Detection of a Local Staphylococcal Infection in Mice with Technetium-99m-Labeled Polyclonal Human Immunoglobulin

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The purpose of this study was to investigate both the ability of ^{99m}Tc-labeled polyclonal human immunoglobulin (HIG) to localize an infection and the modes of action involved in this process. Mice, infected with *Staphylococcus aureus* ATCC 25923 in a thigh muscle, received HIG intravenously. Scintigrams were made 1, 4, and 24 hr later; subsequently the mice were killed and the activity in several organs and thighs was determined. The radiopharmaceutical demonstrated a time-dependent accumulation at the site of infection. It was found that vascular permeability or Fc binding alone could not account for the mode of action of HIG. Neither the origin of Ig (human versus murine) nor the total amount of protein (0.01–1.0 mg Ig per mouse) affected the target-to-background (T/B) ratios. Ratios were not different for leukocytopenic animals. A correlation ($p < 0.001$) was demonstrated between the number of bacteria at the site of infection and the T/B ratio. This was also found after antibiotic treatment ($p < 0.02$).

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Infections are an important cause of morbidity and mortality, especially in immunocompromised patients (*1,2*). Therefore, it is essential to be able to locate the infection and to determine the causative microorganism for optimum antimicrobial therapy. For localization purposes, a variety of radiopharmaceuticals has been developed, such as gallium-67, monoclonal and polyclonal antibodies (*3–8*), and blood granulocytes (*4,9, 10*). The major drawback of the use of monoclonal antibodies in patients is their non-human nature, which eventually leads to all kinds of immunologic reactions. The use of blood granulocytes is limited because of their complexity and the time-consuming preparations before imaging. Polyclonal immunoglobulins lack both of these restrictions, and promising results have been reported. Experimentally many authors have demonstrated localization, especially for deep-thigh infections (*6,7*) and atherosclerosis in rats (*11*). However, they used immunoglobulins labeled with indium-111, a nuclide with a half-life of 67.4 hr, which therefore causes a relatively high radiation burden to the patients. For this reason, it is worthwhile to link the immunoglobulins with a nuclide with a shorter half-life, especially when imaging could then be performed shortly after administration of the radiopharmaceutical. For this reason technetium-99m (^{99m}Tc) was chosen. Combining immunoglobulins with ^{99m}Tc did yield excellent images of inflammatory arthritis in rats (*5*). Furthermore, in a preliminary study about the localization of a thigh infection in mice by various ^{99m}Tc-labeled preparations polyclonal human immunoglobulin was found to be successful. Possible modes of action however were not investigated (*8*).

In the present study, the ability of modified polyclonal human immunoglobulin labeled with ^{99m}Tc to detect a thigh infection with *Staphylococcus aureus* in mice was investigated. Furthermore, the mode of action was studied. Finally, some experiments were performed to see whether the radiopharmaceutical provides information about the severity of the infection.

MATERIALS AND METHODS

Mice

Female, specific pathogen-free Swiss mice weighing 20–25 g (Broekman Institute, Someren, The Netherlands) were used in all experiments. They were housed for one week before the experiments were started. Food and water were given ad libitum.

Microorganisms

Staphylococcus aureus (ATCC 25923) from the American Type Culture Collection, Rockville, MD, was used throughout the study. Some experiments were carried out with *Klebsiella pneumoniae* (ATCC 43816) and *Escherichia coli* (O54) as test organisms. All strains were serum-resistant. Eighteen-hour cultures of these strains were prepared in brain heart infusion

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broth (Oxoid Ltd., Basingstoke, England); aliquots of 1 ml were snap frozen in liquid nitrogen and stored at −70°C in a suspension containing about 10^9 viable bacteria/ml. Just before the start of each experiment, one aliquot of this suspension was rapidly thawed in a waterbath at 37°C.

Cytostatic Drug and Antibiotic

The cytostatic drug cyclophosphamide, obtained from Multi-Pharma B.V., Weesp, The Netherlands, was dissolved in phosphate-buffered saline (PBS, pH 7.5) to final concentrations of 10 and 15 mg/ml. The antibiotic cloxacillin (90.5% activity, Beecham, Amstelveen, The Netherlands) was dissolved in PBS to a final concentration of 10 mg/ml. The minimal inhibition concentration of cloxacillin for *Staphylococcus aureus* ATCC 25923 is 0.25 μg/ml.

Preparation of the Radiopharmaceutical (Technescan HIG)

As radiopharmaceutical human polyclonal immunoglobulin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) labeled with ^{99m}Tc was prepared from a lyophilized kit containing 1 mg 2-iminothiolane modified immunoglobulin and stannous tartrate [Technescan HIG, Mallinckrodt Diagnostica, Petten, The Netherlands (*12*)]. This resulted in extra S-H bonds. Murine immunoglobulin, murine serum albumin and human serum albumin (HSA) were obtained from Sigma (St. Louis, MO) and Fc-fragments from Calbiochem (San Diego, CA). All other preparations were obtained from the Central Laboratory of the Red Cross Blood Transfusion Service and modified according to the procedure described above. Each mouse received 100 μg Ig, corresponding to approximately 40 MBq. A same level of activity was injected in the experiments in which the total amount of protein was changed.

Animal Model

Approximately 1×10^7 colony forming units (CFU) of *S. aureus* suspended in brain-heart infusion broth to a volume of 100 μl were injected into a left thigh muscle. After 18 hr, the mice were anesthetized with a combination of 1 mg fluanison, 0.03 mg fentanyl citrate, (Hypnorm, Janssen Pharmaceutica, Tilburg, The Netherlands), and 0.2 mg diazepam (Valium, Hoffmann-La Roche, Mijdrecht, The Netherlands) per mouse. Immediately afterwards the radiopharmaceutical was injected intravenously into a tail vein. Since each mouse was marked they could be followed individually.

Determination of Bacterial Numbers

The animals were killed by cervical dislocation, the thigh muscle was isolated and homogenized in 5 ml of PBS at 0°C in a tissue-homogenizer (type X-1020 Ystral GmbH, Dottingen, Federal Republic of Germany), and appropriate dilutions were plated onto diagnostic sensitivity test (DST) agar (Oxoid Ltd.). After overnight incubation at 37°C, the numbers of viable bacteria were counted as CFU.

Scintigraphy

One, 4, and 24 hr after administration of the radiopharmaceutical (HIG) scintigraphy was performed using a large field of view gamma camera (Toshiba GCA 40A, Tokyo, Japan) with a low-energy general-purpose parallel-hole collimator. It was connected to a dedicated computer (Medical Data Systems, MDS-A^2, Ann-Arbor, MI) and posterior whole-body images were obtained in a 256 × 256 matrix. The energy peak was set at 140 keV with a window of 20%. The animals were placed in supine position on the camera with both hind legs spread out and fixed with surgical tape.

Determination of Activity at the Site of Infection

On the scintigrams, regions of interest (ROIs), measuring approximately 5 × 5 mm, over the infected and noninfected thighs were drawn. The ratio of the counts for the two ROIs for each mouse was taken as an index of the ability to localize the infection in that particular animal. Furthermore, after the last scintigram, when the animals were killed by cervical dislocation (24 hr after administration of the radiopharmaceutical) various tissues (heart, lungs, liver, kidneys, spleen, infected, and noninfected thigh) were excised. The activity (in counts per 10 sec) in each of these tissues and in a sample of blood was counted in a well type NaI crystal detector connected to a Scaler-Ratemeter (SR3, Nuclear Enterprises, England) and calculated per gram tissue. Once again the ratio of the counts per gram tissue for the two thighs was considered an index of the degree of the uptake, i.e., the ability to localize the infection.

Experimental Design

Leukocytopenia. To induce leukocytopenia, cyclophosphamide was injected intraperitoneally at a dose of 150 mg/kg and 100 mg/kg four days and one day, respectively, before administration of the radiopharmaceutical. The same volume of PBS was given to the control animals (*13*). This treatment resulted in a reduction to 3% of the number of granulocytes and 15% of the number of monocytes found for controls at the time of administration of the radiopharmaceutical (*14*).

Antibiotic Treatment of Infection. Immediately after the first scintigram, to check that there were no initial differences in imaging between the two groups of animals, treatment with cloxacillin or PBS (control) was started. Cloxacillin (100 mg/kg) was given subcutaneously 1, 8, and 23 hr after administration of the radiopharmaceutical. The same volume of PBS was given to the control animals.

Statistical Analysis

Differences between variables were tested for significance with the Mann-Whitney U-test (*15*). The relation between the log number of bacteria present in the inoculum and the accumulation of HIG at the site of infection was analyzed by linear regression. The level of significance was set at 0.05.

RESULTS

The Outgrowth of *Staphylococcus aureus* In Vivo

After an inoculum of approximately 1×10^7 CFU per thigh, the number of bacteria had increased to about 2.5×10^8 CFU per thigh at the time of administration of the radiopharmaceutical (18 hr after infection) and about 4.0×10^8 CFU per thigh 24 hr later. The number of bacteria increased during the course of the experiment.

Reproducibility of the Localization of the Thigh Infection

To investigate whether a reproducible localization with ^{99m}Tc-labeled HIG was found, two groups of 4

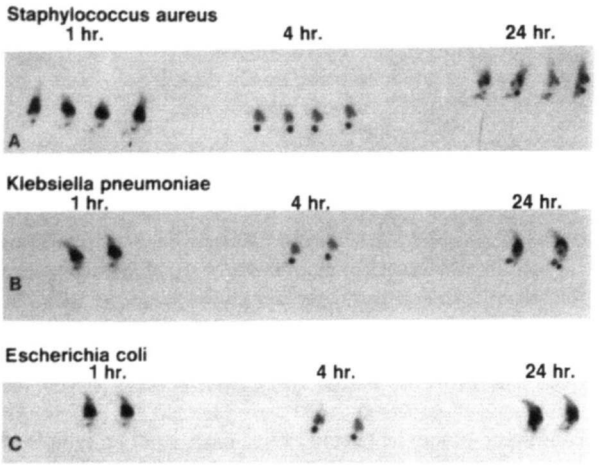

FIGURE 1
Serial scintigrams of mice with a thigh infection of (A) *Staphylococcus aureus* (inoculum about 1×10^7 CFU/thigh), (B) *Klebsiella pneumoniae* (inoculum about 1×10^3 CFU/thigh), or (C) *Escherichia coli* (inoculum about 3×10^7 CFU/thigh). Images were made 1, 4, and 24 hr after the i.v. administration of ^{99m}Tc-labeled polyclonal human immunoglobulin (HIG).

mice were studied. At 24 hr post-administration, the target-to-background (T/B) ratio of both the organ counts (median 4.66, range 4.42–4.79 versus median 4.38, range 3.64–4.77, $p > 0.5$) and the ROI (median 7.67, range 6.26–8.28 versus median 6.44, range 5.1–7.63, $p > 0.2$) were not significantly different. Two mice are presented in Figure 1.

Comparison of Human Versus Murine Ig

To detect any difference in the ratios obtained with ^{99m}Tc-labeled polyclonal human Ig (HIG) and ^{99m}Tc-labeled polyclonal murine Ig, both were compared in mice with a staphylococcal thigh infection. There were no significant differences in the ratios between the two preparations 24 hr after injection of the radiopharmaceutical, not only for the tissue counts (Table 1) but also for the ROI counts (Fig. 2). Since both preparations yielded excellent reproducibility as far as quality of imaging is concerned, further experiments were carried out with the human Ig.

Vascular Permeability

To establish the degree to which vascular permeability determines the ratios, some control experiments were performed with: (a) ^{99m}Tc-HSA; (b) ^{99m}Tc-HSA with extra S-H bonds to mimic HIG since this is enriched with S-H bonds for labeling purposes (*12*); and (c) ^{99m}Tc-murine serum albumin. All three of the radiopharmaceuticals yielded lower ratios ($p < 0.001$), for both the tissue counts (Table 1) and the ROI counts (median values 4.01, 4.19, and 3.09, respectively) 24 hr after administration of the radiopharmaceutical than HIG did. The results of ROI analysis showed that at 1 and 4 hr there was no difference in the ratios found for serum albumin and HIG. But in contrast to the latter, none of the albumin radiopharmaceuticals showed an increase in the ratios between 4 and 24 hr.

Binding of Fc Fragments

Technetium-99m-labeled Fc-fragments were administered intravenously to mice to study possible Fc-mediated binding at the site of infection. As can be seen in Table 1, at 24 hr post-administration a significantly lower ratio of the tissue counts ($p < 0.01$) was obtained for the Fc group compared with the HIG group (Table 1). Median (range between parentheses) of ROI ratios was 4.48 (1.85–6.62) and 7.50 (5.10–8.28), respectively. With regard to the ROI ratios, there were no differences

TABLE 1
Radioactivity Ratios in the Excised Infected (with *Staphylococcus aureus* ATCC 25923) Thigh and the Excised Noninfected Thigh Using Different ^{99m}Tc-Labeled Proteins*

Radiopharmaceutical	Median	Range	n†	p‡
Polyclonal human Ig (HIG)	4.58	3.64–4.79	8	
Polyclonal murine Ig	4.86	3.48–5.62	7	>0.40
Polyclonal human Ig (Fc)	3.39	1.86–4.05	7	<0.01
HSA	3.02	2.35–3.17	6	<0.001
SA (S-H)§	2.29	2.07–2.70	6	<0.001
Murine serum albumin	2.01	1.84–2.17	6	<0.001
Polyclonal human Ig (0.01 mg)	4.17	2.50–5.43	6	>0.35
Polyclonal human Ig (0.1 mg)	4.16	2.61–4.94	6	>0.35
Polyclonal human Ig (1.0 mg)	4.46	3.79–6.10	6	>0.35

The activity (in counts per 10 sec) was measured in excised thighs 24 hr after administration of the radiopharmaceutical and 42 hr after onset of the infection.
† Number of animals.
‡ Compared with polyclonal human Ig, using the Mann-Whitney U-test.
§ Enriched with extra S-H bonds to mimic polyclonal human Ig (HIG).

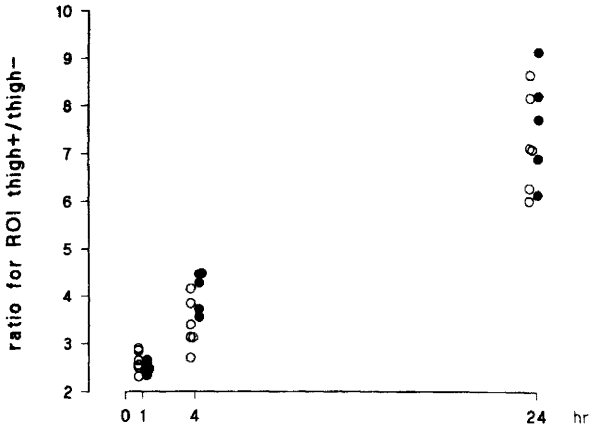

FIGURE 2
Comparison of polyclonal human immunoglobulin (open symbols) and polyclonal murine immunoglobulin (solid symbols) for the detection of a thigh infection with *Staphylococcus aureus* at different intervals after administration of the ^{99m}Tc-labeled radiopharmaceuticals. Each symbol represents one single value of the ratio of the counts obtained by regions of interest analysis for the infected and noninfected thighs.

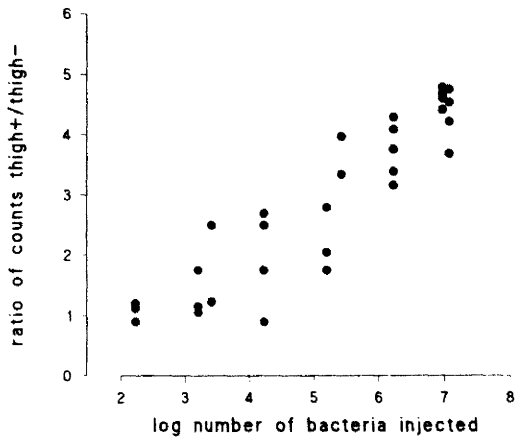

FIGURE 3
Relationship between the log number of bacteria in the inoculum and the detection of the infection expressed as the ratio of the tissue counts for the infected and noninfected thighs at 24 hr post-administration. Each symbol represents one single observation.

between the Fc and HIG groups 1 and 4 hr after administration. However, in the latter the ratio steadily increased, whereas in the former it increased only a little; as a result the Fc group exhibited a significantly ($p < 0.001$) lower ratio 24 hr after administration of the radiopharmaceutical.

Total Amount of Protein in HIG

To study the effect of the amount of protein in HIG on T/B ratios, 0.01, 0.1, or 1.0 mg of modified ^{99m}Tc-Ig was injected into various mice. There appeared to be an improvement in the detection of the infection using 1.0 mg. However, although 1.0 mg of Ig did yield higher ratios, no significant differences in tissue counts could be demonstrated between the different amounts of protein (all differences: $p > 0.35$, Table 1). The same was found after ROI analysis at 24 hr post-administration (median values 7.41 (3.43–8.83), 7.42 (3.42–8.55), and 10.23 (7.56–10.62), respectively.

Effect of the Concentration of Bacteria

Various numbers of *Staphylococcus aureus*, ranging from about 10^2 to 10^8 CFU, were injected into the thigh muscles of different mice. As illustrated in Figure 3, at 24 hr post-administration a linear relationship was observed between the log number of bacteria in the inoculum and the ratios of the tissue counts, i.e., the ability to localize the infection ($r = 0.919$, $p < 0.001$). The same correlation was obtained between the number of bacteria and the ROI ratios.

Effect of Circulating Leukocytes

In order to achieve leukocytopenia, cyclophosphamide was administered to mice. This resulted in a decrease in the number of both monocytes (from 120 to 18 per mm^3 blood) and granulocytes (from 1202 to 37 per mm^3 blood) compared with control animals at the start of the infection (*14*). Although the number of blood leukocytes was significantly reduced, there was no significant ($p > 0.1$) difference at 24 hr post-administration between cyclophosphamide treated and control animals in the ratios of both the tissue counts (median 5.04, range 3.10–11.31, n = 7, median 4.70, range 3.56–7.80, n = 9 respectively) and the ROI counts (median 7.61, range 5.05–39.72, and median 7.38, range 4.0–11.65, respectively).

Effect of Antibiotic Treatment

As far as the ratios for the tissues are concerned, there was no statistical difference between cloxacillin-treated and control (PBS) animals 24 hr after the administration of HIG. However, the ratios for the ROI 24 hr after administration of the radiopharmaceutical were significantly ($p < 0.02$) lower for cloxacillin-treated animals than for control animals (Table 2).

Effect of Infection with Other Microorganisms

A thigh infection with two other microorganisms was established: *Klebsiella pneumoniae* ATCC 43816 and *Escherichia coli* O54. Two mice in each group are presented in Figure 1.

Klebsiella pneumoniae proliferates rapidly in the thigh; after an inoculum of 10^3 CFU per thigh the number of bacteria had increased to 3×10^9 CFU per thigh 42 hr later. The ratios of both the tissue counts (median 5.50, range 3.11–6.49, n = 6) and the ROI counts (median 8.13, range 4.68–17.27) for this microorganism at 24 hr post-administration were higher, although not significantly, than those found for the staphylococcal thigh infection.

TABLE 2
Radioactivity Ratios in Noninfected Mouse Thighs and Infected Thighs (with *Staphylococcus aureus* ATCC 25923) After Treatment with Cloxacillin or PBS (Control)

Treatment	Ratio for excised tissues	Ratio for ROIs	
	hr after HIG administration	hr after HIG administration	
	24	4	24
PBS (control)	4.60 (4.20–5.45)	4.52 (3.90–7.12)	10.60 (7.40–16.23)
Cloxacillin	4.19 (2.35–5.56)	4.80 (3.30–7.48)	7.61 (6.20–8.47)
p*	<0.20	>0.40	<0.02

The ratios are derived from both the excised thighs and the regions of interest on scintigrams. The values represent the median and the range (six mice in each group).
* Result obtained using the Mann-Whitney U-test.

Escherichia coli did not show an increase in numbers at the site of infection; after an injection of 3×10^7 CFU per thigh the number of bacteria was practically the same 42 hr later. The ratios of both tissue counts (median 2.08, range 1.72–2.57, n = 3) and ROI counts (median 2.84, range 2.33–3.06) were significantly ($p < 0.001$) lower at 24 hr post-administration than those found for the staphylococcal thigh infection.

DISCUSSION

The results of the present study show that ^{99m}Tc-labeled modified polyclonal human immunoglobulin can be used to localize a short-term thigh infection with *Staphylococcus aureus* in mice reproducibly. Furthermore, the accumulation of this radiopharmaceutical was dependent on the number of bacteria at the site of infection.

Although various studies on the localization of infections have been carried out, it is still unknown why intravenously administered radiolabeled Ig accumulates at the site of infection in view of the much higher concentration of Ig already present in the circulation. Possible explanations of the mode of action of this preparation are:

1. Increased vascular permeability (*7,16*).
2. Binding of the Fc-part of Ig to Fc-receptors of tissue leukocytes at the site of infection (*3,6,7,17*).
3. Binding of the Ig molecules to blood leukocytes (*3,18*).
4. Binding to microorganisms at the site of infection.

Since a clear relationship was found between the log number of bacteria and the ability to localize the infection in the thigh, vascular permeability could offer an explanation for the mode of action, especially since Selbie and O'Grady have demonstrated a positive correlation between the number of bacteria in the thigh and the swelling of the infected tissue (*19*). Indeed, the study of Morrell et al. (*16*) supported this hypothesis. However, in our study, ^{99m}Tc-labeled HSA did not yield the high T/B ratio found with HIG. It may be argued that HSA is not the proper molecule to determine vascular permeability of HIG, although it has been commonly used in this respect (*5,16*). The molecular weight of HSA (~69,000 D) is much lower than that of immunoglobulin (~160,000 D). To mimic the molecular weight of immunoglobulin more closely, we obtained polymers of HSA using glutaraldehyde. The different size fractions were isolated by Sephadex G-50 gel filtration chromatography. The T/B ratio (tissue count) at 24 hr post-administration found for monomeric HSA (median 2.90, range 1.95–3.64) was higher than that for dimeric (median 2.16, range 1.71–2.60), the latter being higher than that for polymeric HSA (median 1.43, range 1.41–1.44). Obviously the smaller the HSA molecule the higher the ratio. In summary, all these findings suggest that vascular permeability alone can not account for the mode of action of the radiopharmaceutical, as already found by others (*6,7,20*). Since measurement of the swelling of the infected tissue is not really standardized, at least not in our hands, this determination of the inflammatory process was omitted in the present study.

Technetium-99m-labeled Fc-fragments also did not demonstrate the same degree of accumulation as intact Ig (*3,6,11,17*). This indicates that the mode of action of Ig could not be explained exclusively by binding of the Fc-part to the Fc receptors of cells. Nevertheless, both vascular permeability and Fc mediated binding most certainly account for a part of the mode of action of HIG.

Attachment of the HIG to blood leukocytes is another possible mode of action as suggested by Saptogino et al. (*21*). In leukocytopenic animals, a somewhat higher, though not significant, T/B ratio was observed than in control animals. This could have been caused by the higher outgrowth of bacteria at the site of infection in the former group of animals. In fact, the actual

numbers of bacteria in the infected thighs of leukocytopenic animals (approximately threefold compared with control mice) were in agreement with the T/B ratio expected from the linear relationship between the log number of bacteria and this ratio (Fig. 3). Despite the reduction in the number of blood leukocytes, detection of the infection by the immunoglobulin was not really altered, suggesting that binding to blood leukocytes does not play an important role in the mode of action. Fischman et al. (*6*) and Rubin et al. (*22*) also found that leukocytopenia due to the administration of cyclophosphamide did not affect the localization of an infection.

Furthermore, it is possible that the radiopharmaceutical binds to the microorganism, which may be an important factor in the detection of infections by Ig. This may be explained by binding of the F(ab)-part to epitope(s) of the bacterial cell wall, although others (*3, 17*) found less accumulation of activity at the site of infection when F(ab) fragments were used. Experiments to study the impact of F(ab) fragments were performed as well. But, unfortunately, five of six animals died due to pyrogen contamination of the material. The one surviving showed a T/B ratio of 2.36 24 hr after the administration of the radiopharmaceutical. A lower ratio of 1.49 was obtained when F(ab)-material from another immunoglobulin preparation was used. Since various preparations were used, no conclusions could be drawn. However, the study suggested that F(ab) fragments are not responsible for the accumulation at the site of infection. Therefore, further studies with F(ab) fragments were abandoned.

In contrast with ^{99m}Tc-F(ab) preparations, the administration of HIG did not lead to much mortality among the animals. Only one mouse died 4 hr after the administration of HIG, but this may have been caused by the anesthetic drugs.

When we compare our results with those of other studies, especially those of the group of Rubin, Fischman, and Strauss (*3,6,7,11,16,17,20,22*), it may be concluded that several possible modes of action, as mentioned above, can not fully explain the mode of action. Furthermore, the amount of injected Ig did not affect the accumulation of HIG. In leukocytopenic mice the T/B ratios were not different from normal mice. In the present study, a correlation was found between the number of bacteria present at the site of infection and the T/B ratio. This relationship may be used to demonstrate antibiotic efficacy.

Labeled polyclonal human immunoglobulin can be administered to patients. Nevertheless, there are some drawbacks in using this ^{99m}Tc-labeled radiopharmaceutical. In particular, a high activity was observed in the liver and kidneys of the mice, leaving these areas unsuitable for imaging of infections. Furthermore, a relatively high blood background activity was noted, which may bias scintigrams. Fischman et al. (*6*) and Rubin et al. (*22*) reported that ^{111}In-labeled human IgG was effective in detecting an infection in patients, although they also found persistent blood-pool activity. Oyen et al. (*23*) demonstrated that ^{111}In-Ig was very appropriate in the imaging of joint and bone infection in humans. Furthermore, images 24 and 48 hr after administration of the infection proved to be successful despite background activity. However, if radiation exposure can be minimized without interfering with the localization characteristics when using ^{99m}Tc, this isotope bears a significant advantage compared with ^{111}In. Taking this into account, ^{99m}Tc-labeled polyclonal human immunoglobulin may be an effective radiopharmaceutical for the detection of infections in different tissues with relatively low radiation exposure to patients.

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SELF-STUDY TEST
Pulmonary Nuclear Medicine
ANSWERS

(continued from p. 446)

ITEM 1: Safety of Pulmonary Perfusion Imaging

ANSWERS **A,** T; **B,** T; **C,** F; **D,** T; **E,** T

The patient described in Item C, who has only minimal respiratory compromise, can tolerate the usual dose of particles (250,000–500,000). Because the margin of safety is so high (only about 0.1% of the vascular bed is occluded), most patients suffer no adverse effects from perfusion scintigraphy. In the other four situations, a reduced number of particles should be administered.

In pulmonary hypertension, there are obliterative changes in small pulmonary vessels. Administered particles, thus, occlude more proximally in the pulmonary vascular tree and block a larger fraction of the cross-sectional area of the pulmonary circulation. Rarely, death has occurred in patients with pulmonary hypertension immediately after injection of the particles. Thus, injection of fewer particles is desirable. A patient with a surgically-absent lung will have a smaller number of pulmonary capillaries, requiring injection of fewer particles. A patient with a long-standing ventricular septal defect may have a right-to-left shunt secondary to development of pulmonary hypertension. Thus, in addition to the potential risk associated with the pulmonary hypertension, there could be substantial systemic arterial embolization from an injection of ^{99m}Tc-MAA. Although no harmful effects of this systemic embolization have been reported, it seems prudent to inject fewer particles, once it has been determined that the study is absolutely necessary. In patients with very poor respiratory function, the number of particles should be reduced to avoid the unlikely possiblity of even further cardiopulmonary compromise.

The number of particles necessary to ensure adequate statistical quality of perfusion scintigrams is less than that usually employed. It has been shown that artifactual inhomogeneity of perfusion due to use of too few particles is negligible with particle doses of greater than 60,000. Hence, where clinically indicated, the use of a reduced dose of 60,000–100,000 particles will not adversely affect image quality.

References

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2 Heck LL, Duley JW Jr. Statistical considerations in lung imaging with ^{99m}Tc-albumin particles. *Radiology* 1974;113:675–679.

ITEM 2: Ventilation and Perfusion Imaging Sequences

ANSWERS: **A,** T; **B,** F; **C,** T; **D,** F; **E,** T

An advantage of ^{127}Xe, albeit a rather costly and difficult-to-obtain radioactive gas for ventilation imaging, is the relatively high energies of its gamma emissions (172 keV–25% abundance; 203 keV–68%; and 375 keV–18%). Thus ^{127}Xe ventilation imaging may be performed after perfusion imaging with ^{99m}Tc-MAA has been completed. The lower-energy ^{99m}Tc photons will not contribute significantly to the signal detected in the higher-energy ^{127}Xe energy window. The higher photon energy of ^{127}Xe does necessitate the use of a medium- or high-energy collimator for ventilation imaging, however.

Computer subtraction techniques have been employed to correct for "background" ^{99m}Tc scatter in the ^{133}Xe energy window. Unfortunately, the method is difficult to apply and, thus, not routinely used because of uncertainty about the exact fraction of technetium counts to be subtracted from the xenon image and because of errors in spatial registration of the two images. Some nuclear medicine laboratories successfully perform ^{133}Xe imaging after the ^{99m}Tc study by administering a relatively low dose of ^{99m}Tc-macroaggregates (e.g., 1–2 mCi) and a high ^{133}Xe dose (e.g., 20–30 mCi). Under these circumstances, it also is of value to narrow the spectrometer window for imaging of ^{133}Xe, as this will further reduce the scatter contribution.

If the perfusion images are performed first as part of an evaluation for pulmonary embolism, the ventilation study need not be obtained if the perfusion images are normal, because a normal perfusion study effectively excludes the possiblity of embolism.

A major advantage of ^{81m}Kr is its ability to provide ventilation images that are precisely matched to the perfusion images in all standard projections. A typical imaging sequence, following injection of the ^{99m}TC particles, might consist of turning on the oxygen flow through the ^{81}Rb/^{81m}Kr generator to deliver the krypton and obtain the ^{81m}Kr image. Then the oxygen is stopped and, after an interval of approximately 30–45 sec to allow for krypton exhalation and decay, the spectrometer setting of the camera is changed and the ^{99m}Tc image is obtained without moving the patient. Next, the second ^{99m}Tc projection is obtained, the gas flow is restarted, and the matching krypton image is collected. This sequence continues until all of the paired ventilation and perfusion images have been completed.

References

1 Kipper MS, Alazraki N. The feasibility of performing ^{133}Xe ventilation imaging following the perfusion study. *Radiology* 1982;144:581–586.
2 Stein MG, Waxman AD, Ramanna L, Julien P, Chappel M. Postperfusion xenon-133 ventilation scintigraphy with a narrow window. *AJR* 1985;145:511–515.

ITEM 3: The Unilateral Absence of Pulmonary Perfusion

ANSWERS: **A,** T; **B,** T; **C,** T; **D,** F

Pneumonectomy and complete lung collapse (e.g., due to mucous plugging or a malpositioned endotracheal tube) usually would be obvious from the chest radiographs. A central hilar mass (due to bronchogenic carcinoma, lymphoma, or sacroidosis, for example) obstructing pulmonary blood flow is a common cause of these findings. Although high-quality posteroanterior and lateral chest

(continued on p. 499)