353–357.
3. Mock BH, Schauwecker DS, English D, Young KA, Wellman HN. In vivo kinetics of canine leukocytes labeled with technetium-99m-HM-PAO and indium-111-tropolonate. *J Nucl Med* 1988;29:1246–1251.
4. Marcus CS, Kuperus JH, Butler JA, et al. Phagocytic labeling of leukocytes with ^{99m}Tc-albumin colloid for nuclear imaging. *Nucl Med Biol* 1988;15:673–688.
5. deSchrijver M, Streule K, Senekowitsch R, et al. Scintigraphy of inflammation with nanometer-sized colloidal tracers. *Nucl Med Commun* 1987;8:895–908.
6. Streule K, de Schrijver M, Fridrich R. Tc-99m labelled HSA-nanocolloid vs. In-111 oxine labelled granulocytes in detecting skeletal septic processes. *Nucl Med Commun* 1988;9:59–67.
7. Abramovici J, Rubinstein M. Tc-99m nanocolloids: an alternative approach to the diagnosis of inflammatory lesions of bones and joints [Abstract]. *Eur J Nucl Med* 1988;14:244.
8. Morgan JR, Williams LA, Howard CB. Technetium-labelled liposome imaging for deep-seated infection. *Br J Radiol* 1985;58:35–39.
9. Audette M, Buchegger F, Schreyer M, Mach J-P. Monoclonal antibody against carcinoembryonic antigen (CEA) identifies two new forms of cross-reacting antigens of molecular weight 90,000 and 160,000 in normal granulocyte. *Mol Immunol* 1987;24:1177–1186.
10. Joseph K, Hoffken H, Bosslet K, Schorlemmer HU. Imaging of inflammation with granulocytes labelled in vivo. *Nucl Med Commun* 1988;9:763–769.
11. Fischman AJ, Rubin RH, Khaw BA, et al. Detection of acute inflammation with ^{111}In-labeled non-specific polyclonal IgG. *Semin Nucl Med* 1988;18:335–344.
12. LaMuraglia GM, Fischman AJ, Strauss HW, et al. Utility of the indium-111-labeled human immunoglobulin G scan for the detection of focal vascular graft infection. *J Vasc Surg* 1989;10:20–28.
13. Rubin RH, Fischman AJ, Callahan RJ, et al. ^{111}In-labeled nonspecific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989;321:935–940.
14. Oyen WJG, Claessens RAMJ, van Horn JR, van der Meer JWM, Corstens FHM. Scintigraphic detection of bone and joint infections with indium-111-labeled nonspecific polyclonal immunoglobulin G. *J Nucl Med* 1990;31:403–412.
15. Rubin RH, Fischman AJ, Needleman M, et al. Radiolabeled, nonspecific polyclonal human immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67 citrate and technetium-99m-labeled albumin. *J Nucl Med* 1989;30:385–389.
16. Strauss HW, Fischman AJ, Khaw BA, et al. Detection of acute inflammation with immune imaging. In: Chatal J-F, ed. *Monoclonal antibodies in immunoscintigraphy.* Boca Raton, FL: CRC Press; 1989:325–335.
17. Fishman AJ, Rubin RH, White JA, et al. Localization of Fc and Fab fragments of nonspecific polyclonal IgG at focal sites of inflammation. *J Nucl Med* 1990;31:1199–1205.
18. Winkelman J, McAfee JG, Wagner HN Jr, et al. The synthesis of Co-57-tetraphenylporphinesulfonate and its use in the scintillation scanning of neoplasms. *J Nucl Med* 1962;3:249–253.
19. Zanelli GD, Bjarnason I, Smith T, Crawley JCW, Levi AJ, Copeland RI. Technetium-99m labelled porphyrin as an imaging agent for occult infections and inflammation. *Nucl Med Commun* 1986;7:17–24.
20. McAfee JG, Gagne GM, Subramanian G, et al. Distribution of leukocytes labeled with In-111-oxine in dogs with acute inflammatory lesions. *J Nucl Med* 1980;21:1059–1068.
21. Rosebrough SF, McAfee JG, Grossman ZD, Schemancik LA. Immunoreactivity of ^{111}In and ^{131}I fibrin-specific monoclonal antibody used for thrombus imaging. *J Immunol Meth* 1989;116:123–129.
22. Bach J-F, ed. *Immunology*, second edition. New York: Wiley; 1982:76.
23. Epstein AL, Chen F-M, Taylor CR. A novel method for the detection of necrotic lesions in human cancers. *Cancer Res* 1988;48:5842–5848.
24. McCarty DJ, Phelps P, Pyenson J. Crystal-induced inflammation in canine joints. I. An experimental model with quantification of the host response. *J Exp Med* 1966;124:99–114.
25. Steel RGD, Torrie JH. *Principles and procedures of statistics: a biometrical approach*, second edition. New York: McGraw-Hill; 1980:185 and 192.
26. Weiblen BJ, Forstrum L, McCullough J. Studies of the kinetics of indium-111 labeled granulocytes. *J Lab Clin Med* 1979;94:246–255.
27. Hotze A, Ruther W, Briele B, et al. Comparison of ^{99m}Tc-HMPAO labeled leukocytes, ^{99m}Tc-labeled antigranulocyte antibody (AGAB) and ^{99m}Tc-nanocolloid in orthopedic patients with suspected bone infection [in German]. *NucCompact* 1988;19:176–181.
28. Pennington JE. Minireview: Newer uses of intravenous immunoglobulins as anti-infective agents. *Antimicrob Agents and Chemotherapy* 1990;34:1463–1466.
29. Breitz HB, Modell HI, Graham, MM. Localization of inflammation with ^{111}In IgG and ^{99}Tc^{m} albumin colloid labelled leukocytes in a rabbit model. *Nucl Med Commun* 1990;11:737–745.
30. Thakur ML, DeFulvio J, Park CH, et al. Evaluation of Tc-99m-labeled proteins for localization of inflammation: comparison with I-125-TNT and Ga-67 citrate [Abstract]. *J Nucl Med* 1990;31:810.
31. Buscombe JR, Lui D, Ensing G, de Jong R, Ell PJ. ^{99m}Tc-human immunoglobulin (HIG)—first results of a new agent for the localization of infection and inflammation. *Eur J Nucl Med* 1990;16:649–655.
32. Carrio I, Dunker C, Berhá L, Estorch M. Delineation of infection sites by means of ^{99m}Tc-polyclonal immunoglobulin and ^{99m}Tc-antigranulocyte monoclonal antibody studies. *Eur J Nucl Med* 1990;16:426.
33. Eckle I, Reifer R, Hoferichter A, Kolb G, Havemann K. High affinity binding of immunoglobulin G fragments to human polymorphonuclear leukocytes. *Biol Chem Hoppe-Seyler* 1990;371:1107–1111.
34. Becker W, Saptogino A, Wolf F. Tc-99m labelled human polyclonal immunoglobulins for localisation of infectious lesions in humans: uptake mechanism in-vitro [Abstract]. *Eur J Nucl Med* 1990;16:426.