Immunology 1983 **49** 667

Monoclonal antibodies to rat sarcomata

II. A SYNGENEIC IgG_{2b} ANTIBODY WITH ANTI-TUMOUR ACTIVITY

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Accepted for publication 7 March 1983

Summary. The IgG_{2b} monoclonal antibody M10/76, which is specific for the Hooded rat fibrosarcoma MC24, was obtained by fusion of the rat myeloma Y3 Ag 1.2.3 with spleen cells from an MC24 tumour-bearing rat. This antibody has been found to inhibit lung colonization when MC24 cells were injected i.v. into Hooded rats, and when passively transferred into athymic MC24 tumour bearers, it prevented or considerably reduced the incidence of spontaneous lung metastases in more than half the treated animals.

INTRODUCTION

Monoclonal antibodies to tumour antigens are currently under examination to determine their relative merits for use in adjuvant therapy in the treatment of either primary tumours or disseminated disease. Most of the monoclonal antibodies that have been examined for their therapeutic usefulness in man and experimental animals have been directed against tumours of the blood, i.e. leukaemias (Bernstein, Tay & Nowinski, 1979; Kirch & Hammerling, 1981; Miller *et al.*, 1981; Ritz *et al.*, 1981) or lymphomas, (Nadler *et al.* 1980; Young & Hakomori, 1981; Miller *et al.*, 1982). We report here our initial findings on the biological activity of the monoclonal antibody M10/76 (rat IgG_{2b}) a syngeneic tumour-specific monoclonal antibody that is directed against a solid tumour, namely, the Hooded rat fibrosarcoma MC24.

Hybridoma M10/76 was obtained by fusion of the rat myeloma Y3 Ag 1.2.3. (Galfre, Milstein and Wright, 1979) with spleen cells taken from a Lister Hooded/Cbi rat bearing the syngeneic fibrosarcoma MC24 (North *et al.*, 1982). Tests performed *in vitro* on a variety of normal and malignant rat cells have shown that antibody M10/76 binds only to cells of MC24 and when injected intravenously into normal rats it has a long half-life in the blood (North *et al.*, 1982). These data are consistent with a high degree of tumour specificity and suggest that the antigen involved is not present in large quantities in normal rat tissues. Recent work with rodent sacromata clearly implicates the immune system in the prevention of metastatic disease (Eccles & Alexander, 1975; Eccles *et al.*, 1979). To discover if antibodies are able to influence the dissemination of tumour cells, the biological activity of antibody M10/76 has been investigated in relation to its ability to influence (i) colonization of the lung after intravenous injection of tumour cells and (ii) spontaneous metastasis.

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0019–2805/83/0800-0667$02.00

MATERIALS AND METHODS

Animals

Inbred rats of the following strains were maintained in germ-free positive pressure isolators: Lister Hooded/Cbi (RTl^c) and congenitally athymic nude rats derived from a Rowett (rnu/rnu) Lister Hooded/Cbi cross, now at the third backcross generation. All animals were aged 10–12 weeks at the commencement of an experiment.

Tumours and cultures

Two chemically induced fibrosarcomata were used, namely HSNtc, a 3,4-benzpyrene-induced tumour (Currie & Gage, 1973) and MC24, a 20-methylcholanthrene-induced tumour (Eccles, Heckford & Alexander, 1980) both syngeneic to Lister Hooded/Cbi rats. Cell cultures of the above tumours were established by trypsinization of tumour explants and maintained *in vitro* as described previously (North *et al.*, 1982).

Hybridoma production

Procedures for hybridoma production and the purification of monoclonal antibodies M10/76 (IgG_{2b}) and 11/160 (IgG_{2b}) from culture supernatants have been described earlier (North *et al.*, 1982).

Lung colonization assay

Eight Lister Hooded/Cbi rats were each given intravenously 0·5 ml phosphate-buffered saline (PBS) and then four of the animals were challenged i.v. with 10^6 cultured cells of MC24 and four challenged with 10^6 cultured cells of HSNtc. A further eight rats were each given i.v. 0·5 ml PBS containing 180 μg of affinity-purified antibody M10/76 and then challenged with 10^6 cells of either MC24 or HSNtc. Sixteen days later the animals were killed and the lungs removed, weighed and then, after fixing for 24 hr in Bouin's solution, the number of tumour colonies were counted using a dissecting microscope.

Assessment of spontaneous metastasis

Male and female nude Hooded rats were randomly assorted into three groups. On day −1, one group of animals was given via the sub-lingual vein 0·25 ml PBS, the second group was given 100 μg M10/76 in 0·25 ml PBS, and the third group was given 100 μg 11/160 in 0·25 ml PBS. These treatments were repeated at Day 0 immediately after each rat had been challenged intramuscularly with 10^6 cells of MC24. Each rat was treated three times weekly with 0·25 ml PBS or PBS containing 50 μg antibody via the sublingual vein until Day 21, when the tumours were removed by amputation of the hind limb. Samples of tumour tissue were disaggregated using trypsin and the percentage of Fc receptor-positive cells determined using antibody-coated sheep red blood cells. At amputation each of the animals was given 100 μg of antibody or an equivalent volume of PBS and this dose was repeated 24 hr later. After tumour excision the animals received a twice-weekly treatment with antibody at 50 μg/0·25 ml PBS and this was continued until the first of the animals died. At death or at the termination of the experiment each animal was examined for overt metastatic disease. In each case the lungs were removed, weighed, then fixed in Bouin's solution for 24 hr, and the number of tumour colonies was determined.

RESULTS

Effect of antibody M10/76 on lung colony formation

When 180 μg of M10/76 was injected into each of four Hooded rats immediately before challenge with 10^6 cells of MC24, no tumour colonies could be found in the lungs of these rats 16 days later, whereas the animals treated with PBS alone had a tumour burden in excess of 200 colonies/lung (Table 1). Antibody M10/76 did not influence lung colonization by HSNtc, another fibrosarcoma syngeneic to Lister Hooded/Cbi rats, confirming its specificity for the MC24 tumour.

Effect of M10/76 on spontaneous metastasis

Two experiments were performed in nude rats to assess the effect of antibody M10/76 on spontaneous metastasis of MC24, employing the treatment protocols described in 'Materials and Methods', and using as control treatments either PBS or the syngeneic

Table 1. Influence of monoclonal antibody M10/76 on lung colony formation by i.v.-injected rat fibrosarcoma cells

Treatment	No. tumour colonies
PBS + 10^6 MC24	>200, >200, >200, >200
M10/76 + 10^6 MC24	0 0 0 0
PBS + 10^6 HSNtc	>200, >200, >200, >200
M10/76 + 10^6 HSNtc	>200, >200, >200, >200

Table 2. Effect of treatment with monoclonal antibodies on the growth of MC24 in nude rats

Experiment No.	Treatment	Tumour weight (g)	Percent Fc receptor-positive cells
1	PBS	4·05±0·3	6
	M10/76	3·84±0·2	11
	11/160	4·12±0·3	12
2	PBS	2·71±0·2	20
	M10/76	4·47±0·3	30
	11/160	3·76±0·5	15

monoclonal antibody 11/160 (IgG_{2b}) which is specific for the Hooded rat fibrosarcoma HSNtc (North *et al.*, 1982).

In the first experiment, treatment with either of the two monoclonal antibodies did not affect the rate of growth of MC24 as judged by measurement of leg diameters (data not shown) or tumour weights at amputation (Table 2), but the percentage of Fc receptor-positive cells was significantly higher. Measurements of leg diameter were not made in the second experiment, but the tumour weights at amputation (Table 2) in the antibody-treated groups were greater than in the PBS-treated controls. In addition the percentage of Fc receptor-positive cells was higher in the tumours growing in animals treated with M10/76, and this, together with the increased tumour weights in these animals, points to an enhanced infiltration of host cells as a consequence of antibody treatment.

In both experiments, all of the tumour-bearing rats treated with PBS alone (Fig 1, Group B) or antibody 11/160 (Group D) were found at autopsy to be suffering from extensive metastatic disease that was confined to the lungs. The actual lung weights varied considerably and this reflected the size of the tumour colonies in individual animals. Of the 19 animals that had been treated with antibody M10/76, 10 showed a considerable reduction in the number of tumour colonies in the lung (Fig. 1, Group C) and the lung weights were near to or the same as those of non-tumour bearers (Group A). Two of the rats treated with M10/76 were found to be free of metastatic lesions when the experiments were terminated. However, nine of the rats in Group C showed no beneficial effect of treatment with antibody M10/76. All had an extensive tumour burden with lung weights in excess of 6 g and three of these animals died early from lung disease.

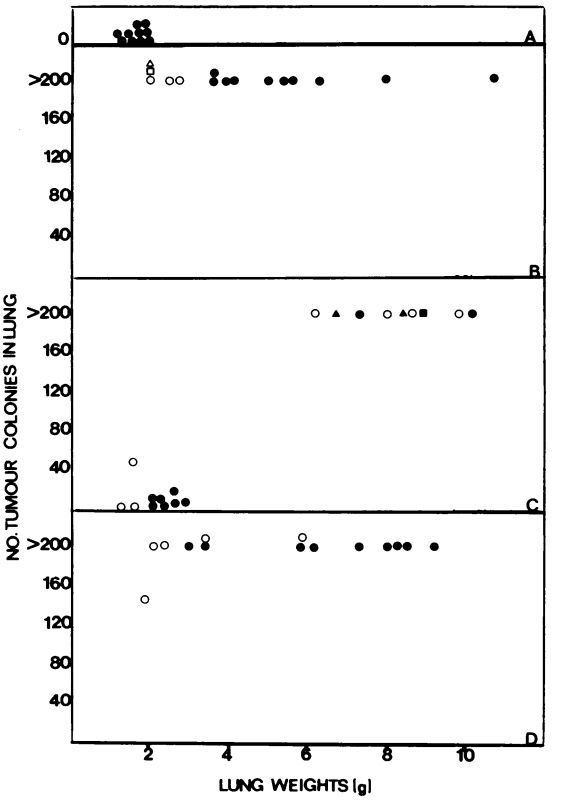

Figure 1. Effect of passively transferred monoclonal antibodies M10/76 and 11/160 on spontaneous metastasis of the Hooded rat fibrosarcoma MC24.

Lungs taken from non-tumour-bearing animals (A) were used as controls. Three groups of nude rats were injected with 0·5 ml PBS alone (B), or 0·5 ml PBS containing affinity purified M10/76 (C) or 11/160 (D). The first experiment was terminated at Day 94 (○) but one animal (□) died at Day 80 and another (△) at Day 82. In the second experiment, one animal died at Day 76 (■), two died at Day 79 (▲) and the remaining animals were killed and examined at Day 81 (●).

DISCUSSION

MC24 is an immunogenic rat sarcoma that rarely metastasizes when grown in immunocompetent animals but shows a high incidence of spontaneous metastases when grown in T cell-deprived or congenitally athymic animals (Eccles *et al.*, 1980). Our aim in the present experiments has been to discover if monoclonal antibodies derived from tumour bearers have biological activity, and for this reason we have used athymic rats because (i) they are defective in host control of tumour dissemination, and (ii) they cannot mount a conventional T cell-dependent response leading to antibody production, the presence of which may obscure the effect of passively transferred monoclonal antibodies.

Our results show that both lung colonization by i.v. injected MC24 cells and dissemination of these cells from the primary i.m. site to the lungs can be specifically restricted by the passive transfer of the syngeneic antibody M10/76. These data point to what may be an important role for syngeneic antibody in the prevention of metastatic disease in normal tumour bearers.

The mechanism for elimination of disseminating tumour cells is currently under investigation. Two possibilities being examined are that opsonization of the tumour cells by specific antibody either prevents the cells leaving the primary tumour site, or facilitates their subsequent trapping and destruction in the lungs. In either case an involvement of host effector cells seems likely, because sera and plasma alone, although containing high titres of M10/76, are not cytotoxic when tested *in vitro*. A likely host effector cell is the macrophage and the finding that tumours grown in antibody-treated animals had an enhanced infiltration of phagocytic cells, is consistent with this possibility. Indeed, Herlyn & Koprowski (1982) have produced strong incriminatory evidence for a macrophage involvement in the antibody-mediated inhibition of growth of human xenografts in nude mice. The second possibility, however, that tumour-cell destruction occurs in the lungs, is consistent with the prevention of lung colonization by i.v.-injected cells and the findings of others (Weiss, Glaves & Walter, 1974; Procter, Auclair & Rudenstam, 1976) that destruction of i.v.-injected 'immunogenic' sarcomata takes place in the lungs.

The failure to influence the occurrence of lung metastases in about half the treated animals was disappointing, but it may reflect a heterogeneity in either the tumour-cell population or in antigen density of the cells perhaps related to cell cycle, and this aspect is under investigation.

ACKNOWLEDGMENTS

This work was supported by a Programme Grant from the Medical Research Council and the Cancer Research Campaign. One of us (S.M.N.) was the recipient of an Institute of Cancer Research Scholarship.

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