Cancer Immunol Immunother (1990) 31: 139–145

Cancer Immunology Immunotherapy

Fate of intravenously administered rat lymphokine-activated killer cells labeled with different markers

Azzam A. Maghazachi and Linda Fitzgibbon

Molecular Immunology Section, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec H4P 2R2, Canada

Received 6 September 1989/Accepted 2 January 1990

Summary. Rat lymphokine-activated killer (LAK) cells, generated by adhering rat splenocytes isolated from the 52% Percoll density fraction to plastic flasks, demonstrate restricted in vivo tissue distribution, localizing in the lungs and liver after 2 h, but redistributing into the liver and spleen 24 h after i.v. administration. However, a different pattern of distribution was observed when this population of LAK cells was labeled with one of four commonly used radioisotopes. For example, LAK cells showed a high distribution into the lungs 30 min after administration when labeled with ^{51}Cr, ^{125}I-dUrd or ^{111}In-oxine, whereas ^{111}InCl-labeled LAK cells showed an equal distribution into the blood, lungs and liver at this time. Two hours after administration, cells labeled with ^{111}In-oxine showed an equivalent distribution into the lungs and liver, those labeled with ^{125}I-dUrd or ^{51}Cr showed a high accumulation in the lungs, whereas those labeled with ^{111}In-Cl entered more into the liver and blood. The pattern of distribution of ^{111}In-Cl- or ^{111}In-oxine-labeled cells was confirmed using gamma camera imaging analysis. By 24 h, LAK cells labeled with ^{111}InCl, ^{111}In-oxine or ^{51}Cr distributed in the liver and spleen in variable concentrations. In contrast, cells labeled with ^{125}I-dUrd were not detected in any organ tested.

This study was paralleled by monitoring the distribution of LAK cells labeled with Hoechst 33342 (H33342) and analyzed for the presence of fluoresceinated cells in different organs either by flow cytometry analysis, or in frozen section. The data indicate that the distribution pattern of LAK cells labeled with ^{111}In-oxine is the closest to the distribution of H33342-labeled cells. Of all the radioisotopes used, ^{125}I-dUrd has the most disadvantages and is not recommended for monitoring the in vivo distribution of leukocytes.

Introduction

Lymphokine-activated killer (LAK) cells have a highly anti-metastatic activity and have been used to treat hepatic and pulmonary metastases of cancer patients with advanced tumors such as melanoma, renal cell carcinoma and colorectal cancer [17, 20–22]. For optimal systemic distribution of LAK cells and for therapy of cancers of various histology, it is highly important to understand the mechanism of in vivo distribution of these cells to help develop protocols for cancer immunotherapy. Lotze et al. have previously shown that killer cells generated by incubating lymphoid cells with T-cell growth factors distributed into the lungs early after i.v. administration, but redistributed into the liver and spleen later on [9]. Similarly, we observed that highly purified LAK cells generated by adherence to plastic flasks [26] have a restricted in vivo tissue distribution, upon labeling them with ^{51}Cr before infusing into syngeneic recipients [11]. These cells, known as adherent LAK cells, were localized in the lungs after 2 h, but redistributed into the liver and spleen 24 h after injection, with no detectable radioactivity in the blood, or any other organ tested [11].

Recently published reports, utilizing either ^{51}Cr [7, 19], ^{111}indium chloride [1], or autoradiographic studies [25] to monitor the distribution of LAK cells, have confirmed these findings. However, we recently observed that LAK cells, labeled with ^{125}I-dUrd were not recovered from any organ tested 24 h after administration (unpublished observation, and this report) suggesting the possibility that LAK cells may be destroyed in vivo during this time, probably because of the unavailability of interleukin-2 (IL-2), presumably a necessary factor for their survival. If this is true, then it suggests that the distribution of ^{51}Cr-labeled LAK cells into the liver and spleen 24 h after administration is probably due to the elution of ^{51}Cr from these cells, with concomitant contamination of the reticuloendothelial tissues. Alternatively, because ^{125}I-dUrd is toxic to leukocytes [4, 6, 16, 24] and elutes easily from the labeled cells [18, 24], the inability to monitor the distribution of ^{125}I-dUrd-labeled LAK cells in the liver and spleen 24 h after administration may be due to the elution of this radioisotope from these cells. Consequently, the cells could not be demonstrated in these organs in spite of their presence.

Offprint requests to: A. A. Maghazachi

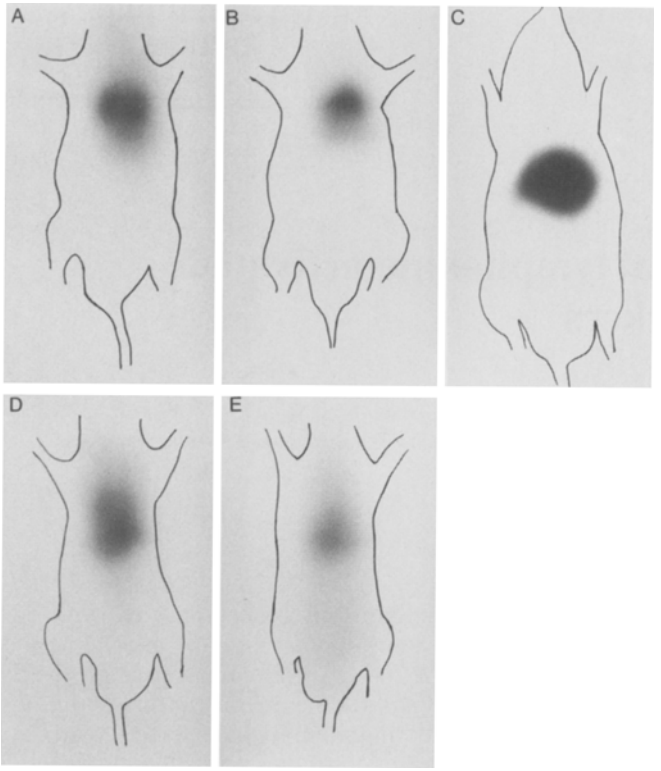

Fig. 1 A–E. LAK cells (10×10^6) were labeled with either 25 μCi ^{111}In-oxine or 100 μCi ^{111}InCl, extensively washed and injected i.v. into syngeneic recipients. **A, B, C** ^{111}In-oxine-labeled cells 30 min, 2 h, or 24 h after injection, respectively. **D, E** ^{111}InCl-labeled cells 30 min, or 2 h after injection, respectively

We therefore undertook this study to resolve this important issue.

Materials and methods

Animals. Pathogen-free male Fischer 344 (F344) rats 4–6 weeks old were obtained from Charles River (Quebec). The rats were maintained in our animal facility at BRI.

Culture medium. Culture medium consisted of RPMI-1640 supplemented with 10% fetal calf serum, 10 U/ml penicillin, 100 μg/ml streptomycin, 1 mM L-glutamine, 1% nonessential amino acids (all from Flow Laboratories Va), and 50 μM 2-mercaphoethanol (Fisher Scientific, Pittsburg, Pa). This is known as complete medium.

Preparation of lymphoid cells. Rat spleen cell suspension was layered over Ficoll/Hypaque (Pharmacia Chemicals, Quebec, Canada), and then centrifuged for 25 min at 600 *g* to remove red blood cells. The erythrocyte-depleted cell preparation was incubated on a nylon-wool column for 1 h at 37° C in a CO_2 incubator to remove B lymphocytes and macrophages. The splenocytes from the nylon-wool column were further separated by layering on Percoll (Pharmacia Chemicals) density fraction. The osmolality of the Percoll was adjusted to 285 mOsm with 10× phosphate-buffered saline and the complete medium to 290 mOsm. Four layers of Percoll were used: 37%, 44%, 52% and 60%. After centrifugation, the cells of the 52% fraction were carefully collected, extensively washed, and then incubated at 1×10^6/ml with 1000 U/ml interleukin-2 (Cetus Corp., Emeryville, Calif). These cells were then allowed to adhere to plastic flasks (Corning, New York) similarly to a previously described method [26]. The mammary adenocarcinoma MADB106 cell line was cultured in complete medium.

Radiolabeling of LAK cells. LAK or MADB106 cells (1×10^7) were labeled with either 100 μCi sodium [^{51}Cr]chromate (^{51}Cr) for 1 h at 37° C, 8 μCi carrier-free ^{111}indium chloride (^{111}InCl), or 8 μCi carrier-free ^{111}indium oxine for 20 min at room temperature, or with 1–3 μCi 5-[^{125}I]iodo-2-deoxyuridine (^{125}I-dUrd) in 5–10 ml complete medium for 4 h at 37° C. All four radioisotopes were purchased from Amersham Canada (Ontario). The cells were extensively washed with complete medium, and their viability was routinely greater than 95% before injection. The labeled cells were suspended in phosphate-buffered saline and 1 ml (containing 4×10^6 cells) was injected into the lateral tail vein. After 30 min, 2 h, or 24 h the animals were sacrificed by cervical dislocation, and the various organs were excised and weighed. All organs except for the lungs and liver were analyzed for organ-associated radioactivity by counting in separate tubes. The radioactivity of the lungs and liver was determined by counting a piece of the organ and then correcting the result according to the weight of the organs. For blood, 1 ml was collected by cardiac puncture and the radioactivity equivalent to the total amount of blood in the rat (routinely estimated to be 10 ml) was calculated. The total incorporation (cpm) of the labeled cells was determined by counting an aliquot of the cells just prior to injection. The radioactivity recovered in each experiment was expressed as the percentage per organ, calculated as the radioactivity (cpm) per organ divided by the total radioactivity injected and multiplied by 100, and per gram of tissue, calculated as the percentage of radioactivity per organ divided by the weight of that organ.

Isolation of LAK cells from various organs. LAK cells were isolated from the liver by perfusion and enzymatic digestion according to a previously described method [11]. Lung cells were isolated by mincing the lungs in stainless-steel mesh, and layering the suspension over a gradient of Ficoll/Hypaque, while spleen cells were prepared as normal.

Gamma camera imaging analysis. Adherent LAK cells (10×10^6) were labeled with 25 μCi ^{111}-In-oxine or 100 μCi ^{111}InCl for 20 min at room temperature. The cells were extensively washed, and then infused i.v. into syngeneic rats. After 0.5 h, 2 h and 24 h, the rats were sacrificed by cervical dislocation and then exposed to X-ray film in the dark for 24 h.

Labeling LAK cells with Hoechst 33342 (H33342). LAK cells (10×10^6) were labeled with 4 μmol H33342 (Calbiochem, La Jolla, Calif) in 15 ml complete medium for 45 min at 37° C. The cells were then washed three times with complete medium and LAK cells (10×10^6) were injected i.v. Two methods were used to evaluate the presence of these cells in various tissues. (a) The various organs were excised after 30 min, 2 h or 24 h, a cell suspension was prepared from these organs and the cells were analyzed for fluorescence using the Coulter EPIC 753 (Coulter Corp., Hialeah, Fla). Control samples included cells prepared from organs injected with unlabeled LAK cells, or with 4 μmol H33342 only. The gates for the flow cytometry were set up according to the following controls: unstained LAK cells, LAK cells stained in vitro with H33342, and a mixture of both. An Argon ion laser operating at 488 nm was used to excite the H33342 dye, and the fluorescent light was transmitted at 530 nm. About 300 000–400 000 events were obtained for each sample. Data analysis was performed using the M. DAS or the EASY 2 system (Coulter Corp.). (b) Frozen sections (8 μm thick) were prepared from the liver and spleen 24 h after injection of H33342-labeled LAK cells. The sections were fixed with 1% formalin and analyzed under a fluorescence microscope (Leitze).

Results

In this study, we utilized cells isolated from the 52% Percoll fraction and adhering to plastic flasks. Previously, adherent LAK cells were generated by incubating 50×10^6/25 ml cells not adhering to a nylon-wool column in T75 plastic flasks with 1000 U/ml IL-2, and after removal of the nonadherent cells, the best cell recovery was

Table 1. In vivo tissue distribution of adherent lymphokine-activated killer (LAK) cells[a] labeled with various radioisotopes

Time after administration	Cells labeled with	Radioactivity[b] (% of injected) in				Lungs:liver ratio[d]	Lungs:blood ratio
		Blood	Spleen	Liver	Lungs		
A. 30 min	^{125}I-dUrd (4)[c]	3±0	1±0 (1±0)	4±1 (1±0)	61±15 (79±22)	15 (132)	21
	^{51}Cr (3)	2±0	1±0 (1±0)	8±2 (1±0)	72± 2 (85± 2)	10 (84)	38
	^{111}InCl (3)	33±5	2±0 (3±0)	27±1 (3±)	31± 1 (35± 1)	1 (11)	1
	^{111}In-ox[e] (3)	14±1	1±0 (2±0)	7±1 (1±0)	60± 6 (74± 9)	9 (74)	4
B. 2 h	^{125}I-dUrd (4)	5±1	2±0 (4±0)	16±0 (4±0)	36± 1 (39± 1)	2 (13)	7
	^{51}Cr (3)	4±0	5±1 (10±1)	17±1 (4±0)	44± 4 (50± 2)	3 (13)	11
	^{111}InCl (3)	19±1	3±0 (5±0)	28±2 (4±0)	16± 0 (19± 1)	1 (5)	1
	^{111}In-ox[e] (3)	9±1	8±1 (15±2)	32±2 (5±1)	30± 1 (35± 1)	1 (7)	3
C. 24 h	^{125}I-dUrd (4)	3±0	1±1 (3±1)	5±1 (2±1)	1± 0 (1± 0)	0.2 (0.5)	0.3
	^{51}Cr (3)	1±0	14±3 (21±3)	37±2 (5±1)	4± 0 (4± 1)	0.1 (0.8)	4
	^{111}InCl (3)	6±0	3±0 (6±1)	27±2 (4±0)	4± 1 (4± 1)	0.1 (1.0)	0.7
	^{111}In-ox[e] (3)	2±0	15±2 (26±4)	42±4 (6±1)	2± 0 (2± 0)	0.05 (0.3)	1

[a] LAK cells were generated by adhering rat splenocytes isolated from the 52% Percoll density gradient to plastic flasks as previously described [11, 26]

[b] Results show the percentage of the administered radioactivity retained per organ; that retained per gram of tissue is shown in parentheses

[c] The numbers of rats used are shown in parentheses

[d] Values show the ratio on a whole-organ basis; ratios calculated per gram of tissue are shown in parentheses

[e] ^{111}In-ox, ^{111}In-oxine

20×10^6 adherent cells at the end of the culture period [26]. However, incubating 50×10^6 cells of the 52% Percoll fraction resulted in the recovery of $(150-200) \times 10^6$ cells/culture, after 5 days of incubation with 1000 U/ml IL-2. This in an enrichment of seven to ten times over that of cells from the nylon-wool column, and led us to incubate 1×10^6 cells rather than 2×10^6/ml at the start of the culture because of crowding of the cells.

Plastic-adherent LAK cells were labeled with various isotopes commonly used to trace leukocytes in vivo and infused i.v. into syngeneic animals. Table 1 shows the tissue localization of adherent LAK cells. The results can be summarized as follows.

At 30 min post-injection (Table 1, A). Cells labeled with ^{125}I-dUrd, ^{51}Cr, or ^{111}In-oxine are largely localized in the lungs. ^{111}InCl-labeled LAK cells are distributed equally in the blood, lungs and liver (lungs: liver or lungs: blood ratio = 1). In contrast, the distribution of ^{125}I-dUrd-, or ^{51}Cr-labeled cells into the blood is negligible, whereas ^{111}In-oxine-labeled cells show an intermediate distribution into this organ. The lungs: liver ratio is highest when LAK cells are labeled with ^{125}I-dUrd, ^{51}Cr or ^{111}In-oxine, and the lungs: blood ratio is highest when the cells are labeled with ^{51}Cr or ^{111}I-dUrd.

At 2 h post-injection (Table 1, B). ^{125}I-dUrd- or ^{51}Cr-labeled cells show a high accumulation in the lungs, confirming previous observations [11], followed by ^{111}In-oxine-labeled cells, whereas significantly less ^{111}InCl-labeled cells enter the lungs. Similar to the distribution 30 min after injection, ^{111}InCl-labeled cells have the tendency to locate in the blood. There was significantly higher distribution into the liver of ^{111}InCl- or ^{111}In-oxine-labeled cells as compared to ^{125}I-dUrd- or ^{51}Cr-labeled cells. Hence, the pattern of distribution at this time point is somewhat similar to that observed 30 min after injection; ^{125}I-dUrd- or ^{51}Cr-labeled LAK cells accumulate highly in the lungs, ^{111}InCl-labeled cells distribute equally into the lungs and blood but predominate in the liver, whereas ^{111}In-oxine-labeled LAK cells distribute equally into the lungs and liver, with intermediate distribution into the blood. The difference between the distribution of ^{111}InCl- and ^{111}In-oxine-labeled LAK cells was monitored using gamma camera imaging. LAK cells were labeled with either of these radioisotopes, infused into syngeneic animals, and their distribution was monitored. Figure 1 shows that ^{111}InCl-labeled LAK cells tend to localize in the liver even after 30 min of administration, whereas most of ^{111}In-oxine-labeled LAK cells localized in the lungs at this time. At 2 h, there was a high accumulation in the liver of both ^{111}InCl- and ^{111}In-oxine-labeled cells. However, ^{111}In-oxine-labeled LAK cells showed a higher distribution into the lungs than did the ^{111}InCl-labeled cells. By 24 h, most of ^{111}In-oxine (Fig. 1) or ^{111}InCl (not shown) was sequestered in the liver and the spleen.

At 24 h post-injection (Table 1, C). At this time point, few, if any ^{125}I-dUrd-labeled LAK cells were observed in any tissue tested. However, LAK cells labeled with any of the other radioisotopes showed a high distribution into the liver and the spleen. The difference between the distribution of LAK cells labeled with ^{125}I-dUrd compared to cells labeled with the other radioisotopes is dramatic and suggests one of two possibilities: (a) LAK cell distribution monitored by ^{125}I-dUrd labeling is genuine, and the inability to recover any labeled cells 24 h after injection is due to the insufficient quantities of IL-2 present in situ, or (b) LAK cell distribution monitored by the other radioisotopes is genuine, and the inability to recover ^{125}I-dUrd-labeled LAK cells may be due to the toxicity and elution of this radioisotope from the cells as previously shown [4, 6, 16, 18, 24]. We have therefore devoted the rest of this work to resolving this issue.

Table 2. Distribution of ^{125}I-dUrd-labeled LAK, or freeze-thawed ^{125}I-dUrd-labeled LAK cells 24 h after i. v. administration

Cells		Radioactivity (% of injected) in			
		Blood	Spleen	Liver	Lungs
LAK	(6)[c]	3.1 ± 0.4	2.0 ± 0.5	4.8 ± 1.0	3.6 ± 1.0
LAK+IL-2[a]	(6)	3.0 ± 0.1	2.0 ± 0.7	4.0 ± 1.0	2.6 ± 0.9
F-T LAK[b]	(6)	2.8 ± 0.3	1.2 ± 0.7	4.8 ± 0.6	2.8 ± 1.0
F-T LAK+IL-2	(6)	2.8 ± 0.2	1.0 ± 0.6	3.6 ± 0.7	2.5 ± 1.1

[a] The rats were treated i. p. with two doses (10 000 U each) of interleukin-2 (IL-2) 8 h apart
[b] ^{125}I-dUrd-labeled LAK cells were frozen and thawed (F-T) in three cycles of liquid nitrogen and a 60° C water bath. The cells were injected i. v. without washing
[c] The numbers of rats used are shown in parentheses

Table 3. In vivo tissue distribution of the mammary adenocarcinoma MADB106 labeled with either ^{111}I-dUrd or ^{111}In-oxine

Time after administration (h)	Cells *labeled with*[a]	Radioactivity (% of injected) in			
		Blood	Spleen	Liver	Lungs
2	^{125}I-dUrd (4)[b]	17.9 ± 3.4	2.6 ± 0.1	18.5 ± 4.4	NT[c]
	^{111}In-ox (4)	16.7 ± 0.1	2.3 ± 0.2	20.6 ± 1.1	2.1 ± 0.1
24	^{125}I-dUrd (4)	3.3 ± 0.2	0.6 ± 0.3	1.6 ± 0.3	0.5 ± 0.1
	^{111}In-ox (4)	5.0 ± 0.6	5.1 ± 2.8	25.4 ± 1.8	2.3 ± 0.5

[a] MADB106 cells were labeled with either 3 μCi ^{125}I-dUrd or 10 μCi ^{111}In-ox. The cells (4×10^6) were injected i. v. into syngeneic rats and the various organs were excised 2 h or 24 h after the injection of the cells
[b] The numbers of rats used are shown in parentheses
[c] NT, not tested

If the inability to recover LAK cells labeled with ^{125}I-dUrd is due to the insufficient amount of IL-2 present in situ, then injection of IL-2 along with the labeled cells should increase the recovery of these cells 24 h after administration. Table 2 shows that there is no difference between the distribution of ^{125}I-dUrd-labeled LAK cells whether or not the rats received two doses (10 000 U each) of IL-2, 8 h apart during the 24-h period of injection. This result excludes the possibility that the inability to recover ^{125}I-dUrd-labeled LAK cells in the liver and spleen 24 h after i. v. administration is due to the lack of IL-2 in situ, and suggests other alternatives. When ^{125}I-dUrd-labeled LAK cells were killed by three rounds of freezing and thawing and then infused i. v., they showed a similar pattern of distribution to viable ^{125}I-dUrd-labeled LAK cells 24 h after administration. This migration pattern was not altered by infusing IL-2 (Table 2). The similarity of distribution between ^{125}I-dUrd-labeled killed or viable cells suggests that ^{125}I-dUrd may be toxic, and may kill the cells in vivo.

If ^{125}I-dUrd is indeed toxic and elutes from the labeled cells in vivo, then labeling cells other than LAK cells (which do not require IL-2 for survival), with this radioisotope should show a pattern of migration similar to that observed with LAK cells. Table 3 shows the in vivo migration of the natural-killer(NK)-resistant MADB106 cell line. ^{111}In-oxine-labeled cells distributed into the blood and the liver at 2 h, but redistributed into the liver and spleen, 24 h after administration. However, those labeled with ^{125}I-dUrd were not recovered from any organ tested 24 h after administration (Table 3). These results clearly indicate that the inability of ^{125}I-dUrd-labeled cells to distribute to different organs is not due to the insufficient amount of IL-2 present endogenously, or to the ability of endogenous NK cells to clear the transferred cells from the system, since MADB106 cells are NK-resistant and do not require IL-2 for optimal growth.

On the other hand, if the distribution of LAK cells labeled with the other radioisotopes is genuine, then this should be confirmed by other methods. For this we used Hoechst(H)33342, a bisbenzamide derivative that binds specifically to DNA and is superior to other fluorescent dyes [2]. Because of this property and its low elution from the cells, adherent LAK cells were labeled with 4 μmol of this dye, washed extensively and infused i. v. into syngeneic animals. Two methods were used to evaluate the presence of H33342-labeled LAK cells in various organs.

a) Various organs were excised and a single-cell suspension was examined by flow cytometry. Table 4 shows the relative number of fluoresceinated cells recovered per organ. It is clear that the lungs: liver ratio, on a whole-organs basis or calculated per gram of tissue, and the lungs: blood ratio closely resemble the ratios obtained when LAK cells were labeled with ^{111}In-oxine. For example, the lungs: liver ratio 30 min after injection is 9 (whole organ) and 74 (per gram of tissue) when ^{111}In-oxine was used to label LAK cells (Table 1), and 6 (whole organ) and 78 (per gram of tissue) when H33342 was used as a label for these cells (Table 4). This is also true when one considers the lungs: liver ratio 2 h, or 24 h after administration. The only exception is the lungs: blood ratio 2 h after injection, which shows that LAK cells labeled with ^{51}Cr have a pattern of distribution closer to that of H33342-labeled cells. In contrast to ^{111}In-oxine and ^{51}Cr, ^{125}I-dUrd has the

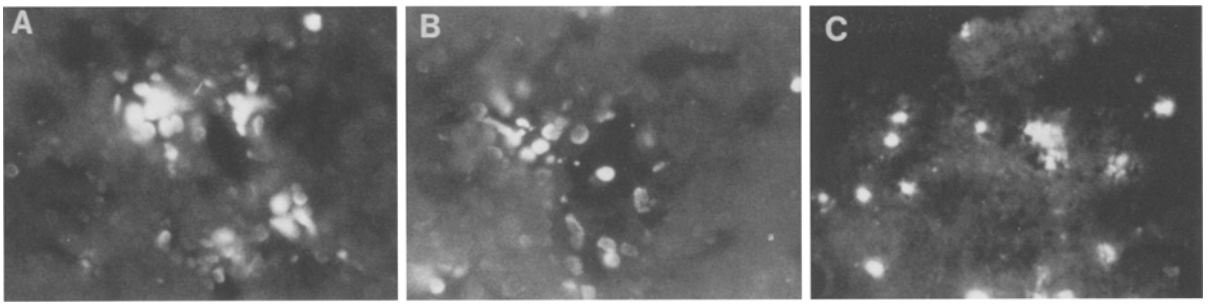

Fig. 2 A–C. LAK cells (10×10^6) were labeled with 4 μmol H33342, extensively washed, and administered i. v. After 24 h frozen sections were made from the liver and the spleen. Notice the bright H33342-labeled LAK cells in the liver parenchyma (**A**), the liver central vein (**B**), and the splenic red pulp (**C**)

Table 4. Distribution of LAK cells labeled with H33342[a]

Time[c]	$10^{-5} \times$ No. LAK cells recovered[b] / $10^{-5} \times$ total no. cells in the organ				Lungs: liver ratio[e]	Lungs: blood ratio[f]
	Blood	Spleen	Liver[d]	Lungs		
30 min	0.03/52	0/1265	0.08/62	0.1/13	5.8 (78)	13.3
2 h	0.01/61	0.12/1070	0.7/44	0.2/14	0.9 (3)	6.0
24 h	0.03/76	4.9/1170	1.3/56	0.01/19	0.02 (0.2)	1.3

[a] Plastic-adherent LAK cells (10×10^6) were labeled with 4 μmol H33342 for 45 min at 37° C, extensively washed and injected i. v. into syngeneic rats

[b] Data from one of three representative experiments showing the number of fluoresceinated cells ($\times 10^{-5}$) recovered: at 30 min: 0.09, 0, 0.94 and 0.15; at 2 h: 0.07, 0.02, 1.07 and 1.6; and at 24 h: 0.09, 0.43, 0.02 and 2.4 in the blood, spleen, lungs and the non-parenchymal layer of the liver respectively. Control rats received 4 μmol H33342 only. The percentage of labeled cells in control animals is: 0.04%, 0.01%, 0.00% and 0.02% in the blood, spleen, lungs and liver respectively. The number of fluoresceinated cells (FC) is calculated as follows: [FC in experimental rats (%) – FC in control rats (%)] × (total number of cells recovered from each organ)/100

[c] Time after which the different organs were excised

[d] Cells were recovered from the non-parenchymal layer of the liver

[e] Lungs: liver ratio on a per organ basis (per gram of tissue) is shown

[f] Lungs: blood ratio on a per organ basis is shown

most disadvantages since most of the labeled cells disappear 24 h after injection, whereas it is clear that H33342-labeled cells are found in the spleen and the liver at this time.

b) The most striking observation is the high accumulation of H33342-labeled LAK cells in the liver and the spleen 24 h after administration. Figure 2 shows that the liver parenchyma, particularly the liver sinusoids and the central vein, are studded with H33342-labeled LAK cells. Similarly, the splenic red pulp and marginal zones show a high influx of LAK cells (Fig. 2). However, LAK cells were absent from the splenic white pulp confirming our earlier observation [11].

Discussion

Lymphocyte circulation, particularly for those with antimicrobial and/or anti-tumor activity, is an important part of the ability of these cells to eradicate the invading particles. In order to trace the cells into the various homing tissues, they should be labeled in vitro before infusing in vivo. Such labeling should theoretically be harmless to the cells, and allow the detection of these cells in various organs. However, this is a difficult task to achieve. Therefore, several methods are described to label lymphoid cells. Butcher and Weissmann [3] suggested that fluorescein isothiocyanate (FITC) can be used to label the cells in vitro. However, FITC was proved to be toxic to the cells when used in high quantities, and the migration of FITC-labeled cells was not different from the migration of those labeled with the radioisotopes [5]. Similarly, rhodamine was also found to be toxic [8].

Radioisotopes, on the other hand, provide a convenient measure of tissue-associated radioactivity after injection of labeled cells. However, it is difficult to prove that the tissue-associated radioactivity is a reflection of viable cells or injured cells releasing the label, which is re-utilized by the host compartments.

Because of their anti-tumor activity, and ability to eradicate tumor metastases in both murine tests and cancer patients [17, 20–22], we previously studied the in vivo tissue distribution of LAK cells [11]. We found that these cells have a restricted pattern of distribution, localizing mainly in the lungs after 2 h but redistributing into the liver and spleen 24 h after injection. We also noticed that LAK cells, purified by procedures other than their adherence to plastic, also showed a restricted pattern of in vivo tissue localization (reviewed in [13]). In those studies ^{51}Cr was used to label LAK cells. A similar pattern of distribution was observed when $^{111}InCl$ [1], autoradiographic studies utilizing [^{3}H]dThd [11, 25], or ^{51}Cr [7, 11, 19] were used to label LAK cells. In our hands $^{111}InCl$-labeled LAK cells were highly distributed into the blood after 2 h and those labeled with ^{125}I-dUrd were not observed 24 h after injec-

tion (data not shown, and this report). Hence, we considered the differential homing of LAK cells labeled with different radioisotopes an important subject to investigate, since the results obtained from these studies will help in developing rational protocols for cancer immunotherapy, and will lead to strategies for manipulating the distribution of the adoptively transferred LAK cells to reach the desired areas in the body. To resolve this issue, LAK cells were labeled with four different radioisotopes, namely: ^{125}I-dUrd, ^{51}Cr, ^{111}In-oxine or ^{111}InCl before infusing them i.v. into syngeneic rats. We found that regardless of the method used to prepare purified LAK cells, i.e. adherent versus non-adherent [12, 13] and (data not shown), these cells have a similar pattern of tissue localization indicating that LAK cells have a characteristic pattern of in vivo distribution. However, we noticed that LAK cells labeled with ^{111}InCl, in contrast to those labeled with the other three radioisotopes, have a strong tendency to enter into the blood. This is true whether the radioactivity was determined by counting the tissue-associated radioactivity or by whole-body gamma camera imaging. The reason behind the tendency of ^{111}InCl-labeled cells to localize in the blood is not clear at the present time. This overestimation, however, is disadvantageous when one is using ^{111}InCl to label LAK cells, and may overshadow its good qualities for imaging analysis [23].

Although, ^{125}I-dUrd demonstrates an acceptable level of monitoring LAK cell distribution early after administration, it has the most disadvantages when LAK cell distribution is monitored at a later time (e.g. 24 h after injection), ^{125}I-dUrd-labeled LAK cells were not recovered from any organ or tissue tested at this time point. Injection of two doses of IL-2 during the 24-h period between the administration of the cells and the recovery of the organs did not result in a higher recovery of labeled cells, suggesting that the inability to observe ^{125}I-dUrd-labeled cells at this time point is not due to the unavailability of IL-2, which presumably is needed to support the growth of these cells. The inability to recover cells labeled with ^{125}I-dUrd 24 h after injection was a common characteristic of this radioisotope since the NK-resistant MADB106 tumor cell line showed a similar pattern of distribution, i.e. an inability to recover any ^{125}I-dUrd-labeled cells 24 h after administration.

The spontaneous release of ^{125}I-dUrd from labeled LAK cells in vitro is similar to the spontaneous release of ^{51}Cr or ^{111}In from these cells; about 20% after 24 h of incubation at 37° C. However, it has been previously shown that ^{125}I-dUrd is toxic to thoracic duct lymphocytes labeled with this radioisotope [24], and that it easily elutes from labeled cells contaminating other tissues, such as the intestine [18, 24]. Although ^{125}I-dUrd did not elute from labeled cells after in vitro incubation, it may elute from these cells after administration in vivo, as suggested by other investigators [4, 6, 18, 24]. This and its low labeling efficiency, especially when used at doses that are not toxic (1 μCi), make it a very inconvenient marker.

The pattern of distribution of LAK cells labeled with these radioisotopes was paralleled when their distribution was monitored after labeling with H33342, which proved to be one of the best labels for lymphocyte migration because of to its high resolution in tissues, low toxicity and low elution from labeled cells [2]. There was a large influx of H33342-labeled LAK cells into the liver parenchyma, accumulating in the liver sinusoids and the central veins. They also accumulated to a considerable extent in the splenic red pulp and the marginal zone. When the distribution of LAK cells labeled with H33342 was compated to their distribution after labeling with the various radioisotopes, it was clear that ^{111}In-oxine shows the closest association, with the exception that 2 h after injection ^{51}Cr-labeled cells showed a distribution in the blood closer to that of H33342-labeled cells. Hence, the distribution of LAK cells labeled with ^{111}In-oxine may reflect the actual localization of LAK cells in various tissues. ^{111}In-oxine is a lipophilic chelating agent that penetrates inside the cells allowing the ^{111}In to bind tightly into the nuclear and cytoplasmic proteins, and it was previously found to be the best radioisotope for monitoring leukocyte distribution in humans [15].

Because it is time-consuming to prepare lymphoid cells labeled with H33342 for flow-cytometric analysis or cross-section examination, we strongly recommend ^{111}In-oxine to be used for monitoring the in vivo tissue distribution of LAK cells. However, we do not recommend the use of ^{125}I-dUrd because of its low labeling efficiency and possible toxicity and elution from the cells.

In summary, the present results support those found by us [11, 13] and by others [1, 7, 19, 25], which showed that LAK cells have a restricted pattern of distribution in both normal [1, 12] and tumor-bearing animals [13, 14], distributing mainly into the lungs and liver early after i.v. administration, but relocating in the liver and spleen later on. These results may explain the inability of LAK cells to eradicate tumor metastases completely in vivo, in spite of their high in vitro antitumor activity. Perhaps, LAK cell therapy may become more effective if the distribution of these cells can be manipulated so they can reach the desired areas in the body, particularly those with tumor growth. We have recently reported that carbohydrates [14], or growth factors such as interferon-γ [10] can modulate the distribution of LAK cells. This area of research may prove to be a vital aspect in the field of adoptive immunotherapy.

References

1. Ames IH, Gagne GM, Garcia AM, John PA, Scatorchia GM, Tumar RH, McAfee JG (1989) preferential homing of tumor-infiltrating lymphocytes in tumor bearing mice. Cancer Immunol Immunother 29: 93
2. Brenan M, Parish CR (1984) Intracellular fluorescent labelling of cells for analysis of lymphocyte migration. J Immunol Methods 74: 31
3. Butcher EC, Weissman IL (1980) Direct fluorescent labeling of cells with fluorescein or rhodamine isothiocyanate. I. Technical aspects. J Immunol Methods 37: 97
4. Cheong L, Rich MA, Eidinoff ML (1960) Introduction of the 5-halogenated uracil moiety into deoxyribonucleic acid of mammalian cells in culture. J Biol Chem 235: 1441
5. Chin GW, Cahill RNP (1984) The appearance of fluorescein-labelled lymphocytes in lymph following in vitro or in vivo labeling: the route of lymphocyte recirculation through mesenteric lymph nodes. Immunology 52: 341

6. Hoffer KG, Hughes WL (1971) Radiotoxicity of intranuclear tritium, ^{125}iodine and ^{131}iodine. Radiat Res 47: 94
7. Hosokawa M, Sawamura Y, Morikage T, Okada F, Yu Z.-Y., Morikawa K, Itoh K, Kobayashi H (1988) Improved therapeutic effects of interleukin-2 after the accumulation of lymphokine-activated killer cells in tumor tissue of mice previously treated with cyclophosphamide. Cancer Immunol Immunother 26: 250
8. Lampidis TJ, Bernal SD, Summerhayes IC, Chen LB (1983) Selective toxicity of rhodamcine 123 in carcinoma cells in vitro. Cancer Res 43: 716
9. Lotze MT, Line BR, Mathisen DJ, Rosenberg SA (1980) The in vivo distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF): implication for the adoptive immunotherapy of tumors. J Immunol 125: 1487
10. Maghazachi AA (1989) In vivo chemotaxis and chemokinesis activities of IFNγ, IFNα and IL-2 for rat lymphokine-activated killer cells. Nat Immun Cell Growth Regul 8: 130 (Abstract)
11. Maghazachi AA, Herberman RB, Vujanovic NL, Hiserodt JC (1988) In vivo distribution and tissue localization of highly purified rat lymphokine-activated killer (LAK) cells. Cell Immunol 15: 179
12. Maghazachi AA, Goldfarb RH, Herberman RB (1988) Influence of T cells on the expression of lymphokine-activated killer cell activity and in vivo tissue distribution. J Immunol 141: 4039
13. Maghazachi AA, Goldfarb RH, Kitson RP, Hiserodt JC, Giffen CA, Herberman RB (1989) In vivo tissue distribution of interleukin-2 activated cells. In: Interleukin-2 and killer cells in cancer. CRC, Florida, p 259
14. Maghazachi AA, Goldfarb RH, Herberman RB (1989) Effect of carbohydrates on the in vivo migration of purified LAK cells. In: Natural killer cells and host defense. Karger, Basel, p 242
15. Marcus CS (1984) The status of indium-111 oxine leukocyte imaging studies. None invasive medical imaging 1: 213
16. Mathias AP, Fisher GA, Prusoff WH (1959) Inhibition of the growth of mouse leukemia cells in culture by 5 iododeoxyuridine. Biochim Biophys Acta 36: 560
17. Mazumdar A, Rosenberg SA (1984) Successful immunotherapy of natural killer-resistant established pulmonary melanoma metastases by the intravenous adoptive transfer of syngeneic lymphocytes activated in vitro by interleukin 2. J Exp Med 159: 495
18. Rannie GH, Donald KJ (1977) Estimation of the migration of thoracic duct lymphocytes to non-lymphoid tissues. A comparison of the distribution of radioactivity at intervals following i. v. transfusion of cells labeled with ^{3}H, ^{14}C, ^{35}S, ^{99m}TC, ^{125}I and ^{51}Cr in the rat. Cell Tissue Kinet 10: 523
19. Rodolfo M, Salvi C, Parmiani G (1989) Influence of the donors' clinical status on in vitro and in vivo tumor cytotoxic activation of interleukin-2-exposed lymphocytes and their circulation in different organs. Cancer Immunol Immunother 28: 136
20. Rosenberg SA, Lotze MT (1986) Cancer immunotherapy using interleukin-2 and interleukin-2-activated lymphocytes. Annu Rev Immunol 4: 681
21. Rosenberg SA, Mule JJ, Spiess PL, Reichert CM, Schwarz SL (1985) Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin-2. J Exp Med 161: 1169
22. Rosenberg SA, Lotze MT, Muul LM, Chang AE, Avis FP, Leitman S, Lineham WM, Robertson CN, Lee RE, Rubin JT, Seipp CA, Simpson CG, White DE (1987) A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 316: 889
23. Sayle BA, Balachandran S, Rogers CA (1983) Indium-111 chloride imaging in patients with suspected abscesses: concise communication. J Nucl Med 24: 1114
24. Sprent J (1976) Fate of H2-activated T lymphocytes in syngeneic hosts. I Fate in lymphoid tissues and intestines traced with ^{3}H-thymidine, ^{125}I-deoxyuridine and ^{51}chromium. Cell Immunol 21: 278
25. Takai N, Tanaka R, Yoshida S, Hara N, Saito T (1988) In vivo and in vitro effect of adoptive immunotherapy of experimental murine brain tumors using lymphokine-activated killer cells. Cancer Res 48: 2047
26. Vujanovic NL, Herberman RB, Maghazachi AA, Hiserodt JC (1988) Lymphokine-activated killer cells in rats. III. A simple method for the purification of large granular lymphocytes and their rapid expansion and conversion into lymphokine activated killer cells (large granular lymphocytes). J Exp Med 167: 15