Chelate Conjugates of Monoclonal Antibodies for Imaging Lymphoid Structures in the Mouse

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Radiolabeling of a mouse monoclonal antibody (MoAb) specific for the mouse histocompatibility alloantigen IA^k expressed by the B lymphocytes of BALB/k and C3H mice but not BALB/c mice was performed by mixing the chelate-labeled anti (α) IA^k MoAb with purified, no-carrier-added ^{111}In citrate. Labeling efficiency was 85–95%, and the labeled αIA^k MoAb retained its antigen binding properties in vitro and in vivo. The organ, spleen, and lymph node distribution of intravenously and subcutaneously administered $^{111}In\alpha IA^k$ MoAb was compared in mice, two IA^k positive and one IA^k negative strains, and to $^{125}I\alpha IA^k$ MoAb in one IA^k positive strain. The $^{111}In\alpha IA^k$ MoAb was more stable in vivo compared to $^{125}I\alpha IA^k$ MoAb, as shown by a much slower excretion and a higher absolute uptake in lymph nodes and spleen. Lymph node to blood ratio was increased twofold by intravenous anti-EDTA MoAb. Subcutaneous injection permitted clear images of the tiny lymph nodes in the mouse. Potential clinical applications of $^{111}In\alpha$ lymphocyte MoAb include localization of normal lymph nodes and T & B cell leukemias and lymphomas, as well as detecting lymphatic metastases of other cancers. Therapy may also be possible using MoAbs labeled with beta-emitting metal ions such as yttrium-90.

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Radioimmunoimaging of tumors currently lacks sufficient sensitivity and specificity for broad clinical application (*1*). Despite the exquisite specificity inherent in the immunoglobulin molecule itself, a number of complex problems relating to the physical, chemical, immunological, and radiopharmaceutical properties of its radiolabeled derivatives remain to be solved before monoclonal antibodies can be used successfully as radiopharmaceuticals.

These difficulties were immediately apparent in the early studies using radioiodine-labeled antisera to rat sarcoma which showed a specificity ratio of only two (*2*), and in subsequent studies with radioiodinated polyclonal anti-carcinoembryonic antigen (CEA) that required background subtraction techniques (*3,4*). The use of radioiodinated affinity purified antibodies (*5*), and more recently monoclonal antibodies (*6*), has shown that simply increasing the purity, specificity, and homogeneity of the antibodies does not provide a complete solution. Some of these unsolved problems include low detection efficiency of the gamma camera for iodine-131 (^{131}I), rapid deiodination of radioiodine-labeled antibodies in vivo resulting in low target concentrations, high background necessitating subtraction techniques, and the inconvenience of frequent radioiodination procedures needed to provide continuous availability in the clinic.

We have previously shown that metal chelate protein conjugates are extremely stable at tracer concentrations in living organisms (*7,8*). More recently, since monoclonal antibodies have become readily available, we have extended our methods to chelate conjugation of immunoglobulins and have also shown them to be stable and to retain their antigen binding capacity in vivo (*9–11*).

In the present study we used a mouse system analogous to a model previously reported by Weinstein et al. for the study of iodine-125- (^{125}I) labeled monoclonal antibodies against the major histocompatibility complex

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(MHC) antigen H-2K^k (*12*). The monoclonal antibody was specific for the mouse MHC alloantigen IA^k, expressed by the B lymphocytes of BALB/k and C3H but not BALB/c mice. This provided a readily identifiable target (normal spleen and lymph nodes) with a normal blood supply and capillaries, in an easily reproducible, economical model. There was also an applicable negative control (BALB/c), in which to compare radioiodinated and chelate conjugated monoclonal antibodies.

MATERIALS AND METHODS

Labeling procedures

Mouse monoclonal antibody (MoAb) 10-3.6 ($IgG2_a$) to the mouse major histocompatability complex (MHC) alloantigen IA^k, present on BALB/k and C3H B lymphocytes but not on BALB/c B lymphocytes was used (*13*). Nonspecific mouse IgG was used as an additional control. Chelate conjugation was performed using bromoacetamidobenzyl ethylenediaminetetraacetic acid (BABE) as described previously (*9*). The labeled MoAb contained ~3.3 chelators per molecule of IgG. The nonradioactive chelate conjugates, 0.15 m*M* in IgG, 0.5 m*M* in chelator, were stored in 20 μl aliquots at −80°C until required. Radiolabeling was performed with a small aliquot (10-50 μl) containing 5-25 nmol of chelator by simply mixing with 50 μl of purified, no-carrier-added, indium-111 (^{111}In) citrate (1-10 mCi) pH 5.0 (*14*). Chelation of ^{111}In was complete in less than 5 min, and it could not be removed from the chelate protein complex even with a large excess (> 1,000-fold) "challenge" of EDTA. The labeling efficiency was measured after each radiolabeling by thin layer chromatography which included an EDTA challenge, and was routinely 85-95%. For some experiments an EDTA "chase" was added to complex any unbound ^{111}In. The radioimmunoglobulin was diluted in phosphate buffered normal saline containing 0.1% human serum albumin, and in some experiments 0.01*M* EDTA and 0.1*M* sodium citrate pH 6.5 for i.v. injection. The specific activity of the ^{111}In-labeled immunoglobulins was 130-1,300 Ci/mmol (~0.87-8.7 μCi/μg).

Radioiodination of anti (α) IA^k MoAb with ^{125}I was done using a minor modification of the chloramine T method (*15*). The ^{125}I αIA^k contained ~0.04 ^{125}I atoms per antibody molecule (0.6 μCi/μg).

Animal biodistribution

In vitro cell binding studies on lymphocytes obtained from the mouse spleen were carried out as described previously (*16*).

Biological half-life determinations were done by whole-body counting with a dual probe scintillation counter,* with flat field collimation, ~2 ft from each crystal. The mean net counts were normalized to the counts obtained immediately after i.v. injection and a semilog plot constructed over 8 to 10 days postinjection. The thyroids were blocked with ~5 m*M* KI in drinking water 4 days prior to and throughout 10 days study in ^{125}I mice.

Organ distribution studies (*17*) were done 24 hr fol-

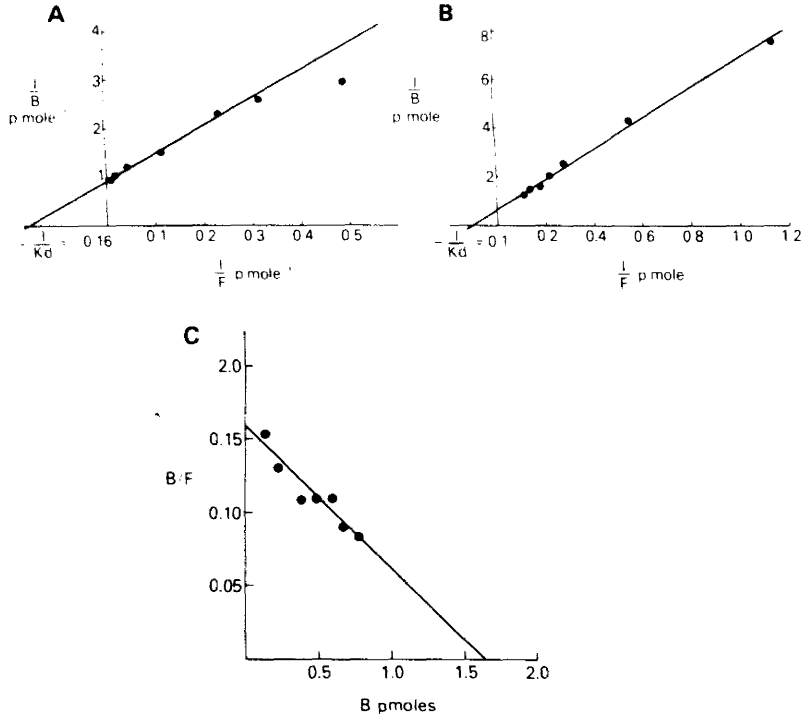

FIGURE 1
Double reciprocal plot of binding of ^{111}InαIA^k MoAb to mouse B-lymphocytes. A: 25°C/60 min; B,C: 4°C/60 min. Scatchard plot (C) indicating single high-affinity binding site. −Ka = 9.5 × 10^{10} M^{-1}; Receptor = 1.8 × 10^5 molecules/cell; % Bound = 5-15% per 10^7 cells. To 10^7 cells/ml (5 ml total volume) in RPMI medium was added 0.1-5.0 pmoles ^{111}InαIA^k. The cells were incubated 1 hr at room temperature, and at 4°C, washed X3, resuspended in a separate tube, and counted to 1% accuracy in scintillation well counter

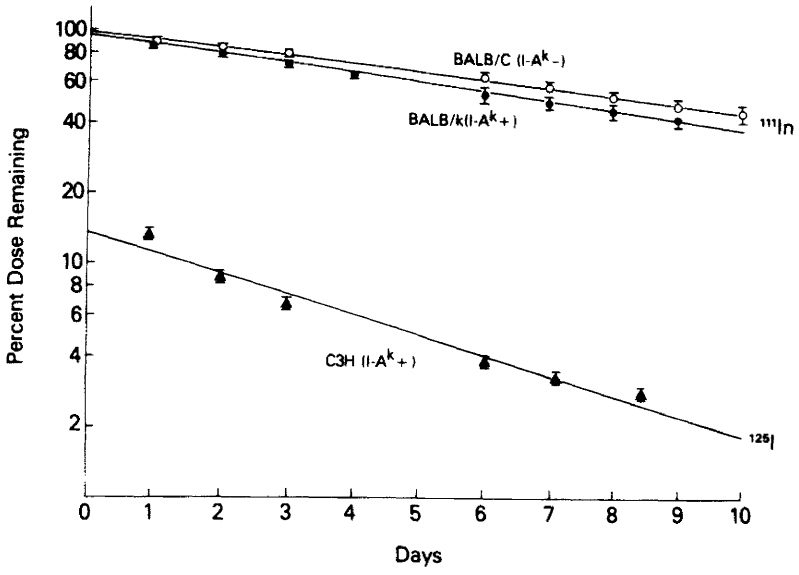

FIGURE 2
Biological $T_{1/2}$ ^{111}In and ^{125}I anti IA^{k}. Decay corrected whole-body counts, normalized to immediately following i.v. injection were plotted daily for 10 days. N = 3 in each group, bars represent ±1 s.d. Excretion of label was significantly faster in IA^{k} positive mice $T^{1}/_{2B}$ 7.5 days compared with $T^{1}/_{2B}$ 9.0 days ($p < 0.01$) suggesting increased metabolism of bound antibody. Iodine-125-labeled αIA^{k} showed a rapid initial excretion of ~80% in 24 hr followed by slower phase with $T^{1}/_{2B}$ = 3.5 days

lowing i.v. or s.c. injection of 3 μCi (0.3–3μg) ^{111}In-labeled monoclonal antibody into BALB/k or C3H mice (IA^{k} positive) and BALB/c control mice (IA^{k} negative). Peripheral lymph nodes were pooled from popliteal, inguinal, axillary, and cervical node chains and counted separately from the mesenteric nodes. Twenty-four hours following s.c. injection into both hind foot pads the popliteal and inguinal node chains draining the hind limbs were removed and counted separately from the nodes draining the upper extremities (axillary and cervical nodes).

In a group of six C3H mice injected i.v. with ^{111}In αIA^{k}, three were injected i.v. with 100 μg of mouse monoclonal anti-EDTA, CHA255,† 2 hr before killing at 24 hr. Organ distribution was performed by excision, weighing, and counting the whole organs in an automatic scintillation well counter, and expressing the results as % of injected dose/g.

In vitro labeling of mouse lymphocytes purified from spleen cells was done as for the cell binding studies by incubating 10^{7} cells in 1 ml with 100 μCi ^{111}InαIA^{k} for 30 min at room temperature. Twenty-four hour organ distribution in C3H mice was determined with the in vitro labeled cells as described for i.v. ^{111}In αIA^{k}.

Animal imaging

Whole-body images of C3H and BALB/c mice were made with a pinhole collimated gamma camera 24 hr following i.v. or subcutaneous (s.c.) injection of 100 μCi of ^{111}In-labeled monoclonal antibody.

RESULTS

The double reciprocal plot of ^{111}In αIA^{k} binding to mouse lymphocytes showed an association constant (K_a) of 9.5×10^{10}, and $\sim 1.8 \times 10^{5}$ binding sites per cell (Fig. 1).

The biological half-life ($T^{1}/_{2B}$) of i.v. ^{111}In αIA^{k} was ~7.5 days in the BALB/k mouse and 9 days in the BALB/c: a difference significant at $p < 0.01$ (Fig. 2). This is consistent with increased metabolism and excretion of the specifically bound antibody compared to the control. Iodine-125αIA^{k} i.v. (Fig. 2) showed an initial rapid excretion of whole-body activity of 80% in the first 24 hr, followed by a slower phase with $T^{1}/_{2B}$ of ~3.5 days. These results were similar to those of Weinstein et al. with ^{125}I anti-H-2K^{k}: 72% excretion in 24 hr, 85% in 48 hr, 89% in 72 hr; $T^{1}/_{2B}$ 3–4 days (*12*). This contrasted with the small amount rapidly excreted (~10%) and the longer $T^{1}/_{2B}$ of ^{111}In αIA^{k}. This difference was probably due to rapid deiodination of ^{125}I αIA^{k} and rapid excretion of the free ^{125}I iodide.

The organ and lymph node distribution of ^{111}In αIA^{k} 24 hr after i.v. injection in BALB/k and BALB/c mice is shown in Fig. 3. The BALB/k spleen, peripheral and mesenteric lymph nodes all had significantly higher concentrations of ^{111}InαIA^{k} than BALB/c ($p < 0.01$).

The % dose per organ, % dose per g, and concentration ratios of ^{111}In αIA^{k} in antigen positive (BALB/k, C3H) and antigen negative (BALB/c) mice as well as ^{111}In-labeled normal mouse immunoglobulin 24 hr after i.v. injection are shown in Table 1. There was a high absolute concentration of ^{111}In α IA^{k} in target organs of antigen positive mice: spleen 116–167%/g (10–13% of total), peripheral nodes 18–24%/g (0.4–1.0% of total) mesenteric nodes 16–17.4%/g (~0.4% of total). The BALB/c lymphoid organs all had significantly lower concentrations ($p < 0.01$). BALB/k and C3H to BALB/c ratios were: spleen 13.6–19.6/1, peripheral nodes 3.8–5.1/1, bone + marrow 3.8–4.2/1. Interestingly, these ratios for

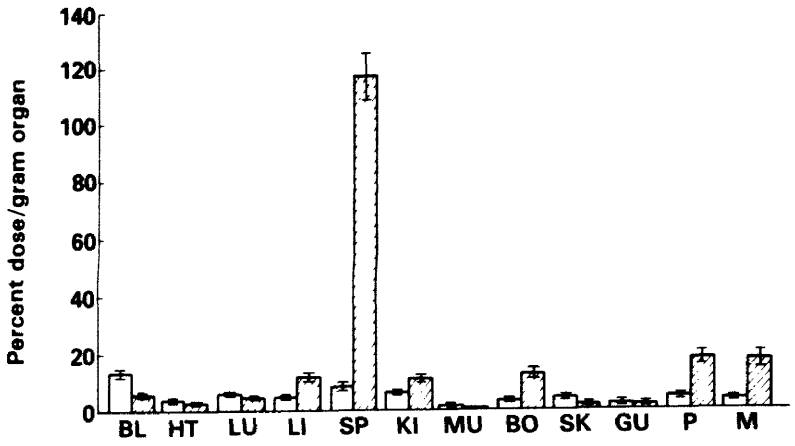

FIGURE 3
Organ and blood concentrations 24 hr following i.v. injection of ^{111}In αIA^k in (▨) BALB/k (Ag positive) and (□) BALB/c (Ag negative) mice. N = 6 in each group, bars = ±1 s.d. (⊢⊣), * = $p < 0.01$ for difference, BL* = blood; HT* = heart; LU = lung; LI* = liver; SP* = spleen; KI* = kidney; MU = muscle; BO* = bone + marrow (femur); SK = skin; GU = gut; P* = peripheral lymph nodes (pooled axillary, cervical and inguinal); M* = mesenteric lymph nodes

liver were 2.1-2.4/1 and blood 0.4-0.6/1 ($p < 0.001$), suggesting increased metabolism of bound antibody by the liver (see Fig. 2). Values reported for ^{125}I-labeled monoclonal anti H-2K^k and ^{125}I-labeled monoclonal anti-Thy 1.1, in antigen-positive mice showed significantly lower uptake in peripheral nodes: 0.9%/g and 4.1%/g, respectively (*12,16*). However, since the blood levels of ^{125}I anti-Thy 1.1 at 20 hr were lower than we observed with ^{111}In αIA^k, the tissue to blood ratios were similar, with the exception of the liver which contained much lower concentrations of ^{125}I anti-Thy 1.1 (*16*).

The distribution of ^{111}In αIA^k 24 hr after s.c. injection (both hind foot pads) is shown in Fig. 4. Again all the lymphoid organs in antigen-positive mice had significantly higher ($p < 0.001$) concentrations, than the antigen-negative mice. The values and ratios for s.c. injection are shown in Table 2. The regional draining popliteal and inguinal nodes (LN) in antigen positive mice had 244-319%/g (1.6-3.0% of total), upper nodes (UN) 11-20%/g (0.3% of total) mesenteric nodes 7.6-17%/g (0.3-0.6% of total) and spleen 57-95%/g (5.0-8.3% of total). These values were 2.4 to 14 times higher than those for antigen-negative mice.

The distribution of ^{125}I-labeled αIA^k following s.c. injection is also shown in Table 2. The absolute concentrations of ^{125}I activity were much lower in the nodes and spleen than ^{111}In, with an ^{111}In to ^{125}I ratio from 3 to 11.6/1. Note that the liver concentration of ^{111}In was six times that of ^{125}I.

Organ distribution of in vitro-labeled lymphocytes is shown in Fig. 5. The labeling yield was from 10-20% of the added activity. The lymphoid organs had approximately the same concentration as for i.v. injection of ^{111}In αIA^k, but the liver uptake was much higher probably due to removal of damaged cells from the circulation. This would produce a high background activity and be a disadvantage in clinical imaging of lesions in the liver. However, as a result the blood levels were somewhat lower producing better lymph node/blood ratios.

The effect of 100 μg i.v. monoclonal anti-EDTA CHA255† on the distribution of ^{111}In αIA^k is seen in Fig. 6. The blood levels were reduced 2.6-fold and peripheral node/blood ratios increased ~twofold ($p < 0.01$). The liver, bone, and marrow activity increased, probably due to removal of immune complex from the circulation by these organs (*29*). There was also a suggestion of a decrease in the spleen, peripheral, and mesentric node concentrations, although this did not reach statistical significance.

Anterior whole-body gamma camera pinhole images are shown in Fig. 7. Twenty-four hours following i.v. injection of 100 μCi ^{111}In αIA^k the spleen is not visualized in the BALB/c mouse (a), but is sharply outlined in the C3H (IA^k positive) mouse (b). Even more striking was the ability to image clearly the popliteal and pelvic lymph nodes (structures 1-2 mm in diam, weighing 1-2 mg) following s.c. injection of the same activity in C3H mice. Specificity was indicated by lack of visualization of the nodes in antigen negative mice (c).

DISCUSSION

The development of monoclonal antibodies (MoAbs) has given a tremendous impetus to the development of specific agents for both the diagnosis and treatment of cancer. Their use as radiopharmaceuticals could provide the targeting specificity required to produce the high absolute uptake and target-to-background ratios necessary for imaging. Although monoclonal antibody technology has reached a high level of development and over 300 murine MoAbs to human leukocyte surface antigens are now commercially available (*18*), the goal of using them effectively as radiopharmaceuticals has been a difficult one to attain. To date, most of the animal and human radiopharmaceutical research has been done with radioiodine labels, either ^{125}I or ^{131}I (*19-21*) and

more recently ^{123}I (*22*). Rapid deiodination in vivo has been a major problem with the use of these agents.

Comparison of the absolute uptakes in our experiments with those of Houston et al. (*16*) using a radioiodine label, showed that the peripheral node concentrations we obtained were ~fourfold higher following i.v. injection. Our findings agree with the results of Weinstein et al. (*12*) in showing the importance of the s.c. route of administration for obtaining locally high concentrations of antibody in the lymphatics.

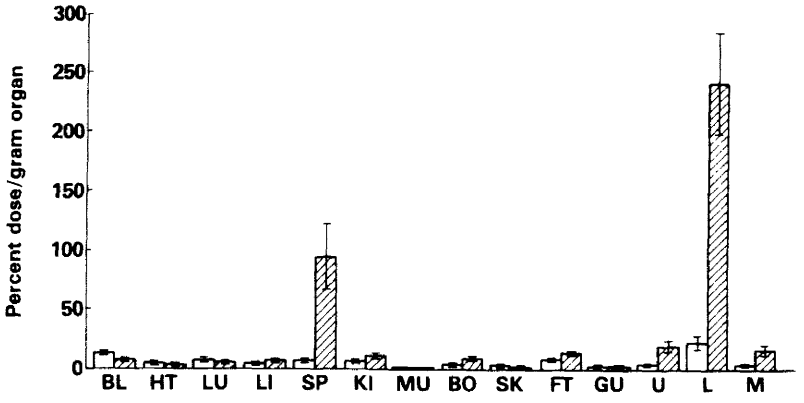

FIGURE 4
Organ and blood concentrations 24 hr following s.c. injection of both hind foot pads with $^{111}In\alpha IA^k$ in (▨) BALB/k (Ag positive) and (□) BALB/c (Ag negative) mice N = 6 in each group; bars = ±1 s.d. (╪), * = $p < 0.001$ for difference; BL* = blood; HT* = heart; LU = lung; LI* = liver; SP* = spleen; KI = kidney; MU = muscle; BO* = bone + marrow (femur); SK = skin; FT = foot; GU = gut; U* = upper cervical and axillary lymph nodes; L* = lower popliteal and inguinal lymph nodes; M* = mesenteric lymph nodes

TABLE 1
[^{111}In]MoAb Intravenous (24 hr)

Mouse injected	Antibody Injected	Tissue*	% dose/organ	% dose/g	Tissue/blood ratio	Experiment/control % dose/g ratio
						BALB/k/BALB/c (αIA^k)
BALB/k (Ag positive) N = 4	$^{111}In\alpha IA^k$	PN	0.4	17.7	2.9	3.8
		MN	1.0	17.4	2.8	4.4
		SP	10.5	116.0	18.7	13.6
		BoM	—	12.6	2.0	4.2
		Li	20.0	12.0	1.9	2.4
		BL	10.9	6.2		0.4
						C3H/BALB/c (αIA^k)
C3H (Ag positive) N = 3	$^{111}In\alpha IA^k$	PN	1.1	23.9	2.9	5.1
		MN	0.5	16.4	.0	4.2
		SP	12.9	166.8	20.1	19.6
		BoM	—	11.3	1.4	3.8
		Li	13.3	10.9	1.3	2.1
		BL	14.5	8.3		0.6
BALB/c (Ag negative) N = 4	$^{111}In\alpha IA^k$	PN	0.2	4.7	0.3	
		MN	0.2	3.9	0.3	
		SP	0.9	8.5	0.6	
		BoM	—	3.0	0.2	
		Li	8.6	5.1	0.4	
		BL	24.5	14.0		
						αIA^k BALB/k/IgG C3H
C3H (Ag positive) N = 3	^{111}In normal mouse IgG	PN	0.1	3.6	0.3	4.9
		MN	0.1	5.0	0.5	3.5
		SP	1.0	13.0	1.3	8.9
		BoM	—	1.5	0.1	8.4
		Li	38.3	21.0	2.0	0.6
		BL	18.0	10.3		0.6

* PN = peripheral nodes (pooled popliteal + inguinal, + axillary + cervical and brachial); MN = mesenteric nodes; SP = spleen; BoM = bone + marrow (femur); Li = liver; BL = blood.

TABLE 2
^{111}In MoAb Subcutaneous (24 hr)

Mouse injected	Antibody injected	*Tissue	% dose/organ	% dose/g	Tissue/blood ratio	Experiment/control % dose/g ratio
						BALB/k/BALB/c (αIA^k)
BALB/k (Ag positive) N = 4	^{111}InαIA^k	LN	1.6	244.2	37.0	11.0
		UN	0.3	19.5	3.0	4.4
		MN	0.6	16.9	2.6	5.3
		SP	8.3	95.0	14.4	12.8
		BoM	—	8.8	1.3	3.3
		Li	10.4	6.9	1.0	1.9
		BL	11.5	6.6		0.5
						C_3H/BALB/c (αIA^k)
BALB/c (Ag negative) N = 4	^{111}InαIA^k	LN	0.2	22.3	1.6	14.3
		UN	0.1	4.4	0.3	2.5
		MN	0.1	3.2	0.2	2.4
		SP	0.7	7.4	0.5	7.7
		BoM	—	2.7	0.2	1.4
		Li	6.1	3.7	0.3	1.7
		BL	24.2	13.8		0.5
						^{111}In/^{125}IαIA^k in C_3H MICE
C3H (Ag positive) N = 3	^{111}InαIA^k	LN	2.9	319.0	49.1	6.2
		UN	0.3	11.1	1.7	3.1
		MN	0.3	7.6	1.2	3.5
		SP	4.9	56.8	8.7	11.6
		BoM	—	3.8	0.6	6.3
		Li	8.9	6.2	1.0	5.6
		BL	11.4	6.5		1.8
C3H (Ag positive) N = 3	^{125}IαIA^k	LN	0.5	51.7	14.0	
		UN	0.1	3.6	1.0	
		MN	0.1	2.2	0.6	
		SP	0.3	4.9	1.3	
		BoM	—	0.6	0.2	
		Li	1.6	1.1	0.3	
		BL	6.5	3.7		

* LN = lower popliteal and inguinal lymph nodes; UN = upper cervical, brachial and axillary lymph nodes; MN = mesenteric lymph nodes; SP = spleen, BoM = bone + marrow (femur); Li = liver; BL = blood.

Reports of the rate of deiodination vary depending on experimental conditions: Weinstein et al., ~72% per day in mice (12), Carrasquillo et al., ~30% per day in humans (*23*). In the present study, ~80% of the radioiodine label on the αIA^k was excreted in the first 24 hr. Since the liver activity was low with radioiodinated antibodies, it is probable that deiodination occurred in this organ with rapid excretion of the free iodide in the urine. This can produce an increased background in the thyroid, stomach, kidney, and bladder.

We have previously demonstrated that ^{111}In chelate-labeled human serum albumin, transferrin, and the polypeptide antitumor antibiotic bleomycin were extremely stable both in vitro and in vivo at tracer concentrations (*7,8*). The results of the present study are also consistent with greater in vitro stability of the ^{111}In chelate than the radioiodine label. The ^{111}In label showed a much lower excretion rate and longer biological $T^1/_2$, as well as a three- to tenfold higher absolute uptake of the antibody in the target.

Stern et al. using diethylenetriaminepentaacetic acid (DTPA) conjugates of monoclonal antibodies showed that these metal chelates were more stable in vivo than iodinated MoAbs (*24*). Scheinberg and Strand (*25*) also noted the instability of radioiodinated monoclonal antibody against the Rauscher virus glycoprotein in mice, and demonstrated improved stability using chelated radiometal conjugated monoclonal antibodies

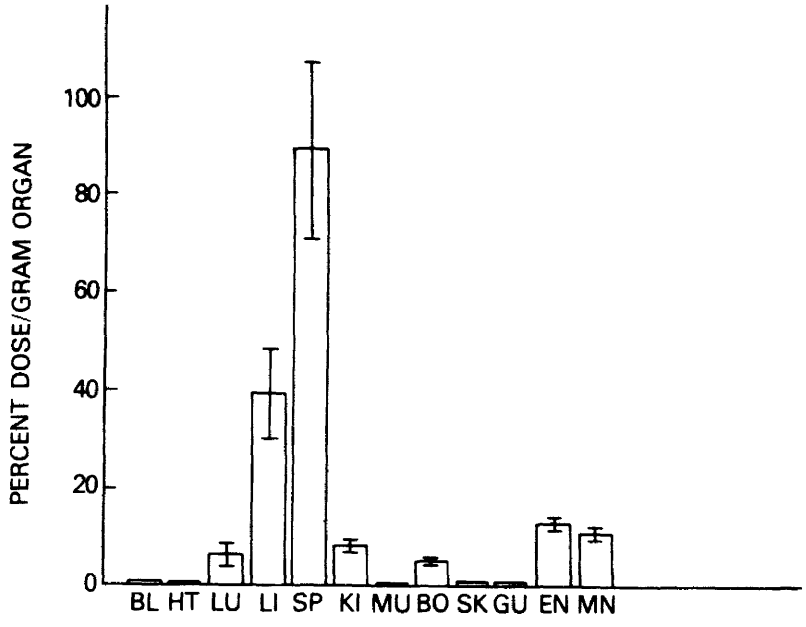

FIGURE 5
Organ distribution 20 hr after i.v. injection of in vitro labeled lymphocytes: BL = blood; HT = heart; LU = lung; LI = liver; SP = spleen; KI = kidney; MU = muscle; BO = bone + marrow (femur); SK = skin; GU = gut; EN = pooled extremity lymph nodes (axillary, inguinal, popliteal); MN = mesenteric nodes

against this determinant (*26*). Rainesbury et al. (*27*) using monoclonal antibodies to human milk fat globule membrane and Fairweather et al. (*28*) using polyclonal anti-CEA chelate labeled with ^{111}In DTPA have reported improved tumor localization in humans.

However, these studies have all shown that the increased stability of the ^{111}In chelate produced not only higher target concentrations but also higher nonspecific blood and liver background activity. Using metal chelate conjugates, it would be possible to label a nonspecific antibody control with a second radiometal with a different gamma energy [for example gallium-67 (^{67}Ga)], for simultaneous injection with specific antibody, labeled with ^{111}In. This would provide the most ideal radiopharmaceutical control for the application of subtraction techniques as the antibodies could be matched for species, class, and subtype. Nevertheless, the problem of differential absorption of the different gamma energies from the two isotopes would still remain a variable.

A more satisfactory blood background reduction technique may be to inject a second antibody just prior to imaging. We have shown that specific polyclonal antisera will produce a 90% lowering of the blood levels within 15 min of administration (*29*). We have recently obtained a mouse monoclonal antibody CHA255† with specificity for ^{111}In EDTA (*30*). In the present study, we have demonstrated that this MoAb effectively lowered the blood concentration of ^{111}In αIA^{k} by a factor of 2.6 and increased the peripheral lymph node to blood ratio by a factor of 2 (Fig. 6). In this experiment there was a slight decrease in the spleen and lymph node concentrations following the anti-EDTA MoAb suggesting that antibody was still available on the cell surface 22 hr

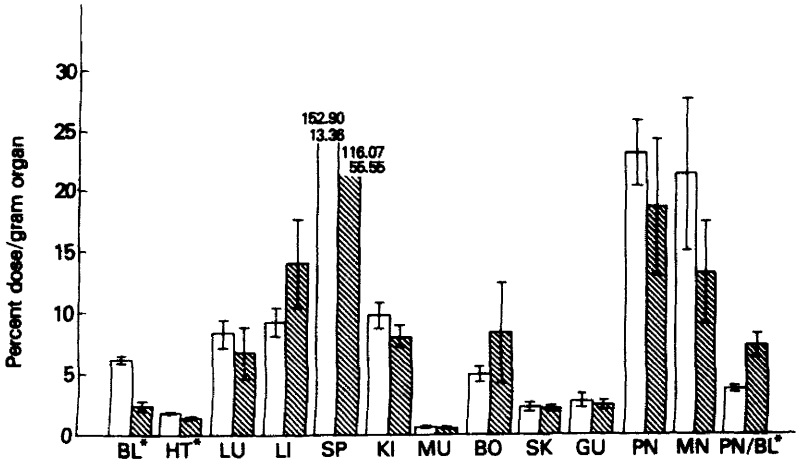

FIGURE 6
24-hr organ distribution of ^{111}InαIA^{k} in C3H (Ag positive) mice with and without 100 μg monoclonal anti-EDTA, CHA255† i.v., 2 hr before killing; BL = blood; HT = heart; LU = lung; LI = liver; SP = spleen; KI = kidney; MU = muscle; BO = bone + marrow (femur); SK = skin; GU = gut; PN = peripheral nodes (pooled axillary, inguinal, popliteal); MN = mesenteric nodes; (1) Peripheral node/Blood concentration ratio. (□) ^{111}In αIA^{k} without anti-EDTA; (▨) with anti-EDTA; (▭) ±1 s.d. * = $p < 0.01$ for difference

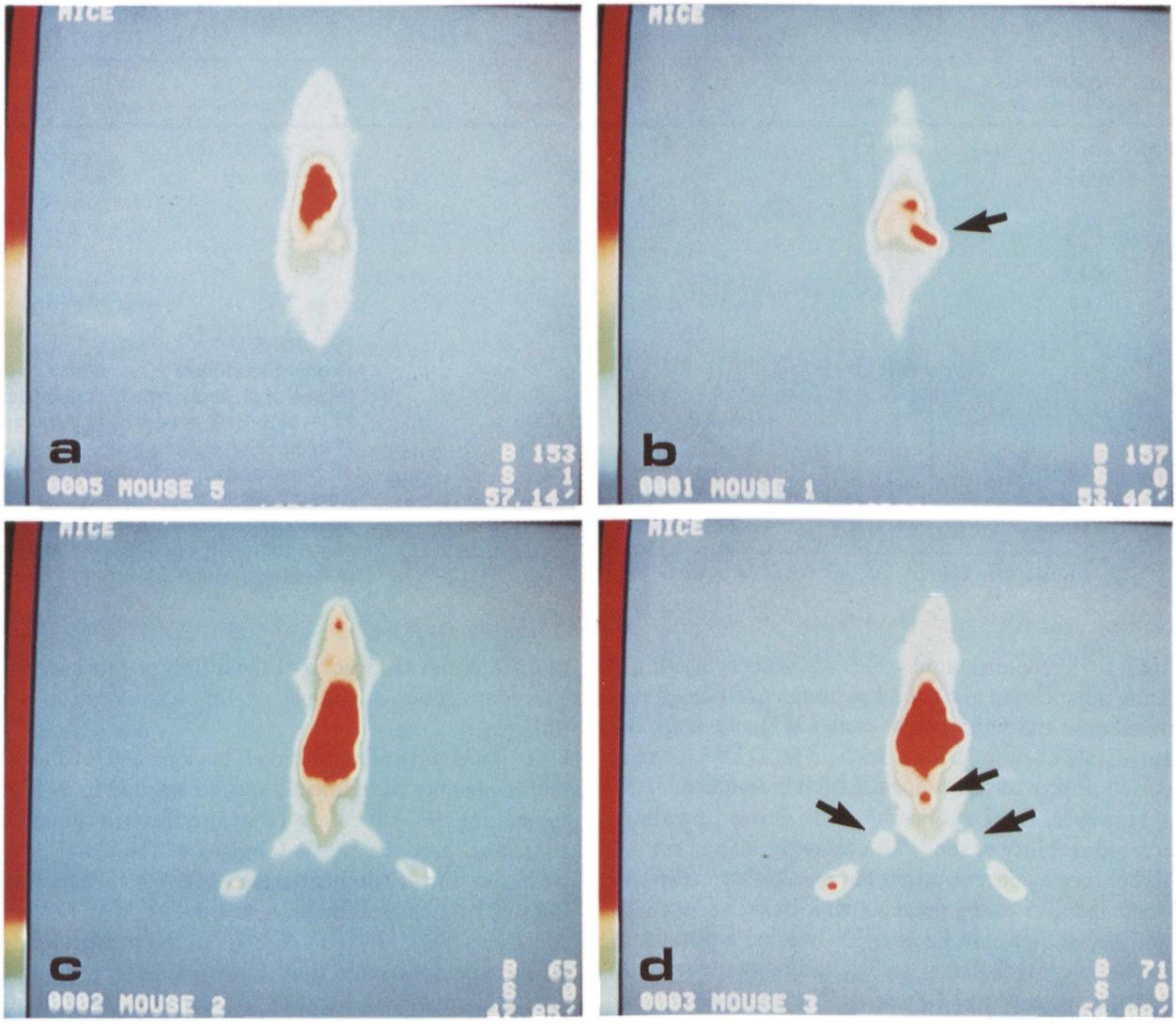

FIGURE 7
Anterior gamma camera pinhole images 24 hr following 100 μCi ^{111}InαIA^{k}; imaging time 60 min, 500K total counts, color scale normalized a = b and c = d. a: i.v. BALB/c (Ag negative) shows mostly blood-pool activity, no spleen visible; b: i.v. C3H (Ag positive) shows spleen sharply outlined (arrow) and less blood pool; c: s.c. BALB/c (Ag negative) blood pool, no lymph nodes evident; d: s.c. C3H (Ag positive), popliteal and pelvic lymph nodes (arrows) clearly visualized

following i.v. injection (Fig. 6). The use of ^{111}InαEDTA MoAb as the second antibody makes it theoretically possible to remove any homologous circulating protein that carries a metal chelate label of this type.

While providing a partial solution to the high blood background problem, the administration of second antibody simultaneously increases the liver background, which is the site of deposition of most of the immune complex. Other approaches will have to be taken to reduce this liver background. In order to take advantage of the abundance of hydrolytic enzymes in the liver, we have attached the chelate to the antibody through a metabolizable ester link. We have shown that the ester link between the chelate and the protein shortens the biological half-life, lowers the liver concentration, and increases the whole-body excretion of an ^{111}In chelate labeled MoAb against a mouse B cell lymphoma, with only a modest decrease in tumor concentration (*31*). Appropriate timing of administration of second antibody with these and other enzyme-cleavable links, may allow some control of deposition in, and metabolism by, the liver, thus enhancing cleavage and excretion only after maximum target concentrations have been achieved.

The images that we have obtained of mouse lymph nodes weighing only approximately 2 mg dramatically illustrate the potential of the technique (Fig. 7). We have previously shown the advantages of ^{111}In colloid for lymphoscintigraphy in animals (*32*), and in this study similar concentrations of MoAb were obtained in lymph nodes (data not shown). However, antibody had the

added advantage of specificity as well as improved absorption from the injection site: 80% for antibody compared with 50% for colloid. Advantages of the s.c. route include the avoidance of circulating antigen and antigen on normal tissue that might complex with the antibody and make it unavailable to the target, as well as allowing more effective use of IgM, which diffuses poorly through blood vessel capillaries in comparison to lymphatic vessels.

An especially attractive application suggested by the present study is the possiblity of using antibodies to normal human lymph structures such as anti-T cell MoAb (anti-leu 1)‡ for imaging normal lymphatic structures and T cell lymphomas and leukemias expressing the leu 1 antigen. Anti-idiotype antibodies directed against B cell leukemias and lymphomas are another especially promising class, since each B cell tumor expresses a unique cell surface Ig common to all members of the malignant clone, making it the closest approximation of a tumor-specific antigen available (*33*). A disadvantage of this approach is that the MoAbs must be "tailor made" for each patient. It is possible using two MoAbs with different labels (e.g., ^{67}Ga and ^{111}In) that lymph node metastases could be detected as a decrease in uptake of normal cell markers coinciding with an increased uptake in markers directed against the tumor cells. In addition to the diagnostic use of ^{111}In chelate-labeled monoclonal antibodies, therapy may be possible with the same chelate-labeled antibodies tagged with a beta-emitting metal ion such as yttrium-90.

FOOTNOTES

* Picker International, 12 Clintonville Rd., Northford, CT 06472.

† Hybritech Inc., 11085 Torreyana Rd., San Diego, CA 92121.

‡ Becton Dickinson Monoclonal Antibody Center, 2375 Garcia Ave., Mountain View, CA 94043.

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