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Comparative Tumour Localization of Antibody Fragments and Intact IgG in Nude Mice Bearing a CEA-producing Human Colon Tumour Xenograft

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Abstract—*The dynamics of distribution of radiolabelled F(ab')2 fragments of a monoclonal anti-CEA antibody have been studied in the nude mouse bearing a CEA-producing human colon carcinoma. Our results showed that the fragment was rapidly cleared from all normal organs reaching about* 1% *of that injected by* 24 hr. *Specific tumour localization occurred as early as* 2 hr *after injection and was complete by* 4 hr. *The amount of fragment localized in tumour was* 4% *of the injected dose, equivalent to that obtained with the intact antibody. Greatly improved tumour:normal tissue ratios were obtained with the fragment compared to intact IgG. However, the residence time of the fragment was much shorter (*24 hr*) than that of intact antibody (more than* 3 days*). Tumour localization indices suggested that fragments were superior to intact IgG at locating tumour specifically. The specificity indices based on lung, spleen and liver were much higher than those for intact antibody, reflecting the lack of Fc-receptor binding of fragments and their reduced excretion by these organs. The 'fragment index' enabled tumour:normal tissue ratios for the fragment and intact IgG to be compared. Together with the distribution study at different time points, this simplifies the task of defining a 'time window' in which tumour imaging and therapy might be optimal.*

INTRODUCTION

RADIOLABELLED antibodies against tumour markers have been shown to specifically localize human carcinomas in both experimental animals [1–3] and in patients by the radioimmunolocalization (RIL) procedure [4, 5]. Immunoradiotherapy using an ^{131}I-labelled monoclonal antibody has also been demonstrated experimentally in nude mice bearing a human tumour xenograft [6]. This animal model is useful for providing a dynamic study of tumour localization and normal tissue distribution, with a view to therapy, and provides a 'framework' in which to study the dosimetry of selected antibodies and fragments.

Antibody fragments, with their faster circulatory clearance, have been investigated with encouraging results, both in experimental animals [1, 7–9] and at the clinical level [10, 11]. Unfortunately fragments have been reported to locate in tumours in relatively smaller amounts than whole antibody [7, 8] and this may detract from their use in therapy. Our study on the dynamics of distribution of F(ab')2 fragments of a monoclonal anti-CEA antibody has provided the first evidence that an equivalent concentration of fragment and intact IgG is capable of localizing in a tumour xenograft.

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MATERIALS AND METHODS

Antibody preparation

The monoclonal anti-CEA (1C12) used in this study has been described previously [12] and chosen for its low cross-reactivity with NCA and its stability on immunopurification and radiolabelling. F(ab')2 fragments were prepared by the method of Lamoyi and Nisonoff [13]. After separation of the digest mixture on Sephacryl S–200, the fractions were analysed by SDS–PAGE using a 7.5% gel. The fraction containing the F(ab')2 was concentrated and dialysed against 0.15M phosphate buffer, pH 7. Both intact 1C12 and the fragment were shown to be immunologically active and relatively homogeneous by electroblotting of the SDS gel onto nitrocellulose paper and overlaying with ^{125}I-labelled CEA (Fig. 1). Intact 1C12 and its F(ab')2 fragment were radiolabelled by the chloramine T method to specific activities of 5.6 and 5.2 μCi/μg respectively. Both labelled preparations were shown to retain immunological activity by solid-phase radioimmunoassay using CEA coupled to amino-cellulose [14]. An excess of 60% activity was retained in each case. As a control,

IgG was isolated from normal mouse serum by protein A – Sepharose affinity chromatography and the F(ab')2 prepared. Radiolabelling of these preparations was carried out as described above.

In-vivo studies

The localization experiments were carried out in nude mice bearing the xenograft tumour MAWI, using the paired label distribution method described by Pressman *et al.* [15]. The MAWI line was derived from a CEA-producing human mucoid adenocarcinoma. The mice, 2–3 weeks after innoculation with MAWI tumour were given injections of 3 μg of intact ^{125}I-labelled 1C12 or 4 μg of ^{125}I-labelled F(ab')2–1C12 together with similar amounts of ^{131}I-labelled non-specific IgG or F(ab')2-IgG. The mice were killed in groups of four at 1, 2, 4 and 7 hr and, at 1, 2, 3, 7 and 13 days after injection. At the same time mice without implanted tumours were similarly treated. Visceral organs were excised, weighed and counted on a dual channel gamma counter (Compugamma, LKB). The following tissues were examined—blood, liver, spleen, kidney, lung, muscle, colon and tumour. The results were expressed as (a) the mean percentage of the injected dose/gram of tissue; (b) tumour:normal tissue ratios; (c) specificity indices—calculated as the tumour:normal tissue ratio for specific antibody divided by the same ratio for the non-specific antibody; and (d) the 'fragment index'—calculated as the tumour:normal tissue ratio for the fragment divided by the same ratio for the intact antibody.

RESULTS

Comparative localization of F(ab')2–1C12 and intact antibody in tumour

The distribution of F(ab')2–1C12 in the tumour is shown in Figs 2 and 3. By 2 hr its concentration had risen to about 3%, and during the following 2 hr to almost 4% of the injected dose. This concentration was then maintained up to 24 hr. Beyond 24 hr there was a marked fall in the amount of fragment in tumour to 1.65% by day 2. Its concentration, however, did not fall below 0.5% of the injected dose up to day 13.

The non-specific fragment also localized in the tumour at a concentration of about 2% of the injected dose at 1 hr, declining steadily to less than 0.1% by day 7 (Fig. 3). In contrast to F(ab')2–1C12, no residual retention of non-specific fragment was observed. Intact 1C12 reached a tumour concentration of approximately 3% of the injected dose by 24 hr and this level was maintained until day 3. By day 7 the concentration of 1C12 fell to 1.3% of the injected dose (Fig. 3).

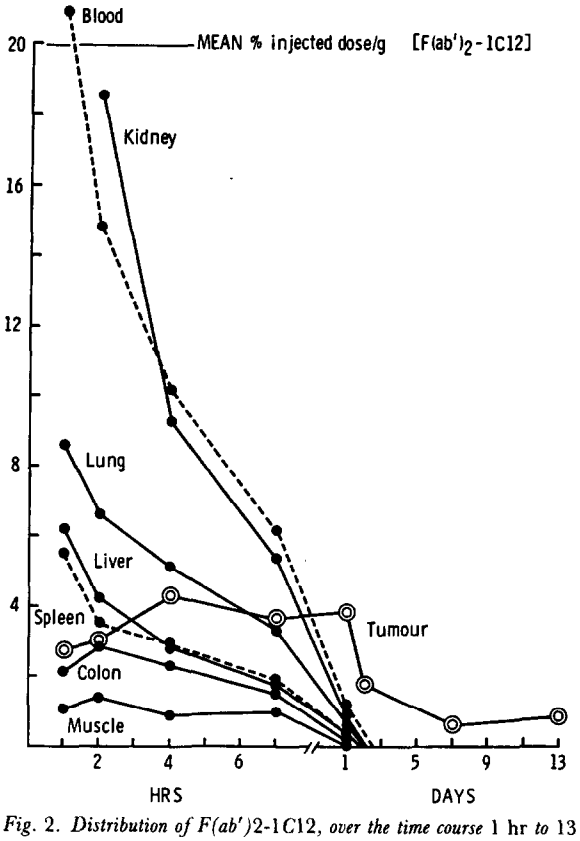

Fig. 2. Distribution of F(ab')2-1C12, over the time course 1 hr to 13 days, in tissues of mice bearing the MAWI colon tumour xenograft.

Distribution in normal organs

The clearance of F(ab')2 fragments of 1C12 in tissues of the tumour-bearing mice are shown in Fig. 2. The concentration of specific label in the kidney reached almost 20% in the first hour and was thereafter in equilibrium with the amount in the blood, where the concentration fell to 1% by 24 hr. By 48 hr the concentration of the fragments in the blood was barely detectable. This contrasts with the blood clearance of intact 1C12 antibody (Fig. 3), where 5% of the injected dose remained in the circulation at 24 hr. The concentration of F(ab')2–1C12 in organs of the reticuloendothelial system (Fig. 2) reached on average 6% of the injected fragment by 1 hr, falling to below 1% by 24 hr. Similar results were seen with the non-specific F(ab')2 fragment (Fig. 4). Distributions of the specific and non-specific fragments in the non-excretory normal organs colon and muscle were again very similar (Figs 2 and 4). Comparative clearances of intact 1C12 from normal tissues are shown in Fig. 5.

Tumour:normal tissue ratios for F(ab')2–1C12 increased rapidly with time and were much higher than those for the non-specific fragment and intact 1C12 (Fig. 6). The 'fragment indices' comparing the tumour:normal tissue ratios for the fragment

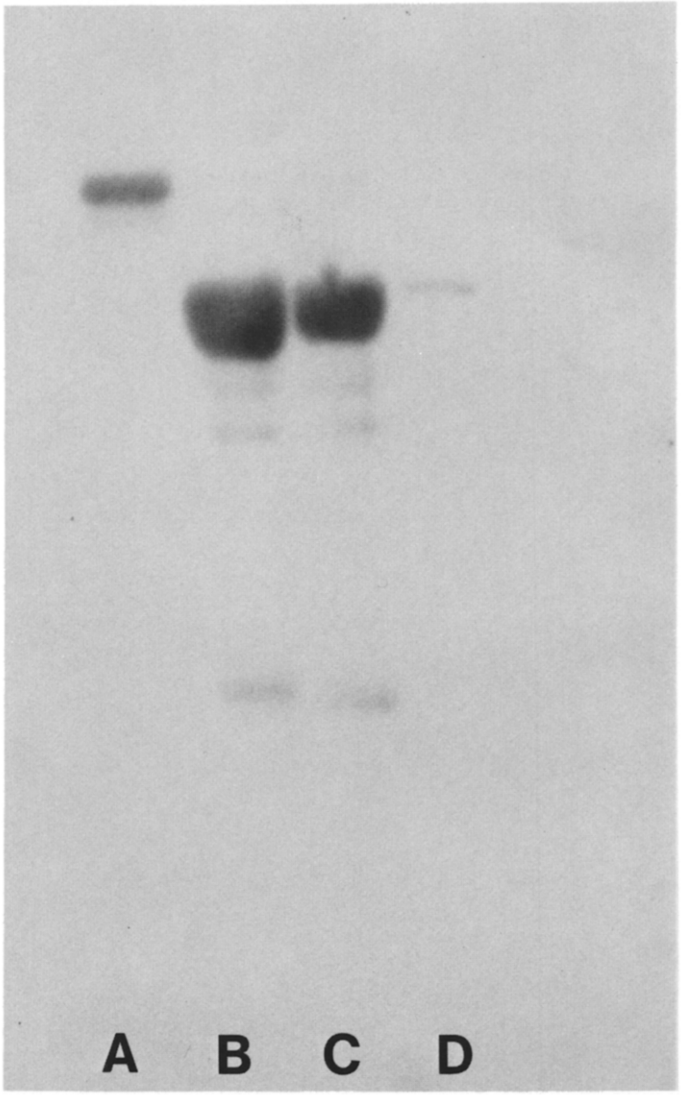

Fig. 1. *SDS–PAGE of immunopurified* 1C12 *(A) and the pepsin digest before (B) and after (C and D) gel filtration on Sephacryl S–*200. *Peak (C) contained F(ab')*2–1C12, *which was shown to be immunologically active by overlay with radiolabelled CEA.*

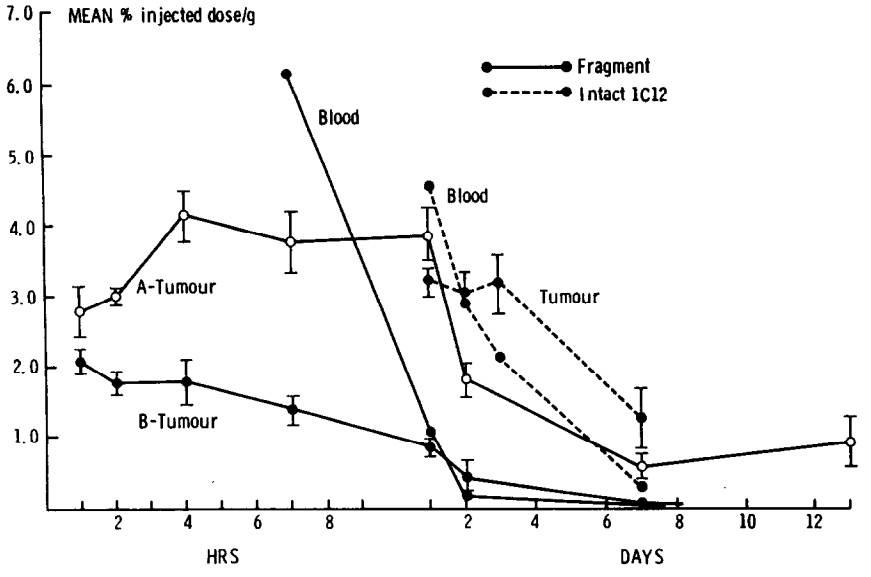

Fig. 3. Relative distribution of F(ab')2–1C12 (curve A) and F(ab')2–IgG (curve B) in tumour and comparison of F(ab')2–1C12 and intact 1C12 antibody in tumour and blood in nude mice bearing the MAWI xenograft.

with the corresponding ratios for intact antibody (see Materials and Methods) show that maximum differences were obtained at 48 hr (Table 1). Mean tumour:blood specificity indices (Table 2) clearly show that the specific fragment is capable of localizing tumour more effectively than the non-specific fragment. More importantly, however, F(ab')2–1C12 shows 4- to 8-fold higher specific localization compared to intact 1C12 at time points beyond 24 hr. High specificity indices based on tumour:normal tissue ratios were also found for liver, lung and spleen (Table 2) and for kidney (Table 3).

Table 1. Fragment index for 1C12 in blood, liver, kidney, lung and spleen from 24 to 168 hr post-injection

Hr	Blood	Liver	Kidney	Lung	Spleen
24	4.3	7.3	2.7	4.1	5.2
48	13.6	12.6	3.4	5.7	14.1
168	10.9	7.1	1.4	2.6	4.0

The fragment index was calculated as (mean tumour:normal tissue ratio for $F(ab')_2$)/(mean tumour:normal tissue ratio for IgG).

Table 2. Specificity indices for F(ab')2–1C12 and intact antibody in blood, liver, lung and spleen from 1 to 168 hr post-injection

	Blood		Liver		Lung		Spleen	
Hr	$F(ab')_2$	IgG	$F(ab')_2$	IgG	$F(ab')_2$	IgG	$F(ab')_2$	IgG
1	0.81 (0.09)		1.1 (0.17)		0.78 (0.07)		1.5 (0.35)	
2	1.21 (0.11)		1.52 (0.10)		0.96 (0.08)		1.58 (0.07)	
4	1.56 (0.07)		2.5 (0.40)		1.3 (0.05)		3.1 (0.08)	
7	1.86 (0.12)		3.1 (1.0)		1.5 (0.11)		3.9 (0.4)	
24	9.9 (1.6)	2.0 (0.19)	11.6 (1.4)	1.25 (0.25)	4.9 (1.0)	1.37 (0.15)	13.6 (0.9)	0.94 (0.22)
48	16.8 (2.6)	2.1 (0.16)	34.1 (2.2)	1.55 (0.16)	9.9 (1.4)	1.69 (0.15)	N.D.	0.80 (0.12)
72	N.D.	3.5 (0.08)	N.D.	2.83 (0.38)	N.D.	2.80 (0.23)	N.D.	1.75 (0.32)
168	26.0 (2.0)	3.7 (0.06)	37.0 (4.5)	1.95 (0.32)	10.7 (0.26)	3.00 (0.50)	N.D.	1.92 (0.05)

The values represent the mean value for each animal group with the standard error in parentheses. N.D. = experiment not carried out or not completed.

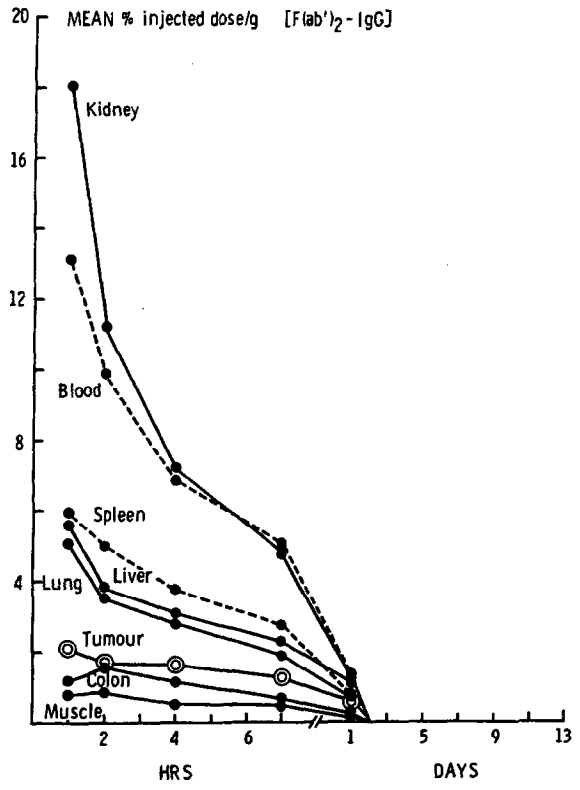

Fig. 4. Distribution of F(ab')2 fragments of non-specific IgG in tissues of mice bearing the MAWI colon tumour xenograft over the time course 1 hr *to* 13 days.

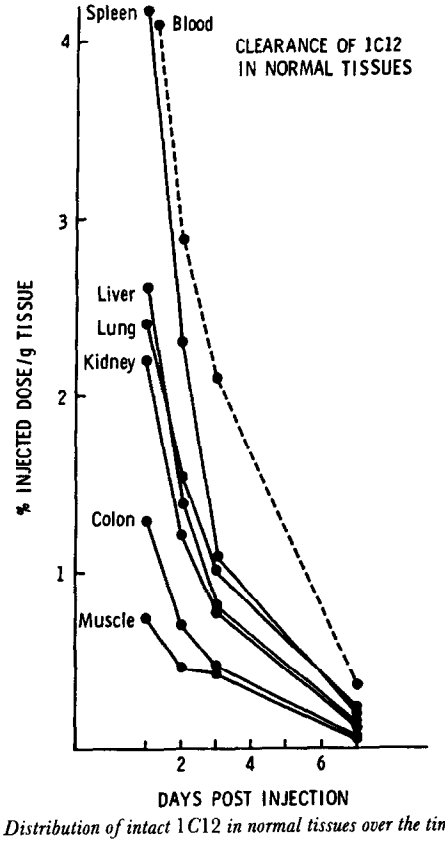

Fig. 5. Distribution of intact 1C12 *in normal tissues over the time course* 1–7 days.

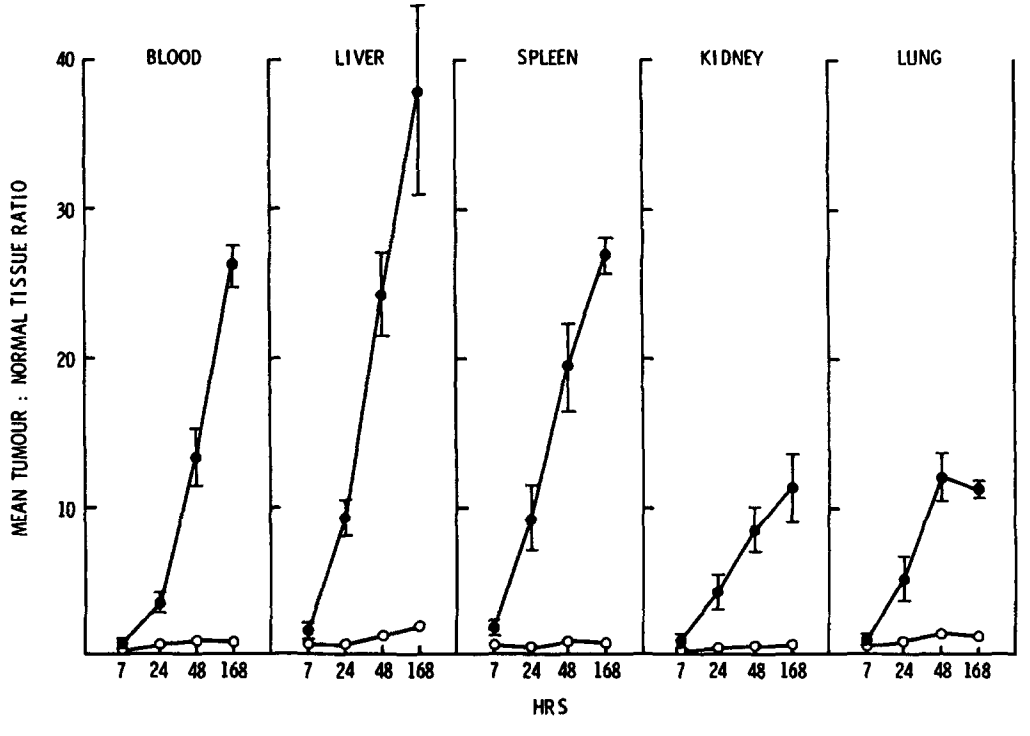

Fig. 6. Comparison of mean tumour:normal tissue ratios for F(ab')2–1C12 and F(ab')2–IgG for blood, liver, spleen, kidney and lung over the time course 7–168 hr.

Table 3. Mean tumour:kidney ratios and specificity indices [based on kidney (S.I.)^K] for F(ab')$_2$–1C12 and intact 1C12

	Tumour:kidney ratio					
	Specific		Non-specific		Specificity index (S.I.)^K	
Hr	F(ab')$_2$	Intact	F(ab')$_2$	Intact	F(ab')$_2$–1C12	Intact 1C12
7	0.63 (0.20)	—	0.29 (0.01)	—	2.17	—
24	4.20 (0.50)	1.5 (0.23)	0.48 (0.05)	0.95 (0.10)	8.75	1.57
48	8.50 (0.51)	2.53 (0.30)	0.55 (0.11)	1.27 (0.21)	15.45	2.00
72	—	4.34 (0.70)	—	1.33 (0.24)	—	3.26
168	11.90 (1.2)	6.21 (1.22)	0.61 (0.09)	1.98 (0.22)	18.65	3.13

The standard error for each animal group is given in parentheses.

DISCUSSION

An important feature of fragments is their inability to bind to Fc receptors on macrophages, Kupfer cells and hepatocytes [16] which should limit their non-specific accumulation in liver and other organs of the reticuloendothelial system [17]. On account of their smaller size, fragments should also be capable of reaching the tumour site and penetrate more easily the capillary network of the tumour [7]. However, one of the drawbacks reported for localization using fragments is an appreciably lower absolute concentration in the tumour, compared to whole antibody [7, 8]. Unfortunately these studies only considered time points beyond 24 hr and did not indicate how long the fragment remained in the vicinity of tumour compared to intact antibody. Our study shows that this could be crucial.

Choosing early time points, the present study has allowed the initial onset of tumour localization to be defined and the distribution of fragment in both tumour and normal tissues to be compared. It is important to note that, contrary to previous reports, accumulation of F(ab')2–1C12 in the tumour was equivalent to that obtained with intact 1C12. However, our study has also shown that the fragment of 1C12 is lost from the tumour site somewhat earlier than is the case with intact antibody.

This difference may, in part, be due to the smaller size of the fragments and their lack of Fc binding so that they tended not to be detained in tumour by an impaired lymphatic drainage to the same extent as intact IgG. The possibility exists that circulatory intervention with a 'second' antibody may increase lattice complex formation and thereby increase the tumour residence time of the fragments.

In this study we have introduced the 'fragment index' to enable the tumour:normal tissue ratios of the fragment to be compared with those of intact antibody. This information, together with knowledge of the change of fragment concentration in tumour, helps to define a 'time window' in which tumour imaging and therapy would be optimal. In the case of 1C12 the fragment indices for blood, liver, kidney, lung and spleen are all highest at 48 hr after injection, unfortunately when absolute amounts of specific fragment in the tumour had started to decline. At 24 hr, however, before this happened, the fragment indices still showed a significant advantage in favour of using the fragment. These results are therefore consistent with the imaging data with F(ab')2-anti-CEA reported by Wahl *et al.* [8], showing that the best tumour localization occurred at 2 days post-injection and was significantly better than that achieved with intact antibody.

Our results suggest that fragments are superior to intact antibodies at localizing tumour and that specific accumulation begins as early as 2 hr after injection and is complete by 4 hr. The high specificity indices based on lung, spleen and liver are particularly revealing as these reflect the lack of Fc-receptor binding and reduced accumulation of fragments in these organs. Likewise the specificity indices based on tumour:kidney ratios show that F(ab')2–1C12 does not accumulate in the kidney. Toxicity in this organ should therefore not be a problem when administering fragments to patients. The possibility of using fragments therapeutically will clearly depend on giving high enough doses to compensate for the premature release at the tumour site.

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