Antigenic Differences between Metastatic and Nonmetastatic BSp73 Rat Tumor Variants Characterized by Monoclonal Antibodies

Siegfried Matzku,1 Achim Wenzel, Sida Liu, and Margot Zöller

Institute of Radiology and Pathophysiology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany

ABSTRACT

Variants AS and ASML of the BSp73 rat tumor differ markedly with respect to morphology and to the capacity for spontaneous metastasis via the lymphatics. Monoclonal antibodies (MAbs) were raised in order to identify surface molecules associated with both phenotypes. Mice were immunized with either whole cells or isolated membranes and hybridoma supernatants were screened according to the criteria of selective binding to cultured cells of the variant used for immunization. Of two MAbs reacting with the nonmetastasizing AS variant, one showed immunostaining of AS tissue. From 11 MAbs binding to the metastatic ASML variant, 6 showed specific staining of ASML tissue, while the remaining 5 MAbs showed cross-reaction with normal rat tissues. According to Western blot data, the 6 MAbs selectively binding to ASML tissue identified at least 2 different antigens, one of them showing up as a complex of 12 bands.

INTRODUCTION

Homotypic contact of tumor cells as well as various types of interaction between tumor cells and normal tissue structures play a decisive role in metastatic spread (recently reviewed in Refs. 1–3). Since cell surface molecules are known to play an important role in these processes (4–6), an identification of surface antigens selectively associated with a metastatic tumor line may help to elucidate the mechanisms conferring metastatic potential to the respective tumor cells. This may be achieved most easily by raising monoclonal antibodies against spontaneously metastasizing tumor cells. The BSp73 tumor (7) represents a promising model, because metastasizing and nonmetastasizing variants have a markedly different morphotype and because they are available as operationally pure populations (8).

In order to identify discriminating molecules on the surface of the variants as comprehensively as possible, we decided to immunize in an xenogeneic species, i.e., BALB/c mice. Using this strategy, a stringent screening procedure was needed in order to identify variant-specific MAbs3 from an abundance of rat-specific MAbs. This was achieved by selecting for MAbs that bind to the variant used for immunization but not to the opposite variant nor to cultured rat fibroblasts. MAbs meeting this criterion were further screened by the immunoperoxidase method using sections from the BSp73 variants as well as from representative normal tissues of the rat.

MATERIALS AND METHODS

Cell Lines and Tumors. Tissue culture lines were kept in RPMI 1640 supplemented with 10% fetal calf serum (GIBCO, Karlsruhe, Federal Republic of Germany), 4 mM glutamine, and antibiotics. Myeloma X63/Ag8.653 was treated with 0.13 mM 8-azaguanine 4 days prior to use. Rat tumor lines BSp6 and BSp12 and variants AS and ASML of the BSp73 rat tumor (all of them being spontaneous BDX tumors) were established and maintained as described previously (7, 9). The autochthonous BSp73 tumor was diagnosed as an adenocarcinoma of the pancreas, but a shift towards anaplastic morphology was noted in the established variant lines (7). Fetal rat (BDX) fibroblasts were obtained by short term culturing of mechanically dispersed fetal tissue from the 17th day of gestation (designated F17). Rat 2A fibroblast (F344 strain) were provided by Dr. H. Ponta (Karlsruhe, Federal Republic of Germany). Cell membranes were prepared as described in Ref. 10. Cultured BSp73 AS or ASML cells were inoculated s.c. into BDX rats (10^5 cells/animals) and tumor tissue was excised when diameters of 10–15 mm were reached.

Immunization. Clones 10AS-7 and 14ASML-1 derived from the respective variants of BSp-73 were used for immunization and testing. Prior to membrane preparation and/or immunization, tumor cells were cultured for three cycles in serum-free Iscove's media (GIBCO) in order to minimize artificial immunization against serum components. Priming of 6-week-old BALB/c mice was done by i.p. injection of 2 × 10^7 living tumor cells in PBS or by s.c. injection (3 sites) of an emulsion of cell membranes (equivalent to 2 × 10^6 cells) in complete Freund's adjuvant. Booster injections were performed 3 and 5 weeks after priming (i.p. or s.c.; incomplete Freund's adjuvant) and 3 days prior to fusion (i.v.; cells in PBS) using the same amounts of cells and membranes, respectively.

Hybridoma Production. According to the method published by Köhler (11), spleen cells from immunized mice and myeloma cells were mixed and fused with PEG 1500 (Boehringer, Mannheim, Federal Republic of Germany). The suspension was transferred into hypoxanthine-aminopterin-thymidine media (Sigma, Munich, Federal Republic of Germany) and distributed in 10 flat-bottomed microplates, which contained 5 × 10^6 feeder cells (rat thymocytes irradiated with 30 Gy).

Testing of Supernatants. Two to 3 weeks after fusion, supernatants from wells with positive growth were collected. Antibody binding to the cell line used for immunization as well as to control cells was tested by ELISA. In short, test cells were collected by trypsinization and conditioned for 2 h in tissue culture media (37°C, 5% CO_2 in air). Cells (1 × 10^5/well) were placed in U-well Immunolon microplates (Greiner, Nürttingen, Federal Republic of Germany), which had been pretreated for 4 h with 5 mg/ml gelatin in PBS. Cells were pelleted by centrifugation and suspended in 50 μl of hybridoma supernatant/well. After overnight incubation at 4°C, pellets were washed three times with 1 mg/ml gelatin in PBS and incubated for 60 min with peroxidase-coupled goat anti-mouse IgG (Dianova, Hamburg, Federal Republic of Germany). After 4 cycles of washing, peroxidase activity was detected by the addition of substrate solution (0.5 mg/ml o-phenylenediamine and 0.32 μl/ml 30% H_2O_2 in phosphate-citrate buffer, pH 5.0). Absorbances were measured in a Titertek scanner (Flow, Bonn, Federal Republic of Germany), the threshold of positivity being set at 2 SD above the absorbance of the negative control.

Production of Monoclonal Antibodies. Cells producing MAbs with the desired binding specificity were expanded in hypoxanthine-thymi- dine media and subject to two cycles of recloneing at limiting dilution (3 and 1 cells/well) in wells containing irradiated thymocyte feeders. MAb-containing supernatants were produced by culturing hybridomas in normal tissue culture media, while larger amounts of MAb IgG were obtained from ascites induced by inoculating 5–10 × 10^6 hybridoma cells into the peritoneal cavity of pristane-treated BALB/c mice. Antibodies were purified by chromatography on protein A and Mono Q columns (Pharmacia, Freiburg, Federal Republic of Germany) as described previously (12). Isotypes were determined by the Ouchterlony technique using specific antisera (Tago, Burlingame, CA).

Immunohistology. Cryostat sections of tumors and normal rat tissues were pretreated with 0.1% phenylhydrazine in order to destroy endogenous peroxidase and with 20% rabbit serum in order to reduce unspecific binding in the subsequent steps. After incubation with hybridoma supernatants, sections were treated with rabbit anti-mouse IgG (anti-H

Received 2/15/88; revised 9/13/88; accepted 11/10/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed.
2 Recipient of a fellowship of the Peoples Republic of China.
3 The abbreviations used are: MAB, monoclonal antibody; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay.

Downloaded from cancerres.aacrjournals.org on August 15, 2017. © 1989 American Association for Cancer Research.
and L chain; Dianova), which had been preabsorbed with rat serum in order to avoid binding of the reagent to endogenous rat immunoglobulin. The mouse F(ab)₂ peroxidase-antiperoxidase complex (Dianova) was added for 30 min and dianaminobenzidine (0.5 mg/ml) was used for staining. Counterstaining was performed with OsO₄. Mouse MAb M.2.7.6 reacting with a human melanoma-associated antigen (kindly provided by D. C. Sorg, Munster, Federal Republic of Germany) was used as a negative control. Staining of sections was scored according to the following criteria: ++, strong staining of >90% of tumor cells or cells in a given normal tissue; +, moderate staining of >50% of cells; —¿, no staining detectable.

Western Blot Analysis. AS cells were detached from plastic by a short treatment with a film of 0.25% trypsin and 3 mM EDTA in calcium and magnesium-free Hanks' medium at room temperature. With the ASML variant, we either collected cells growing in suspension, or we detached adherent cells with 5 ml of the above solution, incubating the cell layer for 5 min at 37°C. Trypsinized cells were conditioned for 3 h in tissue culture medium. Cells were lysed in a buffer containing 0.5% Nonidet P-40 and a cocktail of inhibitors (2 mM phenylmethylsulfonyl fluoride, 2 μM 1,10-phenanthroline, 1 mM iodoacetamide, 1 μg/ml leupeptin [Serva, Heidelberg, Federal Republic of Germany], 1 μg/ml antipain [Boehringer] and 3 mM NaN₃). Lysates were run on a 6% sodium dodecyl sulfate-polyacrylamide gel under nonreducing conditions (12). On each slab gel, molecular weight marker proteins (Sigma) were applied to an extra lane. After separation, proteins were electrophoretically transferred onto nitrocellulose overnight at 4°C. Nitrocellulose sheets were blocked by treatment with a solution of 10% nonfat dry milk powder, 2% bovine serum albumin, and 0.1% Tween20 in PBS for 2 h at 37°C. Individual strips from the nitrocellulose sheet were incubated with hybridoma supernatants (10 ml/lane) for 4 h at room temperature. After 4 cycles of washing with 0.3% Tween 20 in PBS, sheets were treated with peroxidase-coupled goat anti-mouse IgG in blocking buffer (Dianova; dilution, 1:2000) for 2 h at room temperature, and this was followed by the above described washing procedure. Staining was performed with a solution containing 50 mg dianaminobenzidine and 50 μl H₂O₂ in 100 ml PBS. After 10 min at room temperature, sheets were washed with demineralized water and dried.

RESULTS

A total of 6 fusions was performed, using spleens from mice which were immunized with intact 10AS-7 and 14ASML-1 cells (one fusion each) or membranes prepared from these cells (two fusions each). Hybridoma growth was recorded in more than 3000 wells, the supernatants of which were subjected to a screening procedure consisting of an ELISA on cultured AS, ASML, and rat fibroblast cells. The test criterion was significant reaction (i.e., 2 SD above background) with the BSp73 variant used for immunization. Of 563 cultures reacting with these cells, 13 hybridomas showed apparent specificity; i.e., they bound to the variant used for immunization but not to the other variant or to rat fibroblasts. Interestingly, none of these "specific" hybridomas was obtained from fusions of spleen cells immunized with the opposite variant. Hybridomas were expanded and subjected to two cycles of recloning by the limiting dilution method. Table 1 gives ELISA results obtained with supernatants after recloning, the panel of target cells now including both BSp73 variants, two other spontaneous rat tumor lines (BSp6 and BSp12), a rat fibroblast cell line (Rat2A), short term cultured fetal rat fibroblasts (F17), freshly dispersed BDX spleen cells, and a human melanoma line (MeWo). Hybridomas A8.10 and C5.1 produced MAbs reactive with variant AS and another nonmetastasizing BDX tumor line, but not with the metastatic variant ASML. MAbs produced by the other 11 hybridomas showed specific binding to ASML cells, but not to the rest of the cell lines tested. Three hybridomas showing indiscriminate binding to rat cells were used as positive controls in ELISA as well as in subsequent immunohistology experiments. According to immunodiffusion, MAb A8.10 was found to be IgG2a, while the all other MAbs listed in Table 1 were classified as IgG1.

The pattern of expression of MAb-defined antigens in vivo was evaluated by immunohistological analysis of tumor variant tissue as well as of representative normal rat tissues. Cross-reaction of the peroxidase-coupled rabbit anti-mouse IgG reagent with endogenous rat IgG was suppressed by preabsorption with normal rat serum. Mouse MAb M.2.7.6 with specificity for a human melanoma-associated antigen was used as negative control, while MAbs A1.9, C5.3, or D6.10 served as positive controls (data not shown). Immunohistology results are summarized in Table 2, staining intensity being scored according to an arbitrary scale ranging from background (−) to highly positive staining (+++; see "Materials and Methods"). Positive antigen expression on the respective tumor variants was observed with all but one (i.e., C5.1) of the MAbs showing "specific" binding in vitro. Five MAbs were found to define antigens which were markedly expressed on ASML tissue but also on some normal tissues. A1.6 bound to liver and kidney; MAbs D4.6, D5.7, D6.1 bound to liver, kidney, and pancreas; and MAb B5.5 bound to muscle, capillary endothelia, and nerve tissue. Seven MAbs showed operational specificity in the sense that the given detection limit of the method, staining was observed only with the variant tumor tissue used for immunization; i.e., one MAB bound to AS tumor tissue, and the other 6 MAbs bound to ASML tumor tissue. Representative examples of immunohistologies are shown in Figs. 1 and 2, which also illustrate the different levels of staining intensity and the scoring scale. The pattern of staining observed at the cellular level was compatible with antigen presentation at the membrane and possibly also in the cytoplasm but not with deposition of antigens into the extracellular space. Furthermore, Fig. 2c shows an example of positive staining of a spontaneous metastasis (variant ASML) to the lung.

On the basis of these data, it can be concluded that MAbs showing cross-reactivity with normal tissues defined at least 3 different antigens, the similarity in staining patterns obtained with D4.6, D5.7, and D6.1 suggesting a similar if not identical target molecule. In the Western blot, these MAbs stained a single band with a molecular weight of 38,000 (not shown). The MAbs specific for the nonmetastatic variant AS are possibly recognizing two different antigens. This is concluded from the difference in expression in vivo, although the results could also be explained by low binding affinity of C5.1.

To find out how many different antigen molecules are defined by the MAbs "specifically" reacting with ASML tissue in vivo, Western blot analysis was carried out using whole cell lysates of cultured AS and ASML cells (Fig. 3). No band was seen with an isotype-matched MAb (i.e., B40, IgG1) of irrelevant specificity, thus confirming the absence of nonspecific binding of IgG1 to constituents of both types of cell lysate. MAb A2.4 on the contrary stained as much as 8 prominent and 4 feeble bands. An identical set of bands was detected with MAbs B4.4 and B4.10 (not shown). The pattern obtained with MAbs A2.6 and C1.6 suggested that both MAbs possibly bind to components of the A2.4 antigen complex, since staining was observed at positions which were similar, if not identical, to some of the high molecular weight bands seen with A2.4, while the low molecular weight bands were not detected. When lysates were prepared from adherent ASML cells which were conditioned in suspension culture for 3 h after enzymatic detachment, MAbs A2.4 and A2.6, but not C1.6, showed very strong but fuzzy staining at the M, 58,000/54,000 position (not shown). A
ANTIGENS ON METASTATIC RAT TUMOR

Table 1 Cell ELISA: binding of hybridoma supernatant to various target cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MAb BSp73 AS</th>
<th>MAb BSp73 ASML</th>
<th>MAb BSp6</th>
<th>MAb BSp12</th>
<th>MAb Rat2A</th>
<th>MAb F17</th>
<th>MAb Spleen</th>
<th>MAb MeWo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reactive hybridomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1.9</td>
<td>1.30 ± 0.09*</td>
<td>1.49 ± 0.09</td>
<td>1.84 ± 0.10</td>
<td>1.57 ± 0.05</td>
<td>0.43 ± 0.01</td>
<td>—</td>
<td>1.04 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td>C5.3</td>
<td>1.42 ± 0.09</td>
<td>1.40 ± 0.06</td>
<td>1.76 ± 0.09</td>
<td>1.86 ± 0.09</td>
<td>0.84 ± 0.03</td>
<td>—</td>
<td>1.14 ± 0.05</td>
<td>—</td>
</tr>
<tr>
<td>&quot;AS-specific&quot; hybridomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A8.10</td>
<td>1.20 ± 0.07</td>
<td>—</td>
<td>1.30 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C5.1</td>
<td>0.74 ± 0.04</td>
<td>—</td>
<td>0.88 ± 0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>&quot;ASML-specific&quot; hybridomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D6.1</td>
<td>—</td>
<td>1.64 ± 0.09</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D6.6</td>
<td>—</td>
<td>1.71 ± 0.09</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D5.7</td>
<td>—</td>
<td>1.51 ± 0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C1.6</td>
<td>—</td>
<td>1.89 ± 0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B4.4</td>
<td>—</td>
<td>1.80 ± 0.08</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A2.6</td>
<td>—</td>
<td>1.86 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B4.10</td>
<td>—</td>
<td>1.85 ± 0.06</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B5.5</td>
<td>—</td>
<td>1.40 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A2.4</td>
<td>—</td>
<td>1.80 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C4.4</td>
<td>—</td>
<td>1.75 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A1.6</td>
<td>—</td>
<td>1.35 ± 0.07</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Absorbance at 492 nm; mean ± SD of 3 determinations.

DISCUSSION

Evidences accumulating during the past decade foster the consensus that apart from a few exceptional systems, no exquisitely tumor-specific antigens will be identified serologically. Due to its spontaneous origin (7), the BSp73 tumor may not be expected to be exceptionally immunogenic (13, 14). This fact, together with the goal of searching for molecules which are differentially expressed on variant tumor lines differing in morphotype and metastatic phenotype, prompted us to choose an immunization protocol involving a xenogeneic species. By this approach, emphasis was laid on a comprehensive elucidation of molecular differences between variants AS and ASML, but not on the detection of antigens being completely absent from any kind of normal rat tissue. Experiments resulted in the establishment of 7 MAbs which reacted exclusively with the variant used for immunization, while leaving tissue of the other variant essentially unstained. There was a clear predominance of ASML-specific MAbs (6 of 7), which in addition showed strong immunostaining, while the only MAb specifically binding to variant AS tissue showed weak immunostaining. It cannot be excluded that by improving immunohistological methodology, low levels of antigen expression would have been detected in some normal tissues, too. However, this may not represent a problem of central importance, since there is no reason to believe that the functions implemented during the metastatic process and, hence, the surface molecules associated to it should be unique properties of tumor cells.

Further insight into the nature of molecules recognized on ASML cells by the "specific" MAbs came from Western blot data. MAbs A2.4, B4.4, and B4.10 were found to bind to an antigen complex covering the molecular weight range of 42,000–195,000. MAbs A2.6 and C1.6 bound to some, but not all of the high molecular weight components of the A2.4 complex. No binding of these MAbs was recorded in the lower molecular weight range, with the exception of A2.6 staining a diffuse Mr 54,000–58,000 band in lysates of ASML cells harvested by enzymatic detachment. MAb C4.4 detected an unrelated molecule at Mr 86,000.

The relationship within the complex of molecules binding to
A2.4 is unclear at the moment. None of them seems to exhibit unspecific binding to mouse IgG1, since the control MAb showed no staining at all. Enzymatic degradation during and after lysis was hopefully avoided by using a cocktail of inhibitors. The diffuse band(s) at \( M_r 54,000-58,000 \) may have been influenced by the mode of detaching cells from plastic, because they were feeble with ASML cells cultured in suspension but strong with ASML cells detached by trypsin/EDTA treatment. The picture is even more complicated by the point that A2.4 and C1.6, but not A2.6, detected bands in lysates of AS cells which putatively corresponded to some components detected in ASML lysates. This is at variance with data obtained in the ELISA and in immunohistology, since A2.4 and C1.6 showed insignificant binding to AS in both tests. One explanation could be that the respective antigen molecules are not expressed, or are seen only in a masked form, on the surface, while being present at low concentration in the cytoplasm of AS cells. This would lead to negative readings in the ELISA and to subthreshold staining in immunohistology. A similar discrepancy between selective expression of antigens on the surface of metastatic variant cells and indiscriminate presence of the respective bands in Western blots was observed in the 13762NF rat mammary adenocarcinoma using MAb MT10:21 (15).

With respect to the epitopes recognized by A2.4, A2.6, and C1.6, the banding pattern in the Western blot argues in favour of distinctness, since prominent bands of the A2.4 set-up were not detected with A2.6 and C1.6 and since the latter two MAbs, although most likely binding to molecules of the A2.4 antigen complex, produced distinct patterns of staining. However, this assumption awaits confirmation by competition experiments.

Previous biochemical analysis of surface molecules selectively expressed on the AS or ASML variant has revealed two distinct entities (16). A \( M_r 220,000 \) spot obtained with AS cells most likely represented fibronectin, while a \( M_r 170,000 \) glycoprotein found on ASML cells was tentatively categorized as cell adhesion molecule, which may be involved in the peculiar adhesion
Fig. 2. Reactivity of MAb A2.6 with frozen sections of BSp73 variants AS and ASML. A, ASML tissue; B, higher magnification of A visualizing reactivity with virtually all the extranuclear parts of ASML cells; C, metastasis of ASML in the lung; D, negative reactivity on AS tissue. Note also negative reaction with normal tissue in A (upper part) and C (around metastatic nodules). Staining intensity obtained with A2.6 on ASML tissue was scored as +++.

### Properties of this variant (16). Both molecules were found to be secreted. The $M_r$ 170,000 glycoprotein may be related to the A2.4 antigen complex, since a prominent band was detected at this position on blots of A2.4 and A2.6.

Monoclonal antibodies proved to be valuable tools for the characterization of differential antigen expression on primary tumors and metastases. Besides intra- and intertumor heterogeneity, antigenic differences between primary tumors and metastases have been detected in patients (17–19), and molecules which are expressed and/or exposed in correlation with the metastatic potential of variant cell lines have been identified in rodents (15, 20–29). The search for metastasis-associated antigens was particularly successful in well-established model systems. In B16 murine melanoma, MAbs directed against $M_r$ 40,000 and $M_r$ 50,000 molecules (21) or against a set of $M_r$ 31,000, 58,000, and 80,000 proteins (26, 30) were found to inhibit experimental lung metastasis (21, 24). Kimura and Xiang (24) were able to raise syngeneic and xenogeneic MAbs binding to a strong band at $M_r$ 72,000 and two minor bands at $M_r$ 155,000 and 180,000 which were preferentially expressed on highly metastatic clones of B16 (31, 32). In the murine 3LL lung tumor model, Olsson and Forchhammer (23) identified a $M_r$ 45,000 protein, while other groups raised several MAbs (20, 25) detecting the TSP-180 molecular complex composed of $M_r$ 116,000, 134,000, 150,000, 180,000, and 204,000 species, which are expressed with a variable banding pattern (25). In the rat 13762NF mammary adenocarcinoma, Nicholson et al. identified two antigen specificities, one of them being associated with a $M_r$ 580,000 mucin-like molecule (22, 29) and the other with $M_r$ 72,000/73,000 and $M_r$ 120,000 proteins (15).

When these data are compared with results obtained in the BSp73 model, not regarding species differences, different methodological approaches, and lack of precision in the calibration of molecular weights, some striking parallels come into sight. (a)
systems. Direct comparison of MAbs as well as mRNAs coding and C 1.6 were found in other tumor models as well, e.g., in some of the marker molecules previously detected in the other antigens detected in the BSp73 tumor model are related to states (15). In view of this wealth of evidences, it is not unlikely detectability with different MAbs can show considerable varia-

Fig. 3. Western blot of MAbs showing “specific” staining of ASML tissue in immunohistology. Lanes 1, 3, 5, 7, 9, lysates from AS cells. Lanes 2, 4, 6, 8, 10, lysates from ASML cells growing in suspension. Five μl of lyasate were applied per lane, corresponding to 5 × 10^6 cells. A 6% polyacrylamide gel was run under nonreducing conditions. After blotting, nitrocellulose sheets were incubated with lysates from ASML cells growing in suspension. Five μl of lysate were applied per lane, corresponding to 5 × 10^6 cells. A 6% polyacrylamide gel was run under nonreducing conditions. After blotting, nitrocellulose sheets were incubated with multicomponent antigens as identified with MAbs A2.4, A2.6, and C1.6 were found in other tumor models as well, e.g., in B16 melanoma [Mr, 72,000, M, 155,000, M, 180,000 (24)], in the 3L lung tumor [Mr, 116,000, M, 134,000, M, 150,000, M, 204,000 (25)], and in the 13762NF mammary adenocarcinoma of the rat [Mr, 72,000/73,000, M, 120,000 (15)]. (b) The relative strength of individual bands in different cell lines as well as the detectability with different MAbs can show considerable varia-

REFERENCES

Antigenic Differences between Metastatic and Nonmetastatic BSp73 Rat Tumor Variants Characterized by Monoclonal Antibodies

Siegfried Matzku, Achim Wenzel, Sida Liu, et al.