Effects of a ^{99m}Tc-Labeled Murine Immunoglobulin M Antibody to CD15 Antigens on Human Granulocyte Membranes in Healthy Volunteers

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An injectible, ^{99m}Tc-labeled, murine immunoglobulin M antibody to stage-specific embryonic antigen-1 has been developed that can localize infections by binding to CD15 glycoproteins expressed on the cell membranes of human granulocytes in vivo after systemic administration. The purpose of this study was to measure its clinical effects on healthy people. **Methods:** Multiple blood samples were aspirated before and after the intravenous administration of about 125 μg antibody labeled with ~370 MBq (10.0 mCi) ^{99m}Tc in 10 healthy human volunteers. Complete blood cell counts were performed at each time point. Whole-body scans were acquired contemporaneously with a dual-head gamma camera. The fraction of the administered dose at each time point was quantified in 18 regions of interest. Statistical analyses included paired *t* tests. **Results:** Administration was associated with a transient decrease in the concentration of red and white blood cells in the whole blood. The effect always began within 3 min of administration. Its nadir was always reached 15–20 min after administration. There was full recovery with mild overcompensation in about an hour. The hematocrit dropped by a mean of 3.8% ($P < 0.002$), whereas the total white blood cell count fell 44.0% ± 3.1% ($P < 0.001$). The effect was most pronounced on the number of circulating granulocytes, which fell from 5.7 ± 2.1 to 3.2 ±1.3 × 10^3/μL blood. The drop paralleled a decrease in the percentage of whole blood radioactivity bound to the white blood cell membranes, which peaked at 50.4% ± 7.6% at 3 min after injection and then fell to 26.1% ± 9.3% over the next 30 ± 13.4 min before recovering to 40.7% ± 8.2% at 2 h. Image analysis showed that the effect was temporally associated with an increase in the amount of radioactivity within the liver and the spleen. Recovery was associated with a decrease in hepatosplenic radioactivity. No evidence of cell destruction or agglutination could be detected. **Conclusion:** This study confirmed that administration of this radiolabeled antibody is associated with a transient decrease in the number of circulating granulocytes. However, there also seems to be a secondary hemodilutionlike effect on all blood components that has not been reported previously. The effect appears to be clinically silent and very short-lived.

Key Words: anti-stage-specific embryonic antigen-1; infection; imaging; radionuclide

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Both the diagnosis and the treatment of a focal infection require localization of the problem (*1*). Abscesses frequently, but not always, can be visualized with structural imaging techniques (*2*). Phlegmons can be even more challenging to detect. A variety of radiotracers have been developed as alternatives (*3–6*), but several factors make most currently approved nuclear medicine procedures less than ideal (*7*). To be most clinically useful, a test needs to be available around the clock in conventional medical settings and capable of producing a result with relative rapidity. Because few procedures currently meet these criteria, alternative technologies for rapidly imaging infections continue to be developed.

A substantial effort has been made to explore the effectiveness of injectible radiopharmaceuticals that can selectively label white blood cells (WBCs) in vivo after systemic administration without harvesting blood cells from the patient and processing them in vitro (*8,9*). ^{99m}Tc-labeled antibodies appear to be particularly promising for several reasons (*10–13*). ^{99m}Tc is available around the clock in most hospital settings. It is the least expensive radionuclide on the market today (*14*) and has the most favorable imaging characteristics (*15*). Progress in this area has begun to accelerate, placing more and more radiolabeled antibodies in the developmental pipeline.

A ^{99m}Tc-labeled immunoglobulin M (IgM) murine monoclonal antibody to stage-specific embryonic antigen-1 (SSEA-1) has now been developed that, when administered intravenously, binds CD15 antigens expressed on the cell membranes of mature human granulocytes (*16,17*). Very little of the antigen is expressed on other blood cell lines (*16*). As a result of selective binding to glycosphingolipids on the exterior of mature neutrophils (Kd ~0^{-11} mol/L), the radiolabeled antibody should become concentrated in areas of infection (*17*). Theoretically, the fact that neutrophils increase the expression of CD15 antigens when they become activated should increase the amount of specific binding around sites of infection and, as a consequence, enhance the efficacy of the radiopharmaceutical as a diagnostic test (*18*).

In fact, several preliminary trials have now shown that the radiopharmaceutical can be used to localize infections in clinical settings (*19,20*).

The administration of any murine antibody to humans has the potential to produce several specific and nonspecific untoward effects (*21,22*). This particular antibody has not produced human antibodies to mouse antigens in research volunteers However, untoward effects of other anti-SSEA-1 antibodies have included changes in WBC function (*23*). Some inhibit neutrophil bactericidal activity and increase neutrophil adhesion to endothelium (*24*). A study with this particular IgM murine antibody reported that administration produced a "mild leukopenia" shortly after administration in four of five patients with infections (*25*). The effect was sometimes large but apparently transient. A mechanism of action was not determined.

The specific aims of this study included confirming, characterizing and quantifying the effects of this antibody on blood cell counts and function. An attempt was made to elucidate some of the mechanisms of action producing the effects. The safety of the antibody was estimated by systematically observing the clinical responses of the healthy volunteers.

MATERIALS AND METHODS

Accrual and Assessment of Healthy Volunteers

The healthy volunteers who participated in this study are the same persons who were described in a report on the radiation dosimetry of the radiopharmaceutical (*26*). The protocol was performed within a regulatory framework approved by the Committee on Research Involving Human Beings of the University of Pennsylvania. The healthy volunteers were recruited through advertisements in local papers and by word of mouth from other volunteers. Structured medical histories were taken and physical examinations were performed. None of the volunteers had a known history of a health problem that could have significantly affected the biodistribution or elimination of the radioligand at the time of study. None were taking any medicines other than oral contraceptives.

Informed consent was obtained in writing from 4 men and 6 women (age range 21–46 y; mean age 30.2 ± 7.7 y) whose diverse racial demographics tended to reflect those of the local population. All of them were fully employed or full-time students with a mean educational level of 14.8 y (range 12–18 y).

Clinical Assessment Procedures

Because resting, changing position, placing a tourniquet and the stress of venipuncture are known to alter WBC counts in healthy people, intravenous catheters were placed in both forearms early in the course of each study. Baseline blood samples for laboratory studies were not drawn until the healthy volunteers had been at rest in a supine position on the imaging table for at least 25 min. The next eight blood samples that followed were all aspirated while the healthy volunteers remained at rest on the imaging table. The healthy volunteers were more recently physically active when the delayed samples were drawn.

The baseline clinical laboratory tests were dependent measures and included a complete blood cell count with differential (normal range 4.0–11.0 × $10^3/\mu l$), serum electrolytes and liver enzymes. Levels of lactate dehydrogenase (LDH), creatinine, blood urea nitrogen, glucose, cholesterol, triglycerides, albumin and total protein were also assayed. Pregnancy was ruled out with measurements of β-human chorionic gonadotrophin levels. Routine urinalyses and urine toxicology screens were performed after obtaining explicit consent for drug testing. These tests were repeated 1, 4 and 24 h after administration of the radiopharmaceutical. The WBC count and differential were also measured 5 min before injection and 3, 5, 10, 15, 20, 30, 45 and 60 min after administration by aspirating whole blood into vacutainers containing ethylenediaminetetraacetic acid. LDH isoenzymes were run on the last 4 healthy volunteers.

Measurements of Specific Binding

Whole blood was aspirated into vacutainers containing heparin 3, 5, 10, 15, 20, 30, 45 and 60 min after administration, with delayed samples taken 2, 4, 6, 8 and 24 h after administration. The concentration of radioactivity was measured in whole blood and plasma. The fraction of the dose in the whole blood that was bound to WBC membranes was measured at each time point in the last 6 consecutively presenting subjects with instant thin-layer chromatography (ITLC) as previously described (*17,27*). Silica gel-impregnated cellulose strips pretreated with newborn calf serum were used. At each time point, 20 μL whole blood were loaded on ITLC strips and developed in 10 mmol phosphate-buffered saline at pH 7.0 containing 4% ethyl alcohol. The procedures were repeated on 20 μL plasma to correct for nonspecific binding, i.e., the fraction of unbound radioactivity left at the origin along with the cells. The percentage cell binding was defined as the percentage of the radioactivity left at the origin in the whole-blood sample minus the percentage of the activity left at the origin in the plasma sample. The fraction of radioactivity associated with each blood cell type was measured in all 10 healthy volunteers 30 and 60 min after administration. In these studies cells were separated by layering whole blood over a gel gradient and centrifuging at 450 g for 30 min.

Control Procedures for the Clinical Laboratory Studies

The effects of rest and position were measured in a healthy volunteer by aspirating multiple blood samples through an indwelling catheter. The first sample was drawn immediately after inserting the needle in the antebrachium and continued over the next 40 min while the person was at complete rest in a supine position. Follow-up samples were then obtained after moderate exercise.

A sample of whole blood was aspirated from another healthy volunteer who had never been exposed to the radiopharmaceutical. The sample was then fractionated into 12 aliquots. The WBC count and differential were measured in two aliquots with no manipulation. In three pairs of samples, the radiopharmaceutical was added in vitro at one half, one and two times the estimated in vivo concentration in human volunteers, on the basis of an assumed blood volume of 5 L. Another IgG antibody that was not specific to human WBCs was added in two concentrations to the remaining two pairs as another control. The WBC counts, differential cell counts and levels of LDH and LDH isoenzymes were measured with the same procedures that were used in the healthy volunteers. Smears were made and reviewed by clinical pathologists as well as by the investigators.

Radiopharmaceutical

Sterile and pyrogen-free lyophilized kits containing 250 μg anti-SSEA-1 were supplied by the study sponsor (LeuTechô; Palatin Technologies, Inc., Princeton, NJ). Labeling was accomplished in about 15 min with a procedure that has already been described (*28*). Before administration to healthy volunteers, the final product was tested for the presence of free ^{99m}Tc-pertechnetate by ITLC. Free ^{99m}Tc was less than 1% in 9 administered doses and 3.8% in the other 10th.

Emission Images

The radiopharmaceutical was administered through an indwelling catheter in an antecubital vein. The injection was performed as rapidly as possible in 6 healthy volunteers, and drawn out over approximately 2 min in 4 healthy volunteers. Dynamic images of the thorax and abdomen were acquired for 15 s per frame for 20 frames in a 256 × 256 matrix beginning at the time of injection. The first whole-body scan was begun about 6 min after injection. The first 5 scans were acquired back to back for 10 min each. The delayed images were acquired for 20 min at 2, 4, 6, 8, 22, 26 and 30 h after administration.

Image Analysis

As described previously (*29*), a master set of 18 regions of interest (ROIs) was constructed for each healthy volunteer and transposed onto all the images as a single set (Fig. 1). An automated subroutine measured the number of counts in these ROIs. Geometric means for each pair of conjugate ROIs were calculated by multiplying the decay-, attenuation- and background-corrected anterior counts by the corrected posterior counts and taking the square root of the product. The fraction of the injected dose at each time point was then estimated by dividing the corrected geometric mean number of counts in each ROI or urine specimen by the net geometric mean number of counts in the initial whole-body image.

RESULTS

There were no subjective effects of the radiotracer on any of the healthy volunteers. No changes in vital signs could be attributed to a drug effect. There were no changes noted on physical examination. Skin flushing was not observed, even though explicit inspections for such a reaction were made under conditions of good lighting. However, there was a transient effect on the blood cell counts in all the healthy volunteers.

The number of circulating WBCs dropped in all healthy volunteers from a mean (±SD) concentration of 5.7 ± 2.1 to 3.2 ± 1.3 × 10^3μL blood (Table 1). The concentration of granulocytes in the circulation dropped in all healthy volunteers, being detectable on the first sample aspirated 3 min after administration and reaching a nadir within 15–20 min. The relative number of granulocytes decreased from 57.2% ± 10.5% to 35.3% ± 17.7% of total WBC counts, with the absolute number of granulocytes declining from a

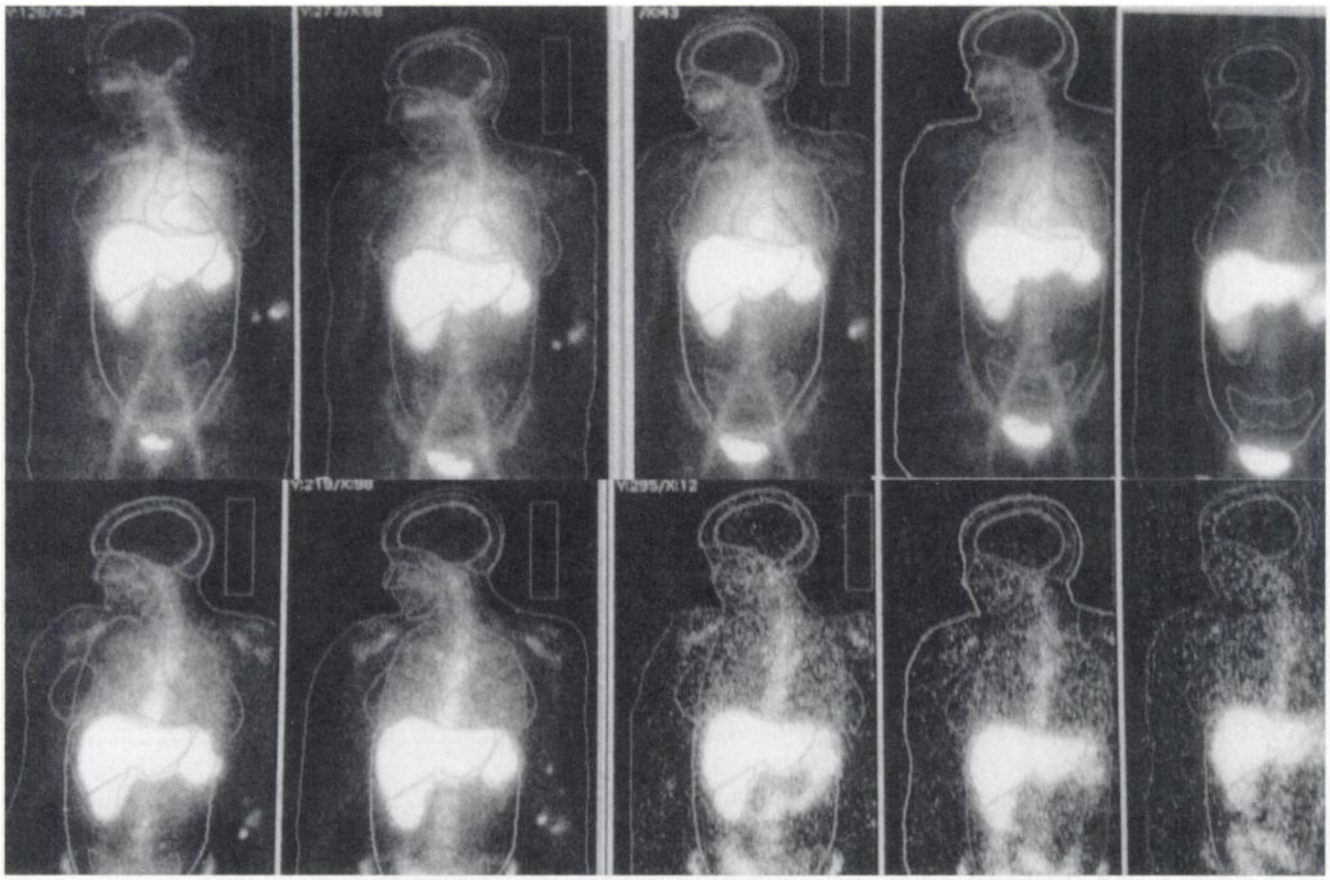

FIGURE 1. Distribution of radioactivity in body as function of time. Images show 10 of 14 whole-body scans in 36-y-old woman. Earliest scan is shown in top left panel. The last, at 30-h mark, is at bottom right.

TABLE 1
Mean of 10 Healthy Human Volunteers: Changes in the Blood with Time

Time after injection (min)	WBC no. ± SD	% PMN ± SD	% Bind ± SD	% Lym ± SD	Hct ± SD	Hgb ± SD
−5	5.7 ± 2.1	57.2 ± 10.5		30.6 ± 11.3	37.3 ± 3.1	12.7 ± 1.1
−3	5.3 ± 1.7	55.0 ± 14.7	50.4 ± 6.8	33.3 ± 14.7	36.7 ± 3.5	12.5 ± 1.2
5	4.5 ± 1.8	51.0 ± 14.2	43.6 ± 5.8	36.8 ± 15.0	36.4 ± 3.2	12.5 ± 1.2
10	3.6 ± 1.5	41.0 ± 16.5	37.1 ± 8.1	48.4 ± 16.4	36.4 ± 3.0	12.3 ± 1.1
15	3.2 ± 1.3	35.3 ± 17.7	29.2 ± 7.4	50.3 ± 18.4	36.2 ± 2.8	12.4 ± 1.1
20	3.7 ± 1.5	42.1 ± 14.8		42.8 ± 13.4	36.2 ± 2.9	12.4 ± 1.1
30	3.6 ± 1.3	34.6 ± 20.9	27.1 ± 8.7	47.7 ± 18.0	35.9 ± 2.5	12.4 ± 1.1
45	4.3 ± 1.7	45.1 ± 23.1	29.0 ± 8.9	41.6 ± 21.6	37.2 ± 2.9	12.7 ± 1.0
60	5.1 ± 2.1	47.3 ± 22.1	32.9 ± 8.2	41.3 ± 22.1	37.1 ± 3.2	12.7 ± 1.2
240	6.2 ± 2.0	62.7 ± 8.6	40.7 ± 7.5*	26.5 ± 9.3	39.5 ± 3.1	13.5 ± 1.1

*Last time point for percentage binding = 120 min.
WBC no. = total number of all white cells × 10^3/μL; % PMN = percentage polymorphonuclear cell number (granulocytes); % Bind = percentage radioactivity associated with granulocyte fraction; % Lym = percentage lymphocyte number; Hct = hematocrit; Hgb = hemoglobin concentration (mg/dL).

mean (±SD) concentration of 3.3 ± 0.6 to 1.1 ± 0.6 × 10^3μL blood. The total number of circulating granulocytes dropped below 200/μL in 2 healthy volunteers, below 400 in another and below 800 in 2 more, for a total of 5 of 10 healthy volunteers whose granulocytes dropped below 1000.

Recovery was rapid. The shape of the time-versus-concentration curves for circulating WBCs was highly similar in all healthy volunteers, as illustrated by graphs of the subjects with the highest and lowest counts in the sample (Fig. 2). There were 6 healthy volunteers who slightly overshot their resting baseline values by 1 h after administration and 1 who did not quite reach it until the next sample was drawn, when the person was sitting up at the 4-h mark.

Differential cell counts, which were frequently verified manually, showed that the effect on the other blood cells was qualitatively similar but less pronounced. As the total number of WBCs dropped with time, the percentage of lymphocytes increased (Table 1). However, the shape of the curves for the absolute concentration of lymphocytes was qualitatively equivalent to the shape of the curves for the absolute concentration of granulocytes, as were the curves for all the other WBC populations. The effects on rare blood cell components, such as the basophils, were often difficult to detect within an individual. Paired *t* tests within individuals, however, were highly significant for the group, with $P < 0.001$ at the nadir and $P < 0.05$ at most other early time points during the first hour of study.

The hemoglobin concentration and the hematocrit also decreased (Table 1). An effect on the number of platelets was difficult to detect in some individuals but not in the mean values for the group. Paired *t* tests showed that the mean platelet count, which dropped by an average of 6.3% within 10 min, was statistically significant ($P = 0.0014$). An index defined by the ratio of the platelet concentration to the hematocrit did not change significantly during the course of the study in any individual at any time point.

In contrast, indices derived by dividing the number of granulocytes per microliter by the concentration of the other blood cell components at each time point showed that the ratios dropped by one half to two thirds in all individuals before recovering.

The 4-h blood samples were drawn without giving the healthy volunteers a prior 30-min rest in a supine position. The platelet count increased by a mean of 5.3% above the

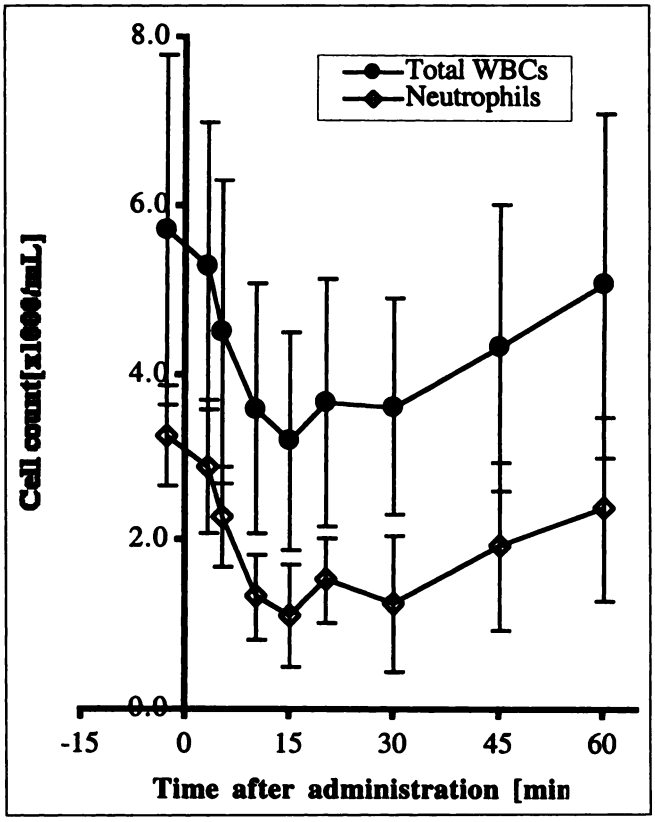

FIGURE 2. Time-activity curve for mean concentration of circulating white blood cells (WBCs). Error bars represent 1 SD. Pattern was similar in all volunteers.

resting supine baseline values at the 240-min mark (paired t test, one-tailed, $P = 0.014$). The granulocyte count increased by a mean of 18.2% ($P < 0.001$). The values were virtually identical to the results of a follow-up study, which showed that WBC counts in the whole blood dropped continuously for 20 min in healthy volunteers placed at bed rest, until the counts stabilized at 82% of the first level immediately after phlebotomy. They then remained stable over the next 20 min, with less than 3% variation.

Examination of the blood smears revealed no evidence of clustering or agglutination. No cell fragments were ever noted. There were no changes in cell morphology.

The decrease in circulating granulocytes was not affected by the rate at which the radiopharmaceutical was administered. There were no differences between the 4 individuals who were injected with a fast bolus and the 6 who received the dose over 2 min. However, when injected slowly, a larger fraction of the dose became trapped in the hub of the intravenous catheter and retained in the cannulated vein.

Cell separation with the Ficoll-Hypaque gradient showed less than 1% of the radioactivity in the lymphocyte and platelet fractions. A mean of 3.9% ± 0.9% was associated with the red blood cell (RBC) fraction. The consistency of these observations across individuals allowed the fraction of the dose bound to any cell in the whole blood to be used as a surrogate measure for the fraction of the dose bound to the granulocytes, with the correction factors for converting whole blood cell binding to granulocyte binding averaging less than 5%. The assumption was validated by showing high agreement between ITLC and the equivalent measurements of the 30- and 60-min samples with the Ficoll-Hypaque gradient; differences were always less than 5%. The fraction of the dose in the whole blood that was bound to the granulocytes was initially high. ITLC in the last 6 individuals showed that the fraction peaked at a mean (±SD) value of 50.4% ± 7.6% on the first sample, which was aspirated 3 min after administration. The percentage of binding then dropped until it reached a nadir of 26.1% ± 9.3% at an average of 30.0 ± 13.4 min after administration. The percentage of whole-blood activity bound to the granulocytes then increased until it had recovered to 40.7% ± 8.2% at 2 h (Fig. 3).

Image analysis confirmed that the radiopharmaceutical was initially distributed in the vasculature. It was eliminated from the whole blood with a mean linear half-life of 8.0 h (monoexponential half-life = 5.7 h). The bone marrow spaces of the axial and proximal appendicular skeleton were well visualized in all individuals.

Activity in the spleen generally peaked on the scans acquired about 25–35 min after administration. The peak splenic activity closely coincided with the nadir of the curves for both the number of circulating granulocytes and the percentage of whole-blood radioactivity bound to the WBCs (Fig. 3).

Radioactivity was taken up rapidly by the liver (Fig. 4A). The fraction of the dose in the liver then increased continuously until it peaked at an average of 40% of the administered dose 35–65 min after injection. The rate of elimination from the liver was usually slower than the rate from the spleen and always much slower than the rates from other organs and tissues (Fig. 4B). The curves for most other organs and tissues showed a peak on the very first whole-body scan and then decreased steadily. There was never any accumulation of activity in the lungs (Fig. 4B).

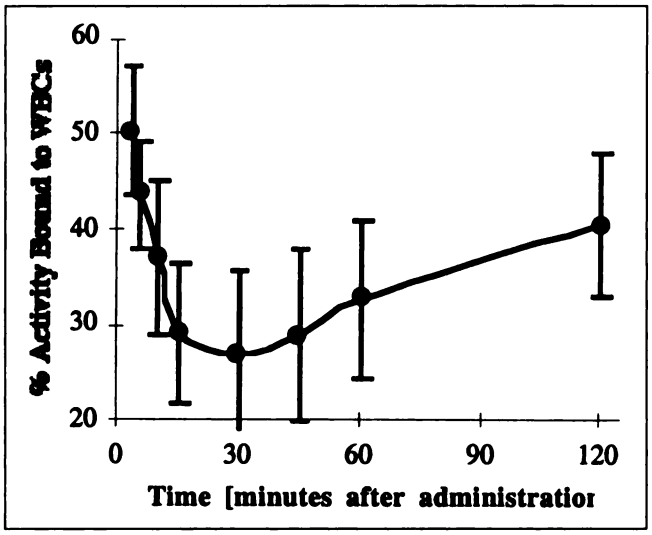

FIGURE 3. Mean time versus % granulocyte binding curve. Data show percentage of radioactivity in whole blood bound to white blood cell (WBC) membranes at each time point. Error bars represent 1 SD.

The measured concentration of WBCs and granulocytes did not change when a single batch of blood was fractionated into 12 aliquots and incubated with variable concentrations of the radiopharmaceutical in vitro. No WBC destruction or agglutination could be detected on microscopic examination in blood samples exposed to the radiopharmaceutical in vitro or in vivo. Neither the level nor the pattern of LDH isoenzymes changed significantly when compared with the variance in measures of control samples. Leukocyte esterase could not be detected in the urine after administration.

DISCUSSION

The results showed that this ^{99m}Tc-labeled murine IgM monoclonal antibody selectively binds WBC lines that are known to express SSEA-1 antigens (*30,31*). The results are particularly encouraging because patients with active bacterial infections should have more granulocytes in circulation than healthy volunteers, and the concentration of CD15 antigens on each individual polymorphonuclear cell should be significantly higher than the concentration on nonactivated granulocytes in healthy volunteers (*18*). The increased availability of antibody binding sites that results from both mechanisms probably explains why the fraction of the administered dose bound to the granulocytes in this population of healthy people was lower at all time points than the

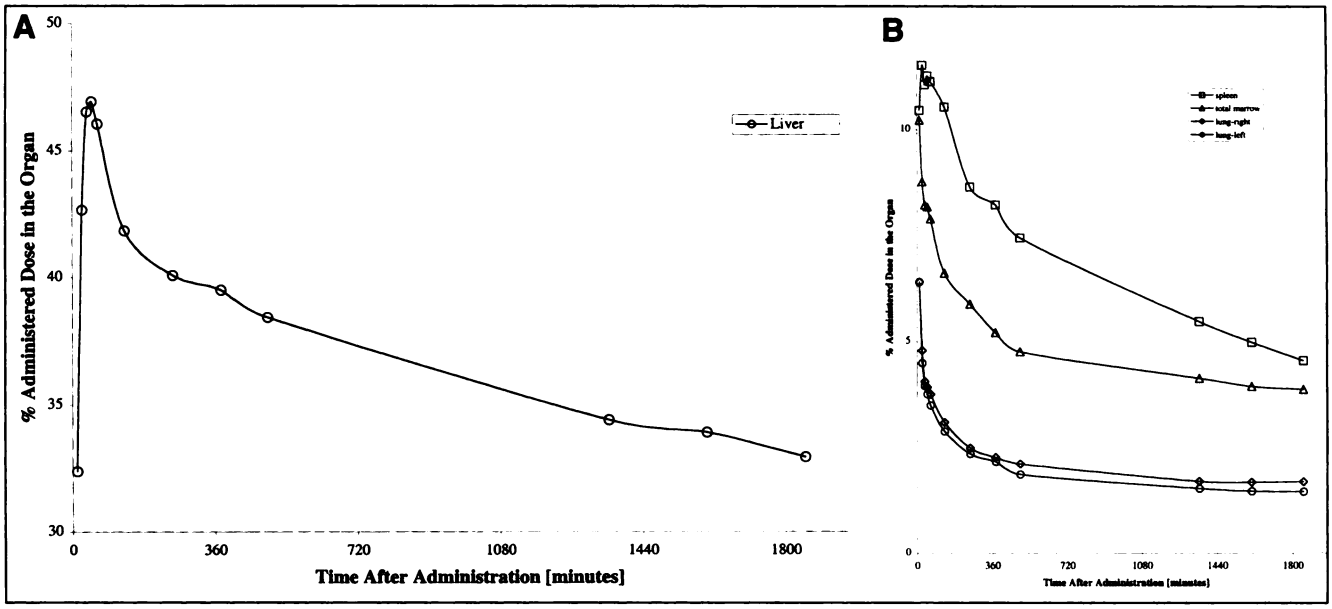

FIGURE 4. Time-activity curves for percentage of administered dose in liver (A) and several other organs of interest (B). Comparisons of Figures 2 and 3 with Figures 1 and 4 are consistent with hypothesis that drop in circulating granulocytes may be partially attributable to transient hepatosplenic sequestration of antibody-coated cells. Curves also show that pulmonic sequestration could not have contributed to transient drop in neutrophils.

values previously reported for a sample of patients with active bacterial infections (*25*).

The findings also confirmed that the administration of this murine IgM antibody to humans produces striking changes in the composition of blood within minutes of injection. Other investigators have already reported that this particular radiopharmaceutical produced a transient mild leukopenia 15 min after injection in four of five patients examined (*25*). This study extends their findings by showing that the phenomenon is more global. The changes in most blood component concentrations were qualitatively similar, although they were much more subtle than the selectively large change seen in the granulocytes. These effects seem like an exaggerated extension of the changes that are seen in drug-free, healthy people when they lie down and rest: the concentration of most RBC and WBC components in the blood drops. This makes it necessary to emphasize that the changes that occurred after administration of the antibody took place after the healthy volunteers had already been at rest on the imaging table for a minimum of 30 min, significantly longer than the 20 min required to re-establish a new steady state after lying down. That is, the 44% drop in the concentration of circulating granulocytes occurred after they had already decreased by a calculated mean of 18.2% from their not-resting baseline values. The 3% change in the hematocrit occurred after it had already decreased by a mean of 5.6%. Transient anticipatory anxiety just before administration with subsequent relief when no subjective side effects were perceived after injection is unlikely to have contributed. It was not observed and cannot account for the magnitude of the decrease when compared with the not-resting values.

Several other mechanisms of action probably contributed to the effect. Some of the decrease in the concentration of blood cell lines that do not selectively express SSEA-1 may be accounted for by the removal from the plasma of granulocytes that do selectively express SSEA-1. At 300–400 $\times$ 10^{-12} L per cell (*32*), the granulocytes constituted a mean of 1.14% of the total blood volume in this population. The removal of 44% of them could have increased the plasma volume by about 0.5%. However, this cannot fully explain the effect on, for instance, the resting platelet counts of about 5% or on the resting hematocrit of about 3%. When coupled with the observation that RBCs are not marginated on the endothelial walls, as are WBCs, this suggests that some blood cell components must have been sequestered in the extravascular space. When linked with the observation that 3.9% of the circulating radioactivity was associated with the erythrocytes, part of the effect may be accounted for by retardation of antibody-coated RBCs by the liver and spleen. However, this seems unlikely to fully account for the same phenomenon in the platelets, because the mean drop of more than 5% was significantly larger than the average fraction of the administered dose associated with the platelets, which was always less than 1% in this sample.

Comparison of Figures 2 and 3 with Figure 4 indicates that the shapes of the blood time-activity curves reflect the inverses of the curves for the liver and spleen. It follows that temporary hepatosplenic sequestration of antibody-coated blood cells could account for some of the drop in circulating blood cells as well as the decrease in the fraction of radiolabeled granulocytes in the circulation. The reticuloendothelial system is known to retard the transit of blood cells coated with other particles and antibodies.

No other reservoirs of sequestered radioactivity could be seen on the images to explain the drop. Specifically, as shown in Figures 1 and 4B, there was no increased retention of WBCs in the lungs, as has been reported for other conditions (*33*) and as a result of administering other anti-SSEA-1 IgM antibodies to animals (*31*). It is possible that antibody binding produced increased WBC adherence to the blood vessel walls, so that when blood was drawn, fewer cells were counted because more remained marginated to the vessel wall. This would be consistent with previous investigations showing that anti-CD15 antibodies increase neutrophil adhesion to endothelium (*14*). However, this mechanism is unlikely to explain the effect on the RBC parameters and may not fully account for the effect on the WBCs.

Several other potential causes of the decreased granulocyte counts were virtually ruled out. The finding did not appear to represent a technical artifact. Labeling aliquots of whole blood in vitro with various concentrations of the antibody indicated that tagging the cells with the radiopharmaceutical did not cause the Coulter counter (Beckman Coulter, Brea, CA) to misidentify granulocytes as another cell type. Many of the clinical samples were reread manually, which always confirmed that the phenomenon was real.

Cell lysis seems unlikely for several reasons. Neither agglutination nor cell fragmentation was ever observed on the slides. The transient drop did not produce meaningful changes in the LDH isoenzyme patterns. Leukocyte esterase was not detected in the urine after administration.

Whatever the mechanism, there were no exceptions to this effect in healthy people, in contrast to some patients with infections who showed little or no effect on their WBCs. The difference probably reflects the relatively rapid and constant release of granulocytes from the bone marrow of patients with active infections. It is possible that new granulocytes are added to the circulation so rapidly in otherwise healthy patients with infections that the antibody-induced removal of some granulocytes cannot be detected easily. The difference may also reflect a normal lag time before a peripheral signal can induce a bone marrow response in healthy people. If this is so, then the findings suggest that the response time in healthy people is very rapid, on the order of about 20 min, with a range of 10–45 min, but slower than in patients with infections whose immune systems have already been activated. This is consistent with the observation that the curve for the recovery of the total number of granulocytes in the circulation preceded the recovery of the number of antibody-bound granulocytes in circulation at all time points, as shown by a comparison of Figures 2 and 3. The fact that 6 of 10 healthy volunteers overshot their resting baseline values in less than 1 h also suggests that the bone marrow is capable of replacing the coated cells faster than the liver and spleen release them back into the circulation.

This finding is not inconsistent with alternative explanations for the recovery in the percentage of cell binding that suggest that delayed in vivo radiolabeling of freshly demarginated cells from the bone marrow with free antibody in the plasma contributes to the recovery curves for the percentage of cell binding (Fig. 3). This explanation seems attractive, because the concentration of CD15 antigens on granulocytes is usually highest right after release from the bone marrow in healthy people. However, preliminary data in other healthy volunteers suggest that binding by previously unbound but circulating antibody to newly demarginated granulocytes may not account for much of the recovery (ML Thakur PhD, unpublished data, 1998). Release of labeled cells seems more likely. Regardless, the lag is more evidence of the robustness and rapidity with which the bone marrow can respond to a challenge with this antibody.

Whatever the mechanism, the phenomenon appears to be clinically silent in healthy people. It did not produce a subjective effect on any of the healthy volunteers and was not associated with any objective changes on physical examination. The lack of a clinically meaningful effect on patients with infections has already been reported (*25*).

The granulocytes that were affected have very short half-lives of less than 10 h in the circulation anyway (*34–36*). Circulating cells constitute only a small fraction (1/25–1/30) of the total pool of mature granulocytes in the body (*37,38*). It follows that even if all the circulating granulocytes were destroyed by the radiolabeled antibody, the problem still would not be clinically meaningful, because mobilization from reserves in the bone marrow would quickly replace them (*39*). This speculation is consistent with the observation that rapid recovery from other iatrogenic causes of transient WBC drops prevents similar phenomena from becoming clinically significant. It follows that the transient changes in the blood produced by this murine antibody should not harm otherwise healthy patients with infections, although further research may be required before extrapolating a presumption of safety to some patients with compromised bone marrow function.

CONCLUSION

This study confirmed that the administration of this radiolabeled murine IgM antibody to humans produces a pronounced drop in the number of circulating granulocytes and transient decreases in the concentration of most other blood cell components. The effect appears to be clinically silent and partially attributable to temporary hepatosplenic sequestration. Rapid responses by the bone marrow make it highly unlikely that the phenomenon is clinically meaningful when the antibody is given in this dose range.

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