USE OF ^{99m}Tc AS A RADIOISOTOPIC LABEL TO STUDY THE MIGRATORY PATTERNS OF NORMAL AND NEOPLASTIC CELLS

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We have employed ^{99m}Tc as a radioisotopic label to study the migratory patterns of syngeneic and xenogeneic lymphocytes and erythrocytes in mice. Maximum splenic and hepatic localization of lymphocytes and erythrocytes was attained 1–2 hr following intravenous injection. The mean liver/spleen ratio was 6.2 with syngeneic murine thymocytes and 14.0 with human peripheral blood lymphocytes. When syngeneic murine erythrocytes were administered intravenously approximately equal numbers of cells localized in the liver and spleen. In contrast, when xenogeneic sheep RBC were given, there was markedly increased hepatic localization as reflected by a mean liver/spleen ratio of 99.6. There was a linear relationship between the number of lymphocytes or erythrocytes injected and gamma counts recorded in the liver and spleen.

Technetium-99m also could be employed as a label to study the distribution of Sarcoma I cells in syngeneic A/J mice. The greatest number of tumor cells localized in the liver and lungs in a ratio approximating unity and this was maximal at 4 hr. Significant numbers of counts also were detected in the kidneys, less in the spleen, and relatively few in the peripheral blood.

These studies are the first to show that ^{99m}Tc can be used as a label to follow the organ distribution of both normal and neoplastic nucleated cells. The high specific activity, rapid labeling time, lack of release from dead and injured nucleated cells, and ready availability of the radionuclide are significant advantages which suggest that ^{99m}Tc may have wide applicability in both experimental and clinical studies of cell migration.

Lymphocyte homing patterns have been extensively investigated using ^{51}Cr. Cells labeled with this radionuclide have been employed in studies of murine allograft reactivity (*1–3*), compartmental localization of lymphocytes in normal (*4–6*) and antigen-stimulated mice (*7*), and recirculation of subpopulations of murine lymphocytes (*8*). Chromium-51-labeled cells also have been used in humans to study survival time (*9,10*) and homing (*11–13*) of normal and neoplastic lymphocytes (*14*). The high spontaneous release, low specific activity, and relatively long half-life (28 days) with high radiation exposure and low available gamma photon flux per microcurie have limited the clinical applications of this radionuclide in gamma imaging to its occasional use as an erythrocyte tag for spleen scans. On the other hand, ^{99m}Tc, a high specific activity, metastable gamma emitter of short half-life (6 hr) and high photon flux has rapidly become the most widely used radioisotope for diagnostic gamma imaging (*15*). Technetium-99m-labeled compounds have been used to scan liver, brain, lung, thyroid, bone marrow, joints, gastrointestinal tract, and cardiovascular system. Erythrocytes can be labeled with ^{99m}Tc (*16,17*) and these have been used to study organ distribution following intravenous administration (*16*), determination of red cell volume (*18*), the quantitative assessment of hemolytic antibody (*19*), and as indicator cells in the mixed hemadsorption reaction (*20*). We have described a method for labeling nucleated cells with ^{99m}Tc (*21,22*) and have developed assays for cell-mediated immunity (*21, 23–26*) and lymphotoxin activity (*27*) employing ^{99m}Tc-labeled target cells. We now present data show-

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ing that ^{99m}Tc can be used as a radioisotopc label to study the migratory patterns of lyphocytes, erythrocytes, and tumor cells.

MATERIALS AND METHODS

Mice. BALB/c and CFW mice of either sex weighing approximately 18–20 gm were obtained from Carworth Farms, New City, N.Y. A/J mice were obtained from the Jackson Memorial Laboratory, Bar Harbor, Me.

Labeling of nucleated cells. Thymic lymphocyte suspensions were prepared by mincing BALB/c thymuses, suspending them in cold Hanks' Balanced Salt Solution (HBSS), and passing the fragments through progressively higher-gage needles attached to a 12-ml syringe. Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained by eluting a New England Nuclear molybdenum–technetium generator with 0.9% saline. Labeling was accomplished by adding 5 mCi of ^{99m}Tc and 100 μg of Na_2CrO_4 to a suspension containing 50×10^6 cells in 2 ml of HBSS and allowing them to incubate for 10–15 min at 37°C. Following this, the valence of ^{99m}Tc was reduced by the dropwise addition of 0.3 ml of a sterile solution of 0.2% $SnCl_2 \cdot 2H_2O$ dissolved in freshly prepared acid citrate dextrose (30 gm $Na_3C_6H_5O_7$, 0.15 gm NaH_2PO_4, and 2.0 gm dextrose/liter of distilled H_2O, pH adjusted to 7.4). After an additional 15-min incubation at 37°C, the cells were sedimented by centrifugation at 400 G for 15 min and the unbound radioisotope removed by washing them three times in HBSS. The labeled cells, having a viability of 88–98% as determined by trypan blue exclusion (*28*), were adjusted to a final concentration of 2×10^6/ml. The same procedure was used to label human peripheral blood lymphocytes which had been separated by Ficoll-Hypaque density gradient centrifugation (*29*) and Sarcoma I, a transplantable murine fibrosarcoma of strain A/J origin, which had been maintained in tissue culture. The uptake of ^{99m}Tc by nucleated cells was generally in the range of 1–2% of the total initial radioactivity used for labeling. Subcellular fractionation of labeled cells revealed that this was distributed to the nuclear, mitochondrial, and microsomal fractions and that following the third wash with HBSS the amount of cell-associated radioactivity remained constant over 48 hr (*26*).

Labeling of erythrocytes. Sheep and mouse erythrocytes were labeled by adding 1 mCi of ^{99m}Tc in 0.2 ml of 0.9% NaCl to a pellet containing 6.4×10^9 washed RBC and allowing them to incubate for 10–15 min at 37°C. Following this the ^{99m}Tc was reduced by the addition of 0.3 ml of $SnCl_2 \cdot 2H_2O$, the cells incubated for an additional 15 min, washed three times in phosphate-buffered saline (pH 7.4) to remove unbound radioisotope, and the final concentration adjusted to $4 \times 10^5 - 4 \times 10^9$ cells/ml for dose studies or 4×10^7 cells/ml for time studies.

Homing studies. Groups of four to five mice were injected through the lateral tail vein with 0.25 ml of labeled cells and bled from the retro-orbital sinus immediately prior to killing. Since it has been well established that intravenously injected lymphocytes home primarily to the liver and spleen (*4–8,30–32*), reticuloendothelial activity was assessed by removing these organs and counting their total radioactivity. Means and standard errors (s.e.) were calculated from pooled data. The liver-to-spleen (L/S) ratio provides a useful index of phagocytic function (*30,32*) and for this reason it has been employed frequently in studies of lymphocyte homing. In our experiments liver/spleen ratios were calculated for each individual animal and the mean and s.e. were determined from the pooled values of each experimental group.

RESULTS

Localization of lymphocytes and thymocytes. The hepatic and splenic localization of murine thymic and human peripheral blood lymphocytes are summarized in Table 1. Groups of four BALB/c mice were injected i.v. with 5×10^5 ^{99m}Tc-labeled BALB/c thymus or human peripheral blood lymphocytes and killed at 10 and 30 min and 1, 2, 3, and 4 hr following injection. Radioactivity was determined at the same time (4 hr) for all groups in order to correct for decay. Maximum splenic and hepatic localization was attained 1–2 hr following i.v. injection and remained constant thereafter. Although equal numbers of murine and human lymphocytes were used, higher counts were obtained with human cells and this was due to their greater labeling efficiency in this set of experiments. The liver/spleen ratio ranged from 4.1 to 8.8 for murine lymphocytes (mean 6.2) and from 12 to 17 for human lymphocytes (mean 14.0) indicating that xenogeneic cells were preferentially taken up by the liver.

The relationship between the number of lymphocytes injected and their splenic and hepatic localization is presented in Fig. 1. Groups of four BALB/c mice were injected i.v. with varying numbers of cells and killed after 1 hr. There was a linear relationship between the number of lymphocytes injected and gamma counts recorded in the liver and spleen. Although the absolute number of counts in each organ increased with cell number, the liver/spleen ratio remained constant for both murine (mean 7.1, range 6.5 to 8.0) and human lymphocytes (mean 17.5, range 17 to 19).

TABLE 1. SPLENIC AND HEPATIC LOCALIZATION OF ^{99m}Tc-LABELED MURINE AND HUMAN LYMPHOCYTES AT VARYING TIMES FOLLOWING INTRAVENOUS INJECTION INTO MICE

	Mouse			Human		
Time*	Spleen (cpm ± s.e.)	Liver (cpm ± s.e.)	L/S†	Spleen (cpm ± s.e.)	Liver (cpm ± s.e.)	L/S†
10 min	2,625 ± 250	22,932 ± 462	8.8 ± 0.6	12,768 ± 1,984	177,008 ± 23,350	14 ± 2
30 min	3,563 ± 915	22,926 ± 6,399	6.3 ± 0.3			
1 hr	4,634 ± 964	25,439 ± 4,046	5.6 ± 0.4	17,081 ± 3,207	212,862 ± 5,546	13 ± 3
2 hr	5,277 ± 663	31,312 ± 535	6.0 ± 0.6	9,327 ± 7,575	82,148 ± 5,947	12 ± 2
3 hr	3,983 ± 781	25,035 ± 4,372	6.3 ± 0.2			
4 hr	5,644 ± 399	23,404 ± 1,847	4.1 ± 0.3	9,300 ± 1,037	152,446 ± 1,010	17 ± 3

* Groups of four mice were injected i.v. with 5×10^5 cells, killed at the times indicated, and radioactivity in the whole spleen and liver was determined.
5×10^5 murine thymocytes gave 232,589 cpm and 5×10^5 human lymphocytes gave 558,292 cpm.
† The liver/spleen (L/S) ratio was calculated by dividing cpm of the liver by cpm detected in the spleen.

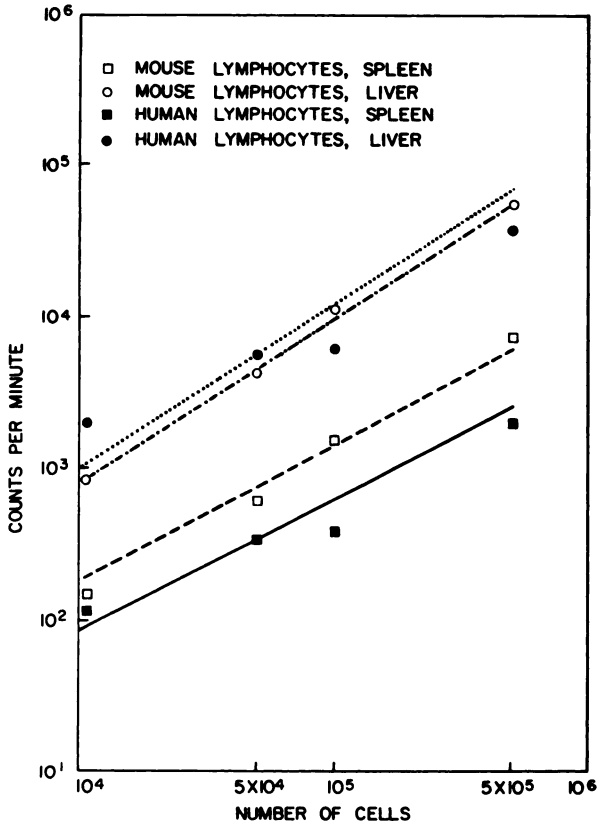

FIG. 1. Splenic and hepatic localization of ^{99m}Tc-labeled human and mouse lymphocytes. Groups of four BALB/c mice were injected i.v. with varying numbers (10^4–10^6) of ^{99m}Tc-labeled cells and killed after 1 hr.

Localization of erythrocytes. The hepatic and splenic localization of syngeneic CFW and xenogeneic sheep erythrocytes are summarized in Table 2. Groups of four CFW mice were injected i.v. with 10^7 ^{99m}Tc-labeled CFW or sheep RBC and killed at 30 min and 1, 2, 4, and 24 hr following injection. All samples were counted at the same time (24 hr) in order to correct for decay. Maximum splenic and hepatic localization was attained 30–60 min following i.v. injection. When syngeneic erythrocytes were used, approximately equal numbers of cells localized in the liver and spleen and the mean liver/spleen ratio was 1.2. In contrast, when xenogeneic sheep RBC were employed, there was markedly increased hepatic localization as reflected in liver/spleen ratios ranging from 77 to 134 (mean 99.6).

The relationship between the number of erythrocytes injected and their splenic and hepatic localization is presented in Fig. 2. Groups of CFW mice were injected i.v. with varying numbers of cells (10^6 to 10^9) and killed after 1 hr. As expected, there was proportionality between the number of erythrocytes injected and the number of gamma counts recorded. The liver/spleen ratios ranged from 2.7 to 4.8 (mean 3.9) when syngeneic erythrocytes were used and from 19 to 98 (mean 60.3) with xenogeneic sheep RBC.

Localization of tumor cells. Technetium-99m also can be employed as a radioisotopic label to study the dissemination of intravenously injected tumor cells (Table 3). Groups of four A/J mice were injected i.v. with 5×10^5 ^{99m}Tc-labeled Sarcoma I cells which had been propagated in tissue culture. Mice were killed at 4, 8, and 24 hr following injection and gamma counting for ^{99m}Tc was carried out at 24 hr in order to correct for decay. Five $\times$ 10^5 cells gave an average of 1,770,000 cpm. The greatest number of tumor cells localized in the liver and lungs in a ratio approximating unity and this was maximal at 4 hr. Quite unexpectedly, none of these mice developed hepatic or pulmonary metastases despite the fact that as few as 10^3 labeled cells injected subcutaneously resulted in progressively growing tumors leading to the death of the host. If, however, the liver or lungs of i.v. injected mice were minced up into small fragments and injected subcutaneously into A/J mice, these recipients developed progressively growing tumors which eventually killed the

host animals. Significant numbers of counts also were detected in the kidney, less in the spleen, and relatively few in the peripheral blood.

DISCUSSION

In the studies reported in this paper we have demonstrated that ^{99m}Tc can be used as a radioisotopic label to determine the splenic and hepatic localization of lymphocytes, erythrocytes, and tumor cells following intravenous injection. Chromium-51 previously has been employed for this purpose (*1–14, 30–34*) but release and subsequent reutilization of radioisotope occurs and may constitute a serious problem (*35*). Since most of the data on the migratory patterns of cells have been obtained with ^{51}Cr as a radioisotopic label, it was essential to determine whether there were significant differences in the distribution of ^{51}Cr and ^{99m}Tc-labeled cells. In an extensive series of experiments, we have established that lymphocytes and erythrocytes labeled with ^{99m}Tc have similar migratory patterns as cells labeled with ^{51}Cr (*36*). The liver/spleen ratios essentially were identical when either ^{51}Cr- or ^{99m}Tc-labeled cells were employed. Although ^{51}Cr may not necessarily be the proper reference point, the similarity in homing patterns of cells labeled with it and ^{99m}Tc should allow direct comparison of previously reported data and that obtained in future studies with ^{99m}Tc-labeled cells.

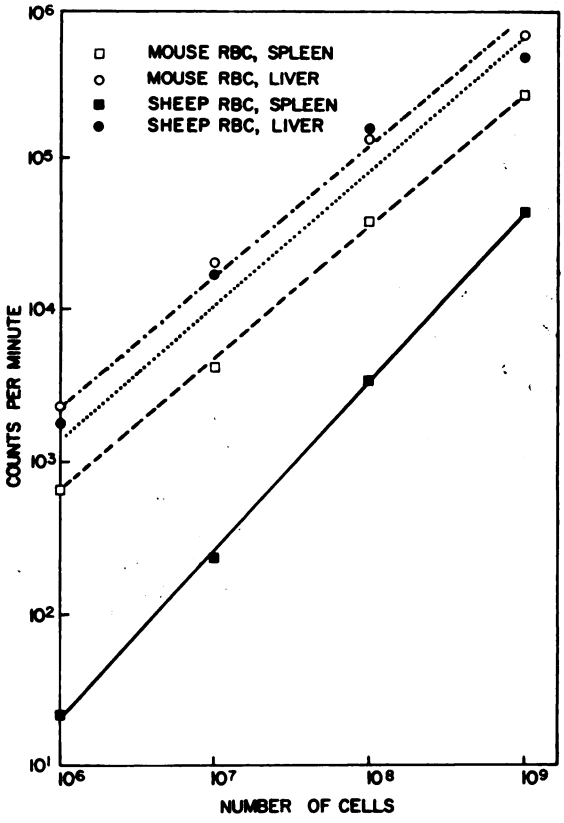

FIG. 2. Splenic and hepatic localization of ^{99m}Tc-labeled sheep and mouse erythrocytes. Groups of four CFW mice were injected i.v. with varying numbers (10^6–10^9) of ^{99m}Tc-labeled CFW or sheep RBC and killed after 1 hr.

TABLE 2. SPLENIC AND HEPATIC LOCALIZATION OF ^{99m}Tc-LABELED SYNGENEIC (CFW) AND XENOGENEIC (SHEEP) ERYTHROCYTES AT VARYING TIMES FOLLOWING INTRAVENOUS INJECTION

	Syngeneic			Xenogeneic		
Time*	Spleen (cpm ± s.e.)	Liver (cpm ± s.e.)	L/S†	Spleen (cpm ± s.e.)	Liver (cpm ± s.e.)	L/S†
30 min	9,430 ± 1,970	21,360 ± 960	2.5 ± 0.5	975 ± 42	129,460 ± 5,925	134 ± 12
1 hr	16,660 ± 2,405	15,847 ± 2,650	1.0 ± 0.2	1,677 ± 390	117,360 ± 4,442	77 ± 16
2 hr	17,307 ± 915	14,460 ± 1,900	0.8 ± 0.1	1,157 ± 207	96,590 ± 3,617	88 ± 14
4 hr	12,650 ± 1,002	9,317 ± 610	0.7	802 ± 105	76,767 ± 1,370	100 ± 15
24 hr	5,092 ± 1,062	4,830 ± 922	1.1 ± 0.3	540 ± 162	47,397 ± 4,505	99 ± 18

* Groups of four mice were injected i.v. with 10^7 cells, killed at the times indicated, and radioactivity in the whole spleen and liver was determined.
10^7 CFW erythrocytes gave 71,787 and 10^7 sheep RBC gave 203,730 cpm.
† The liver/spleen (L/S) ratio was calculated by dividing the cpm of the liver by cpm detected in the spleen.

TABLE 3. ORGAN DISTRIBUTION OF ^{99m}Tc-LABELED SARCOMA I CELLS IN A/J MICE FOLLOWING INTRAVENOUS INJECTION*

Time†	Liver	Lung	Kidney	Spleen	Blood
4 hr	363,359 ± 16,379	382,098 ± 22,433	131,710 ± 3,298	21,467 ± 3,169	6,549 ± 330
8 hr	340,030 ± 20,038	389,190 ± 31,053	127,530 ± 8,569	21,265 ± 1,426	4,278 ± 334
24 hr	259,025 ± 44,115	185,623 ± 8,437	83,416 ± 136	26,866 ± 1,979	3,735 ± 410

* 10^5 Sa I cells gave 438,000 cpm.
† Groups of four mice were injected i.v. with 5×10^5 cells, killed at the times indicated, and radioactivity was determined in either the whole organs indicated or in 0.4-ml aliquots of blood.

Iodine-125-iododeoxyuridine recently has been used as a radioisotopic label to study the distribution of tumor cells (*35,37,38*) and this appears to have significant advantages over ^{51}Cr. Only dividing cells incorporate this radionuclide, however, and this is a serious limitation. Furthermore, good gamma imaging has not to our knowledge been reported using ^{125}I-labeled tumor cells. We have compared the localization of ^{125}I-iododeoxyuridine and ^{99m}Tc-labeled Sarcoma I cells and have found that they are similarly distributed. At the present time we have no explanation why intravenously administered cells failed to form tumors whereas as few as 10^3 cells given subcutaneously resulted in progressively growing tumors. This observation together with previous reports on the fate of circulating tumor cells (*30,40*) suggest that there are important host defense mechanisms which can eliminate malignant cells. Although the short half-life of ^{99m}Tc is an advantage for diagnostic nuclear scanning, it does limit the time during which cells can be counted to approximately 72 hr following i.v. injection. The use of longer-lived radionuclides of technetium such as ^{95m}Tc ($T_{1/2}$ = 60 days) or ^{96}Tc ($T_{1/2}$ = 4.3 days) would circumvent this problem. Nevertheless, the high specific activity, rapid labeling time, lack of release from dead and injured cells (*21,26*), and ready availability of the radionuclide are significant advantages which suggest that ^{99m}Tc may have wide applicability in both experimental and clinical studies of cell migration.

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