In Vitro and In Vivo Properties of Human/Mouse Chimeric Monoclonal Antibody Specific for Common Acute Lymphocytic Leukemia Antigen

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A human/mouse chimeric monoclonal antibody specific for a common acute lymphocytic leukemia antigen was efficiently obtained by ligating human heavy-chain enhancer element to the chimeric heavy- and light-chain genes. Cell binding and competitive inhibition assays of both radioiodine and indium-111- (^{111}In) labeled chimeric antibodies demonstrated in vitro immunoreactivity identical with that of the parental murine monoclonal antibodies. The biodistribution of the radiolabeled chimeric antibody in tumor-bearing nude mice was similar to that of the parental murine antibody. Tumor accumulation of radioiodinated parental and chimeric antibodies was lower than that of ^{111}In-labeled antibodies, probably because of dehalogenation of the radioiodinated antibodies. Indium-111-labeled chimeric antibody clearly visualized xenografted tumor. These results suggest that a human/mouse chimeric antibody can be labeled with ^{111}In and radioiodine without the loss of its immunoreactivity, and that chimeric antibody localizes in vivo in the same way as the parental murine antibody.

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Since the establishment of hybridoma technology, several monoclonal antibodies (MoAbs) which recognize cancer-associated antigens have been used for in vitro serodiagnosis, immunoscintigraphy, and targeted therapy (*1–4*). The MoAbs currently employed are of murine origin, and have intrinsic limitations for in vivo use, such as immunogenicity when exposed to the human immune system and ineffective interaction with human effector cells. Although the use of human MoAbs may resolve these problems, the stable production of human MoAbs is still quite difficult and only a few human MoAbs are available at present (*5,6*).

Immunoglobulin molecules consist of variable and constant region domains (*7*). Recent advances in gene technology make possible the exchange of mouse constant region domains with human constant region domains, to construct a human/mouse chimeric antibody (Ab) (*8,9*). Human/mouse chimeric Abs may reduce or eliminate the problems of murine MoAbs mentioned above. By ligating the human heavy-chain enhancer element to chimeric light- and heavy-chain genes, we have been able to produce stably and efficiently human/mouse chimeric Abs which react with a common acute lymphocytic leukemia antigen (cALLA) (*10*). To determine whether the exchange of constant region domains affects the immunologic characteristics of labeled MoAb, we compared the in vitro and in vivo properties of radiolabeled parental murine Ab with those of a human/mouse chimeric Ab.

MATERIALS AND METHODS

Cells

Manca cells (SK-DHL-2, human B-lymphoblastoid cell line) (*11*), known to express cALLA, were cultured in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY) and 0.03% L-glutamine.

Monoclonal Antibodies

Parental murine MoAb NL-1 (IgG_{2a}), specific for cALLA, was secreted by a murine hybridoma generated by a standard method (*12*). A rearranged murine heavy-chain variable region gene was cloned from a murine hybridoma which secreted NL-1. A constant region gene of the human γ_1 heavy chain, containing human heavy-chain enhancer element, was cloned from human plasma cell leukemia ARH77 cells. These two genes were linked together to construct a human/mouse chimeric heavy-chain gene. A rearranged murine light-chain

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variable region gene, cloned from the same hybridoma, was joined to a constant region gene of human *k* light chain, and human heavy-chain enhancer element was incorporated. A non-producer mouse myeloma cell line X63Ag8.653 was transfected by these chimeric heavy- and light-chain genes. The transfectoma thus obtained produced 10–30 μg/ml of chimeric NL-1 Ab in serum-free medium and chimeric Ab was purified by applying the culture medium to a Protein-A affinity column.

MoAb 59A (IgG_1) and OST7 (IgG_1), which recognized human thyroglobulin and alkaline phosphatase (*13*), respectively, were used as control Abs.

Radiolabeling of MoAbs

MoAbs were radioiodinated by the chloramine-T method (*14,15*). Purified antibodies (40 μg) in 0.3 *M* phosphate-buffer (PB), pH 7.5, and iodine-125 (^{125}I) (0.3 mCi) for protein labeling (Amersham International plc, Buckinghamshire, UK) were mixed with 2.0 μg of chloramine-T (Nakarai Chemicals, Kyoto, Japan) dissolved in 0.3 *M* PB. After five min of reaction in a total volume of 200 μl, ^{125}I-labeled MoAb was separated from free ^{125}I by Sephadex G-50 gel chromatography. The radiochemical labeling yield ranged from 60% to 70%, and the specific activity was ~5 mCi/mg.

MoAbs were labeled with indium-111 (^{111}In) with the use of a bifunctional chelating agent, diethylenetriaminepentaacetic acid (DTPA), as previously described (*16*). First, MoAb solution (1 mg/ml) in 0.1 *M* $NaHCO_3$ was mixed with cyclic DTPA anhydride in DTPA/Ab molar ratios ranging from 5 to 15 for 1 hr at room temperature. Then, unconjugated DTPA was separated by Sephadex G-50 gel chromatography. The DTPA/Ab conjugation ratio was 1.4:1 for parental murine NL-1 and 0.7:1 for chimeric NL-1 Abs. DTPA-conjugated Ab in 0.2 *M* citrate buffer was mixed with ^{111}In-chloride and allowed to stand for 1 hr at room temperature. The labeling efficiency, determined by Sephadex G-50 gel chromatography, was more than 95% for each Ab.

In each experiment, aliquots of labeled Ab were analyzed by Sephadex G-50 column chromatography and high-performance liquid chromatography (HPLC) using TSK-G3000SW gel (Tosoh Co., Tokyo, Japan) to assess the presence of Ab aggregates and free radioiodine or ^{111}In.

In Vitro Binding Assay

Radiolabeled parental murine and chimeric NL-1 Abs (30,000 cpm/100 μl) were incubated with increasing concentrations of Manca cells (5×10^4 to 4×10^6 for ^{125}I-labeled Ab and 5×10^4 to 1×10^7 for ^{111}In-labeled Ab) suspended in 100 μl of phosphate-buffered saline (PBS) in 5.7×46 mm microcentrifuge tubes for 1 hr at 4°C. After centrifugation, the supernatant was aspirated and the radioactivity bound to cells was counted in an auto-well gamma counter.

For the competitive inhibition assay, radiolabeled parental or chimeric NL-1 Ab (30,000 cpm/50 μl) and increasing amounts of unlabeled Ab (50 μl) were incubated with 1×10^6 Manca cells in 100 μl PBS for 1 hr at 4°C. After centrifugation, the radioactivity bound to the cells was counted, and inhibition curves were plotted. Binding affinity constants of both Abs to Manca cells were calculated by Scatchard plot analysis (*17*).

In Vivo Biodistribution Study and Scintigraphy of Tumor-Bearing Mice

Female BALB/c athymic nu/nu mice were inoculated subcutaneously with 5×10^6 Manca cells. Tumors weighing 500–800 mg 2–3 wk after transplantation were used in this study. For radioiodinated Abs, nonradioactive iodine was administered to mice beginning one day before the injection of labeled Abs until the end of the experiment. Nude mice were injected into the tail vein with 1 μCi of ^{125}I- or ^{111}In-labeled parental, chimeric, or control Abs. The Ab dose was adjusted to 10 μg per mouse by the addition of the corresponding unlabeled Ab. At designated times after the injection, groups of mice were killed by ether inhalation. Biodistribution data were expressed as percentages of injected dose per gram of tissue and tumor-to-normal tissue ratios. Specific localization of labeled MoAb in the tumor was evaluated by a localization index, defined as the tumor-to-blood ratio of specific Ab divided by that of the control 59A Ab.

Xenografted tumors were imaged by the injection of 50 μCi of ^{131}I- or ^{111}In-labeled chimeric NL-1 Ab. At 24, 48, and 96 hr after the injection, mice were anesthetized by intraperitoneal injection of sodium pentobarbital and imaged using a gamma camera equipped with a 4-mm pinhole collimator.

For comparison, images of tumor-bearing mice were obtained after injections of isotype-matched 59A and chimeric OST7 Abs.

RESULTS

In Vitro Reactivity of Radiolabeled Antibodies

When Manca cells were used as target cells, ^{125}I-labeled parental murine NL-1 Ab and human/mouse chimeric NL-1 Ab showed almost identical binding curves (Fig. 1), whereas significant binding was not observed with control ^{125}I-labeled 59A Ab. This binding

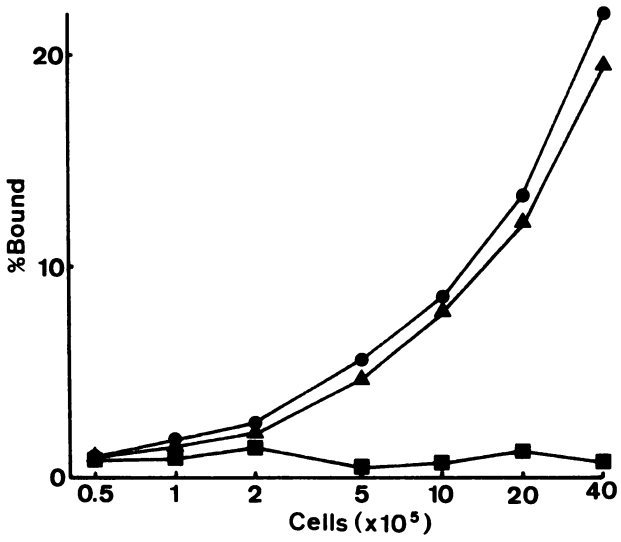

FIGURE 1
Binding of ^{125}I-labeled MoAbs to Manca cells. Iodine-125-labeled parental (●), chimeric NL-1 Ab (▲) or control 59A Ab (■) was incubated with increasing concentrations of Manca cells for 1 hr at 4°C. Percentage of radioactivity bound to cells is plotted against the number of cells.

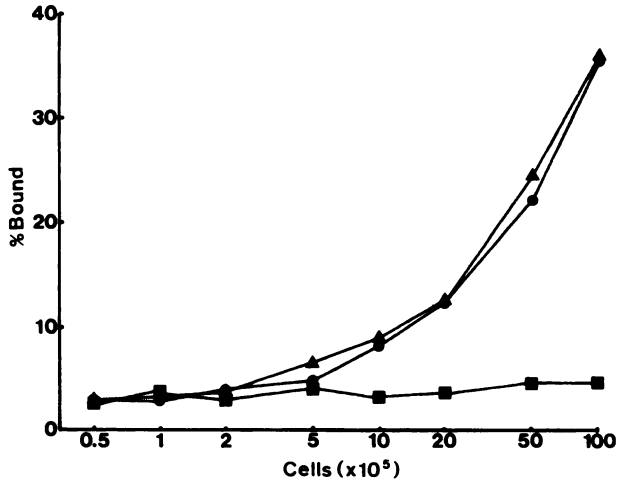

FIGURE 2
Binding of ^{111}In-labeled MoAbs to Manca cells. Cell binding assay was performed by the same procedure as in Figure 1. (●) Indium-111-labeled parental NL-1 Ab, (▲) ^{111}In-labeled chimeric NL-1 Ab, and (■) ^{111}In-labeled control 59A Ab.

of ^{125}I-labeled chimeric NL-1 Ab to Manca cells was dose-dependently inhibited by both unlabeled parental and chimeric NL-1 Abs (data not shown). The binding of ^{125}I-labeled parental NL-1 Ab to Manca cells was similarly inhibited by unlabeled parental and chimeric NL-1 Abs (data not shown), but no inhibition was seen with the control 59A. Affinity constants derived from Scatchard plot analysis were calculated as 1.7×10^8 M^{-1} for parental NL-1 Ab and 2.0×10^8 M^{-1} for chimeric NL-1 Ab.

Both parental and chimeric NL-1 Abs were similarly conjugated with ^{111}In using DTPA as a bifunctional chelating agent. Indium-111-labeled parental and chimeric NL-1 Abs had almost identical curves of binding to Manca cells (Fig. 2), and there was not a significant difference in immunoreactivity between radioiodinated and ^{111}In-labeled NL-1 Abs. The binding of ^{111}In-labeled chimeric NL-1 Ab to Manca cells was similarly inhibited by unlabeled parental and chimeric NL-1 Abs, but not by control 59A Ab (data not shown).

In Vivo Biodistribution of ^{125}I-labeled Abs

Biodistribution data of ^{125}I-labeled parental murine and chimeric NL-1 Abs in nude mice bearing Manca cells are listed in Table 1 expressed as percentages of injected dose per gram of tissue and shown in Figure 3 as tumor-to-normal tissue ratios. Although the data are quite varied, ^{125}I-labeled parental and chimeric NL-1 Abs had similar distribution patterns except on Day 1, when ^{125}I-labeled chimeric Ab showed a higher tumor uptake than parental NL-1 Ab. From Day 2 to Day 7, there was no significant difference in tumor-to-blood, -liver, -intestine, -spleen and -lung ratios of either parental or chimeric NL-1 Ab, and tumor-to-normal tissue ratios increased from Day 2 to Day 7. Tumor-to-blood ratios of control 59A Ab remained unchanged, so there was a relative increase in localization indices of the specific Abs with time. Localization indices reached 2.11 and 2.15 on Day 4 and 3.08 and 2.88 on Day 7 for parental and chimeric NL-1, respectively, indicating the specific localization of ^{125}I-labeled paren-

TABLE 1
Biodistribution of ^{125}I-labeled Parental Murine and Chimeric Human/Mouse NL-1 Antibodies in Nude Mice Bearing Manca Cells

%ID/G Tissue*	Day 1	Day 2	Day 4	Day 7
Blood				
Parental Ab	12.39 ± 1.64†	11.65 ± 0.70	4.58 ± 0.74	1.59 ± 0.28
Chimeric Ab	17.70 ± 1.64	12.14 ± 0.65	6.90 ± 1.69	1.99 ± 0.06
Liver				
Parental Ab	3.44 ± 0.54	2.83 ± 0.24	0.99 ± 0.17	0.31 ± 0.05
Chimeric Ab	3.90 ± 0.27	3.09 ± 0.33	1.55 ± 0.40	0.49 ± 0.06
Intestine				
Parental Ab	1.36 ± 0.08	1.26 ± 0.12	0.40 ± 0.05	0.14 ± 0.02
Chimeric Ab	1.07 ± 0.03‡	0.97 ± 0.14	0.51 ± 0.09	0.18 ± 0.02
Spleen				
Parental Ab	4.16 ± 0.81	3.25 ± 0.85	0.88 ± 0.12	0.31 ± 0.04
Chimeric Ab	4.40 ± 0.84	3.28 ± 0.43	1.20 ± 0.20	0.37 ± 0.04
Lung				
Parental Ab	7.60 ± 1.04	4.77 ± 0.21	2.01 ± 0.40	0.73 ± 0.10
Chimeric Ab	7.70 ± 0.42	5.84 ± 0.39	3.28 ± 0.84	1.08 ± 0.12
Tumor				
Parental Ab	2.85 ± 0.47	3.62 ± 0.53	2.70 ± 0.74	0.99 ± 0.14
Chimeric Ab	5.33 ± 0.23§	4.23 ± 0.35	4.08 ± 1.18	1.51 ± 0.21

* Data are shown as a percentage of injected dose per gram of tissue in four mice.
† mean ± s.e.
‡ $p < 0.05$ compared with parental Ab.
§ $p < 0.01$ compared with parental Ab.

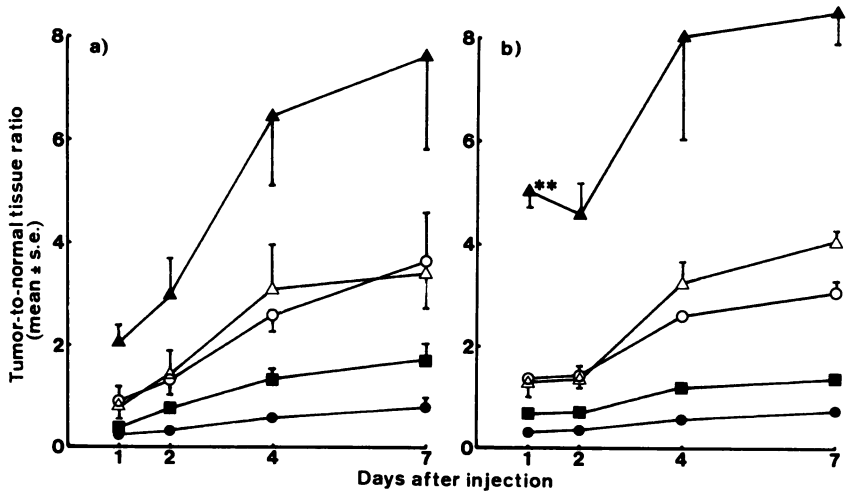

FIGURE 3
Tumor-to-normal ratios for Manca cells xenografted in nude mice after injection of ^{125}I-labeled parental (A) and chimeric NL-1 Ab (B). (●) tumor-to-blood ratio, (○) tumor-to-liver ratio, (▲) tumor-to-intestine ratio, (△) tumor-to-spleen ratio and (■) tumor-to-lung ratio. ** = $p < 0.01$ compared with parental Ab. Vertical bars indicate s.e.

tal and chimeric NL-1 Abs to tumor sites. However, tumor-to-blood ratios of both Abs were too low for imaging. Xenografted tumor was not clear using ^{131}I-labeled chimeric NL-1 Ab.

In Vivo Biodistribution of ^{111}In-labeled Abs

Both ^{111}In-labeled parental and chimeric NL-1 Abs showed almost identical tissue distribution from Day 1 to Day 4 (Table 2 and Fig. 4). Tumor-to-normal tissue ratios of both ^{111}In-labeled NL-1 Abs increased from Day 1 to Day 4, when compared to the ^{111}In-labeled control 59A Ab. Localization indices increased from 1.69 and 1.63 on Day 1 and 2.48 and 2.21 on Day 2 to 4.40 and 3.55 on Day 4, for parental and chimeric NL-1 Abs, respectively. In comparison with radioiodinated Abs, absolute tumor uptake and tumor-to-blood ratios of ^{111}In-labeled NL-1 Abs were sufficient for imaging, and the xenografted tumor was clearly demonstrated by ^{111}In-labeled chimeric NL-1 Ab (Fig. 5A). However, there was no significant tumor uptake when ^{111}In-labeled chimeric OST7 (Fig. 5B) or 59A Ab (data not shown) were used.

DISCUSSION

Advances in gene technology have made it possible to manipulate immunoglobulin genes and to produce a human/mouse chimeric MoAb (*8,9*). Chimeric Ab has advantages over murine MoAb when in vivo use is required. A few chimeric Abs have been produced and

TABLE 2
Biodistribution of ^{111}In-labeled Parental Murine and Chimeric Human/Mouse NL-1 Antibodies in Nude Mice Bearing Manca Cells

%ID/G Tissue*	Day 1	Day 2	Day 4
Blood			
Parental Ab	6.46 (6.15–6.87)†	4.76 (4.67–4.92)	1.54 (1.19–1.73)
Chimeric Ab	7.42 (7.10–7.73)‡	4.96 (4.43–5.64)	2.14 (1.65–2.81)
Liver			
Parental Ab	16.81 (14.26–19.38)	13.24 (12.19–14.99)	10.12 (9.83–10.55)
Chimeric Ab	12.16 (10.76–13.05)*	10.11 (9.83–10.36)*	8.56 (7.83–9.29)*
Intestine			
Parental Ab	2.40 (2.30–2.58)	2.26 (2.18–2.31)	1.42 (1.26–1.58)
Chimeric Ab	2.92 (2.64–3.05)‡	2.65 (2.22–2.89)	1.48 (1.47–1.51)
Spleen			
Parental Ab	9.47 (7.34–12.10)	7.48 (7.08–7.84)	6.63 (5.88–7.55)
Chimeric Ab	7.57 (6.11–10.25)	6.95 (6.36–7.55)	5.35 (4.56–6.83)
Lung			
Parental Ab	4.07 (3.68–4.30)	3.64 (3.26–3.75)	2.33 (1.95–2.62)
Chimeric Ab	4.63 (4.53–4.80)‡	3.97 (3.76–4.29)	2.62 (2.22–3.25)
Tumor			
Parental Ab	7.31 (6.67–7.78)	9.89 (8.45–10.26)	6.91 (6.53–7.43)
Chimeric Ab	8.09 (7.78–8.25)	9.14 (8.57–9.93)	7.72 (6.11–9.09)

* Data are shown as a percentage of injected dose per gram of tissue in three to four mice.
† mean (range).
‡ $p < 0.05$ compared with parental Ab.

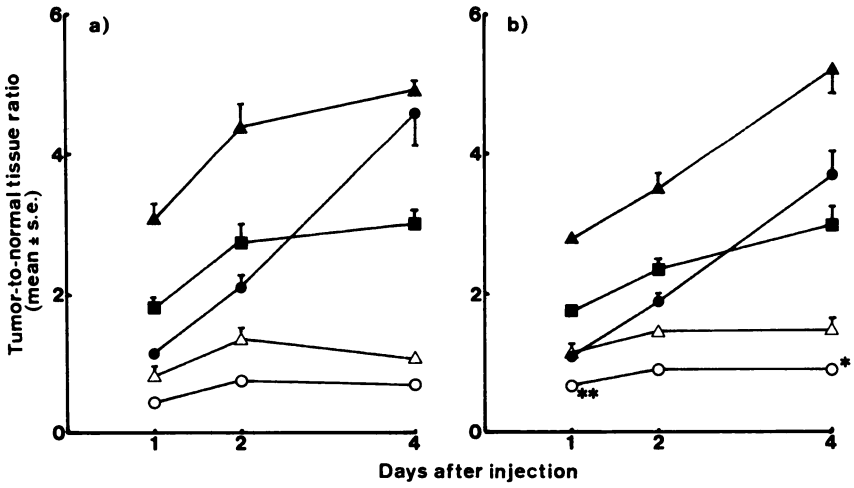

FIGURE 4
Tumor-to-normal tissue ratios for Manca cells xenografted in nude mice after injection of ^{111}In-labeled parental (A) and chimeric NL-1 (Ab (B). (●) tumor-to-blood ratio, (○) tumor-to-liver ratio, (▲) tumor-to-intestine ratio, (△) tumor-to-spleen ratio and (■) tumor-to-lung ratio. * = $p < 0.05$ compared with parental Ab and ** = $p < 0.01$ compared with parental Ab. Vertical bars indicate s.e.c.

their characteristics have been reported (*18,19*). However, the usefulness of genetically engineered Abs is limited by the difficulty of producing a large amount of Ab, especially in view of the clinical findings that the detection rate of metastatic tissues increases with the dose of Ab administered (*20*). Twenty to 40 mg of unlabeled MoAb have been added to 1 mg of radiolabeled MoAb in the imaging of patients with malignant melanoma or colorectal cancer (*20,21*).

CALLA is a common antigen of acute non-T, non-B lymphocytic leukemia, and parental murine MoAb NL-1 recognizes cALLA (*10*). A human/mouse chimeric MoAb specific for cALLA has been efficiently constructed by ligating human heavy-chain enhancer elements into chimeric heavy- and light-chain genes. Transfectomas thus obtained secrete almost the same amount of Ab as the parental murine hybridoma, which is more than that of previous investigations. The in-

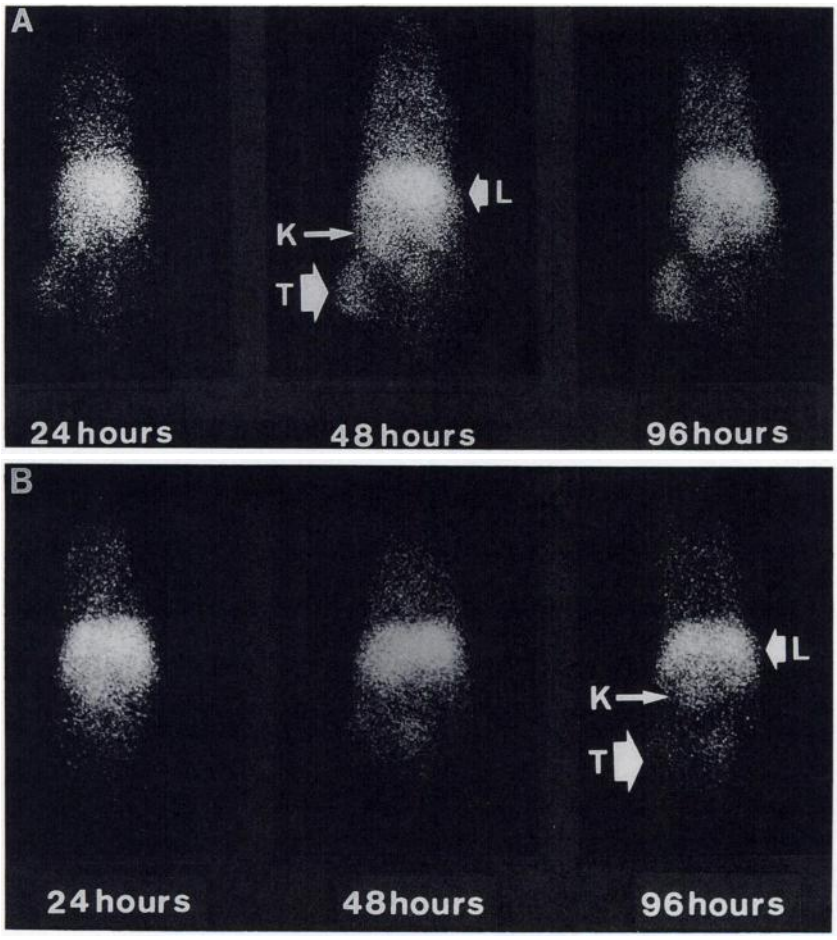

FIGURE 5
Scintigrams of a mouse bearing Manca cell xenograft. Images were obtained 24, 48, and 96 hr after the injection of ^{111}In-labeled chimeric NL-1 Ab (A) or ^{111}In-labeled cOST7 Ab (B). Chimeric OST7 Ab was used as an isotype-matched control Ab. T = tumor; L = liver; and K = kidney.

creased ADCC activity of this chimeric NL-1 Ab has been reported by Nishimura et al. (*10*).

In vitro binding and competitive inhibition assays using Manca cells, which express cALLA on their surface membranes, indicated that ^{125}I-labeled chimeric NL-1 Ab had immunoreactivity similar to that of the parental murine NL-1 Ab. The two Abs competed efficiently for binding sites regardless of which was the labeled substrate. It seems clear that the replacement of murine constant region domain with its human counterpart did not reduce or alter the original immunoreactivities. Indium-111 has many advantages over radioiodine for clinical use and has been mostly employed for the labeling of anti-tumor MoAbs (*20–22*). After conjugation with cyclic DTPA anhydride, ^{111}In was efficiently coupled to Ab through chelation with DTPA. The binding curves of ^{111}In-labeled parental and chimeric NL-1 Abs to Manca cells were identical with those of radioiodinated Abs, and no loss of immunoreactivity was seen with either NL-1 Ab.

In vivo biodistribution studies demonstrated that ^{125}I- and ^{111}In-labeled chimeric NL-1 Abs were distributed in almost the same manner as parental NL-1 Ab. Tumor-to-normal tissue ratios and localization indices of both radiolabeled Abs increased with time, indicating specific localization in tumor sites. However, the amount of both radioiodinated NL-1 Abs accumulated in tumor sites was quite small and xenografted tumors in a nude mouse were not demonstrated clearly by ^{131}I-labeled chimeric NL-1 Ab. In contrast, tumors could be clearly seen when ^{111}In-labeled chimeric NL-1 Ab was used. Liver and spleen retention of both ^{111}In-labeled NL-1 Abs was high. HPLC and Sephadex G-50 gel chromatography showed no colloidal indium (data not shown), and this high liver and spleen deposition seemed to be due to the intrinsic properties of indium-labeled Abs (*23*).

CALLA is known to modulate after the Ab binds to cell-surface antigens. Since the formed Ag-Ab complex enters the cell (*24–26*), radioiodinated Ab seems to be dehalogenated during this process and free iodine leaves the cell. Thus, radioactivity at tumor sites is reduced. In the imaging of human cutaneous T-cell lymphoma, the rapid clearance of ^{131}I-labeled T101 Ab and the loss of ^{131}I-tracer from T101 has been described, and skin lesions were not imaged with ^{131}I-labeled T101, whereas good visualization was obtained with ^{111}In-labeled T101 (*22*). These findings indicate that a human/mouse chimeric Ab specific for cALLA retains its original immunoreactivity and can be used for in vivo tumor targeting. Radioiodinated chimeric Ab also seems to be dehalogenated, indicating that ^{111}In-labeled chimeric Ab is superior to radioiodinated Ab for the localization of tumors, if the target antigen of a given MoAb is known to modulate. Because of its reduced immunogenicity and improved interaction with human effector cells, the clinical application of a human/mouse chimeric Ab appears promising.

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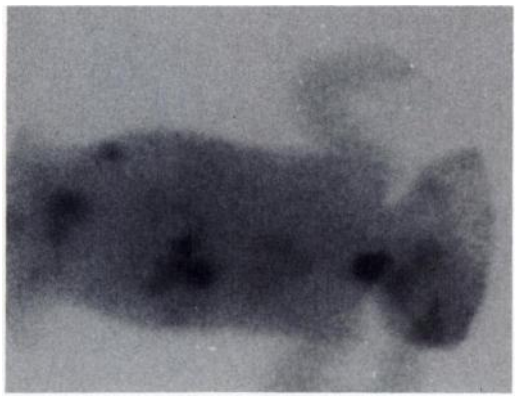

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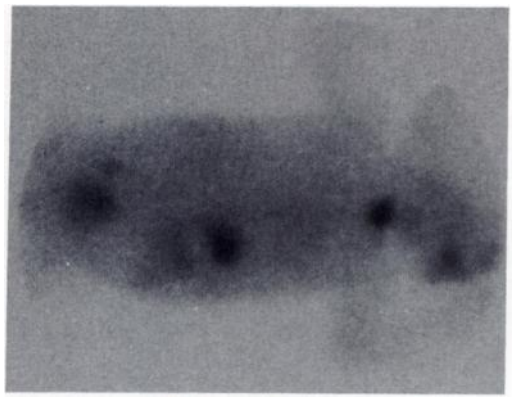

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FIRST IMPRESSIONS

PURPOSE:
Patient with recidive short-time rectal bleeding was given 50 mBq [^{99m}Tc]pertechnetate intravenously. Scintigrams were registered at 5- and then at 10-min intervals. Activity was found in the stomach and, simultaneously, in a well-defined round form in the lower right abdominal region. Increased uptake of the tracer in the right lower addominal region gave some indication of Meckel's diverticulum. The patient was subject to surgery and Meckel's diverticulum was proven histologically.

TRACER:
[^{99m}Tc]pertechnetate

ROUTE OF ADMINISTRATION:
Intravenously

TIME AFTER INJECTION:
5, 10, 15, and 25 min postinjection

INSTRUMENTATION:
Gamma camera

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