IN VITRO AND IN VIVO LABELING OF RABBIT BLOOD LEUKOCYTES WITH ^{67}Ga-CITRATE

Richard L. Burleson, Merrill C. Johnson, and Harold Head

Walter Reed General Hospital and Walter Reed Army Institute of Research, and Walter Reed Army Medical Center, Washington, D.C.

The labeling characteristics of rabbit blood leukocytes with ^{67}Ga-citrate both in vitro and in vivo have been defined. Both granulocytes and lymphocytes were consistently labeled by the in vitro incubation of anticoagulated whole blood with ^{67}Ga-citrate. Minimal uptake by red blood cells occurred. Both the absolute and relative uptake of the radionuclide by granulocytes as compared with lymphocytes was greater in media-1640 than in anticoagulated whole blood. Maximal uptake of label by leukocytes occurred within 1 hr, was independent of temperature of incubation and type of anticoagulant, and occurred at a concentration of approximately 200 μCi ^{67}Ga/10 ml of heparinized blood. Comparative studies with ^{51}Cr demonstrated that ^{67}Ga produced a label on both granulocytes and lymphocytes equal to or greater than the label obtained with ^{51}Cr and that there was minimal loss of label by ^{67}Ga-labeled cells with in vitro incubation. Blood leukocytes labeled in vitro with ^{67}Ga offer an excellent gamma-emitting radioactive tracer that can be used to develop a technique for the scintigraphic detection and localization of abscesses.

The application of radioisotope-imaging techniques to abscess detection has recently shown encouraging results. The concepts of radioactive labeling of leukocytes concentrated in an area of septic inflammation in vivo (*1*) and of labeling leukocytes in vitro and allowing them to concentrate the radioactivity in the septic area by their migration in vivo (*2*) have been validated. Gallium-67, a gamma-emitting radionuclide that concentrates in lysosomal-like granules within cells (*3*), may improve these techniques. The following experiments, which were done as preliminary studies to obtain labeled leukocytes as radioactive carriers for the scintigraphic detection and localization of septic inflammatory processes in rabbits (*4*) define the specific affinity of rabbit blood leukocytes for ^{67}Ga-citrate.

EXPERIMENTS AND RESULTS

In vitro labeling of gradient isolated blood granulocytes and lymphocytes with ^{67}Ga-citrate and ^{51}Cr. Female New Zealand white rabbits weighing between 3 and 5 kg were used for all experiments*. Rabbits were anesthetized with Innovar-Fentanyl, 0.24 mg/kg, and Droperidol, 1.2 mg/kg, i.m. After surgical isolation of the right femoral vein, 20–60 ml of whole blood were drawn into a plastic syringe containing Na heparin† (1 ml/20 ml of blood, 1,000 μ/ml) through a 20-gage cannula. The blood was transferred into 50-ml plastic tubes (Falcon, Oxnard, Calif.) for further manipulation.

Populations of lymphocytes and granulocytes were isolated from a Ficoll (Pharmacia, Uppsala, Sweden), sodium diatrozoate (Hypaque, Winthrop Laboratories, New York, N.Y.), albumin gradient, by a technique modified from Perper (*5*). The gradient was prepared by serially layering 2.0 ml of albumin (2.0 ml Pentex albumin + 0.35 ml normal saline), 10 cc Ficoll-Hypaque solution (24 part 9.0% Ficoll and 10 parts 34.0% Hypaque), and 30 ml cell suspension (10 ml heparinized blood diluted 1:3 with

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For reprints contact: Richard L. Burleson, Peter Bent Brigham Hospital, 721 Huntington Ave., Boston, Mass. 02115.

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† Preservative-free heparin, courtesy of Medical Chemical Corp., Melrose Park, Ill.

media RPMI-1640*, Microbiological Associates, Bethesda, Md.) in a 50-ml plastic tube. Each gradient was centrifuged for ½ hr at 600 g. The two interface layers, media-Ficoll containing lymphocytes and Ficoll-Albumin containing granulocytes, were harvested with a Pasteur pipette. The cells were washed with media, and the cell button was treated with Tris-NH_4 Cl (1 part Tris buffer, 9 parts 85% NH_4 Cl; 20 ml added to the cell button, incubated at 37°C for 5 min with agitation) to remove erythrocytes. The leukocytes were then washed two times and 2×10^8 cells were resuspended in 10 ml of media. Differential counts on the final cell suspension showed greater than 90% granulocytes in the granulocyte fraction and greater than 95% lymphocytes in the lymphocyte fraction.

One hundred microcuries of each isotope, ^{51}Cr (^{51}Cr-sodium chromate, 1.50 mEq Na_2 Cr O_4/ml, 0.166 mCi/ml, Abbott Laboratories, Chicago, Ill.) and ^{67}Ga (^{67}Ga-citrate 3.3 mCi in 1.7 ml pH 4.5–7.5, New England Nuclear, Boston, Mass.) were added and the cell-isotope mixture incubated for 1 hr in a 37°C water bath with agitation. The cells were washed three times to remove unbound isotope and again suspended in 10 ml of media. Cell counts were performed with an electronic particle counter (Coulter Electronics, Hialeah, Fla.) and scintillation counting was performed with a gamma scintillation detector (Packard Instrument, Downer Grove, Ill.) on duplicate 1.0-ml aliquots of the final cell suspension.

The cell-bound radioactivity was calculated and expressed as microcuries of isotope uptake by 2×10^8 cells. Calibration curves using 1 μCi of each isotope in 1.0 ml of media gave 1.65×10^6 cpm/μCi ^{67}Ga and 1.32×10^5 cpm per μCi ^{51}Cr. Baseline to infinity windows were used for each isotope; the baseline used for ^{67}Ga was 50 keV and the baseline used for ^{51}Cr was 250 keV. In five experiments the cell label on granulocytes with ^{67}Ga was 2.8 ± 0.5 s.e. μCi, an uptake of 2.8% of the original ^{67}Ga added to the cell suspension. With ^{51}Cr the granulocyte label was 2.2 ± 0.5 μCi, a 2.2% uptake. In one experiment the lymphocyte population was also labeled. Gallium-67-citrate produced a label of 0.47 μCi per 2×10^8 cells; the ^{51}Cr label was 0.26 μCi/2×10^8 cells. With ^{67}Ga the lymphocyte label was approximately one-sixth the label obtained on granulocytes; with ^{51}Cr the lymphocyte label was one-eighth of that obtained on granulocytes.

The degree of isotope leak from the labeled granulocytes was estimated in three experiments. After scintillation counting was performed, the cell suspensions were incubated for 24 hr at 37°C in a 95% O_2 atmosphere. The cells were centrifuged, washed, resuspended in 1.0-ml media, and scintillation counted. The incubation procedure was repeated for an additional 24 hr; the cells were centrifuged, washed, and counted. The percentage of radioactivity lost into the discarded supernate, corrected for radioactivity lost by decay, was calculated from the radioactivity measured in the cells. The ^{67}Ga-labeled granulocytes lost 3.3 ± 1.8% of their original radioactivity at 24 hr compared with 30 ± 8.8% for granulocytes labeled with ^{51}Cr. At 48 hr ^{67}Ga-labeled granulocytes had lost 13 ± 3.3% of their label; ^{51}Cr-labeled granulocytes lost 34 ± 1.6%. The precise contribution of cell death, as opposed to "label leak", to label loss could not be quantified because cell clumping rendered accurate cell counting technically impossible.

In vivo labeling of blood granulocytes and lymphocytes with ^{67}Ga-citrate. Two rabbits were given ^{67}Ga-citrate, 100 μCi/kg, i.v. in the marginal vein of the right ear. Twenty-four hours later 20 ml of heparinized blood were obtained from each rabbit. Populations of granulocytes and lymphocytes were separated by double gradient centrifugation, washed, and suspended in media. Cell and scintillation counting were performed on duplicate 1.0-ml aliquots. Granulocytes were labeled with 0.36 ± 0.01 μCi/2×10^8 cells and lymphocytes with 0.030 ± 0.01 μCi/2×10^8 cells, demonstrating the capability of circulating leukocytes to be labeled in vivo by circulating ^{67}Ga-citrate.

In vitro labeling of blood granulocytes and lymphocytes in whole heparinized blood with ^{67}Ga-citrate. Twenty milliliters of whole heparinized blood were obtained from two rabbits. Two hundred microcuries of ^{67}Ga-citrate were added to the whole blood. After 1 hr incubation at room temperature (23°C) the blood was centrifuged at 2,000 g for 30 min. The plasma and buffy coat were removed. Populations of lymphocytes and granulocytes were isolated from the leukocyte-rich plasma by double gradient centrifugation. Five-milliliter specimens of the packed red blood cells were washed three times. Each population of cells—erythrocytes, granulocytes, and lymphocytes—was resuspended, and cell and scintillation counting performed on duplicate 1.0-ml aliquots. The mean label of granulocytes was 0.15 ± 0.01 μCi/2×10^8 cells, of lymphocytes was 0.71 ± 0.12 μCi/2×10^8 cells, and of red blood cells was 2.0 ± 0.2 $\times 10^{-4}$ μCi/2×10^8 cells. Lymphocytes labeled 3,600 times greater than red blood cells and granulocytes 700 times greater than red blood cells with ^{67}Ga-citrate.

* Media RPMI-1640 was used for all cell washings and suspensions.

Determination of optimum conditions for in vitro in blood labeling of leukocytes with ^{67}Ga-citrate. The optimum conditions for labeling leukocytes in whole blood were established by evaluating the effects of (A) anticoagulants—heparin and Na citrate, (B) temperature of incubation, (C) time of incubation, and (D) concentration of ^{67}Ga-citrate on the degree of labeling of leukocytes incubated with ^{67}Ga-citrate in anticoagulated whole blood.

Blood was obtained from the right femoral vein and anticoagulated with either heparin or Na citrate. Gallium-67-citrate was added; after incubation the labeled cells were recovered by a modification of Jacobson's Dextran sedimentation procedure (*6*). The blood was mixed 1:1 with 3% Dextran (500,000 molecular weight) in minimal essential media (Flow Laboratories, Rockville, Md.). The mixture was allowed to settle for 15 min, and the plasma fraction was decanted; the cells were recovered by centrifugation. Each cell button was treated with Tris-NH_4 Cl to remove red blood cells, washed three times, and the cells were resuspended in 5 ml of media. Duplicate 1.0-ml aliquots were taken for cell and radioactive counting. Excepting the variable under consideration, all experiments were conducted with heparinized blood, an incubation temperature of 23°C, an incubation time of 1 hr, and a concentration of 200 μCi ^{67}Ga/10 ml of blood.

In five experiments leukocytes labeled equally well in heparinized blood, mean 0.60 ± 0.15 μCi/2 × 10^8 cells, and in citrated blood, mean 0.54 ± 0.10 μCi/2 × 10^8 cells. In four experiments the temperature of incubation had no effect on leukocyte labeling with ^{67}Ga-citrate. Mean microcurie uptake per 2 × 10^8 cells at 5°C was 0.39 ± 0.08, at 23°C was 0.53 ± 0.09, and at 37°C was 0.44 ± 0.08. In four experiments, maximum labeling of leukocytes occurred after 1-hr incubation time; at 1 hr mean microcurie uptake per 2 × 10^8 cells was 0.33 ± 0.16 and did not increase with longer incubation (Table 1). In three experiments the leukocyte label with ^{67}Ga increased with an increase in the concentration of ^{67}Ga-citrate. Maximum label occurred at a concentration of 200 μCi/10 ml of blood with a mean label of 0.41 ± 0.03 μCi/2 × 10^8 cells. Addition of greater amounts of ^{67}Ga did not increase the label significantly (Table 2).

TABLE 1. RABBIT BLOOD LEUKOCYTE LABELING WITH ^{67}Ga

Time of in vitro incubation	
Time (hr)	μCi/2 × 10^8 leukocytes*
0.5	0.28 ± 0.17
1.0	0.33 ± 0.16
3.0	0.33 ± 0.15
5.0	0.25 ± 0.06

* mean ± s.e.

TABLE 2. RABBIT BLOOD LEUKOCYTE LABELING WITH ^{67}Ga

Concentration of ^{67}Ga-citrate	
μCi/10 ml blood	μCi/2 × 10^8 leukocytes*
25	0.03 ± 0.01
50	0.13 ± 0.02
100	0.17 ± 0.02
200	0.41 ± 0.03†
300	0.60 ± 0.06†
400	0.40 ± 0.02

* mean ± s.e.
† $p > 0.3$ (student's t test).

DISCUSSION

Gallium-67-citrate has recently undergone extensive investigation as a tumor-scanning agent (*7*). It has been established that ^{67}Ga is taken up by the macrophage as well as tumor cells and that its intracellular localization is on lysosome-like structures (*3,8*). These factors indicate that ^{67}Ga should be an excellent agent for labeling leukocytes—cells rich in lysosomes.

The studies reported here confirm that both granulocytes and lymphocytes can be labeled with ^{67}Ga-citrate in blood, both in vitro and in vivo. Comparative studies with ^{51}Cr show ^{67}Ga-citrate to be a superior labeling agent for leukocytes; ^{67}Ga produced as much radioactivity per cell, showed less loss of label with time, and labeled red blood cells to a minimal degree.

The observations that purified granulocytes suspended in media-1640 label to a greater degree than granulocytes in in vitro heparinized blood and that the relative degree of labeling of granulocytes and lymphocytes is different in media-1640 and blood are unexplained at this time. Binding of ^{67}Ga to plasma proteins (*9*) in blood may decrease the availability of ^{67}Ga for intracellular localization, thereby producing a lower label in blood (*10*); or cell membrane damage during the process of preparing an isolated population of granulocytes may change the uptake characteristics of either the isolated cell membrane or the lysosomal membranes, thereby producing a greater cell label in media-1640.

Labeling of unseparated leukocytes with ^{67}Ga-

citrate, with minimal labeling of red blood cells, was readily accomplished in vitro by incubating anticoagulated whole blood for 1 hr at 5°C with 200 μCi ^{67}Ga-citrate/10 ml of blood. This technique provides a useful method of labeling blood leukocytes with a gamma-emitting radionuclide that may then be used to study the migration patterns of labeled leukocytes in vivo.

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