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Syngeneic Anti-idiotypic Antibody Prevents Localization of a Murine Monoclonal Antibody in Human Tumour Xenografts

Malcolm V. Pimm and Robert W. Baldwin

BALB/c mice were immunized against syngeneic mouse monoclonal antibody (Mab) 791T/36 to produce anti-idiotypic antibody. To examine the effect of this antibody on tumour localization of the Mab, serum from these mice was transferred to nude mice with human tumour xenografts and distribution was studied with I-125 labelled Mab. Serum containing anti-idiotypic antibody prevented tumour localization of the Mab. This finding has implications for the clinical use of human or humanized Mab since, if these evoke anti-idiotypic antibody, this alone may be sufficient to prevent tumour targeting.
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INTRODUCTION

PATIENTS given mouse monoclonal antibodies (Mabs) to tumour associated antigens for immunoscintigraphy or therapy frequently produce anti-mouse antibody (AMA) [1]. AMAs can be a limitation to repeated administration of Mabs because they form immune complexes with the further doses of Mab, and these complexes are cleared to the liver or spleen [2, 3].

AMA may contain anti-species, anti-isotype and anti-idiotypic antibody [4–7], but the relative contributions of these to the perturbation of distribution is unclear. Animal models are needed to investigate this question [8, 9]. Anti-idiotypic AMA against 791T/36 (directed against a human tumour associated gp72 antigen) was generated in syngeneic (BALB/c) mice by injection of 791T/36–ricin toxin conjugate. In the mice the distribution of radiolabelled 791T/36 was altered, with hepatic clearance of the label [9]. Thus it would appear that anti-idiotypic antibodies alone can affect the distribution of Mab. It is unclear whether this would also affect localization of the Mab in tumour, or whether the affinity of Mab for the tumour target antigen would be sufficient to overcome the formation and eventual clearance of immune complexes with the anti-idiotypic antibody in the circulation. An analogous situation is the formation of immune complexes between Mab and circulating carcinoembryonic antigen (CEA), where tumour localization is not affected because of different affinities of the Mab for tumour-associated CEA compared with circulating CEA [10].

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It would be difficult to devise a model to determine directly the effect of anti-idiotypic AMA on tumour localization of Mabs to human tumours, because immunologically competent animals are needed to generate AMA, and these cannot support the growth of human tumour xenografts. Consequently, we transferred serum from mice with AMA into nude mice with human tumour xenografts and examined the localization of radiolabelled 791T/36 Mab in the tumours.

MATERIALS AND METHODS

Anti-idiotypic AMA was generated in BALB/c mice by two intraperitoneal injections of 1 mg/kg 791T/36-ricin toxin A chain immunotoxin [9] 10 days apart. Mice were bled out after a further 10 days and serum stored at −20°C. The presence in the serum of antibody capable of preventing binding of fluorescein isothiocyanate (FITC) labelled 791T/36 Mab against 791T tumour target cells was confirmed by a flow cytometric blocking assay [9]. Thus 0.1 ml test serum reduced the mean linear fluorescence (MLF) obtained by treating 2×10^5 791T cells with 100 ng FITC-791T/36 from 64 to14, while control mouse serum had no effect (MLF 77).

Groups of three athymic nude mice with subcutaneous xenografts of osteosarcoma 788T [11] were injected twice 2 h apart with 1 ml test or control mouse serum. Six hours after the second injection these mice and an additional group of untreated mice were injected intravenously with 10 μg I-125 labelled 791T/36 (specific activity 10 MBq/mg) [11]. All mice were killed after a further 4 days, dissected and radioiodine was measured in weighed samples of blood, tumour and organs. This time was

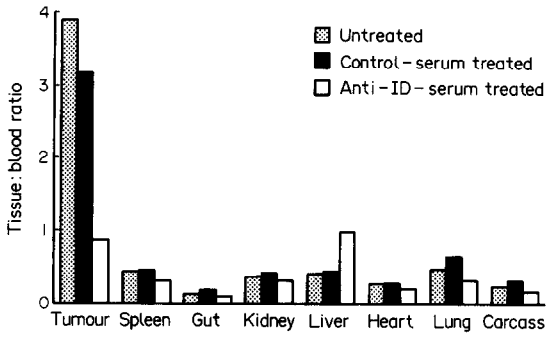

Fig. 1. Distribution of I-125 labelled 791T/36 Mab in nude mice with human osteosarcoma xenografts. Mice were untreated or had been pretreated with normal mouse serum or serum from BALB/c mice with anti-idiotypic antibody to the 791T/36 Mab. Tumours in untreated mice (three per group) weighed 1.02, 1.33 and 1.48 g, those in control-serum treated and immune-serum treated weighed 1.28, 2.78 and 3.11, and 1.4, 1.82 and 3.25 g, respectively.

chosen based on previous observations that in this system maximum discrimination between tumour and normal tissues is achieved after several days [11]. A tissue to blood ratio was calculated for each organ as the ratio of the count rate per gram of tissue to that per gram of blood.

RESULTS

Untreated mice and mice treated with control-serum showed the characteristic tumour localization previously reported with this Mab in this model [11], with mean tumour tissue to blood ratios after 4 days of 3.91 (SE 0.70) and 3.18 (0.14), respectively, which were not significantly different by t test (Fig. 1). In contrast, mice pretreated with immune-serum showed much lower levels, with a mean tumour tissue to blood ratio of 0.88 (0.04) ($P < 0.001$ and $P < 0.05$ compared with mice treated with normal serum and untreated controls, respectively). Liver levels were increased in these mice to a mean ratio of 0.99 (0.07), compared with 0.44 (0.04) in mice treated with normal serum and 0.40 (0.01) in untreated mice ($P < 0.01$ compared with both groups).

The whole body retentions of radioiodine in the three groups of mice were virtually the same: 12.7 (0.16), 10.8 (1.84) and 10.1 (0.09)% in untreated, control-serum treated and immune-serum treated mice, respectively.

DISCUSSION

This and our previous study [9] have shown that AMA, even restricted to an anti-idiotypic response only, can perturb the distribution of Mab, with hepatic clearance of immune complexes and reduced tumour localization. The presence of anti-idiotypic antibody in donor serum was shown by ability to react with the combining site of the 791T/36 Mab by blocking of a subsequent reaction with target antigen expressed on the surface of 791T cells.

Previous tests in BALB/c mice showed accelerated loss of radiolabel from mice with anti-idiotypic AMA as a consequence of clearance of immune complexes [9]. In the present study the whole body retention of radiolabel was virtually the same in the three groups of mice, although low at only about 10% of the dose after 4 days. Mice with xenografts showing tumour localization of the 791T/36 Mab catabolize the antibody more quickly than control mice [12]. Thus the low whole body retention of the radiolabel is probably due to accelerated catabolism following tumour localization in untreated and control-serum treated mice, but accelerated whole body catabolism following clearance of immune complexes in mice treated with anti-idiotypic serum. The net consequence was similar whole body survival of radioiodine in the three groups of mice but different distributions of label, with tumour discrimination being effectively destroyed in test serum treated mice.

This finding with anti-idiotypic AMA is significant since many attempts are being made to produce human or humanized (chimeric) Mabs to overcome the problem of AMA production [13–15]. Patients given murine Mabs can produce anti-idiotypic AMA [4–7], but it is not yet known whether those given human or chimeric antibodies do so. If they do, our study suggests that this alone is sufficient to prevent effective tumour localization of these Mabs.

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