Cancer Immunol Immunother (1997) 44: 179–188

ORIGINAL ARTICLE

Robert M. Sharkey · Thomas M. Behr
M. Jules Mattes · Rhona Stein · Gary L. Griffiths
Lisa B. Shih · Hans J. Hansen · Rosalyn D. Blumenthal
Robert M. Dunn · Malik E. Juweid
David M. Goldenberg

Advantage of residualizing radiolabels for an internalizing antibody against the B-cell lymphoma antigen, CD22

Received: 23 August 1996 / Accepted: 7 February 1997

Abstract LL2 is an anti-CD22 pan-B-cell monoclonal antibody which, when radiolabeled, has a high sensitivity for detecting B-cell, non-Hodgkin's lymphoma (NHL), as well as an antitumor efficacy in therapeutic applications. The aim of this study was to determine whether intracellularly retained radiolabels have an advantage in the diagnosis and therapy of lymphoma with LL2. In vitro studies showed that iodinated LL2 is intracellularly catabolized, with a rapid release of the radioiodine from the cell. In contrast, residualizing radiolabels, such as radioactive metals, are retained intracellularly for substantially longer. In vivo studies were performed using LL2-labeled with radioiodine by a non-residualizing (chloramine-T) or a residualizing method (dilactitol-tyramine, DLT), or with a radioactive metal (^{111}In). The biodistribution of a mixture of ^{125}I (non-residualizing chloramine-T compared to residualizing DLT), ^{111}In-labeled LL2 murine IgG2a or its fragments [F(ab′)$_2$, Fab′], as well as its humanized, CDR-grafted form, was studied in nude mice bearing the RL human B-cell NHL cell line. Radiation doses were calculated from the biodistribution data according to the Medical International Radiation Dose scheme to assess the potential advantage for therapeutic applications. At all assay times, tumor uptake was higher with the residualizing labels (i.e., ^{111}In and DLT-^{125}I) than with the non-residualizing iodine label. For example, tumor/blood ratios of ^{111}In-labeled IgG were 3.2-, 3.5- and 2.8-fold higher than for non-residualizing iodinated IgG on days 3, 7 and 14, respectively. Similar results were obtained for DLT-labeled IgG and fragments with residualized radiolabels. Tumor/organ ratios also were higher with residualizing labels. No significant differences in tumor, blood and organ uptake were observed between murine and humanized LL2. The conventionally iodinated anti-CD20 antibody, 1F5, had tumor uptake values comparable to those of iodinated LL2, the uptake of both antibodies being strongly dependent on tumor size. These data suggest that, with internalizing antibodies such as LL2, labeling with intracellularly retained isotopes has an advantage over released ones, which justifies further clinical trials with residualizing ^{111}In-labeled LL2 for diagnosis, and residualizing ^{131}I and ^{90}Y labels for therapy.

Key words B-cell non-Hodgkin's lymphoma · Radioimmunodetection · Radioimmunotherapy · Anti-CD22 monoclonal antibody · Internalization · Radioactive metals · Residualizing iodine label

Supported in part by USPHS grants from the National Institutes of Health CA39841 and CA60039 and a fellowship from the Deutsche Forschungsgemeinschaft DFG (Be1689/1-1/2; TM Behr)

R. M. Sharkey · T. M. Behr · J. Mattes · R. Stein · R. D. Blumenthal · R. M. Dunn · M. E. Juweid · D. M. Goldenberg (✉)
The Garden State Cancer Center, 520 Belleville Avenue, Belleville, NJ 07109, USA
Fax: (201) 844 7020

G. L. Griffiths · L. B. Shih · H. J. Hansen
Immunomedics Inc., Morris Plains, NJ 07950, USA

Introduction

Since the fundamental work with polyclonal anti-(carcinoembryonic antigen) IgG in animal and human studies [15, 17], numerous antibodies against a variety of different antigens have been developed and tested in animal models and in clinical settings. Whereas, in solid tumors, the success of radioimmunotherapy is still limited [7, 16], in lymphoma it is becoming a third mode of therapy in addition to chemotherapy and external-beam radiation [9–11, 18, 21, 22, 33, 34].

Our group has developed a monoclonal antibody directed against the CD22 antigen of B-cell, non-Hodgkin's lymphoma [30, 44]. High sensitivities in the diagnosis and the staging of lymphoma (e.g., as ^{99m}Tc-labeled Fab′ fragment [1, 2, 8, 28]) have been observed, as well as partial to complete remissions when the ^{131}I-labeled IgG of its F(ab′)$_2$ fragment was used therapeutically [18, 21]. An important property of LL2 is its rapid internalization [42]. Earlier studies have shown that iodinated antibodies are metabolized quickly with subsequent release of low-mo-

lecular-mass metabolic products from the cell [14, 29]. In contrast, it is well known that radioactive metals are retained intracellularly [12, 13, 27, 32, 35, 45]. Hence, it is to be expected that such metals, or other forms of intracellularly retained radiolabels, possess an advantage over non-residualizing released ones (e.g., a conventional iodine label) in diagnosis, as well as in therapy with LL2. Residualizing forms of radioiodine also have been developed and introduced into preclinical animal models [36, 45]. Thus, the aim of this study was to determine whether residualizing forms of radiolabels may have advantages over released forms in the targeting and therapy of B-cell, non-Hodgkin's lymphoma with the anti-CD22 LL2 in a nude-mouse-human-B-cell xenograft model. These findings were presented previously in part in abstract form [5, 41].

Materials and methods

Antibodies

LL2 (or Immu-LL2, originally named EPB-2), is a murine IgG2a monoclonal antibody that reacts with the CD22 antigen of B cells and non-Hodgkin's B-cell lymphoma [30, 44]. Intact IgG was isolated from ascites-grown hybridoma cells. Its $F(ab')_2$ fragment was prepared by pepsin digestion separation from undigested IgG by protein A and exhaustive ultrafiltration. The Fab′ fragment was prepared from $F(ab')_2$ by dithiothreitol reduction, followed by iodoacetamide blocking and purification by gel filtration. The development and characteristics of the humanized form of LL2 (hLL2) were described recently [24]. Humanized LL2 was shown to bind to Raji cells with an equivalent afffinity to murine LL2. The anti-CD20 monoclonal antibody, 1F5, was obtained from the American Type Culture Collection (ATCC).

All final reagents were analyzed for purity by size-exclusion high-pressure liquid chromatography (HPLC) and sodium dodecyl sulfate/polyacrylamide gel electrophoresis under reducing and non-reducing conditions.

Isotopes and radiolabeling procedures

Iodine-125 was purchased as sodium iodide in 10 μM NaOH, iodine-131 in 0.1 M NaOH, and indium-111 as $^{111}InCl_3$ in 0.05 M HCl from NEN DuPont (N. Billerica, Mass.). Radioiodination to a specific activity of 10–14 mCi/mg was performed with $Na^{125}I$ or $Na^{131}I$, using a minor modification of the chloramine-T or iodogen method described previously [47]. This modification substituted sodium phosphate buffer for borate buffer in the labeling procedure. Antibodies labeled by these methods are referred to as non-residualizing conjugates.

For ^{111}indium labeling, isothiocyanate benzyldiethylenetriaminepentaacetic acid (SCN-Bz-DTPA) conjugates of LL2 IgG, $F(ab')_2$ and Fab′ were prepared as described previously [4, 19, 39]. Labeling conditions were established that permitted more than 95% ^{111}In-incorporation, thereby eliminating the need for further purification. However, excess DTPA was added at the end of the 1-h incubation period to scavenge any unbound radioactive metal. The final specific activity for ^{111}In-labeled antibodies was approximately 5 mCi/mg. Labeling with iodinated dilactitoltyramine (DLT) was described previously [45]. The specific activity of the radioiodinated antibodies prepared by the DLT method was 1–2 mCi/mg. Antibodies labeled by any one of these methods are referred to as residualizing conjugates.

All labeled antibodies were administered within 3 h of their preparation. The quality of each preparation was tested by instant thin-layer chromatography and HPLC on a Bio-Sil SEC-250 gel filtration column (300 × 7.8 mm; BioRad Laboratories, Richmond, Calif.), and detected with an in-line radioactivity detector (Beckman, Irvine, Calif.). No aggregates were detectable, and the amount of unbound radioisotope was less than 5% in each preparation. Immunoreactivity of the labeled LL2 IgG or fragments was evaluated by binding to an immunoadsorbent containing an anti-idiotype antibody (WN) [25]. These previous studies showed this method gave similar results to those obtained by a direct cell-binding assay. Radiolabeled LL2 binding to this immunoadsorbent for these studies was between 85%–95%.

Lymphoma cell line

RL cells were a generous gift from Dr. John Gribben, Dana-Farber Cancer Institute (Boston, Mass.). Cells were tested for reactivity with purified antibodies using an indirect immunofluorescent assay. Briefly, washed cells (100 μl; 5×10^6 cells/ml) were mixed with 25 μl antibody at 10 μg/ml and incubated at 4 °C/30 min. The cells were then washed with buffer followed by incubation with 100 μl fluorescein-conjugated goat anti-(mouse IgG). Analysis by flow cytometry showed a 32.1% reactivity with LL2 anti-CD22, and an 80.9% reactivity with the anti-CD20 mAb, 1F5.

Animal model and biodistribution studies

Studies were performed in 4- to 6-week-old female nu/nu mice purchased from Harlan (Hsd: athymic nude-nu; Indianapolis, Ind.) or from Taconic [Tac:Cr:(NCr)-nufBR; Germantown, N.Y.]. Since previous studies had indicated that these strains of nude mice were susceptible to a wide variability in blood clearance of intact murine IgG2a (i.e., but not with fragments), with altered enhanced spleen and liver accretion [40], each of these animals received a total of 200 μg unlabeled irrelevant murine IgG2aκ (UPC-10; Sigma Immunochemical, St. Louis, Mo.) added to the labeled antibody to reduce this effect. Humanized LL2 IgG1 also had an altered biodistribution in these strains of nude mice when compared to murine IgG1, but not as severe as that of murine IgG2a. It was subsequently discovered that, in the Swiss nude mice strain (Tac:N:NIHS-nufDF, Taconic), the radiolabeled murine LL2 IgG2a and humanized LL2 IgG1 had similar blood clearance and only a slightly enhanced splenic uptake in comparison to murine IgG1. Therefore, this strain did not require excess murine IgG2a to normalize the blood clearance and splenic and hepatic uptake. Furthermore, studies in the Swiss nude mice, bearing 0.5–5.0 g RL tumor xenografts, also demonstrated a similar biodistribution and tumor uptake between a protein dose of 2 μg and 200 μg radioiodinated murine LL2 IgG (data not shown). Thus, the minor difference between the protein dose administered for the various radiolabeled products tested in these studies (e.g., 1 μg and 9 μg, see below) was not considered a significant factor contributing to the outcome of these studies.

Animals were injected subcutaneously with approximately 1×10^7 cells in a 200-μl cell culture suspension. Tumor growth became visible after about 4–6 weeks in only about 40%–60% of the animals. Once tumors became visible, some would grow at a very rapid rate (e.g., from approximately 100 mg to more than 2 g within 7–10 days), whereas in other animals growth was minimal. Targeting studies were initiated when suffcient numbers of animals had visible tumor growth. Thus, tumor sizes were highly variable in all of these studies, with a majority of tumors in excess of 0.5 g. The average tumor sizes are given in the tables and figure legends for each study. Targeting studies were initiated when there were suffcient numbers of animals to include a minimum of three animals per assay time, but more often a total of four or five animals were studied at each interval.

Radiolabeled antibodies were injected intravenously into the tail vein. A total of approximately 8–20 μg radiolabeled antibody protein (i.e., 5–10 μCi ^{125}I; 25–90 μCi ^{111}In) was injected per animal. Animals were co-injected with a mixture of either ^{125}I- and ^{111}In-labeled or ^{125}I-DLT- and ^{131}I-chloramine-T- or iodogen-labeled mAb. Windows were set for each radionuclide, and the backscatter of the ^{111}In- or ^{131}I-window in the ^{125}I window was corrected. The mice were

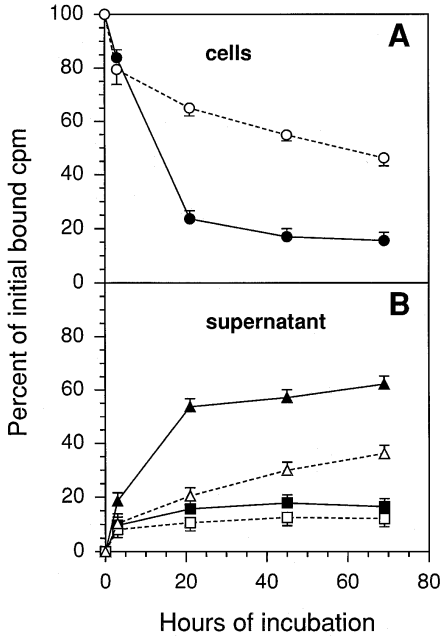


Fig. 1 A, B The evaluation of the processing of ^{125}I-labeled LL2 (anti-CD22) by RL B-cell lymphoma cells in vitro was carried out according to Hanna et al. [18]. Briefly, the antibody was labeled with either dilactitol-tyramine (DLT; ○, △, □) or by conventional chloramine-T iodination (●, ▲, ■). After an initial 2-h incubation at 37 °C, unbound mAb was washed away and, after replenishment of media, the fate of the bound mAb was followed by 37 °C for 3 days. A Retention of the radioactivity by the cells; B radioactivity released into the supernatant eithe intact (□, ■,) or degraded (△, ▲). Means ± standard deviations of triplicates are shown. The DLT LL2 was retained by the cells much longer than the conventional iodine lablel, which was degraded and excreted relatively rapidly

necropsied at 4 h, 1, 3, and 7 days for F(ab′)$_2$ and additionally at 14 days for IgG. Fab′ was studied at 1, 4, and 24 h. At the prescribed times, animals were anesthetized with sodium pentobarbital and then bled by cardiac puncture. After cervical dislocation, the animals were dissected. The amount of activity in the tumors and tissues (liver, spleen, kidney, lung, and blood) was determined by gamma scintillation counting, using an injection standard to account for physical decay to calculate the percentage of the injected dose per gram (%ID/g) and tumor/nontumor ratios. The localization ratio was defined as the %ID/g tumor of the residualizing radiolabeled antibody divided by the %ID/g of the non-residualizing antibody in the tumor. The localization index is defined as (%ID/g tumor residualizing/%ID/g blood residualizing)/(%ID/g tumor non-residualizing/%ID/g blood non-residualizing). The localization index normalizes the localization ratio for differences in the percentage of the residualizing versus non-residualizing radiolabeled antibody in the blood. The number of animals for each study is presented in the tables and figure legends. Comparisons of the residualizing and non-residualizing radiolabels were made by a two-tailed, paired *t*-test (95% confidence interval), since the two radiolabels were co-administered.

The radiation doses to the tissues were calculated as self-to-self doses from the biodistribution data according to the Medical Internal Radiation Dose scheme, modified to a mouse model, as published previously [39]. Absorbed doses projected for ^{90}Y-LL2 were based on ^{111}In-labeled LL2 biodistribution data, whereas absorbed doses for ^{131}I-LL2 were based on either ^{125}I- or ^{131}I-LL2 data.

Results

Use of residualizing labels of LL2 in vitro

We demonstrated previously that LL2 is internalized [42]. In contrast to antibodies that are radioiodinated by conventional means (i.e., non-residualizing), residualizing radiolabels, such as DLT, are lysosomally trapped after catabolism of the antibody to which they were originally conjugated, and thus should be retained in cells longer than when the non-residualizing method is used. Figure 1 shows the in vitro antibody retention results obtained with iodinated-DLT-LL2 in comparison to a conventional iodine radiolabel in the RL cell line. As expected, the DLT-LL2 was retained much longer by the RL cells, with a slow release of catabolic products. Similar results were obtained with other B-cell lymphoma cell lines, namely Raji, Daudi, and Ramos [20], and with ^{111}In-DTPA-LL2 (data not shown).

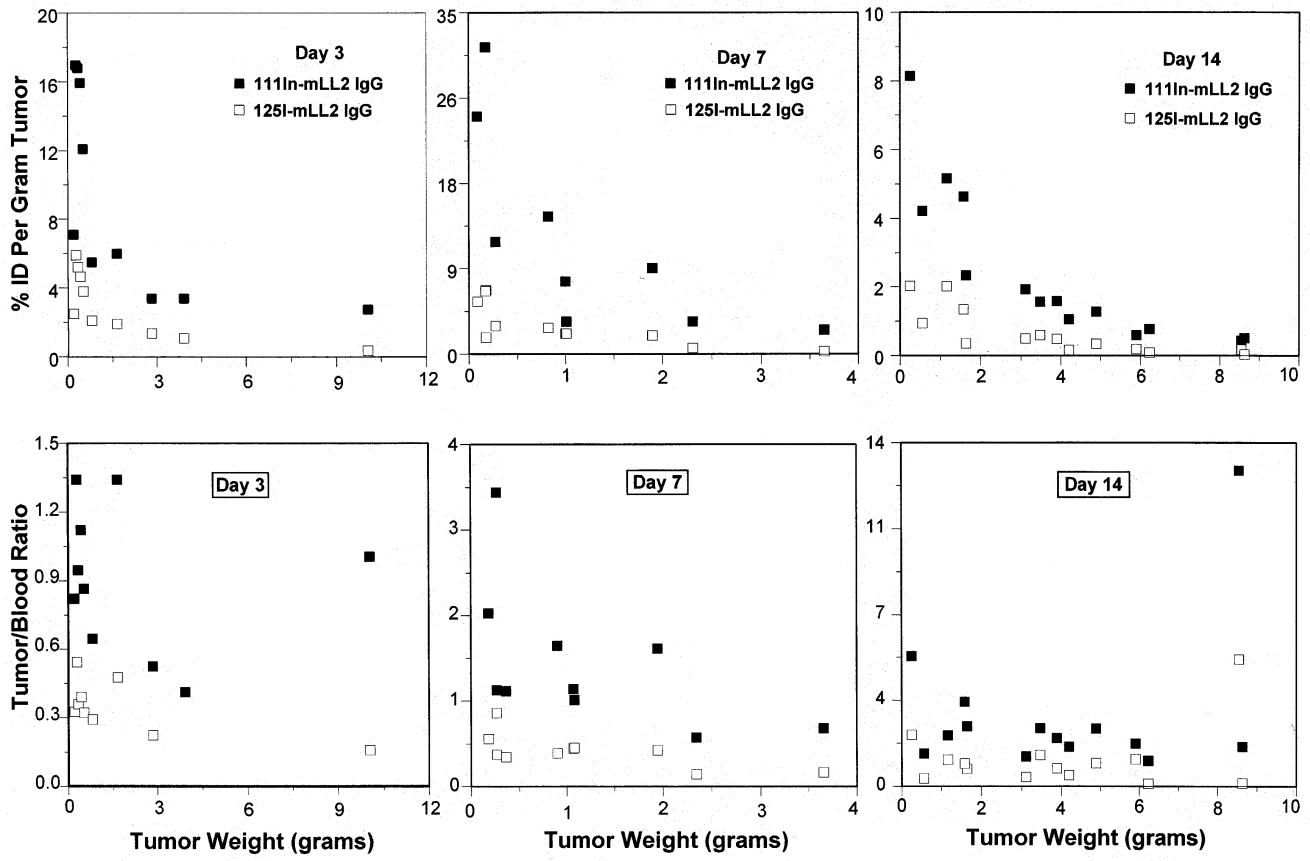
Biodistribution of iodinated and radioactive metal-linked murine LL2

Two separate studies were performed to compare the biodistribution of non-residualizing ^{125}I-LL2 IgG and ^{111}In-IgG. In each study, ^{125}I- and ^{111}In-labeled LL2 murine

Table 1 Comparison of ^{125}I- and ^{111}In-labeled murine LL2 IgG2a targeting in nude mice bearing RL human B-cell lymphoma xenografts. Taconic NIHS mice were injected with a mixture of 10 μCi ^{125}I- (1 μg) and 40 μCi (9 μg) ^{111}In-labeled antibody containing an additional 200 μg irrelevant murine IgG2a, UPC-10. The data combined from two separate studies are shown here. Values in parentheses are the numbers of animals. Tumor weights (g) were 3.35 ± 1.44 (range 1.3–4.5), 2.10 ± 2.45 (range 0.04–6.3), 2.11 ± 3.06 (range 0.20–10.1), 1.24 ± 1.16 (range 0.2–3.7), and 3.86 ± 2.74 (range 0.25–8.6) at the respective times shown below. *NS* not significant

Time after injection	Localization ratio		Localization index	
	^{111}In-LL2 IgG/^{125}I-LL2 IgG	*P*[a]	^{111}In-LL2 IgG/^{125}I-LL2 IgG	*P*[b]
4 h[c]	1.2 ± 0.2	NS (4)	1.1 ± 0.2	NS
1 day[c]	1.9 ± 0.6	0.03 (5)	1.7 ± 0.5	NS
3 days	3.4 ± 1.5	0.001 (10)	3.6 ± 1.3	< 0.001
7 days	4.3 ± 1.0	0.006 (10)	3.5 ± 0.7	0.001
14 days	4.4 ± 2.1	0.001 (15)	3.1 ± 1.5	0.002

[a] Comparison of percentage of the injected dose (%ID)/g of the ^{111}In-LL2 to ^{125}I-LL2 in the tumor



IgG2a (approximately 1.0 μg and 9.0 μg, respectively) were co-injected into RL-xenograft-bearing Taconic NIHS nude mice together with 0.2 mg irrelevant murine IgG2a per animal. Although a higher percentage of the injected dose per gram of tumor was seen in the first experiment, because of the smaller sized tumors in this study (see below), no significant difference was observed between the LI and LR these 2 studies, and thus these data were combined (Table 1). Figure 2 summarizes the individual paired observations for the percentage of the injected dose per gram of tumor and tumor/blood ratios for each of the radiolabels on days 3, 7 and 14.

At 4 h after injection, there was no significant difference between the tumor uptake of the non-residualizing and residualizing labeled LL2 (i.e., the localization ratio), but thereafter the accretion of the residualizing LL2 in the tumor was significantly higher than that of the non-residualizing LL2 (Table 1). An inverse relationship between tumor uptake and mass was defined that was more pronounced with the ^{111}In-labeled LL2 (Fig. 2). A similar relationship between tumor mass and tumor/blood ratio was seen on days 3 and 7, but by day 14, this relationship was not well-defined. Neither the localization ratio nor the localization index was significantly influenced by tumor size. No significant difference was found in the rate of blood clearance for the ^{125}I- or ^{111}In-LL2, but %ID/g blood was influenced by tumor size (i.e., the larger the tumor size, the lower the blood concentration). As expected, the percentage of the injected dose in the liver and spleen was

Fig. 2 Percentages of injected dose per gram of tumor (*upper panels*) and tumor/blood ratios (*lower panels*) are shown for the paired observations with ^{111}In-labeled (■) and non-residualizing ^{125}I-labeled (□) murine LL2 IgG in nude mice bearing RL B-cell lymphoma xenografts on days 3, 7, and 14. The scales for the various graphs differ

somewhat higher (approximately 1.2- to 2.0-fold) for the ^{111}In-labeled LL2 IgG. Despite higher liver accretion for ^{111}In-LL2 IgG, on days 3 and 7 the enhanced uptake of ^{111}In-LL2 IgG in the tumor produced significantly higher tumor/liver ratios (1.8 ± 0.6 versus 1.1 ± 0.4 on day 3 and 3.2 ± 0.8 versus 1.4 ± 0.5 on day 7 for the ^{111}In-LL2 IgG versus the ^{125}I-LL2 IgG, respectively; $P < 0.05$ for each). At all other assays times, the tumor/liver ratios for the two radiolabels were not significantly different; however, by day 14, the average tumor/liver ratio for the ^{125}I-LL2 had exceeded that for ^{111}In-LL2 IgG, albeit not significantly (3.1 ± 0.8 versus 2.5 ± 1.3, $P = 0.494$).

The calculated radiation doses to the larger tumors were between two- and fivefold lower than those obtained with the smaller tumors. On the basis of the biodistribution of the ^{111}In-LL2 IgG, radiation absorbed doses predicted that, if ^{90}Y-labeled LL2 were used, it would have a two- to 3.5-fold advantage over the non-residualizing iodinated form with respect to the tumor/blood radiation absorbed dose ratios. The tumor/liver ratios were comparable between the two isotopes in animals with large tumors, but 2.2-fold higher for ^{90}Y in animals with small tumors, because of higher antibody uptake in smaller tumors.

Table 2 Comparison of ^{125}I- and ^{111}In-labeled murine LL2 F(ab′)$_2$ and Fab′ targeting in nude mice bearing RL human B-cell lymphoma xenografts. NIHS nude mice were injected with a mixture of 30 μCi ^{125}I- (2.5 μg) and 60 μCi (12 μg) ^{111}In-labeled F(ab′)$_2$, or a mixture of 15 μCi (1.5 μg) ^{125}I-Fab′ with 40 μCi (8 μg) ^{111}In-Fab′. Values in parentheses are the numbers of animals. Tumor weight (g) for the F(ab′)$_2$ study was 2.92±3.57 (0.43–8.2), 0.79±0.62 (0.41–0.62), 0.37±0.30 (0.03–0.67), and 0.50±0.35 (0.2–1.0); for the Fab′ study, 0.80±0.39 (0.4–1.3), 0.86±0.39 (0.4–1.4), and 1.13±1.42 (0.3–3.64) at the respective times shown below. *NS* not significant

Time after injection	Localization ratio		Localization index	
	^{111}In-LL2/^{125}I-LL2	P[a]	^{111}In-LL2/^{125}I-LL2	P[b]
F(ab′)$_2$				
4 hours	0.9±0.1	NS (4)	0.9±0.1	NS
1 day	1.8±0.2	0.002 (4)	1.3±0.1	0.008
3 days	4.4±1.3	0.003 (4)	1.8±0.6	0.050
7 days	4.0±1.6	0.017 (4)	4.6±2.0	0.017
Fab′				
1 hour	1.6±0.1	0.002 (3)	0.7±0.05	0.002
4 hours	2.1±0.1	<0.001 (6)	1.1±0.04	0.006
1 day	6.6±0.7	0.008 (5)	2.9±0.4	0.001

[a] Comparison of %ID/g of the ^{111}In-LL2 to ^{125}I-LL2 in the tumor
[b] Comparison of tumor/blood ratio of ^{111}In-LL2 to that obtained with ^{125}I-LL2

Biodistribution of iodinated and radioactive metal-linked murine LL2 fragments

At comparable tumor sizes, the %ID/g uptake in the tumor was lower with F(ab′)$_2$ and Fab′ fragments than with IgG and, consistent with the earlier findings, the %ID/g in the tumors was higher for radioactive-metal-labeled F(ab′)$_2$ and Fab′ than for the respective conventionally iodinated conjugates (Table 2). ^{111}In-LL2 Fab′ fragments had a similar tumor, liver, and spleen uptake to that of the bivalent fragments over the 1 day this was tested. However, Fab′ was cleared from the blood more quickly, resulting in a tumor/blood ratio of 6.6±1.6 within 1 day, whereas the tumor/blood ratio for the ^{111}In-LL2 F(ab′)$_2$ took 3 days to reach this same level (i.e., 6.1±1.3). The tumor/blood ratio for the ^{125}I-LL2 Fab′ on day 1 was only 2.7±1.0 and for the ^{125}I-LL2 F(ab′)$_2$ was 3.5±0.6 on day 3, giving the ^{111}In-labeled fragments an approximately 2- to 3-fold higher tumor/blood ratio compared the non-residualizing ^{125}I-LL2 fragments at these times (LLI, Table 2).

Although tumor uptake favored ^{111}In-labeled fragments, the significantly higher uptake in the other normal organs yielded more favorable tumor/nontumor ratios for the non-residualizing ^{125}I-LL2 fragments. This was most pronounced for the kidney uptake, where ^{111}In-LL2 F(ab′)$_2$ and Fab′ 1 day after injection was 29.2±4.5 and 72.4±8.3 %ID/g, respectively, which was nearly 70 times higher than the tumor/nontumor ratios of the radioiodinated fragments. Indeed, tumor/kidney ratios for the ^{111}In-labeled fragments never exceeded 0.5:1, whereas tumor/kidney ratios for the non-residualizing iodinated fragments was above 1.0 within 1 day. Liver uptake for the ^{111}In-labeled fragments also resulted in 4- to 10-times higher tumor/liver ratios for the ^{125}I-labeled fragments.

Radiation dose estimates from the ^{111}In-LL2 biodistribution predicted that ^{90}Y-LL2 F(ab′)$_2$ would deliver 4.6-fold higher doses/mCi than the ^{131}I-LL2 F(ab′)$_2$, but when corrected for blood doses, only a 1.3-fold dose advantage was achieved. ^{90}Y-LL2 Fab′ was predicted to have a 2.1-fold tumor/blood dose advantage over ^{131}I-LL2 Fab′, but this advantage was overshadowed by renal doses of over 150 times that of ^{131}I-Fab′.

Residualizing forms of iodine (DLT)

Although proteins that are directly radioiodinated by conventional means (e.g., chloramine-T or iodogen) will yield products that, upon catabolism, will release iodotyrosine, radioiodination can be performed with derivatives that remain internalized even after catabolism. We have shown that DLT-conjugated iodine, when coupled to antibodies, produces residualizing iodinated products [45]. Iodinated DLT conjugates of LL2 IgG were therefore also tested. Except for the slightly faster blood clearance of the ^{125}I-DLT-LL2 than the ^{131}I-LL2 over the first 3 days, most of the other normal tissues had an identical concentration of each radiolabel (data not shown). Figure 3 shows the %ID/g in the tumor and tumor/blood ratios for the two radiolabels. The percentage uptake in the tumor for the DLT-LL2 was similar to that observed for the ^{111}In-LL2 IgG, and followed a similar inverse relationship according to tumor size. At all assay times, significantly higher localization and indices were seen for LL2 labeled by the residualizing DLT-LL2 IgG (Table 3). Dosimetry from these biodistribution studies revealed a 3.5-fold higher dose delivered to the tumor for the ^{131}I-DLT-LL2 IgG compared to non-residualizing ^{131}I-LL2 IgG. Tumor/blood absorbed dose ratios favored the DLT by 5:1.

Residualizing versus released radiolabels of humanized LL2 IgG

Figure 4 shows the results of a paired-radiolabel biodistribution study of non-residualizing ^{125}I-hLL2 and ^{131}I-DLT-hLL2, as well as a separate study using ^{111}In-labeled hLL2. The humanized and murine forms of LL2 IgG had similar biodistribution properties and tumor uptake. Thus, the same

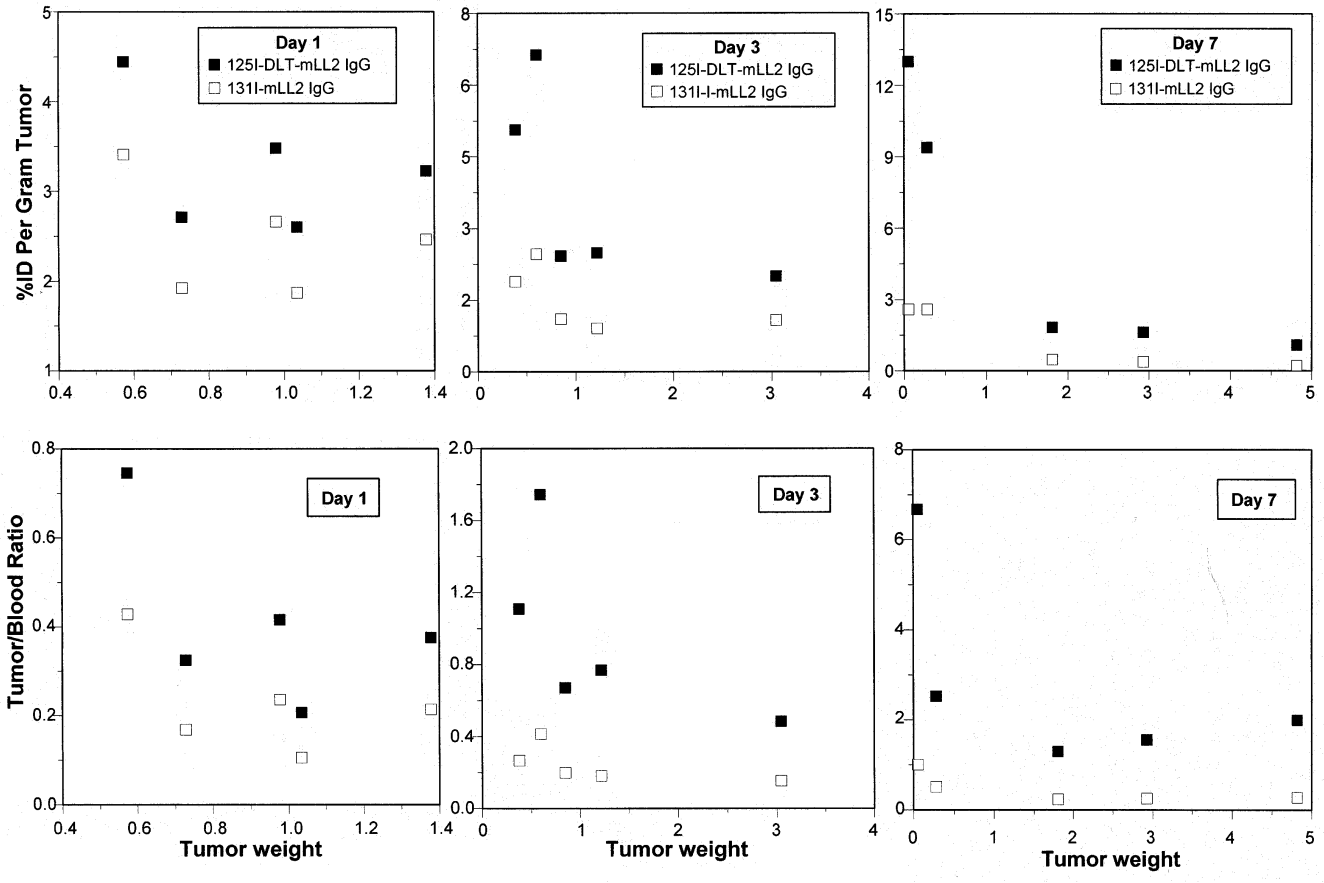


Fig. 3 Percentage of the injected dose per gram of tumor (*upper panel*) and tumor/blood ratios (*lower panel*) are shown for the paired observations with residualizing ^{125}I-DLT-labeled (■) and non-residualizing ^{131}I-labeled murine LL2 IgG (□) in nude mice bearing RL B-cell lymphoma xenografts on days 1, 3, and 7. The scales for the various graphs differ.

advantage of the residualizing over the non-residualizing label was observed in both of these studies. In the paired analysis, tumor uptake was significatly higher with the residualizing ^{131}I-DLT-hLL2 than with the non-residualizing hLL2 (Table 3). As shown in Fig. 4, the uptake (%ID/g)and tumor/blood ratios for the ^{111}In-labeled hLL2 were similar to those achieved by the DLT-hLL2 within the same range of tumor sizes. However, because of the wide range of tumor sizes and small number of samples, a statistical comparison of the ^{111}In-hLL2 to the two other radioiodinated hLL2 agents was not performed.

The dosimetry for the humanized LL2 compared favorably to that of the murine form of LL2, and a similar advantage of the residualizing label was observed. For example, in comparison to the non-residualizing ^{131}I-hLL2, the radiation dose to the tumor was 3.3- and 4.7-fold higher for the ^{131}I-DLT-hLL2 and ^{90}Y-hLL2, respectively. Compared to non-residualizing ^{131}I-hLL2, the tumor/blood absorbed dose ratio was 3.6- and 2.3-fold higher for the ^{131}I-DLT-hLL2 and ^{90}Y-hLL2, respectively.

Comparison of the anti-CD20 antibody 1F5 and anti-CD22 LL2

A paired-radiolabel study was performed to compare the targeting of an anti-CD20 antibody to that of LL2 (anti-CD-22). By flow cytometry, the RL cells expressed more CD20 than CD22, so better targeting with the CD20 antibody seemed possible. However, 1F5, which is reportedly a non-internalizing antibody [31], had similar tumor uptake to that seen with non-residualizing ^{125}I-LL2 (Fig. 5).

Flow-cytometry studies were performed on cells used to implant these tumors, as well as on a cell suspension prepared from 1- to 2.5-g tumors (6–8 weeks of tumor growth). There was no difference in the expression of either CD20 or CD22 in the cells taken from tissue culture or the xenograft. Histological examination of the lymphoma xenografts revealed relatively poor vascularization and a high degree of necrosis. Thus, physiological factors, in accordance with the observed strong dependence of tumor uptake upon tumor size, may affect the cells accessibility to the antibody.

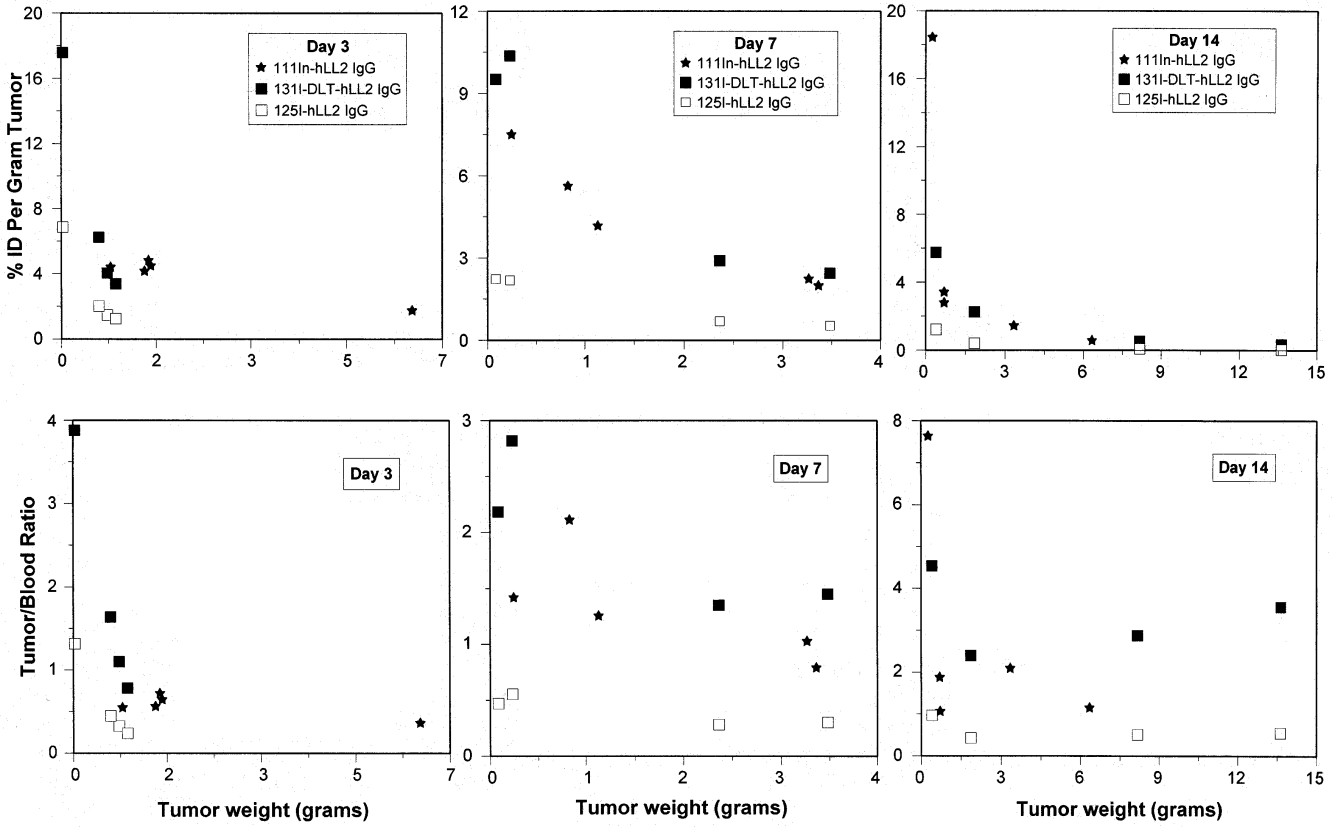
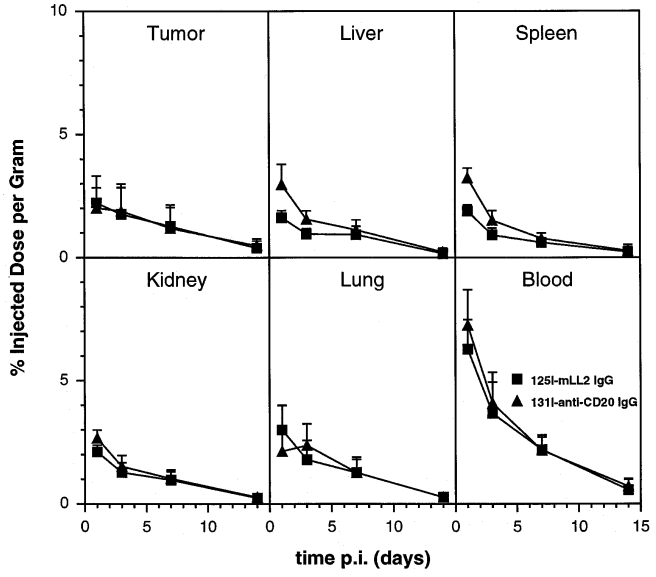


Fig. 4 Percentage of the injected dose per gram of tumor (*upper panels*) and tumor/blood ratios (*lower panels*) are shown for the paired observations with residualizing ^{111}In-labeled (★), ^{131}I-DLT-labeled (■), and non-residualizing ^{125}I-labeled humanized LL2 IgG (□) in nude mice bearing RL, B-cell lymphoma xenografts on days 3, 7, and 14. Animals were co-injected with the ^{131}I-DLT-hLL2 and ^{125}I-hLL2, whereas the ^{111}In-labeled hLL2 was injected in a separate group of NIHS animals. These animals received 40 μCi (9 μg) ^{111}In-diethylene-trianinepentaacetate-hLL2 IgG without any additional unlabeled IgG. The scales for the various graphs differ. Refer to Table 3 for further details

Fig. 5 Biodistribution of non-residualizing, radioiodinated murine LL2 (anti-CD22) compared to 1F5 (anti-CD20) in RL-bearing nude mice (tumor sizes: 0.43±0.18 g, 1.79±1.05 g, 0.24±0.12 g, and 0.38±0.11 g at 1, 3, 7, and 14 days respectively)



Discussion

Radioimmunotherapy of hematological malignancies, especially B-cell, non-Hodgkin's lymphoma, appears to be a potentially new treatment modality [10, 16, 34]. Until now, ^{131}I conjugates of pan-B-cell antibodies (anti-CD20, anti-CD37, etc.) have mostly been used for this purpose [34]. Our group has been studying an anti-CD22 antibody, LL2. Using this mAb, high sensitivities in the detection of B-cell, NHL have been reported, such as with ^{99m}Tc-labeled Fab′ fragments of LL2 [1, 2, 8, 28]. In addition, partial and complete remissions in the treatment with ^{131}I-LL2 IgG and its F(ab′)$_2$ fragment have been reported [18, 21]. Shih et al. [42] first showed the rapid internalization of LL2 after it had bound to the CD22 molecule on the cell membrane, with subsequent metabolic degradation and release of low-molecular-mass compounds (most likely monoiodotyrosine according to Geissler et al. [14]). Other studies have reported the potential advantage of using residualizing radiolabels with antibodies that internalize [20, 35, 36,

Table 3 Comparison of residualizing ^{125}I-dilactitol-tyramine(DLT)-labeled to non-residualizing ^{131}I-labeled murine and humanized LL2 IgG targeting in nude mice bearing RL human B-cell lymphoma xenografts. Harlan mice were injected with a mixture of 10 μCi ^{125}I-DLT-murine LL2 (1 μg) and 25 μCi (2 μg) ^{131}I-labeled murine LL2 containing an additional 200 μg irrelevant murine IgG2a, UPC-10. Tumor weights (g) were 0.94 ± 0.31 (0.6–1.4), 1.22 ± 1.07 (0.4–3.0), and 3.18 ± 1.52 (1.8–4.8) at their respective times. For the humanized LL2, NIHS mice were injected with a mixture of 20 μCi (21 μg) ^{131}I-DLT-hLL2 IgG and 10 μCi (1 μg) ^{125}I-hLL2 IgG without additional IgG. Tumor weights (g) were 1.36 ± 1.24 (0.09–3.0), 1.091 ± 0.86 (0.2–2.1), 0.81 ± 0.14 (0.7–1.0), 1.54 ± 1.66 (0.08–3.5), 6.0 ± 6.1 (0.4–13.6) at the respective times. Numbers of animals shown in parentheses

Time after injection	Localization ratio		Localization index	
	I-DLT-LL2 IgG/I-LL2 IgG	P[a]	I-DLT-LL2 IgG/I-LL2 IgG	P[b]
Murine				
1 day	1.3 ± 0.05	< 0.001 (5)	1.8 ± 0.1	0.007
3 days	2.4 ± 0.4	0.022 (5)	3.8 ± 0.5	0.016
7 days	6.3 ± 0.9	0.001 (3)	4.4 ± 0.5	0.019
Humanized				
4 h	1.1 ± 0.06	0.037 (4)	1.1 ± 0.05	0.03
1 day	1.6 ± 0.2	0.025 (4)	1.7 ± 0.2	0.023
3 days	2.8 ± 0.2	0.043 (4)	3.3 ± 0.3	0.047
7 days	4.4 ± 0.3	0.032 (3)	4.8 ± 0.2	0.012
14 days	5.8 ± 1.4	0.032 (4)	5.6 ± 0.7	0.005

[a] Comparison of %ID/g of the I-DLT-LL2 to I-LL2 in the tumor
[b] Comparison of tumor/blood ratio of I-DLT-LL2 to that same ratio obtained with I-LL2

45]. Although most of this evidence has been obtained in vitro, recent in vivo studies by Stein et al. [45] and Reist et al. [36] have shown that residualizing radiolabels have an advantage over non-residualizing ones for internalizing antibodies. Therefore, the major purpose of these studies was to determine whether a similar advantage could be achieved with LL2 in human lymphoma xenografts growing in nude mice.

Our in vivo study establishes an advantage of radioactive metals, as well as of residualizing forms of the iodine label (such as DLT), in the RL subcutaneous lymphoma model, when compared to conventionally (non-residualizing, iodogen or chloramine-T) iodinated LL2. Significantly higher tumor uptake for the residualizing radiolabels was detected as early as 1 day after injection of radiolabeled IgG, but it was more pronounced by day 3. Fab′ fragments showed an advantage as early as 1 h after injection and, by 24 h, the localization ratio for the residualizing Fab′ was comparable to the IgG obtained within 3 days. Although the percentage injected dose per gram was inversely related to tumor size, the localization ratio and index were not size-dependent. Thus, tumors from as small as 0.03 g to as large as 5–14 g showed similar differences in the percentage uptake of the residualizing and non-residualizing radiolabels. Owing to the dependence of the percentage uptake in the tumor on size, the radiation doses absorbed were 3- to 8-fold higher with ^{90}Y than with the non-residualizing ^{131}I label. This enhanced retention of tumor uptake for the residualizing radiolabels resulted in an overall average of 2- to 3.5-fold higher absorbed dose to the tumor compared to the blood, strongly suggesting that improved therapeutic benefit may be obtained when using ^{90}Y-LL2 over conventionally radioiodinated LL2. No major differences were found between the murine and the humanized, CDR-grafted form of LL2, with respect to tumor targeting and tissue distribution. This is consistent with preliminary clinical results that have suggested similar biodistribution and tumor targeting with conventionally radioiodinated humanized LL2 in comparison to the murine LL2 [21]. Thus, clinical trials with ^{111}In/^{90}Y-labeled hLL2 are in progress that include imaging studies to compare the dosimetry for ^{131}I- and ^{90}Y-hLL2 IgG (using ^{111}In-hLL2 as a surrogate for ^{90}Y-hLL2).

The most favorable dosimetric results were observed for a residualizing form of radioiodine (DLT), where a fivefold higher tumor/blood radiation dose was found for the whole IgG. This is probably due to a combination of long retention of the DLT in the tumor tissue, a comparably fast clearance of the radiolabel from other tissues, and a long physical half-life of ^{131}I. Unfortunately, the labeling effficiencies of DLT (< 10%) are not yet suitable for a larger-scale clinical application [45].

Although residualizing conjugates may optimize tumor accretion for an internalizing antibody, careful consideration must also be given to the biodistribution of the conjugates in normal tissues to determine the optimal conjugate. In this model system, most tumor/nontumor ratios were consistently higher with the residualizing conjugate. However, there were some instances where tumor/nontumor ratios for the residualizing conjugate were not higher than for the non-residualizing conjugate. For example, the tumor/liver absorbed dose ratio in animals with large RL xenografts was similar for ^{90}Y- and ^{131}I-LL2 IgG. Owing to very high renal uptake, the tumor/kidney ratio was substantially higher for the ^{131}I-LL2 Fab′ than with ^{90}Y-LL2 Fab′. Although methodology has been developed to reduce renal accretion of antibody fragments radiolabeled with residualizing radioactive metals [4], it is uncertain whether it will be sufficient to provide greater opportunity for using radioactive-metal-labeled antibody fragments therapeutically [3, 6]. Since the degree to which a residualizing conjugate will optimize tumor accre-

tion will vary according to how far the physiology of the tumor allows adequate access to the individual tumor cells, the rate of internalization and fate of the radiolabel after intracellular catabolism, and the specificity of the antibody and stability of the conjugate, which will affect tumor and normal tissue uptake, it is not certain that a residualizing conjugate will be the optimal radiolabel for all model systems where the antibody is known to internalize.

Unlike in vitro studies, where a detailed analysis of the fate of antibodies bound to tumor cell surfaces can be examined readily, an identical analysis is more difficult in vivo. Thus, the in vivo finding of a higher tumor accretion than with a non-residualizing conjugate is not in itself direct proof that the mechanism responsible for this phenomenon is internalization of the antibody within the tumor, with retention of the radiolabel. Several reports have described higher tumor uptake with radioactive-metal-labeled antibody conjugates than with iodinated antibody [31, 46]. Although a number of factors can explain higher tumor accretion with a radioactive-metal-labeled than with radioiodinated antibody, including the possibility of dehalogenation of conventionally radioiodinated antibodies, these observations may also be attributed in part to an unappreciated internalization of the antibody. Our in vitro studies with many different cell lines suggest that virtually any antibody that is capable of binding to the cell surface can internalize, but the rate of internalization can vary widely, from just a few minutes to several hours or even days [20, 23, 43, 45]. Thus, without an in vivo analysis similar to that obtained in vitro, the mechanism responsible for the observation that the residualizing conjugate yields higher tumor accretion than the non-residualizing conjugate can only be inferred.

Our original hypothesis considered the possibility that the percentage uptake in the tumor for a residualizing conjugate may increase over time, given a continuous supply of antibody entering the tumor and if newly synthesized antigen is expressed on the cell surface, as was shown in vitro [42]. Failure to demonstrate this phenomenon in the in vivo studies could be related to the rapid growth of these tumors, which reduces the percentage of the injected dose when expressed on a weight basis. As expected, the relationship between tumor mass and tumor uptake followed an inverse relationship, similar to that described in other tumor models [26, 38]. When the percentage injected dose is considered as a function of the total tumor mass, the amount of ^{111}In- and DLT-LL2 in the tumors remained constant, whereas the non-residualizing ^{125}I-LL2 decreased over time. Larger tumors also have more necrosis, and histological examination of these tumors revealed a relatively poor vasculature. Similar observations were made by Schmid et al. [37]. Thus, physiological properties of lymphoma xenografts are the most likely cause of the relatively low accretion of anti-lymphoma antibodies in these studies. This may also explain why the anti-CD20 antibody targeted identically to the non-residualizing radioiodinated LL2, even though the CD20 antigen is more highly expressed on this cell line. This issue is being investigated.

Summarizing, for rapidly internalizing antibodies, such as LL2, intracellularly retained radiolabels may have a 2- to 3-fold advantage over released ones. This suggests the use of radioactive metals (indium or technetium) for radioimmunodetection, and either yttrium or other residualizing labels for therapeutic applications. The targeting capability of anti-CD20 and anti-CD22 monoclonal antibodies was similar, at least in the lymphoma model investigated. Studies comparing the targeting and dosimetry of indium-labeled with results for iodinated humanized LL2 in patients, as well as a comparison of the therapeutic efficacy of ^{90}Y- and ^{131}I-labeled hLL2, are in progress.

Acknowledgements The expert technical assistance of R. Aninipot is gratefully acknowledged. Furthermore, we thank M. Przybylowski, D. Varga and P. Andrews for preparations of the antibodies, radiolabeling, and quality control, and S. Chen for tissue-culture assistance. We are also grateful for the expertise of K. Sides for preparation of the histological specimens. We are indebted to Dr. Susan Thorpe, University of South Carolina, for providing DLT.

References

1. Baum RP, Niesen A, Hertel A, Adams S, Kojouharoff G, Goldenberg DM, Hör G (1994) Initial clinical results with technetium-^{99m}-labeled LL2 monoclonal antibody fragment in the radioimmunodetection of B-cell lymphoma. Cancer [Supp]73: 896–899
2. Becker W, Behr T, Cumme F, Rößler W, Wendler J, Kern P, Gramatzki M, Kalden JR, Goldenberg DM, Wolf F (1995) Ga-67 citrate versus Tc-^{99m}-labelled LL2-Fab′-fragments (anti-CD22) in the staging of B-cell-non-Hodgkin's lymphoma (NHL). Cancer Res 55 [Supp]: 5771–5773
3. Behr TM, Goldenberg DM (1996) Improved prospects for cancer therapy with radiolabeled antibody fragments and peptides? J Nucl Med 37: 834–836
4. Behr TM, Sharkey RM, Juweid ME, Blumenthal RD, Dunn RM, Griffiths GL, Bair HJ, Wolf FG, Becker WS, Goldenberg DM (1995) Reduction of the renal uptake of radiolabeled monoclonal antibody fragments by cationic amino acids and their derivatives. CancerRes 55: 3825–3834
5. Behr TM, Sharkey RM, Juweid ME, Aninipot R, Mattes MJ, Stein R, Griffiths GL, Goldenberg DM (1995) Residualizing (indium, yttrium) versus released (iodine) isotopes in radioimmunodetection and therapy with internalizing antibodies (abstract 71). J Nucl Med 36: 20P
6. Behr TM, Sharkey RM, Blumenthal RD, Sgouros G, Dunn RM, Alisauskus R, Griffiths G, Goldenberg DM (1996) Overcoming nephrotoxicity in therapy with radiometal-conjugated fragments and peptides: improvement of their therapeutic efficacy. (abstract 374) J Nucl Med 37: 96P
7. Behr TM, Sharkey RM, Juweid ME, Dunn RM, Vagg RC, Swayne LC, Siegel JA, Goldenberg DM (1997) Phase I/II clinical radioimmunotherapy with an ^{131}I-labeled anti-CEA murine IgG monoclonal antibody. J Nucl Med (in press)
8. Blend MJ, Hyun H, Kozloff M, Levi H, Mills GQ, Gasparini M, Buraggi G, Hughes L, Pinsky CM, and Goldenberg DM (1995) Improved staging of B-cell non-Hodgkin's lymphoma patients with ^{99m}Tc-labeled LL2 monoclonal antibody fragment. Cancer Res 55: 5764s–5770s
9. Carrasquillo JA, Kramer B, Fleisher T, Perentesis P, Boland CJ, Foss F, Rotman M, Reynolds RC, Mulshine JL, Camera L, Frincke J, Lollo C, Neumann RD, Larson SM, Raubitschek A (1991) ln-111 versus Y-90 T101 biodistribution in patients with hematopoietic malignancies. J Nucl Med 32: 970
10. Cox JD (1994) Lymphomas and leukemia. In: Cox JD (ed) Moss' radiation oncology - rationale, technique, results, 7th edn. Mosby - Year Book, St Louis, pp 795–826

11. DeNardo SJ, DeNardo GL, O'Grady LF, Macey DJ, Mills SL, Epstein AL, Peng J-S, McGahan JP (1987) Treatment of a patient with B-cell lymphoma by I-131 Lym-1 monoclonal antibodies. Int J Biol Markers 2: 49–53
12. Duncan JR, Welch MJ (1993) Intracellular metabolsim of indium-111 DTPA labeled receptor targeted proteins. J Nucl Med 34: 1728–1738
13. Franano FN, Edwards WB, Welch MJ, Duncan JR (1994) Metabolism of receptor targeted 111In-DTPA-glycoproteins: identification of ^{111}In-DTPA-ε-lysine as the primary metabolic and secretory product. Nucl Med Biol 21: 1023–1034
14. Geissler F, Anderson SK, Venkatesan P, Press O (1992). Intracellular catabolism of radiolabeled anti-μ antibodies by malignant B-cells. Cancer Res 52: 2907–2915
15. Goldenberg DM (ed) (1990) Cancer imaging with radiolabeled antibodies. Kluwer, Boston, Mass
16. Goldenberg DM (ed) (1995) Cancer therapy with radiolabeled antibodies. CRC, Boca Raton, Fla.
17. Goldenberg DM, DeLand F, Kim E, Bennett S, Primus FJ, van Nagell JR, Estes N, De Simone P, Rayburn P (1978) Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N Engl J Med 298: 1384–1388
18. Goldenberg DM, Horowitz JA, Sharkey RM, Hall TC, Murthy S, Goldenberg H, Lee RE, Stein R, Siegel JA, Izon DO, Burger K, Swayne LC, Belisle E, Hansen HJ, Pinsky CM (1991) Targeting, dosimetry and radioimmunotherapy of B-cell lymphomas with iodine-^{131}-labeled LL2 monoclonal antibody. J Clin Oncol 9: 548–564
19. Griffiths GL (1995) Radiochemistry of therapeutic radionuclides. In: Goldenberg DM (ed) Cancer therapy with radiolabeled antibodies. CRC, Boca Raton, Fla.
20. Hanna R, Ong GL, Mattes MJ (1996) Processing of antibodies bound to B-cell lymphomas and other hematological malignancies. Cancer Res 56: 3062–3068
21. Juweid M, Sharkey RM, Markowitz A, Behr T, Swayne LC, Hansen HJ, Shevitz J, Leung S, Rubin AD, Herskovic T, Hanley D, Goldenberg DM (1995) Treatment of non-Hodgkin's lymphoma with radiolabeled murine, chimeric, or humanized LL2, an anti-CD22 monoclonal antibody. Cancer Res 55: 5899s–5907s
22. Kaminski MS, Zasadny KR, Francis IR, Milik AW, Ross CW, Moon SD, Crawford SM, Burgess JM, Petry NA, Butchko GM, Glenn SD, Wahl RL (1993) Radioimmunotherapy of B-cell lymphoma with [^{131}I]anti-B1 (anti-CD20) antibody. N Engl J Med 329: 459–465
23. Kyriakos RJ, Shih LB, Ong GL, Patel K, Goldenberg DM, Mattes MJ (1992) The fate of antibodies bound to the surface of tumor cells in vitro. Cancer Res 52: 835–842
24. Leung S-O, Goldenberg DM, Dion AS, Pellegrini MC, Shevitz J, Shih LB, Hansen HJ (1995) Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific leukemia/lymphoma antibody, LL2. Mol Immunol 32: 1413–1427
25. Losman MJ, Leung SO, Shevitz J, Shukla R, Haraga L, Shih LB, Goldenberg DM, Hansen HJ (1995) Development and evaluation of the specificity of a rat monoclonal anti-ld antibody, WN, to an anti-B-cell lymphoma monoclonal antibody, murine LL2. Cancer Res 55: 5978–5982
26. Moshakis V, McIlhinney RAJ, Raghaven D, Neville AM (1981) Localization of human tumor xenografts after i.v. administration of radiolabelled monoclonal antibodies. Br J Cancer 44: 91–99
27. Motta-Hennessy C, Sharkey RM, Goldenberg DM (1990) Metabolism of indium-111-labeled murine monoclonal antibody in tumor and normal tissue of the athymic mouse. J Nucl Med 31: 1510–1519
28. Murthy S, Sharkey RM, Goldenberg DM, Lee RE, Pinsky CM, Hansen HJ, Burger K, Swayne LC (1992) Lymphoma imaging with a new technetium-^{99m} labelled antibody, LL2. Eur J Nucl Med 19: 394–401
29. Naruki Y, Carrasquillo JA, Reynolds JC, Maloney PJ, Frincke JM, Neumann RD, Larson SM (1990) Differential cellular catabolsim of ^{111}In, 90Y and ^{125}I radiolabeled anti-CD5 monoclonal antibody. Nucl Med Biol 17: 201–207
30. Pawlak-Byczkowska EJ, Hansen HJ, Dion AS, Goldenberg DM (1989) Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma. Cancer Res 49: 4568–4577
31. Pimm MV, Perkins AC, Baldwin RW (1985) Differences in tumour and normal tissue concentrations of iodine- and indium-labelled monoclonal antibody. II. Biodistribution studies in mice and human tumour xenografts. Eur J Nucl Med 11: 300–304
32. Press OW, FarrAG, Borroz Kl, Anderson SK, Martin PJ (1989) Endocytosis and degradation of monoclonal antibodies targeting human B-cell malignancies. Cancer Res 49: 4906–4912
33. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Poster B, Matthews DC, Gooley T, Bernstein ID (1995) Phase 11 trial of ^{131}I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphoma. Lancet 346: 336–340
34. Press OW, Eary JF, Appelbaum FR, Bernstein ID (1995) Treatment of relapsed B cell lymphomas with high dose radioimmunotherapy and bone marrow transplantation. In: Goldenberg DM (ed) Cancer therapy with radiolabeled antibodies. CRC, Boca Raton, Fla, p 229
35. Press OW, Shan D, Howell-Clark J, Eary J, Appelbaum FR, Matthews D, King DJ, Haines AMR, Hamann P, Hinman L, Shochat D, Bernstein ID (1996) Comparative metabolism and retention of iodine-125, yttrium-90, and indium-111 radioimmunoconjugates by cancer cells. Cancer Res 56: 2123–2129
36. Reist CJ, Archer GE, Kurpad SN, Wikstrand CJ, Vaidyanathan G, Willingham MC, Moscatello DK, Wong AJ, Bigner DD, Zalutsky MR (1995) Tumor-specific anti-epidermal growth factor receptor variant III monoclonal antibodies: use of the tyramine-cellobiose radioiodination method enhances cellular retention and uptake in tumor xenografts. Cancer Res 55: 4375–43821
37. Schmid J, Möller P, Moldenhauer G, Dörken B, Bihl H, Matzku S (1993) Monoclonal antibody uptake in B-cell lymphomas: experimental studies in nude mouse xenografts. Cancer Immunol Immunother 36: 274–280
38. Sharkey RM, Primus FJ, Goldenberg DM (1987) Antibody protein dose and radioimmunodetection of GW-39 human colon tumor xenografts. Int J Cancer 39: 611–617
39. Sharkey RM, Motta-Hennessy C, Pawlyk D, Siegel JA, Goldenberg DM (1990) Biodistribution and radiation dose estimates for yttrium- and iodine-labeled monoclonal antibodies IgG and fragments in nude mice bearing human colonic tumor xenografts. Cancer Res 50: 2330–2336
40. Sharkey RM, Natale A, Goldenberg DM, Mattes MJ (1991) Rapid blood clearance of immunoglobulin G2a in nude mice. Cancer Res 51: 3102–3107
41. Sharkey RM, BehrT, Mattes MJ, Stein R, Shih LB, Goldenberg DM (1995) Enhanced retention of a radiometal-labeled internalizing antibody for cancer radioimmunotherapy (abstract 2911). Proc Am Assoc Cancer Res 36: 489
42. Shih LB, Lu HHZ, Xuan H, Goldenberg DM (1994) Internalization and intracellular processing of an anti-B-cell lymphoma monoclonal antibody, LL2. Int J Cancer 56: 538–545
43. Shih LB, Thorpe SR, Griffiths GL, Diril H, Ong GL, Hansen HJ, Goldenberg DM, Mattes MJ (1994) The processing and fate of antibodies and their radiolabels bound to the surface of tumor cells in vitro: a comparison of nine radiolabels. J Nucl Med 35: 899–908
44. Stein R, Belisle E, Hansen HJ, Goldenberg DM (1993) Epitope specificity of the anti-B cell lymphoma monoclonal antibody, LL2. Cancer Immunol Immunother 37: 293–298
45. Stein R, Goldenberg DM, Thorpe SR, Basu A, Mattes MJ (1995) Effects of radiolabeling monoclonal antibodies with a residualizing iodine radiolabel on the accretion of radioisotope in tumors. Cancer Res 55: 3132–3139
46. Stern P, Hagan P, Halpern S, Chen A, David G, Adams T, Desmond W, Brautigam K, Royston I (1982) In: Mitchell MS, Oettgen HF (eds) Hybridomas in cancer diagnosis and treatment. Raven Press, New York, p 245
47. Weadock KS, Sharkey RM, Varga DC, Goldenberg DM (1990) Evaluation of a remote radioiodination system for radioimmunotherapy. J Nucl Med 31: 508–511