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Letter to the Editor

Distribution of IgM Monoclonal Antibody in Mice with Human Tumour Xenografts: Lack of Tumour Localization*

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WITH a number of anti-human tumour monoclonal antibodies being produced [1] one of their potential applications is *in vivo* tumour targeting for diagnostic or therapeutic applications [2]. The majority of antibodies so far evaluated have been mouse antibodies of IgG1 [3], IgG2a [4] or IgG2b [5, 6] isotypes, but there is little information on tumour localization potential of IgM antibodies. In the present study the IgM C/14/1/46/10 antibody, which defines the Y hapten carbohydrate antigen and reacts with 96% of human colorectal carcinomas [7], has been examined for localization into human colorectal carcinoma xenografts in immunodeprived mice.

Antibody was isolated from ascites fluid from BALB/c mice with the C/14/1/46/10 hybridoma by lentil lectin affinity chromatography with α-methyl mannoside elution [8]. Immunoelectrophoretic analysis of the preparation at 300 μg/ml using a rabbit anti-mouse serum showed a single precipitation arc in the IgM region, and Ouchterlony radial immunodiffusion against rabbit anti-mouse Ig subclass antisera (Miles Laboratories, Stoke Poges, Bucks, U.K.) detected only IgM. The yield of antibody was 0.6 mg/ml of ascitic fluid. Control IgM was prepared from normal mouse serum using the same method.

The preparations were labelled with ^{125}I to 1 μCi/μg using ^{125}I pre-labelled Bolton and Hunter reagent [9], or by the iodogen [10] or chloramine T [11] methods for incorporation of ^{125}I from Na^{125}I. Normal mouse serum IgM was labelled with ^{131}I by the iodogen technique. Labelled preparations were characterized by gel filtration on Sephacryl S300 with normal mouse serum calibration [6] and by direct binding assays against cultured HCT-8 or HRT-18 colon carcinoma cells [6]. HCT-8 and HRT-18 cultured cell lines were also used to initiate xenografts in immunodeprived CBA mice [6]. Purified antibody was labelled with fluorescein isothiocyanate (FITC) to a fluorescein:protein molar ratio of 3:1 [6] and reaction against colon carcinoma cells examined in a FACS IV flow cytofluorimeter (Becton Dickinson, Sunnyvale, Cal, U.S.A.) [6]. Specificity of the reaction was assessed in competitive binding tests in which FITC-C/14 antibody was mixed with increasing amounts of unlabelled antibody [6].

S300 gel filtration of ^{125}I-C/14 added to normal mouse serum showed the major peak of radioactivity in the first protein peak, known to contain IgM (Fig. 1). The labelled antibody bound significantly to HCT-8 or HRT-18 colon carcinoma target cells, a mean of 49% of counts ($n = 3$) binding with the Bolton and Hunter labelled preparations, 33% ($n = 4$) with iodogen and 36% ($n = 1$) with chloramine T. There was virtually no binding (<3%) to antigen-negative 791T control cells. Following injection of ^{125}I-C/14 prepara-

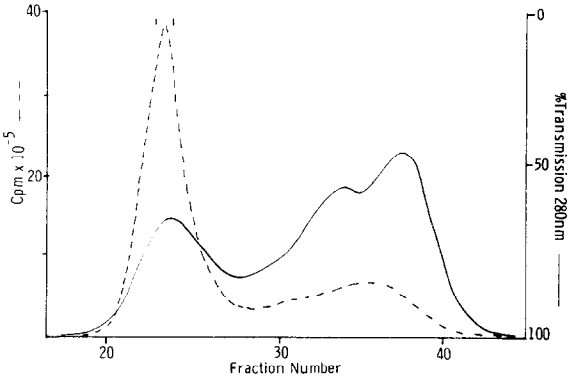

Fig. 1. Sephacryl S300 gel filtration of ^{125}I-C14 antibody added to normal mouse serum. One millilitre run on a 90×1.5 cm column. Peak fractions of ^{125}I were taken for some of the in vivo studies.

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tions or their S300 peaks into tumour-xenografted mice there was no clear localization of the radiolabel into tumour tissue (Fig. 2). Tumour levels of radiolabel were no higher than many normal organs, and in two tests with paired labelled ^{125}I-C/14 antibody and ^{131}I-normal mouse IgM, tumour to blood ratios of the two labels were virtually identical. Average whole body survival of ^{125}I label was 22% at day 1 and 7.5% at day 3.

To confirm that C/14-defined antigen continued to be expressed in colon carcinoma xenografts, cells were prepared from HCT-8 xenograft tumour by trypsin digestion and reacted with FITC-C/14. Reactivity was comparable to that seen with cultured cells (Fig. 3A) and unlabelled antibody competed quantitatively with FITC labelled in its reaction with both cell preparations.

To examine the possibility that antibody failed to tumour localize because of neutralization by circulating antigen, the ability of tumour-bearer mouse serum to neutralize FITC-C/14 antibody was assessed, but there was no neutralization with nine individual serum preparations (Fig. 3B). In addition, sera 1 and 3 days after injection of ^{125}I-C/14 into HCT-8 xenograft-bearing mice were examined in binding tests against HCT-8 target cells. The ^{125}I in these serum preparations bound

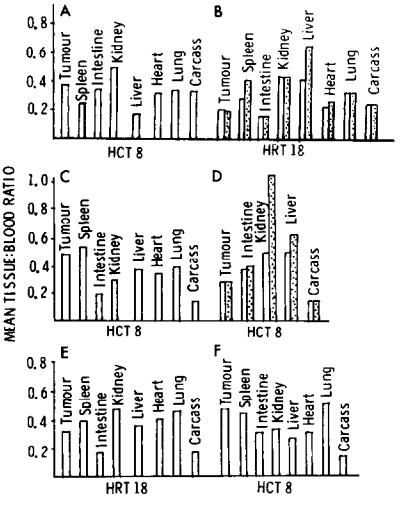

Fig. 2. Distribution of ^{125}I-C/14 and ^{131}I-normal IgM in mice with colorectal carcinoma xenografts. Aliquots (2-10 μg) of preparations were injected i.p. or i.v. Drinking water was supplemented with sodium iodide throughout. A mean of 3-6 mice per group was used. Tissue:blood ratio $= \frac{\text{cpm } radioiodine/\text{g } tissue}{\text{cpm } radioiodine/\text{g } blood}$. *(A) Iodogen-labelled preparation; killed day 3. (B) Iodogen-labelled preparations i.v.; killed day 3. (C, D) S300 peaks of iodogen-labelled preparations i.p.; killed day 1. (E) Chloramine T-labelled preparation i.p.; killed day 3. (F) Bolton-Hunter-labelled preparation i.p.; killed day 3.* □ ^{125}I-C/14; ▨ ^{131}I-*normal IgM.*

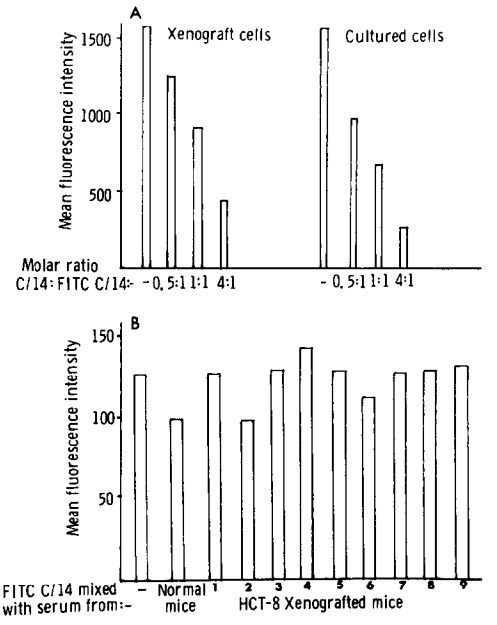

Fig. 3. Flow cytofluorimetric analysis of HCT-8 colon carcinoma cells and FITC-C/14 antibody. (A) 2 × 10^5 cells from tissue culture or xenografted tumour were mixed with excess (3 μg) FITC-C/14 alone or with increasing amounts of unlabelled C/14 antibody. Total reaction volume, 0.5 ml. Analysed using forward angle light scatter to gate on tumour cells. FITC-antibody reacts against both cell preparations; but unlabelled antibody competes. (B) Cells from tissue culture were incubated with non-excess (0.6 μg) FITC-C/14 alone or pre-incubated with 0.1 ml normal nouse serum or sera from mice with established (mean diameter, 2 cm) HCT-8 xenografts. Serum fails to neutralize antibody.

to HCT-8 target cells, although with reduced efficiency (17 and 17% of counts bound) compared with injected preparations (42 and 32% respectively).

These studies have failed to show clear localization of the C/14 antibody in appropriate human tumour xenografts. This cannot be accounted for by lack of reactivity of labelled preparations, since following ^{125}I incorporation by three different techniques antibody retained integrity, shown by gel filtration studies, and antigen recognition, shown by binding to appropriate target cells. Neither can it be explained by lack of antigen expression in developing xenografts, or neutralization of antibody by excess circulating tumour-derived antigen such as inhibited xenograft localization of a radiolabelled IgG2a anti-HLA antibody in human tumour xenografts [12]. In addition it is unlikely that poor vascularization of tumours could account for the localization failure, since radiolabelled anti-CEA antibody of the IgG1 isotype shows significant localization into the same xenografts [13]. It is more likely that the present failure to tumour localize is a reflection of the poor extravasation of IgM antibodies. Thus it is established that IgM extravasates more slowly

[14] and less extensively [15, 16] than IgG. These factors together with the short whole-body half-life of IgM, already established with polyclonal IgM preparations [17] and also shown in the present studies, could account for inefficient tumour localization.

In contrast to the present study, there is one positive report of tumour localization of monoclonal IgM antibody and F(ab')2μ fragment into an intramuscularly transplanted mouse teratoma [18]. However, the situation here was clearly complex, since the antibody's target antigen was expressed at least as highly in normal kidney and brain as in tumour. Since only tumour showed antibody localization it is probable that this particular mouse tumour showed abnormally increased access for antibody to antigen [18], even for intact IgM. However from the present study with C/14 antibody and human tumour xenografts, the indication is that tumour localization of IgM monoclonal antibodies may be much less clear cut than that already widely demonstrated with IgG antibodies and human tumours either as xenografts or clinically. It may be more appropriate to prepare IgG rather than IgM antibodies, for example by isolation of immunoglobulin class switch variants from IgM producer hybridoma lines [19, 20].

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