Growth inhibition of experimental glioma grafts by monoclonal antibody treatment

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Summary. The effects of 14AC1 monoclonal antibody (McAb) on 79FR-G-41 rat glioma cells in vitro, on the formation of metastases in lung by antibody coated glioma cells, and on the growth of glioma grafts in BALB/c-nu/nu mice were investigated.

The 14AC1 antibodies – isotyped as IgG2a – were obtained from a hybridoma clone established after fusion of X63-Ag8.653 myeloma cells and spleen cells of BALB/c mice hyperimmunized with 79FR-G-41 glioma cells. Antibody treatment of glioma cells in vitro caused evident cell surface alterations and pronounced growth depression of most cells. However, a few tumor cells remained unchanged in morphology and continued to proliferate. Moreover, 14AC1 antibodies drastically reduced lung metastasis by pretreated and i.v. delivered glioma cells. Additionally, 14AC1 antibodies suppressed the growth of transplanted rat gliomas in nude mice as evidenced by a longer latency period and a smaller volume of glioma grafts in treated than in control tumor bearers. Nevertheless, glioma grafts showed accelerated growth after termination of antibody treatment.

Further experimental investigation is required in order to identify the precise mechanisms of the effects of McAbs on tumor cells in vitro and in vivo.

Key words: Experimental gliomas – Monoclonal antibodies – Immunotherapy – Serotherapy – Block of metastasis – Growth inhibition

Introduction

The considerable difficulties in the demonstration of tumor-associated antigens have been overcome by the development of a monoclonal antibody technique by Köhler and Milstein (1975). From recent data using monoclonal antibodies (McAbs), human and experimental brain tumors have been demonstrated to express glioma-associated antigens (de Tribolet and Carrel 1980; Schnegg et al. 1981; Seeger et al. 1981; Wikstrand and Bigner 1982; Coakham et al. 1982; Stavrou and Süss 1982; Stavrou et al. 1983). Moreover, radiolabeled McAbs generated against glioma-associated antigens of human and experimental gliomas were used for tumor radioimmunodetection by external scintigraphy. These studies indicated that (a) glioma xenografts are permeable to monoclonal immunoglobulins (IgG2a and IgG2b) and (b) iodinated McAbs provide useful reagents for radioimaging of gliomas in vivo (Bourton et al. 1984; Stavrou et al. 1985a, b).

The possibility of applying molecules with defined specificities for clinical purposes has reactivated the interest in immunotherapy of cancer using native McAbs or drug-antibody conjugates. Numerous experimental applications of McAbs to cancer treatment involve their use as carrier molecules for radionuclides, drugs, and toxins of different origin (Vitetta et al. 1983; Bullard and Bigner 1985). In addition, native McAbs have been shown to have direct tumoricidal effect through antibody mediated immunological mechanisms (Capone et al. 1984).

We have established hybridoma clones secreting McAbs directed against the tumor cell line 79FR-G-41 which was derived from a chemically induced malignant rat astrocytoma (Stavrou et al. 1980, 1983). It has been reported that the McAb 14AC1 of this series causes in vitro cell surface changes and growth delay of cultured tumor cells bearing relevant target antigens (Lederer et al. 1984; Stavrou et al. 1984). The same McAb brings about glioma cell lysis in vitro by complement-dependent and antibody dependent cell-mediated cytotoxicity (Bilzer et al. 1984).
In the present study, we investigated the effect of 14AC1 monoclonal immunoglobulins on glioma cells in vitro, on lung colony formation of glioma cells, and on the growth of experimental glioma grafts in BALB/c-nu/nu mice.

**Materials and methods**

**Animals.** BALB/c and BALB/c-nu/nu mice used for ascitic fluid production and glioma transplantation were taken from our barrier maintained colony.

**Glioma cell line.** The establishing of the rat glioma cell line 79FR-G-41 used in this study as well as morphological and immunological characteristics have been described elsewhere (Stavrou et al. 1980, 1983).

**Myeloma cell line.** The BALB/c myeloma line X63-Ag8.653 (Kearney et al. 1979) were used for fusion experiments and production of ascitic fluid for control experiments.

**Hybridoma cell line.** The production of the hybridoma clone 14AC1 secreting antibodies, which bound 79FR-G-41 glioma cells but did not react with normal rat cells, has been reported previously (Stavrou et al. 1983, 1984). Furthermore, no reaction could be found with radiation-induced rat osteosarcoma cells (unpublished data). The antibody belongs to the IgG2a isotype as shown in agar immunodiffusion using relevant antisera (Stavrou et al. 1985a). Hybridoma supernatants were centrifuged at 1000 g and stored at -20°C.

**Production of ascitic fluid.** Adult BALB/c mice pretreated with tetramethylpentadecane (Pristan, Ega Chemicals, Steinheim, FRG) were inoculated i.p. with 10^7 14AC1 hybridoma cells or X63-Ag8.653 myeloma cells. Ascitic fluid was collected 2 weeks after cell injection. The cells were spun down at 1000 g, Pristan was removed, and the cell-free supernatant was stored at -20°C.

**Treatment of glioma cells in vitro by 14AC1 antibodies.** Glioma cells (5 x 10^5) were seeded into Leighton tubes (Nunc) and incubated with culture medium (Dulbecco’s modified Eagle’s medium, DMEM) containing 10% fetal bovine serum (FBS) for 24 h at 37°C to attach to the underlayer. Cell cultures in exponential growth phase were washed three times with phosphate-buffered saline (PBS) followed by incubation with DMEM containing 140 ug 14AC1 antibody/ml and 10% FBS. The 14AC1 antibody was purified from heat-inactivated (57°C, 30 min) ascitic fluid over a Protein A-Sepharose column (Pharmacia). As control glioma cells were incubated with the same amount of nonspecific mouse IgG. The final cell density was determined 48 h after the beginning of treatment and expressed as a percentage of the untreated control value. In some experiments, 2 h after immunoglobulin incubation cells were washed three times, fixed with 1% glutaraldehyde solution in PBS and dehydrated through a series of ethanol and isomylacetate of increasing concentrations. Specimens were dried by the CO2 critical point method, coated with gold and examined in a Cambridge S4-10 scanning field emission microscope.

**Lung colony formation assay.** Trypsinized 79FR-G-41 glioma cells (1 x 10^5) were incubated for 1 h at 37°C with 5 ml of cell-free hybridoma supernatant (containing approximately 10 ug antibody/ml) or with control medium containing the same amount of polyclonal mouse anti-rat IgG, and no mouse IgG at all, respectively. After centrifugation (10 min at 150 g) cells were resuspended in PBS and 10^6 cells were injected into the tail veins of 6-to 8-week-old male BALB/c-nu/nu mice. After 3 weeks the mice were killed and the lungs, kidneys, livers, and other organs examined for metastasis, which were visible on the surface as white nodules.

**Treatment of glioma xenografts in nude mice.** For each experiment, 6- to 8-week-old BALB/c-nu/nu mice were inoculated s.c. in the flank with 10^7 79FR-G-41 tumor cells in 0.3 ml DMEM containing 10% FBS. After tumor cell inoculation the animals received an i.p. injection of 0.5 ml ascitic fluid diluted 1:3 in PBS. Experimental animals received 14AC1 ascitic fluid (approximately 1 mg antibody/ml), and control animals received X63-Ag8.653 ascitic fluid with the same amount of nonspecific mouse IgG, or no treatment at all. Each experimental group consisted of 10 animals.

In the first experiment the ascitic fluid injection was repeated every 2nd day during the observation period. In the second experiment the 14AC1 antibody was administered every 2nd day for 18 days followed by dividing the animals in two groups: one group was treated further with 14AC1 ascitic fluid, and the other group was observed without any treatment. The observation was performed for 10 days with or without antibody application. In the first and the second experiment tumor sizes were monitored every 3rd day. The 3 major diameters of the tumors were measured and the results were recorded as tumor volume. The differences in tumor growth curves between experimental and control animals were statistically evaluated using a method of least-squares regression. Fragments of glioma grafts grown in experimental and control animals were fixed and processed as usual for light microscopy. Additional tumor specimens were used for identification of effector cells (Adams et al. 1984).

**Results**

**Surface changes of glioma cells in vitro by 14AC1 antibody treatment.** The McAb 14AC1 induced morphological changes of glioma cells in vitro with severe alterations of cell membrane and detachment from the culture dishes. The aliquot of detached cells 48 h following the first incubation of glioma cultures with 14AC1 antibodies amounted to 63%-84%. The remaining attached cells preserved their common morphological appearance and continued to proliferate. The floating cells lacked commonly seen structures such as microvilli and membrane ruffling. Intercellular contacts appeared to retract. The antibody caused considerable agglutination and clotting of surface texture of the target cells, without membrane damage, however (Fig. 1). Therefore it was of interest to find out whether the antibody that induces surface changes and detachment in vitro would also affect adhesion in vivo.

**Effect of 14AC1 antibody treatment on lung colony formation by glioma cells.**

The condition of the cell surface and the resulting attachment of glioma cells circulating in the blood to vascular endothelia is a requirement for metastasis formation and tumor growth. Cells of the glioma line 79FR-G-41 were preincubated with McAb 14AC1 as described above and injected into adult BALB/c-nu/nu mice. All mice injected with glioma cells developed visible metastasis in the lungs 3 weeks after injection.