Eur J Cancer Clin Oncol, Vol. 18, No. 6, pp. 579–588, 1982.
Printed in Great Britain.

0277-5379/82/060579-09$03.00/0

Identification of an Oncofetal Antigen (gp90) on Murine B16 Melanoma Cells

CHIEKO NISHIO,* YOSHIFUMI ISHII,† KEITA ISHII†‡ and KOKICHI KIKUCHI†
*Departments of *Dermatology and †Pathology, Sapporo Medical College, S1 W17, Sapporo 060, Japan*

Abstract—*A xenoantiserum to murine B16 melanoma cells was developed by immunizing rabbits with cultured B16 melanoma cells. This antiserum could, after extensive absorption with normal C57BL/6 mouse tissues, react with syngeneic (B16) and allogeneic (HP) melanomas as well as with other murine neoplasms, including syngeneic 75S adenocarcinoma, allogeneic myeloma and leukemic T cell lines. The antiserum also cross-reacted with syngeneic fetal fibroblasts and with an allogeneic fetal fibroblast cell line (SC-1) either uninfected or infected with murine leukemia virus (MuLV). Immunoprecipitated material from B16 melanoma cell-surface glycoproteins that had been labeled with [^{125}I] by lactoperoxidase and purified by a Lentil lectin column was analyzed by one-dimensional SDS- and two-dimensional polyacrylamide gel electrophoresis, which disclosed an acidic glycoprotein with a molecular weight (mol. wt) of* 90 K daltons. *Absorption studies suggested that the* 90 K *mol. wt gylcoprotein represented the oncofetal moiety expressed in murine medanoma, carcinoma and fetal tissues. When the amount of this antigen in developing C57BL/6 mouse fetuses was measured by absorption assays, we found that it was expressed strongly in those fetuses just before birth. Binding and absorption studies demonstrated that the* 90 K *mol. wt glycoprotein, while being expressed on a variety of fetal and neoplastic cells in mice, did not exist at detectable levels in normal tissues of adult C57BL/6 mice, including tissues of the thymus, lymph node, spleen, liver, brain, lung and kidney.*

INTRODUCTION

MURINE B16 melanoma cells possess melanoma-associated antigens (MAA) capable of eliciting both cellular and humoral immune reactions in syngeneic hosts [1, 2]. These antigens, particularly those bound to the cell membrane, are presumed to play a major role in the host defence against this tumor. The membrane-bound MAA on murine B16 melanoma cells have been serologically defined by using syngeneic [3] or xenogeneic antisera [4, 5] raised against those cells, but their exact nature and molecular characteristics have not been well documented, and thus warrant further investigation. In this study we employed xenoantiserum raised against cultured B16 melanoma cells in order to define the cell-surface antigens of B16 melanoma cells by indirect immunoprecipitation with this reagent, followed by one-dimensional SDS- (SDS-PAGE) or two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). The data obtained indicate that B16 melanoma cells express a glycoprotein with a molecular weight (mol. wt) of 90 K daltons, which is shared among a variety of murine malignant and fetal cells.

Accepted 10 December 1981.
‡Correspondence and reprint requests should be sent to: Yoshifumi Ishii, M.D., Department of Pathology, Sapporo Medical College, S1 W17, Sapporo 060, Japan.

MATERIALS AND METHODS

Tumors

B16 melanoma and 75S mammary adenocarcinoma were maintained *in vivo* by serial subcutaneous transplantation in syngeneic C57BL/6 inbred mice. Allogeneic Harding Passey (HP) melanoma was also maintained in ICR mice in the same manner as described above.

Cell cultures

The B16 melanoma cell line (F10S) was cloned and maintained in a culture with F10 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS). The allogeneic mouse myeloma (X-63-Ag-8-653, NS-1) and leukemic T cell (BW 5147) lines were cultured in RPMI-1640 medium plus 10% FBS. Fetal fibroblasts

derived from C57BL/6 embryo and fetal fibroblast cell line (SC-1, kindly provided by Dr. A. Ishimoto, Kyoto University) derived from wild mouse embryo were cultured in the same RPMI-1640 medium with 10% FBS. Human melanoma (HMV-I, HMV-II) and carcinoma (Hela) cell lines maintained in cultures were also used.

Cell suspension

A single cell suspension of tumor cells was prepared mechanically from transplanted solid B16 and HP tumors or from 75S carcinoma by treatment with 0.1% trypsin at 37°C for 30 min. A monolayer of cultured melanoma, carcinoma or fetal cells was also detached from the culture bottle by the trypsin treatment. The detached cells were washed twice with RPMI-1640 medium and cultured for 20 hr at 37°C in a 90 × 20 mm Petri dish (Termo Co., Tokyo, Japan), where re-attachment of these cells was markedly suppressed. Cell suspensions were also prepared from C57BL/6 mouse spleens, lymph nodes and thymuses by the method previously described [6].

Antisera

Xenoantisera to B16 melanoma cells were developed by immunizing rabbits intravenously with 2×10^7 of the cultured B16 cells (F10S) weekly for 4 weeks. The immunized cells were harvested with 0.25% trypsin, washed twice with phosphate-buffered saline (PBS) and cultured in chemically defined F10 medium without FBS for 3 hr. The rabbits were bled 5 days after the last injection and the sera were collected, heat-inactivated and absorbed sequentially with equal volumes of C57BL/6 mouse red cells, pooled spleen and thymus cells and pooled tissue homogenate obtained from lung, heart, kidney, brain and liver tissues of C57BL/6 mice until the antiserum no longer reacted with C57BL/6 mouse thymus and spleen cells by immunofluorescence. This serum was referred to as anti-B16 melanoma xenoantiserum (XAB16) in this study. Syngeneic anti-B16 melanoma serum (SAB16) was prepared by repeated subcutaneous immunization of C57BL/6 mice with 1×10^6 of F10S cells pretreated with mitomycin C (30 μg/ml) at 37°C for 30 min. Goat antisera prepared against the purified proteins gp70 and p30 of Rauscher MuLV (murine leukemia virus) were obtained from the Virus Center Program of the National Cancer Institute, National Institutes of Health, through the courtesy of Dr. C. H. Granatek, M. D. Anderson Hospital and Tumor Institute.

Immunofluorescence

The binding of antibody to antigens on the cell surface was studied by indirect membrane immunofluorescence[7]. Either XAB16 (1 : 10 dilution) or SAB16 (1 : 5 dilution) was used as the first reagent. After incubation of cells with these antisera for 30 min at 4°C, the cells were washed 3 times with PBS and were reincubated with Fab_2 fragments of fluorescein-conjugated antibodies to either rabbit IgG or mouse IgG at 4°C for 30 min. Each stain was applied for viable cells and the percentage of cells with surface fluorescence was counted under a Leitz Ortholux II microscope with an incident u.v. illuminator.

Absorption study

Unabsorbed XAB16 serum (1 : 10 dilution) was absorbed once or twice with an equal volume of either normal, fetal or neoplastic cells and tissue homogenates at 4°C for 30 min. The mixture was then centrifuged at 2000 ***g*** for 15 min and the supernatant was tested for residual antibody activity on B16 cell-surface antigens by radioimmunoprecipitation (RIP) and SDS-polyacrylamide gel electrophoresis (SDS-PAGE), as described below.

Cell-surface iodination

Plates of a confluent monolayer of cultured B16 melanoma cells (F10S) were washed 3 times with PBS and covered with 2 ml of PBS, to which 2 mCi Na[^{125}I], 100 μg lactoperoxidase (Sigma Chemical Co., St. Louis, MO) and 200 μl of 0.03% hydrogen peroxide were added [8]. Hydrogen peroxide was added 4 times at 3 min intervals. After incubation at 20°C for 15 min, the fluid was discarded and the plates were washed 3 times with 50 ml of PBS. The labeled monolayer was covered with 4 ml of 2% Lubrol-PX (Nakarai Chemicals, Kyoto, Japan) in PBS and antigen extraction was continued at 4°C for 1 hr. The supernatant fluid was then collected, spun at 4000 ***g*** for 20 min, filtered through a Millipore 0.45 μm membrane and applied onto a Sepharose 4B column (1.8 × 20 cm) coupled with *Lens culinaris* hemagglutinin (LcH: Pharmacia Fine Chemicals, Uppsala, Sweden). The column was extensively washed at a flow rate of 15 ml/hr with 0.1 M Tris–HCl (pH 8.0) containing 0.2% Lubrol-PX and 0.5 M NaCl. The bound material was eluted from the column by adding 4% methyl-D-mannoside [9], dialysed against PBS containing 0.2% Lubrol-PX and subjected to RIP experiments. Approximately 20% of the radioactivity added to the column was recovered in the bound fraction.

Radioimmunoprecipitation

Aliquots of the labeled glycoprotein preparation (approximately 100,000 cpm) prepared from labeled B16 melanoma cells by LcH-coupled Sepharose 4B column chromatography were mixed with 10 μl of the antiserum or with preimmune normal serum and were incubated at 4°C for 20 hr. Antigen–antibody complexes were precipitated by adding 100–200 μl of 10% *Staphylococcus aureus* Cowan I (SACI: The Enzyme Center Inc., Boston, MA) to the mixture [10]. After 30 min at 37°C, the bacteria were washed 3 times with normal saline containing 0.2% Lubrol-PX and immune complexes bound to the bacteria were dissociated by heating them at 100°C for 4 min in 150 μl of 50 mM Tris–HCl (pH 7.0) containing 2% SDS with or without 5% 2-mercaptoethanol (2-ME)(SDS sample buffer), or in 50 μl of 9.5 M urea containing 0.2% Nonidet P-40 (NP-40), 5% 2-ME and 8.5% Pharmalyte (pH 3 : 10 5%, pH 8 : 10.5 2%, pH 2.5 : 5 1%, pH 5 : 8 0.5%; Pharmacia Fine Chemicals, Uppsala, Sweden) (isoelectric focusing (IEF) sample buffer). Each sample solubilized in SDS or IEF sample buffer was applied to either one-dimensional SDS-PAGE or 2-D PAGE, as described below.

SDS-PAGE

SDS-PAGE (0.1% SDS and 10% polyacrylamide tube gel) was performed by Laemmli's method [11]. The gel was electrophoresed at 3 mA/tube until a bromophenol blue marker reached the end of the gel. The gel was then stained with Coomassie brillant blue and cut into 2 mm slices, which were then counted in a Packard gamma counter. In some experiments SDS-polyacrylamide slab gel electrophoresis was performed and radioactivity in the slab gel was visualized by the radioautographic technique [12]. The protein standards and their mol. wts were phosphorylase B (94 K), bovine serum albumin (67 K), ovalbumin (43 K), carbonic anhydrase (30 K), trypsin inhibitor (20.1 K) and alpha-lactalbumin (14.4 K).

2-D PAGE

2-D PAGE was performed by a slight modification of O'Farrell's method [12]. In our experiments we used IEF tube gels that contained 8 M urea, 0.2% NP-40 and 8.5% Parmalyte (pH 3 : 10 5%, pH 8 : 10.5 2%, pH 2.5 : 5 1%, pH 5 : 8 0.5%), instead of 9.2 M urea, 2% NP-40 and 2% Ampholines. By using this modification, a pH gradient in the basic side of the gel was preserved, resulting in a good resolution of proteins, with isoelectric points (pI) ranging from 8.2 to 2.8. The first dimension separation was performed on IEF gels, while the second dimension separation was done on 10% SDS-polyacrylamide slab gels.

RESULTS

Reactivity of XAB16 with different cell types

Table 1 shows the reactivity of XAB16 with different cell types as defined by indirect membrane immunofluorescence. The antiserum, which was extensively absorbed with normal lymphocytes and pooled tissue homogenates from adult C57BL/6 mice, reacted strongly with cultured as well as freshly excised B16 melanoma cells and less strongly with allogeneic HP melanoma and syngeneic 75S carcinoma cells. On the other hand, human melanoma and carcinoma cell lines were entirely negative in fluorescence. XAB16 serum weakly but clearly cross-reacted with murine myeloma and leukemic T cell lines, but was entirely nonreactive with leukocytes obtained from the thymuses, lymph nodes and spleens of normal adult C57BL//6 mice. The antiserum was, in addition, reactive with syngeneic fetal fibroblasts and with a fibroblast cell line (SC-1) either uninfected or infected with murine leukemia virus (MuLV), suggesting that XAB16 detects antigen(s) unrelated to MuLV

Table 1. *Reactivity of XAB16 with various cell types as defined by immunofluorescence*

Cell types	Immunofluorescence*
Murine melanoma	
B16 (Fresh)	+
B16 (Cultured)	+
HP (Fresh)	+
Murine carcinoma	+
Murine myeloma	
NS-1	+
X-63-Ag-8-653	+
Murine T cell leukemia (BW 5147)	+
Murine fetal fibroblasts	
Syngeneic	+
Allogeneic (SC-1)	
uninfected	+
infected†	+
C57BL/6 adult normal cells‡	
Thymus	−
Spleen	−
Lymph node	−
Human melanoma (HMV-I, II)	−
Human carcinoma (Hela)	−

*More than 50% of fluorescent cells were positive (+) and less than 3% were negative (−).

†SC-1 cells were infected with either xenotropic or amphotropic MuLV.

‡Cells were obtained from normal 8-week-old C57BL/6 mice.

but shared among a wide variety of fetal and neoplastic cells in mice.

Characterization of XAB16-defined cell-surface antigen(s)

For partial characterization of XAB16-defined antigen(s) we used an RIP technique and reacted XAB16 with B16 melanoma cell-surface glycoproteins that had been labeled with [^{125}I] by lactoperoxidase and purified by affinity chromatography on a LcH-coupled Sepharose column. SDS-PAGE analysis of the immunoprecipitates formed between XAB16 and the labeled B16 melanoma glycoproteins clearly demonstrated that XAB16 precipitated a single glycoprotein with mol. wt of 90 K daltons (gp90), as estimated on the basis of the electrophoretic mobilities of the protein standards on SDS gels (Fig. 1). This 90 K mol. wt component appeared to consist of a single polypeptide rather than sulfhydryl-linked subunits since it ran almost identically under both reducing and nonreducing conditions. Whereas XAB16 clearly precipitated the 90 K mol. wt component from the labeled B16 melanoma glycoproteins, preimmune normal serum did not precipitate any definite peaks from the same glycoprotein preparation.

A 2-D PAGE profile of this 90 K mol. wt glycoprotein is shown in Fig. 2. The XAB16 serum again precipitated the 90 K mol. wt component from labeled B16 melanoma glycoproteins, showing a charge heterogeneity with a pI between 3.2 and 4.5.

In order to determine the tissue distribution of the gp90 precipitable by XAB16 from B16 melanoma cells, aliquots of the unabsorbed XAB16 (1 : 10 dilution) were absorbed once or twice with an equal volume of tissue homogenates prepared by a Virtis blender homogenizer from a variety of tumor, normal and fetal tissues, and their residual capacity to precipitate the gp90 glycoprotein was determined by RIP and SDS-PAGE. As can be seen in Fig. 3, the unabsorbed XAB16 precipitated two major glycoproteins with mol. wts of 120 K and 90 K daltons from the labeled B16 melanoma cell glycoproteins. When it was absorbed with normal tissues from the livers, brains, lungs, kidneys, spleens and thymuses of C57BL/6 mice, the 120 K component completely disappeared, suggesting that the 120 K glycoprotein is a normal cellular component present on both murine melanoma and normal tissues. Whereas anti-gp90 antibody activity in XAB16 could not be reduced by absorption with normal tissues, it could be absorbed out with B16 melanoma tumor. Although allogeneic HP melanoma and syngeneic S75 carcinoma tissues, as well as syngeneic fetal (17 days) tissue homogenates, were less effective in removing anti-gp90 antibody from XAB16, almost complete absorption was possible when sufficient amounts of these tissue homogenates were used for absorption. In contrast, human melanoma (HMV-I) and carcinoma (Hela) cells could absorb neither the antibody to the 120 K component or that to the 90 K component.

Quantitation of gp90 in developing C57BL/6 fetuses

XAB16, which was absorbed with normal lymphocytes and pooled tissue homogenates from adult C57BL/6 mice, was further absorbed with an equal volume of tissue homogenates prepared from whole C57BL/6 mouse embryos with different gestational ages. The residual antibody activity was measured by RIP using [^{125}I]-labeled B16 melanoma cell glycoproteins. The data were expressed in terms of the percentage inhibition of the binding reaction caused by absorption of the antiserum with fetal tissue homogenates. As the results shown in Fig. 4 indicate, there was a gradual increase in absorption capacity for anti-B16 melanoma activity in the XAB16 of C57BL/6 fetuses parallel to their gestational ages, lasting from 15 to 20 days.

Relationship of antigens defined by xenogeneic and syngeneic anti-B16 melanoma sera

Whereas XAB16 precipitated the 90 K mol. wt glycoprotein (gp90) from the labeled B16 melanoma glycoprotein preparation, SAB16 could not precipitate any components from the same sample with either SACI or rabbit anti-mouse immunoglobulin serum as the precipitating reagent (data not shown). Therefore we employed a blocking technique using these two antisera, in which the cultured B16 melanoma cells were first treated with either XAB16 or SAB16 at 4°C for 30 min and then, after washing, were subjected to staining with reciprocal antisera by immunofluorescence. By means of this procedure it is possible to determine whether both antisera are directed to the same or closely associated antigens on the B16 melanoma cells. The results shown in Table 2 indicate that XAB16 had no blocking effect on the subsequent binding of SAB16 to B16 melanoma cells or vice versa, suggesting that each antiserum detects a distinct antigen system on B16 melanoma cells.

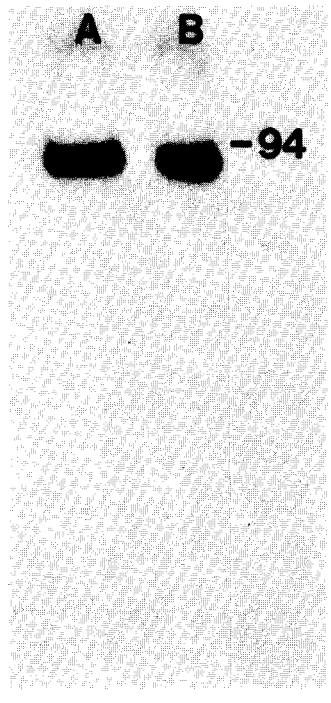

Fig. 1. SDS-PAGE profiles of the immunoprecipitates formed between XAB16 and [^{125}I]-labeled B16 melanoma cell-surface glycoproteins that had been purified by LcH affinity chromatography. The samples were electrophoresed under reducing (A) and non-reducing conditions (B).

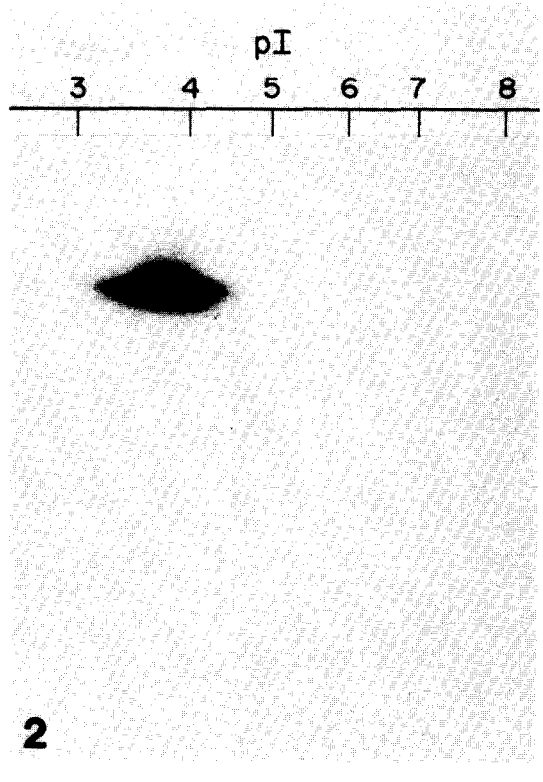

Fig. 2. 2-D PAGE profile of the immunoprecipitate formed between XAB16 and [^{125}I]-labeled B16 melanoma cell-surface glycoproteins. The first dimension separation was by IEF (the acidic side being on the left and the basic side on the right). The second dimension separation was by SDS-PAGE on 10% gel (from top to bottom).

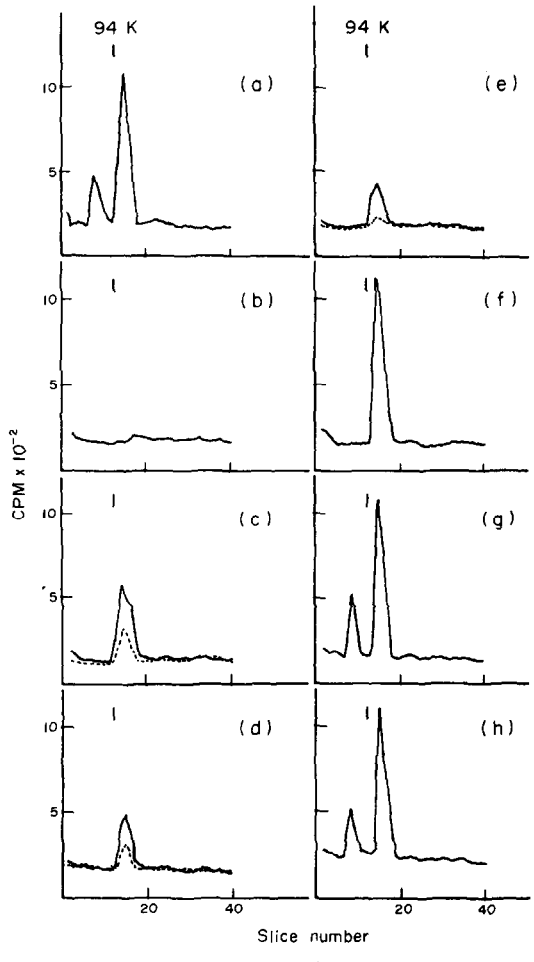

Fig. 3. SDS-PAGE profiles of [^{125}I]-labeled B16 melanoma cell-surface glycoproteins that reacted with XAB16 either unabsorbed (A) or absorbed once (—) or twice (--) with B16 (B), HP (C) and 75S (D) murine tumor cells and with syngeneic C57BL/6 fetal (E) and adult normal cells (F). Normal tissues, including liver, brain, spleen, thymus, kidney and lung, were used for absorption and gave almost identical results as indicated in Fig. 3F. The antiserum was also absorbed with human carcinoma (Hela) cells (G) and melanoma (HMV-I) (H).

Table 2. Immunofluorescent blocking experiments using xenogeneic (XAB16) and syngeneic (SAB16) anti-B16 melanoma antisera

1st reagent	2nd reagent	Membrane fluorescence*
XAB16	SAB16	+
XAB16	NMS†	–
NRS‡	SAB16	+
SAB16	XAB16	+
SAB16	NRS	–
NMS	XAB16	+
NRS	NMS	–
NMS	NRS	–

*Cultured B16 melanoma cells were pretreated with the 1st reagent at 4°C for 30 min and then were retreated with the 2nd reagent. Cells were stained by immunofluorescence with Fab_2 fragments of the fluorescein-labeled antibody to the 2nd reagent.
†Normal C57BL/6 mouse serum.
‡Normal rabbit serum.

Relationship of gp90 on B16 melanoma cells to MuLV viral components (gp70 and p30)

To determine whether the gp90 antigen detected by XAB16 on B16 melanoma cells is related to MuLV viral components, such as gp70 or p30, sequential immunoprecipitation studies were performed using XAB16 and xenoantisera to the purified gp70 or p30 of Rauscher MuLV. For this purpose the labeled B16 glycoproteins (100,000 cpm) were preabsorbed twice with 25 μl of packed SACI on which 50 μl of undiluted XAB16, anti-gp70, anti-p30 or normal preimmune serum had been loaded. After incubation at 4°C for 2 hr the mixtures were centrifuged at 4000 *g* for 20 min, after which the supernatants were col-

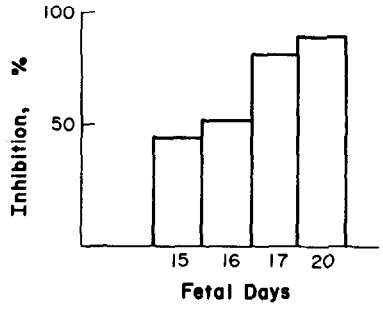

Fig. 4. The absorption capacities for anti-B16 melanoma activity in XAB16 of syngeneic C57BL/6 fetuses at 15–20 days of gestation. The antiserum (× 10 dilution) was absorbed with an equal volume of fetal homogenates and residual reactivity was measured with [^{125}I]-labeled B16 melanoma cell glycoproteins. The data were expressed as the percentage reduction of the binding activity of XAB16 after absorption with respective fetal tissue homogenate, as estimated by the following formula:

$$\frac{\text{cpm bound to XAB16} - \text{cpm bound to XAB16 absorbed with fetal homogenates}}{\text{cpm bound to XAB16} - \text{cpm bound to normal rabbit serum}} \times 100.$$

Table 3. Reactivity of XAB16 with [^{125}I]-labeled B16 melanoma glycoproteins immunodepleted with either XAB16 or antisera to the gp70 and p30 of Rauscher MuLV

Immunodepletion with	Specific binding to XAB16*
Normal goat serum	7215 (0)†
Goat anti-gp70	6672 (7.5)
Goat anti-p30	7188 (0.4)
Normal rabbit serum	7352 (0)
XAB16	1470 (80.0)

*The radioactivity (cpm) bound to XAB16 was subtracted by that bound to preimmune normal serum.
†The percentage reduction of radioactivity bound to XAB16 after immunodeplition with respective antiserum is in parentheses.

lected and subjected to immunprecipitations with XAB16. As the results shown in Table 3 indicate, neither anti-gp70 nor anti-p30 serum could deplete the antigen detected by XAB16, suggesting that XAB16 recognizes the antigen distinct from the gp70 or p30 of MuLV.

DISCUSSION

It has been suggested that experimental murine tumors induced chemically or by viruses express a variety of embryonic antigens either immunogenic or nonimmunogenic to the host's immune system [13–18]. In the case of murine B16 melanoma, however, the existence of such an oncofetal moiety has not been reported as far as we know, although there have been many reports suggesting the presence of oncofetal antigens on human malignant melanoma [19, 20].

The present study has shown that murine B16 melanoma cells express an oncofetal antigen widely shared among a variety of murine fetal and neoplastic cells. Since the XAB16 used in this study for detecting such an oncofetal antigen was raised against cultured melanoma cells, the question may arise as to whether the antigen detected could be part of the FBS. This possibility, however, is unlikely, due to the fact that freshly excised B16 melanoma tissue also contains this antigen. For the same reason, this antigen is unlikely to be a neoantigen acquired during the culturing process. Moreover, MuLV-associated viral components, which might exist in murine B16 melanoma cells, do not account for our data since XAB16 reacted equally with the SC-1 murine fetal fibroblast cell line either uninfected or infected with MuLV. This was further confirmed by sequential immunoprecipitation studies, indicating that the XAB16-defined antigen was immunodepleted by neither the antiserum to gp70 nor by that to the p30 of Rauscher MuLV. In addition, the XAB16-defined antigen is clearly different from brain-associated membrane antigens such as MBA-2, INMA or Thy-1 [21] since C57BL/6 mouse brain tissue failed to remove the reactivity of XAB16 with B16 melanoma cells. A question still remains to be answered, however, as to whether the antigen is associated with fetal brain, as has been suggested in the case of human melanoma [19].

The oncofetal antigen defined by XAB16 on B16 melanoma appears to be similar to that detected by pregnant retired breeder serum on cultured murine fetal fibroblasts and a variety of murine tumor cell lines including colon carcinoma and HP melanoma [18]. In our experiments we found that the XAB16-defined antigen was expressed on syngeneic (B16) and allogeneic (HP) melanomas as well as on other neoplasms, including syngeneic 75S adenocarcinoma, allogeneic myeloma and leukemic T cells. This antigen appears to be expressed strongly on syngeneic fetuses just before birth, since 20-day-old fetus had the highest absorption capacity for anti-B16 activity in XAB16. A similar observation has also been made by Cleveland *et al.* [18], who showed that 19-day-old fetal cells are more effective for absorbing the anti-HP melanoma antibody found in pregnant retired breeder serum than are 14-day-old fetal cells.

SDS-PAGE analysis of the immunoprecititates formed between unabsorbed XAB16 and labeled B16 melanoma cell-surface glycoproteins demonstrated two major glycoproteins with mol. wts of 120 K and 90 K daltons respectively. Whereas the former, 120 K glycoprotein appeared to be a normal cell-surface constituent as evidenced by absorption studies with normal tissues, the latter, 90 K glycoprotein (gp90) could not be depleted by absorption of the antiserum with normal tissues but could indeed be depleted with fetal and tumor tissues, indicating that gp90 represents one of the oncofetal moieties expressed on various neoplasms in the murine system. Several studies concerned with the molecular aspects of melanoma MAA defined by either syngeneic [3] or xenogeneic antisera [4, 5] raised against B16 melanoma cells have been reported. Bystryn *et al.* [3], using syngeneic antisera, detected an antigen expressed largely on B16 melanoma cells and to a lesser extent on syngeneic adult fibroblasts. This antigen has been considered to have a mol. wt greater than 150 K daltons. Poskitt *et al.* [4], on the other

hand, described a B16 MAA with a mol. wt of 20–25 K daltons which appeared to be specifically expressed on B16 cells. This low mol. wt antigen, though detected by xenoantisera, has also been shown to be immunogenic to syngeneic C57BL/6 mice, resulting in the production of anti-B16 antibody in those animals. These reported B16 melanoma-associated antigens seem to be different from the gp90 described herein due to the differences in molecular characteristics and tissue distribution. The gp90 antigen may, however, be related to the glycoprotein of the apparent mass of 100 K daltons considered by Bramwell and Harris [22] to be invariably linked to the malignant trait in their study of various tumor types, since both glycoproteins have high affinity to LcH or concanavalin-A and show similar molecular weight and isoelectric point characteristics.

More recently, Bhavanandan *et al.* [23], using xenoantisera to B16 melanoma cells, have identified B16 MAA, which has the general characteristics of sialoglycoproteins. Its molecular weight as estimated by gel filtration is 375 K daltons, but when it was subjected to prolonged treatment with SDS and analyzed by SDS-PAGE, these authors found a smaller subunit which migrates in SDS gels slightly faster than phosphorylase B (94 K). The gp90 antigen detected by our antiserum seems to have some similarities to the B16 MAA described by Bhavanandan *et al.* [2] since both can interact with LcH or concanavalin-A and also have similar electrophoretic mobilities in SDS gels. The MAA defined by their antiserum, however, has been shown to exist in B16 melanoma cell nuclei in a much higher concentration than in the plasma membrane.

It may be of interest to compare MAA between murine and human melanoma. One of the MAA detected by either xenoantisera [20, 24] or monoclonal antibody [25, 26] on human melanoma cells has been demonstrated to have a mol. wt of around 90–97 K daltons and to be widely shared among various human malignancies, including melanoma and carcinoma. The oncofetal nature of this antigen has not been well established, but it appears to be similar to the gp90 antigen on murine melanoma cells in its tissue distribution and molecular characteristics. Both antigens are glycoproteins with a mol. wt of around 90 K as estimated by SDS-PAGE, and are widely shared among a variety of solid tumors, including melanoma and carcinoma. It might be possible that such a component is the common constituent of the melanoma cell membrane in various species, including mice and man.

Acknowledgement—We wish to thank Dr. K. Jimbow for providing available materials in this study.

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