Eur J Cancer Clin Oncol, Vol. 20, No. 4, pp. 515-524, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Quantitative Evaluation of the Localization of a Monoclonal Antibody (791T/36) in Human Osteogenic Sarcoma Xenografts*

M. V. PIMM and R. W. BALDWIN
Cancer Research Campaign Laboratories, University of Nottingham, Nottingham NG7 2RD, U.K.

Abstract—*Monoclonal antibody 791T/36 (IgG2b) directed against human osteogenic sarcoma-associated antigens has been used to examine quantitative aspects of* in vivo *tumour localization into osteogenic sarcoma xenografts. With small doses of antibody (<40 μg) the extent of localization consistently correlated with xenograft size, an average of 36% of the total body burden of radiolabelled antibody being present/g tumour after 2 or 3 days. Parallel studies with labelled control IgG2b showed a similar correlation, but only 7% of the total body radiolabel was localized/g tumour. With the 791T/36 antibody, the tumour:blood ratio did not increase with tumour size except with the largest xenografts (800–900 mg), where up to 30–40% of the total body radioactivity was localized within the xenografts and this was sufficient to produce a measurable decrease in its blood level. There was also a significant correlation between the dose of injected radiolabelled antibody and the amount localized in xenografts but only up to a dose of about 100 μg, beyond which the amount localized was not directly proportional to the injected dose. The maximum level of radiolabelled antibody localized in xenografts was 70 μg antibody/g tumour tissue. It was not possible to displace radiolabelled antibody already localized in xenografts by systemic administration of a large dose of unlabelled antibody. The rate of localization of antibody was such that maximum uptake, measured as the absolute amount of localized antibody, was seen 2–4 days after injection. After this time the absolute amount of antibody in the tumours declined, but not as rapidly as the blood and whole body levels, so that the tumour:blood ratio continued to increase with time.*

INTRODUCTION

THE LOCALIZATION of antitumour monoclonal antibodies in human tumours developing as xenografts in immunodeprived mice has been reported [1–4] and these studies form the basis of a number of clinical trials on the localization of antibodies in primary and metastatic malignant tumours [5–10]. Antibody 'localization' in this context is generally interpreted as a greater uptake of radiolabelled antibody in malignant tissue than in normal organs when compared on a weight basis. In addition, it must be shown that this uptake is not due to non-specific accumulation of antibody at the tumour site, and this has been assessed in parallel studies with either labelled normal immunoglobulins or an unrelated monoclonal antibody, preferably of the same isotype as the monoclonal antibody in question.

Previous studies with a mouse monoclonal antibody (791T/36) to a human osteogenic sarcoma line showed localization of radiolabelled antibody in xenografts of a range of human osteogenic sarcomas, including the original immunising cell line 791T [3]. There was a greater uptake of radiolabelled antibody into tumour than normal tissues or blood and control mouse immunoglobulin showed no such tumour localization. Clinically, localization of radiolabelled 791T/36 antibody was shown, by external imaging techniques, in a primary human osteogenic sarcoma [11] and in both primary and metastatic deposits of colorectal carcinoma [5]. In a therapeutic context, conjugates of this antibody and a number of

Accepted 19 October 1983.
*This work was supported by a grant from the Cancer Research Campaign, U.K.

anticancer and immunomodulating agents have been prepared and these are being evaluated for *in vitro* and *in vivo* therapeutic effectiveness. For example, conjugates of the 791T/36 monoclonal antibody with vindesine showed selective cytotoxicity towards 791T target cells, when compared with cells not expressing the 791T-defined antigen [12], and these conjugates can significantly retard 791T xenograft development [13].

In view of the potential importance of the 791T/36 antibody for diagnostic and therapeutic applications, further knowledge of the quantitative aspects of its tumour localization would be of value. These studies were carried out in mice with 791T xenografts using radiolabelled 791T/36 antibody and normal mouse immunoglobulin to assess the relationship between xenograft size and the extent of antibody deposition in the tumours. The relationship between the dose of antibody and its extent of tumour localization was also examined, together with the rate of antibody localization.

MATERIALS AND METHODS

Tumour cell line

The human osteogenic sarcoma cell line 791T was grown as a monolayer in Eagle's medium supplemented with 10% foetal calf serum. For *in vivo* injection cells were harvested with trypsin and washed in serum-free medium.

Mice and xenografts

CBA mice (Bantin and Kingman, Hull, U.K.) were thymectomized at 3–4 weeks of age and up to 20 weeks later received 9 Gy whole body γ-irradiation from a ^{60}Co source. The lethal effect of radiation was prevented by intraperitoneal injection of 200 mg/kg cytosine arabinoside (Cytosar, Upjohn, Sussex, U.K.) 2 days before irradiation [14]. Xenograft growth of the 791T osteogenic sarcoma was initiated by inoculation of 1×10^6 cells subcutaneously in the flank into mice irradiated not more than 5 weeks previously. Mice were maintained on sterile bedding with sterilized food and water, and held in isolator cabinets (Vickers Pathoflex Isolators, Basingstoke, U.K.).

Anti-osteogenic sarcoma monoclonal antibody 791T36

Hybridoma 791T/36, clone 3 [15] was used as the source of antibody, the hybridoma being maintained as an ascitic growth in BALB/c mice.

Purification of 791T/36 antibody and normal mouse IgG2b

791T/36 monoclonal antibody (IgG2b) was isolated from hybridoma ascites fluid as previously described [3] using Protein A Sepharose affinity chromatography. Control normal mouse IgG2b was isolated from normal mouse serum by a similar procedure [3].

Radioiodination of 791T/36 monoclonal antibody and control mouse immunoglobulin

791T/36 monoclonal antibody and control IgG2b were labelled with either ^{125}I or ^{131}I (Radiochemical Centre, Amersham, U.K.) to 0.25–1.0 μCi/μg of protein using iodogen as previously described [3].

In vivo *antibody distribution studies*

Tumour xenografted mice were inoculated i.p. with radiolabelled antibody or control immunoglobulin (2 μg–2 mg/mouse). Drinking water was supplemented with 0.1% w/v NaI throughout the experiment. One to seven days after antibody injection mice were killed and dissected. Blood, tumours and visceral organs were weighed and counted for radioactivity in a LKB Wallac 80000 Gamma Sample Counter. The residual carcass was cut into pieces of suitable size for counting and the whole counted as five samples. The distribution of radiolabelled antibody and/or normal mouse IgG2b calculated from these measurements was expressed in several ways: (a) the weight (μg) of antibody or control immunoglobulin/g tumour or blood; (b) the proportion of the whole body radioactivity present in the total tumour; (c) the proportion of the injected radioactivity present in the total tumour; (d) the proportion of the whole body radioactivity present/g tumour tissue and per g blood; (e) a tumour:blood (T:B) ratio, calculated as:

$$\frac{\text{counts/min radiolabel/g tumour}}{\text{counts/min radiolabel/g blood}};$$

(f) a tumour localization index (L.I.), calculated as:

$$\frac{\text{tumour to blood ratio of radiolabelled antibody}}{\text{tumour to blood ratio of radiolabelled control IgG2b}}$$

RESULTS

Relationship between tumour mass and localization of monoclonal antibody

Mice bearing subcutaneous xenografts of osteogenic sarcoma 791T were injected i.p. with a mixture of 10 μg of ^{131}I-labelled 791T/36 monoclonal antibody and 10 μg of ^{125}I-labelled control mouse IgG2b and killed 3 days later. For each, the radioactivity in the tumour and in the whole of the remaining body was counted. As

shown in Fig. 1, there was a statistically significant correlation ($r^2 = 0.88$, $P < 0.001$) between the proportion of the total body count of ^{131}I (the 791T/36 label) present within the tumours and their mass. From the slope of the regression line it was calculated that 34% of the total body radioactivity was present/g tumour tissue. There was also a statistically significant correlation ($r^2 = 0.62$, $P < 0.001$) between the proportion of the total body count of the control IgG2b ^{125}I label within the tumours and their weights, but here only 7% of the total body count was present/g tumour tissue.

In a series of five further experiments, groups of mice with 791T xenografts of a range of sizes were injected with doses of between 2 and 40 μg of [^{125}I]-791T/36 monoclonal antibody and killed 2 or 3 days later for analysis. In all experiments (Table 1) there was a statistically significant correlation between the proportion of the total body count of ^{125}I within the tumours and the weights of the tumours. Analysis of the data from this series of experiments showed that the tumours contained between 25% and 44% of the total body count/g tissue, the mean (including the test in Fig. 1) being 36 ± 3%/g. Although there was a significant correlation between the proportion of the total body radioactivity within the tumour and the weight of tumour in all five experiments, in only one (experiment 5, Table 1) was there a significant correlation between the proportion of the initially injected radioactivity that had localized within the tumours and tumour weights. This could be accounted for by the marked variation between individual mice in the clearance of injected antibody radioactivity. For example, in the first experiment shown in Table 1 between 6.3 and 26.0% of the injected ^{125}I was present in the bodies of individual mice when they were killed 3 days after antibody injection. In only one of the five experiments (experiment 4), however, was there a significant correlation between the clearance of the injected labelled antibody and the weights of the xenografts.

Since, as shown above, the amount of injected radiolabelled antibody (expressed as a proportion of the body count) taken up into the xenograft was proportional to the weight of the tumour, and there was no increased uptake/g tumour tissue as the tumour size increased, it would be expected that the tumour:blood ratio (T:B) of radiolabel [T:B = (count ^{125}I/g tumour)/(count ^{125}I/g blood)] would remain constant. In three experiments, however (Nos 1, 3 and 5, Table 1), there was a significant increase in the T:B ratio as the tumour sizes increased. However, there was also a significant drop in the level of radioactivity/g blood expressed as a proportion of the total body count as tumour size increased in three of these five tests (Nos 1–3). This indicates that the increase in the T:B ratio of radiolabelled antibody as tumour size increased was due to the decline in blood levels as the antibody was taken up into the tumour rather than to an increase in the amount of antibody taken up per unit weight of tumour tissue.

Relationship between antibody dose and localization into xenografts

The data presented in Table 1 show that doses of between 2 and 40 μg of radiolabelled 791T/36 antibody gave effective localization into tumour xenografts. There was no indication that an

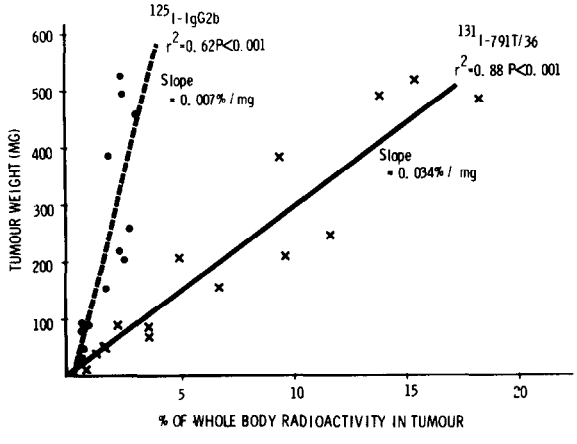

Fig. 1. Correlation between weights of individual 791T xenografts and the proportion of the total body ^{125}I-control IgG2b and ^{131}I-791T/36 monoclonal antibody localized within the tumours. Fourteen individual xenograft-bearing mice were injected with a mixture of radiolabelled control immunoglobulin and antibody and killed for analysis after 3 days.

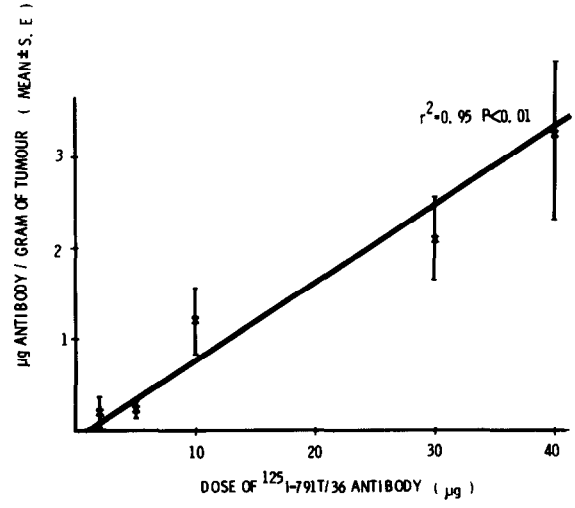

Fig. 2. Correlation between ^{125}I-791-T/36 antibody dose and amount of antibody localized in 791T xenografts. Groups of 4–9 mice were injected with increasing amounts of labelled antibody and killed for analysis 2 or 3 days later.

Table 1. In vivo *localization of* ^{125}I-791*T* 36 *monoclonal antibody in* 791*T osteogenic sarcoma xenografts*

Experiment	^{125}I-Antibody dose (μg)	Day of analysis*	Tumour wt (mg)	% injected activity in whole body	Radioactivity in tumour as % of: whole body count	injected dose	T:B ratio†	Radioactivity/g blood as % of total body count
1	30	3	56	19.5	1.5	0.29	0.74	35.9
			133	12.8	5.7	0.73	1.32	32.7
			167	25.0	8.6	2.18	1.80	28.5
			231	26.0	15.2	4.08	1.85	35.5
			246	15.5	10.0	1.57	1.72	23.8
			482	21.0	16.8	3.36	1.81	18.3
			645	13.0	20.7	2.70	1.46	21.0
			688	6.3	20.6	1.31	2.89	10.3
			808	8.4	38.0	3.20	2.84	16.6
		r^2‡	-	0.44	0.85	0.20	0.59	0.77
		P	-	>0.05	<0.001	>0.1	<0.02	<0.01
2	40	3	159	19.7	9.5	1.89	1.52	39.5
			230	19.0	14.0	2.67	1.86	32.7
			403	23.0	20.8	4.92	1.63	31.5
			563	7.2	23.0	1.70	1.86	22.2
			744	14.0	27.0	3.86	N.T.	N.T.
			997	3.9	27.0	1.09	1.46	19.0
		r^2	-	0.64	0.85	0.05	0.13	0.85
		P	-	>0.05	<0.001	>0.5	>0.5	<0.05
3	5	3	292	11.7	10.3	1.21	1.53	22.9
			328	13.9	13.7	1.92	1.74	24.1
			439	13.2	15.1	1.99	1.55	22.2
			560	14.5	23.9	3.49	2.06	20.6
			647	8.3	24.5	2.05	2.10	18.0
		r^2	-	0.19	0.94	0.37	0.73	0.88
		P	-	>0.1	<0.01	>0.1	0.05	<0.02
4	2	2	87	39.1	1.87	0.73	0.59	36.0
			97	40.1	4.02	1.61	1.03	39.8
			151	33.0	5.67	1.89	0.96	39.5
			290	30.6	9.59	2.88	0.93	35.0
		r^2	-	0.98	0.98	0.85	0.10	0.31
		P	-	>0.02	0.01	>0.05	>0.5	>0.1
5	10	2	121	77.0	3.0	2.39	0.63	40.0
			249	23.0	6.2	1.46	0.76	32.4
			437	32.1	10.9	3.59	0.85	29.6
			961	31.1	35.0	18.00	1.33	27.5
		r^2	-	0.01	0.98	0.91	0.99	0.67
		P	-	>0.05	<0.01	<0.05	<0.01	>0.1

*With respect to labelled antibody injection.

†$\frac{\text{counts/min } ^{125}\text{I/mg tumour}}{\text{counts/min } ^{125}\text{I/mg blood}}$.

‡ r = coefficient of correlation with tumour weights.

'excess' of antibody had been reached, since the extent of tumour localization and T:B ratios were similar with all of the antibody doses tested. This is further illustrated by the results in Fig. 2. Here part of the data in Table 1 has been re-expressed to show the absolute amount of radiolabelled 791T/36 antibody present/g tumour tissue in groups of mice in relation to the dose of antibody injected. There is a statistically significant, positive correlation between these two values: the larger the dose of antibody, the greater the absolute amount of antibody localized in the xenografts.

To examine further the relationship between antibody dose and its tumour localization, groups of 4 mice with 791T xenografts (mean wt, 301 mg) were injected with increasing amounts (10 μg–2 mg) of labelled antibody and killed after 3 days (Fig. 3). With 10 μg of antibody there was a mean of 0.76 μg localized/g tumour tissue, and these mice had a mean T:B ratio of 1.64. With increasing doses (100 μg and above), although the

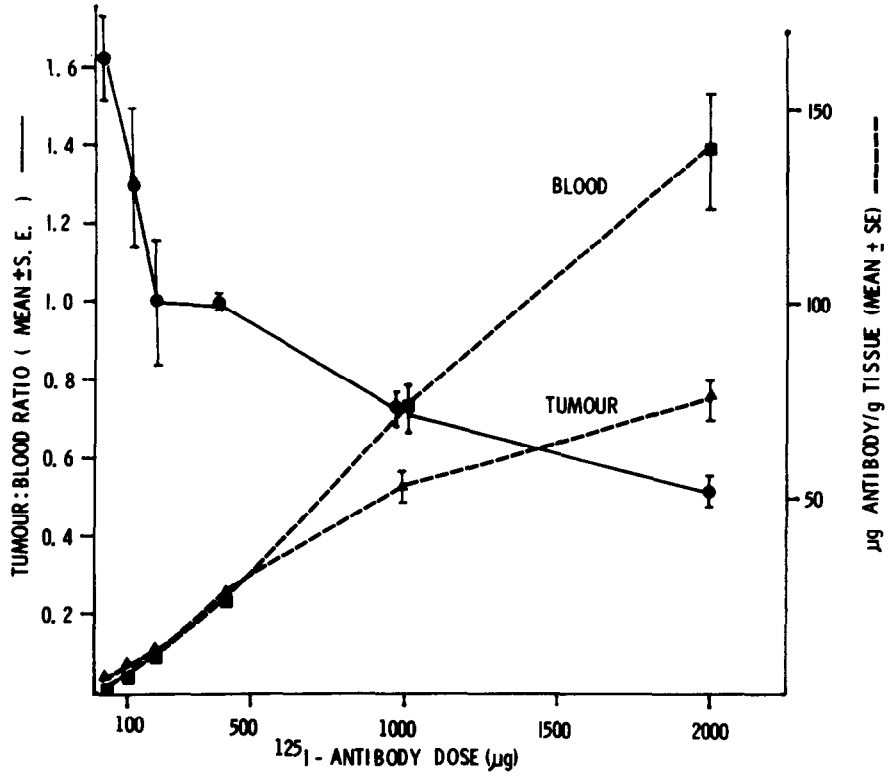

Fig. 3. Influence of dose of ^{125}I-791T/36 *antibody on tumour and blood levels in* 791T *xenograft-bearing mice. Groups of 4 mice were injected with between* 10 *and* 2000 μg *of labelled antibody and killed for analysis after* 3 days.

blood levels of antibody increased proportionally, there was not a corresponding increase in the amount of antibody localizing in the xenografts. Consequently, the T:B ratio declined progressively with increasing antibody doses, reaching only 0.52 with a 2-mg dose (Fig. 3).

Rate of antibody localization into 791T *xenografts*

Previously all 791T xenograft-bearing mice injected with radiolabelled 791T/36 antibody were killed for analysis after 2 or 3 days. This was based on previous findings that effective tumour localization had occurred by this time [3]. In a more precise analysis of the kinetics of this tumour localization, groups of mice with 791T xenografts (mean wt, 166 mg) were injected with a mixture of ^{131}I-labelled 791T/36 antibody and ^{125}I-control IgG2b (10 μg of each) and killed 1–7 days later for analysis. As shown in Fig. 4A, the proportion of the injected ^{125}I-normal IgG2b in the xenografts was 4%/g after 1 day, declining to 2% over the next 6 days (half-life, 6 days). The blood levels of radioactivity was 14% of the injected dose/g 1 day after injection, declining with a half-life of 4.4 days. The decline in blood levels of radiolabelled control IgG2b was slightly faster than that in the tumour, and this resulted in an increase in the T:B ratio from 0.31 at day 1 to 0.45 at day 7. The whole body survival of radiolabel was 52% at day 1, declining with a half-life of 4.6 days, comparable to the blood half-life.

Analysis of the simultaneous distribution of ^{131}I-791T/36 monoclonal antibody (Fig. 4B) showed a mean level of 6.9% of the injected dose/g tumour tissue after day 1, rising to 8.9% at day 2, remaining at this level to day 4 and then declining to 3.3% at day 7. The blood level (10.2%/g at day 1) declined with a half-life of 2.6 days. The relative changes in tumour and blood levels resulted in an increase in the T:B ratio from a mean of 0.67 at day 1 to 2.8 at day 7, representing an increase in L.I. from 2.16 (day 1) to 6.20 (day 7). The mean whole body survival of ^{131}I-radiolabel was 35% at day 1, with a subsequent half-life of 2.8 days, comparable to the blood half-life.

The data in Fig. 4 show overall distribution patterns of labelled control IgG2b and 791T/36 antibody, but it is difficult to appreciate from this figure any changes in the body distribution of the surviving radiolabels during their continual excretion. Consequently an additional analysis of this data was carried out to examine the tumour and blood levels of the radiolabels in relation to their whole body survivals. As shown in Fig. 5A, with ^{125}I-control IgG2b the proportion of the total body count of ^{125}I present in tumour tissues remained constant over the 7-day period, at about

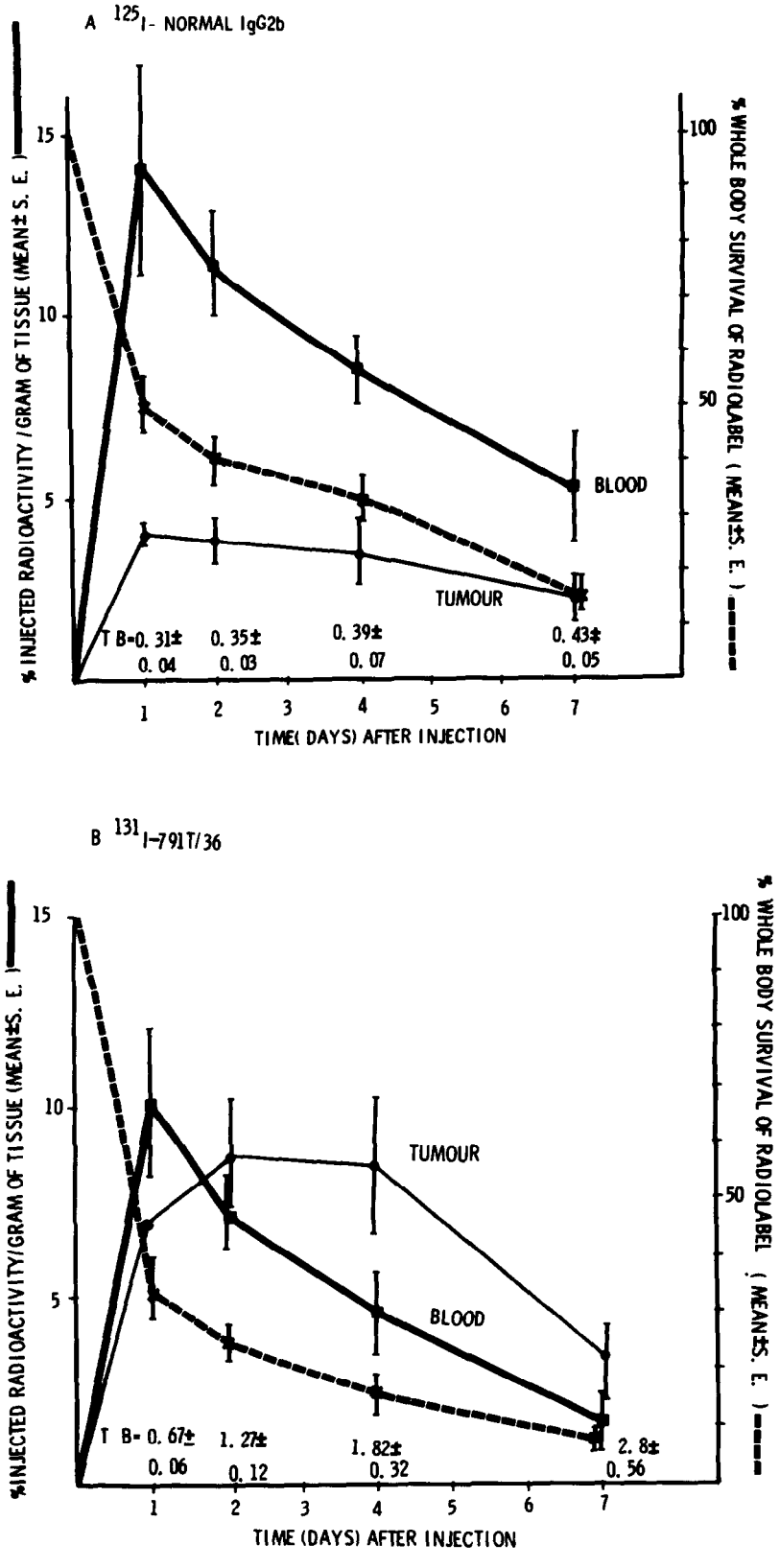

Fig. 4. Time course study of tumour, blood and whole body levels of (A) ^{125}I-control IgG2b and (B) ^{131}I-791T/36 antibody in 791T xenograft-bearing mice. Groups of 4–6 mice were injected with a mixture of radiolabelled control immunoglobulin and antibody and killed for analysis after 1–7 days. Results expressed as % injected radioactivity/g tissues.

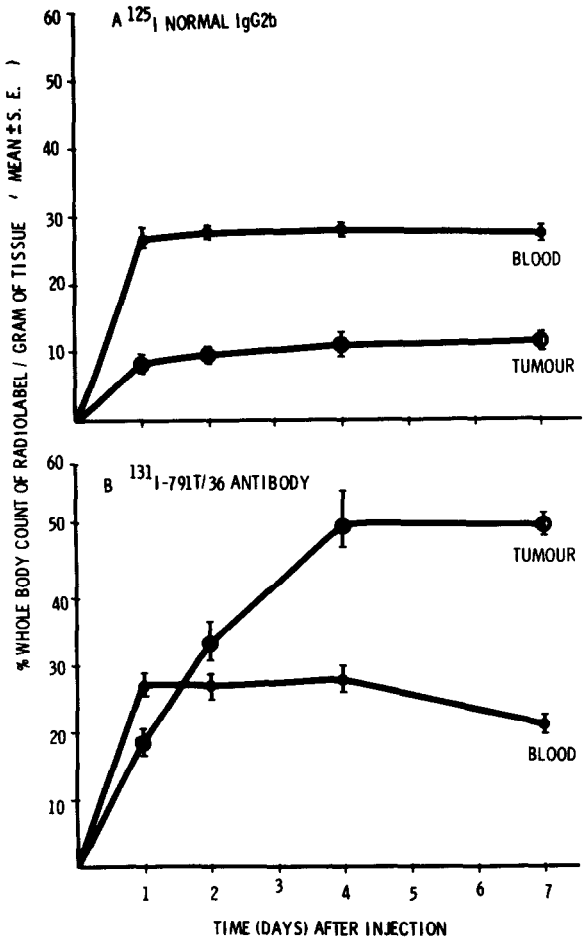

Fig. 5. Time course of tumour and blood levels of (A) ^{125}I-normal IgG2b and (B) ^{131}I-791T/36 antibody in 791T xenograft-bearing mice. As in Fig. 4, results are expressed as % whole body counts/g tissues.

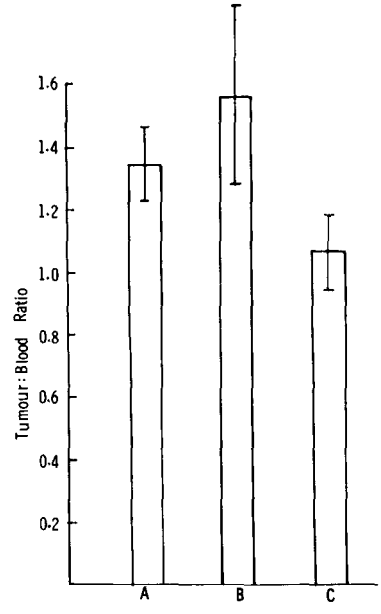

Fig. 6. Influence of a large dose of unlabelled 791T/36 antibody on xenograft retention of previously localized ^{125}I-791T/36. (A) 10 μg ^{125}I-791T/36 i.p. day 0, killed day 3; (B) 10 μg ^{125}I-791T/36 i.p. day 0, killed day 5; (C) 10 μg ^{125}I791T/36 i.p. day 0, 2 mg unlabelled 791T/36 i.p. day 3, killed day 5. 4–5 mice/group; no statistically significant difference between groups.

10% of the whole body count/g tissue, and similarly the blood level also remained constant at about 28%. In contrast, the tumour level of ^{131}I-791T/36 antibody increased for each of the first 4 days, with an average doubling time of 1.8 days and reaching a peak of about 50% of the whole body count/g tissue, and this was maintained to day 7. The blood levels of ^{131}I-791T/36 antibody were comparable to those of the normal IgG2b, at 27%/g over the first 4 days but falling slightly by the seventh day.

Equilibrium between tumour-localized radiolabelled antibody and blood-borne antibody

A test was carried out to determine whether 791T/36 monoclonal antibody, which had localized in 791T xenografts, was in a state of dynamic equilibrium with antibody in the blood. This was examined by attempting to displace radiolabelled antibody, already localized in tumour xenografts from a small initial dose of labelled antibody. Groups of control mice injected with 10 μg ^{125}I-791T/36 antibody and killed 3 or 5 days later showed effective localization of radiolabel in xenografts (mean T:B ratios = 1.35 and 1.56 respectively). Mice also injected with 10 μg ^{125}I-791T/36 antibody but which had received an intraperitoneal injection of 2 mg of unlabelled 791T/36 antibody 3 days later were killed after a further 2 days, i.e. 5 days after the initial ^{125}I-791T/36 antibody injection. These mice still had radiolabelled 791T/36 antibody in the xenografts at levels comparable (mean T:B ratio = 1.06) to those of mice injected only with the labelled antibody (Fig. 6).

DISCUSSION

The objective of these studies with the 791T/36 monoclonal antibody was to assess the extent and rate of antibody localization into 791T xenografts. With small doses of antibody there was a direct proportionality between the mass of the xenografts and the amount of antibody localized. Since this analysis required assays on individual rather than groups of mice, it was difficult to examine directly the correlation between the proportion of the injected dose of antibody localized and the xenograft size, since individual mice showed differences in the clearance of radiolabelled antibody (Table 1) and therefore in the amount of antibody potentially capable of tumour localization. This was overcome by normalizing the tumour radioactivity counts with respect to whole

body survival, and this analysis showed a good correlation between tumour weight and localization of antibody. An average of 36% of the surviving radiolabel was present/g tumour tissue 2 or 3 days after its injection. Taking an average whole body survival of labelled antibody at this time as 22% (the mean of all mice in Table 1 and Fig. 4B), an average of 8% of the injected dose of radiolabelled antibody was present/g tumour tissue 2–3 days after injection, and this value is comparable to other tumour-localizing antibodies in other systems [4, 9, 16, 17].

In many localization studies the absolute levels of antibody in tissues is not reported, and tumour levels of radiolabel have been compared only with those in other organs. However, in many localization models there is no corresponding 'normal' organ with which to compare antibody localization levels and so a conventional comparison is that with blood levels, to give a tumour:blood (T:B) ratio. Exceptions include a Rauscher murine erythroleukaemia, where uptake of radiolabelled monoclonal antibody into leukaemic spleens has been compared with uptake into normal spleens [18]. Here a leukaemic spleen:normal spleen ratio of 7.4:1 was seen when whole spleens were counted, but isolated spleen cells showed a ratio as high as 63:1. The maximum T:B ratio was 1.6:1, not as high as that seen with the 791T xenograft model, where the maximum T:B ratios found in this and previous studies [3] were approaching 3:1. This value with 791T/36 antibody, while higher than that seen in some systems (e.g. 0.8 with antimelanoma antibody [9]), is, however, lower than that reported in other systems: e.g. 6.5:1 with an antimelanoma monoclonal antibody [19], 9:1 with teratoma monoclonal antibody [1], 18:1 with an anti-milk fat globule monoclonal antibody and breast carcinoma xenografts [20]. This T:B ratio is not, however, a good indication of the extent of antibody localization in tumour, since factors tending to reduce blood levels but with no influence on the uptake of antibody in the tumour will increase the T:B ratio. These factors could include prolonged periods before analysis, so that blood levels have fallen appreciably; the use of antibodies or fragments with particularly short half-lives; and the use of particularly large xenografts. In these studies the T:B ratio increased with time after antibody injection (Fig. 4), 7 days after injection being the maximum time examined (T:B ratio = 2.80). This increase was due to a decline in blood levels rather than to increased uptake of antibody into the xenograft. In other systems, where higher T:B ratios have been reported, blood levels of radiolabelled antibody fell faster than that seen with 791T/36. For example, Hedin *et al.* [4] found a whole body survival of anti-CEA monoclonal antibody of 7.6–9% of the injected dose after 4 days (in contrast to the 15% in our studies) and T:B ratios of up to 7:1, although the absolute level of antibody deposited in the tumour was no higher than that with the 791T system. With IgM monoclonal antibody Levine *et al.* [16] found a T:B ratio of up to 10:1 after 5 days, but blood levels of labelled antibody (1% of the injected dose/g) were lower than that with 791T/36 (4%/g).

Our studies emphasize, also, that the size of tumour can influence T:B ratios, since in some cases a direct correlation between this ratio and tumour weight was observed (Table 1). The probable interpretation here is that sufficient radiolabelled antibody had been taken into the tumours to produce a reduction in blood levels and therefore increased T:B ratios. Similarly, in their studies with a monoclonal antibody to a Rauscher murine erythroleukaemia, Scheinberg and Strand [18] found that the uptake of antibody into leukaemic spleen produced a decline in blood levels so that the blood half-life was only one-twentieth of that in control mice. The implication from these considerations is that an antibody with short biological half-life (e.g. IgM, Fab or F(ab)2 fragments) injected into mice with comparatively large large xenografts will result in high T:B ratios after several days.

The rate of antibody localization in the 791T xenograft system was such that greatest localization was seen several days after antibody injection, and similar results have been reported with other solid tumour xenografts [1, 4, 16, 17, 19]. Other tumours, particularly those more vascular than solid subcutaneous xenografts, show more rapid antibody uptake. For example, maximum uptake of monoclonal antibody into spleens of leukaemic mice was seen within 6 hr of injection of radiolabelled monoclonal antibody [18]. In some clinical situations effective imaging of tumour localization within minutes has been reported [8]. However, this rapidity of localization is not necessarily accompanied by a greater extent of antibody uptake than that seen in more slowly localizing situations. For example, in the Rauscher murine leukaemia [18], as already mentioned, the peak T:B ratio was lower than that with the 791T xenografts.

In examining the relationship between antibody localization in the 791T xenografts and antibody dose, there was direct proportionality between the amount of antibody injected and the amount localized in the xenograft, but only with smaller doses of antibody (<100 μg). Beyond this dose there was progressively less uptake into the xenografts, and 1–2 mg of antibody was sufficient

to virtually saturate the tumour, the T:B ratio of the xenograft falling from a mean of 1.6 (10 μg antibody) to 0.52 (2 mg antibody). As tumours became saturated with antibody there was about 70 μg antibody/g tumour tissue. The studies on the rate of antibody localization (Fig. 4) indicate that this tumour level would be reached within 2 days and maintained for a further 2 days. The indication from this is that doses of 2 mg of 791T/36 antibody injected at 3–4-day intervals would maintain tumour levels at up to 70 μg antibody/g tumour tissue. This regime of administration would probably be necessary with antibody conjugated to antitumour agents to maintain the highest intratumour level of the drug. In this context it is pertinent to examine whether antibody localized in tumour xenografts is in a state of dynamic equilibrium with blood-borne antibody. But in the present studies there was no displacement of the radiolabelled antibody already localized in the xenografts by an excess of unlabelled antibody (Fig. 6). The implication is that there is no simple dynamic equilibrium between antibody deposited in the tumour and that circulating in the blood, and therefore it seems unlikely that antibody that has delivered an antitumour agent at the tumour site could be replaced by a further antibody–drug conjugate. This supposes that a drug–antibody conjugate would localize as effectively as free antibody. There are few data available on this point, although it has been shown with radiolabelled 791T/36 antibody conjugated to vindesine that tumour localization is as efficient as with free antibody and that the conjugate produces growth-inhibitory effects [13]. Imaging of patients injected with ^{131}I-labelled anti-CEA antibody conjugated to the same drug has also shown effective tumour localization of radiolabel [21]. In comparison, conjugates of interferon with 791T/36 antibody also showed xenograft localization characteristics comparable to those of free antibody [22].

Specific conclusions that can be drawn from the 791T xenograft model are that antibody localization is proportional to the size of tumours and also to the dose of antibody, although the tumours can become saturated with about 70 μg antibody/g tissue. The rate of localization is relatively slow, requiring 2–4 days before maximum uptake is achieved. More generally, it is clear that further studies are necessary on the kinetics of monoclonal antibody localization into tumours. More quantitative studies on the absolute levels of antibody deposited in tumours is necessary, particularly in comparing one antibody with another, since a simple T:B ratio gives no real indication of the true extent of antibody localization. Many studies have concentrated on subcutaneous xenografts, but tumours at other, visceral sites may have greater blood flow and therefore more rapid, and possibly greater, antibody localization. These continued studies may facilitate the further development of anti-tumour monoclonal antibodies for effective tumour detection and therapy.

Acknowledgements—This work was carried out with the skilled technical assistance of Mrs. S. J. Gribben, Mr. D. G. Fox, Mrs. E. Jacobs and Mrs. J. E. Bullock.

REFERENCES

1. MOSHAKIS V, MCILHINNEY RAJ, RAGHAVAN D, NEVILLE AM. Localization of human tumour xenografts after i.v. administration of radiolabelled monoclonal antibodies. *Br J Cancer* 1981, **44**, 91–99.
2. BALDWIN RW, PIMM MV. Antitumor monoclonal antibodies for radioimmunodetection of tumors and drug targeting. *Cancer Metast Rev* 1983, **2**, 89–106.
3. PIMM MV, EMBLETON MJ, PERKINS AC *et al.* *In vivo* localization of anti-osteogenic sarcoma 791T monoclonal antibody in osteogenic sarcoma xenografts. *Int J Cancer* 1982, **30**, 75–85.
4. HEDIN A, WAHREN B, HAMMARSTRÖM S. Tumor localization of CEA-containing human tumors in nude mice by means of monoclonal anti-CEA antibodies. *Int J Cancer* 1982, **30**, 547–552.
5. FARRANDS PA, PERKINS AC, PIMM MV *et al.* Radioimmunodetection of human colorectal cancers using an anti-tumour monoclonal antibody. *Lancet*, 1982, **ii**, 397–400.
6. MACH JP, BUCHEGGER F, FORNI M *et al.* Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinoma by external photoscanning and tomoscintigraphy. *Immunol Today* 1981, **2**, 239–249.
7. BERCHE C, MACH J-P, LUMBROSO J-D *et al.* Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: first clinical rsults using radio-labelled monoclonal antibodies against carcinoembryonic antigen. *Br Med J* 1982, **285**, 1447–1451.

8. EPENETOS AA, MATHER S, GRANOWSKA M *et al.* Targeting of iodine-123-labelled tumour associated monoclonal antibodies to ovarian, breast and gastrointestinal tumours. *Lancet* 1982, **ii**, 999–1004.
9. LARSON SM, BROWN JP, WRIGHT PW, CARRASQUILLO JA, HELLSTROM I, HELLSTROM KE. Imaging of melanoma with I-131-labeled monoclonal antibodies. *J Nucl Med* 1982, **24**, 123–129.
10. SMEDLEY HM, FINAN P, LENNOX ES *et al.* Localization of metastatic carcinoma by a radiolabelled monoclonal antibody. *Br J Cancer* 1983, **47**, 253–259.
11. FARRANDS PA, PERKINS A, SULLEY L *et al.* Localization of human osteosarcoma by anti-tumour monoclonal antibody 791T/36. *J Bone Joint Surg (Am)* 1983, **65**, 638–640.
12. EMBLETON MJ, ROWLAND GF, SIMMONDS RG, JACOBS E, MARSDEN CH, BALDWIN RW. Selective cytotoxicity against human tumour cells by a vindesine–monoclonal antibody conjugate. *Br J Cancer* 1983, **47**, 43–49.
13. BALDWIN RW, PIMM MV, EMBLETON MJ *et al.* Monoclonal antibody 791T/36 for tumor detection and therapy of metastases. M. D. Anderson Symposium, Houston, TX, 1983.
14. STEEL GG, COURTNEY VD, ROSTOM AY, Improved immune suppression technique for the xenografting of human tumours. *Br J Cancer* 1978, **37**, 224–230.
15. EMBLETON MJ, GUNN B, BYERS VS, BALDWIN RW. Antitumour reactions of monoclonal antibody against a human osteogenic sarcoma cell line. *Br J Cancer* 1981, **43**, 582–587.
16. LEVINE G, BALLOU B, REILAND J, SOLTER D, GUMERMAN L, HAKALA T. Localization of I^{131} labelled tumor specific monoclonal antibody in the tumor bearing BALB/c mouse. *J Nucl Med* 1980, **21**, 570–573.
17. GAFFAR SA, PANT KD, SCOCHAT D, BENNETT SJ, GOLDENBERG DM. Experimental studies of tumour radioimmunodetection using antibody mixture against carcino-embryonic antigen (CEA) and colon specific antigen-p(CSAP). *Int J Cancer* 1981, **27**, 101–105.
18. SCHEINBERG DA, STRAND M. Kinetic and catabolic considerations of monoclonal antibody targeting in erythroleukaemic mice. *Cancer Res* 1983, **43**, 265–272.
19. CHOSE T, FERRONE S, IMAI K *et al.* Imaging of human melanoma xenografts in nude mice with a radiolabeled monoclonal antibody. *JNCI* 1982, **69**, 823–826.
20. RAINSBURY R, WESTWOOD J. Tumour localization with monoclonal antibody radioactively labelled with metal chelate rather than iodine. *Lancet* 1982, **ii**, 1347–1348.
21. FORD CHJ, NEWMAN CE, JOHNSON JR *et al.* Localization and toxicity studies of a vindesine-anti-CEA conjugate in patients with advanced cancer. *Br J Cancer* 1983, **47**, 35–42.
22. PELHAM JM, GRAY JD, FLANNERY FR, PIMM MV, BALDWIN RW. Conjugation of α interferon to human osteogenic sarcoma monoclonal antibody 791T/36. *Cancer Immunol Immunother* 1983, **15**, 210–216.