Monoclonal Antibodies to Human Sarcoma and Connective Tissue Differentiation Antigens

Carl Feit, Arie H. Bartal, Barbara Fass, Yuri Bushkin, Carlos Cordon Cardo, and Yashar Hirshaut

Laboratory of Immunodiagnosis, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

The use of monoclonal antibodies to distinguish human sarcoma from carcinoma cells has been explored. Spleen cells from a BALB/c mouse immunized with a human malignant fibrohistiocytoma were fused with cells of the mouse P3U1 plasmacytoma cell line. Antibodies were then screened for reactivity against human sarcoma and carcinoma cells growing in culture. This work has yielded 2 immunoglobulin G monoclonal antibodies VIE4 and VIF3 which, respectively, reacted with 85% (17 of 20) and 90% (18 of 20) of sarcoma lines tested but with none of eight carcinoma cell line preparations. Reactivity against normal fibroblasts was also demonstrated. By immunofluorescence, the antigens detected by the two antibodies appear to have distinctive intracellular distributions. Immunoprecipitation with VIF3 has shown that it is detecting a protein with a molecular weight of 70,000. When tested against pathological frozen tissue sections, VIF3 reacted with four of 11 and VIE4 with three of 11 human sarcomas but with none of ten carcinomas tested. VIF3 occasionally bound to normal adult connective tissues, whereas no such reactivity was seen with VIE4. These antibodies appear to be directed to fibroblastic markers associated with sarcomas and connective tissue differentiation antigens.

INTRODUCTION

While the first immunological investigations of human cancer were initiated more than 50 years ago, progress in defining tumor-distinctive antigens in humans has been slow. In 1968, Morton and coworkers (2, 12, 20) described an antigen common to human sarcomas using patient sera as sources of antibody. This work led to our own extensive investigations of allogeneic and syngeneic responses of sarcoma patients to their tumors (5, 6, 13, 17, 19). Eventually, we identified 3 sarcoma-associated antigens of which 2, S1 and S3, are heterophile in nature and the other, S2, is an oncofetal marker (6, 13, 17). The nature and pathogenetic significance of the sarcoma-associated antigens remain to be further defined. However, there has been general disappointment with the lack of specificity of tumor markers detected with patient sera.

Recently, methods for generating mouse monoclonal antibodies have been developed (8). These permit reagents of high titer and specificity to be produced in almost limitless quantities. The principal challenge faced in the use of this technology is not in securing a sufficient number of monospecific antibodies but in developing means for selecting those antibodies with "relevant" specificities which warrant further characterization. Given the impasse reached in immunological studies of human tumors using patient sera, there is increasing interest (7, 9, 16) in developing monoclonal antibodies to human tumor markers. This paper reports the results of our initial studies directed to the detection of sarcoma-related antigens using monoclonal antibody methods.

MATERIALS AND METHODS

Human Sarcoma Tissues. A human malignant fibrohistiocytoma was obtained directly from the operating room at the MSKCC, New York, NY. The tumor was dissected out of surrounding normal tissues, immediately minced, passed through a sterile mesh, and then passed through needles of diminishing size to 25 gauge. Aliquots of the cell suspension were frozen and stored at −20° to be used for immunizations.

Immunization Protocol. A sarcoma cell suspension (0.5 ml) containing 1 × 10⁶ cells mixed with 0.5 ml of incomplete Freund's adjuvant was injected s.c. into each of 5 BALB/c mice (The Jackson Laboratory, Bar Harbor, ME). Using the cell suspension only, mice received rejections of 0.5 ml of the sarcoma cells at biweekly intervals 4 additional times. They then were permitted to rest for 2 months before being given a final booster immunization prior to fusion.

Production of Hybridoma. The mouse used for the studies reported in this paper received a booster immunization, i.e., 4 days before the fusion attempt. On the day of fusion, 15 × 10⁶ spleen cells were mixed with 3 × 10⁷ cells of the mouse P3U1 plasmacytoma cell line in the presence of 0.2 ml of 40% Polyethylene Glycol 4000 (Sigma Chemical Co., St. Louis, MO) prepared in PBS containing in g/liter: CaCl₂ (0.1):KCl (0.2):KH₂PO₄ (0.2):MgCl₂ · 6H₂O (0.1):NaCl (9.0):Na₂HPO₄·7H₂O (2.16) with 15% dimethyl sulfoxide (Fisher Scientific, Pittsburgh, PA). After 2 min at room temperature, the polyethylene glycol was slowly diluted with 10 ml of RPMI-1640 and 15% fetal calf serum (Media Lab., MSKCC). The cells were then spun down at 500 × g for 10 min. The pellet was resuspended in RPMI-1640 containing 15% fetal calf serum and 2% hypoxanthine:aminopterin:thymidine (Media Lab., MSKCC) and plated out in 96-well plates (Flow Laboratories, MacLean, VA). Twenty-one days postfusion, supernatants from wells containing hybridomas were obtained for screening. Cloning of selected hybridomas was performed by the limiting dilution method (11).

Determination of Immunoglobulin Type. The immunoglobulin type of specific monoclonal antibodies was determined by using heavy chain-specific fluoresceinated goat anti-mouse antisera (Meloy Laboratories, Springfield, MA) in an immunofluorescence assay and similar unlabeled goat antisera for testing by the Ouchterloney method (Cappel Laboratories, Downingtown, PA).

Ascites Production. BALB/c mice were initially primed with 0.5 ml of 2,6,10,14-tetramethylpentadecane (Pristane; Aldrich Chemical Co., Milwaukee, WI) injected i.p. One week later, 1 to 5 × 10⁹ hybridoma cells were injected into the abdomen. When ascites developed, the fluid was tapped and then divided into aliquots and frozen at −20°.

Slide Preparation for Immunofluorescence. Twenty-five μl of a tumor cell suspension (5 × 10⁶ cells/ml) were placed in each of the wells on plastic-coated multwell slides (Hendley-Essex, Sussex, England). These slides were tapped with a small glass microscope slide and then air-dried. This procedure was repeated three times. After air-drying, the slides were then incubated in PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
slides were incubated at 37° in an atmosphere of 5% CO2 for 48 hr. This
process permits the antigen-bearing cell to adhere to the glass and
spread out. In this way, antigen distribution can subsequently be more
readily determined. The slides were then washed twice with PBS, air
dried, fixed in acetone for 10 min, and stored at -20°.

Cell Lines. Methods used for establishing tumor cell lines have been
described previously (4, 18). The variety of cell lines used in this study is
shown in Table 2. A total of 20 human sarcoma cell lines, 8 carcinoma
lines, and 6 fibroblastic cell lines originating from the normal skin of
cancer patients was used in the study. Two of the cell lines, one from
a patient with leiomyosarcoma (ALMS) and the other (1) from a patient
with breast carcinoma (ALAB), were used for initial screening to select
hybridomas producing antibodies to sarcomas as distinguished from
carcinomas. The other cell lines were used to establish more definitively
the specificity of selected antibodies. All cell lines were checked routinely
for Mycoplasma using Mycotrin-TC (Hana Media, Inc., Berkeley, CA).
All lines used in these experiments were Mycoplasma free.

Indirect Immunofluorescence. Details of the methods used are pub-
lished elsewhere (6). Briefly, diluted supernatant or ascitic fluids were
placed on the wells of prepared slides which were then incubated for 1
hr at 37°. After incubation, slides were washed twice for 5 min with PBS
followed by a short immersion in distilled water. After complete drying,
1:20 diluted IgG and IgM (specific affinity-purified goat, anti-mouse
immunoglobulin) conjugated with fluorescein isothiocyanate (Meloy Lab-
oratories, Springfield, MA) were placed on the wells, and slides were again
incubated for 1 hr at 37°. Following the second incubation, the wash
was repeated, and slides, still wet, were then placed for 5 min in
Evan’s blue (0.005%). This step counterstains those portions of the
cells which have no antigen and diminishes nonspecific autofluorescence.
Finally, the slides were washed again, dried, covered with 50% glycerol,
and examined using a Leitz Ortholux II microscope equipped with Ploem
optics.

ELISA. Viable cell monolayers to be used in an ELISA assay were
prepared from 2 tumor cell lines established previously, one derived from
a human osteogenic sarcoma (WOSg2a) and the other from a breast
carcinoma (ALAB) (1). The details of the ELISA performed in our labo-
ration were published previously (3).

Immunoaffinity precipitation. Monoclonal antibodies were tested for
their ability to precipitate surface components of the human osteoar-
soma cell line MOS-229. A rubber policeman was used to harvest 2 x 10°
cells from culture plates. Cells were washed twice with PBS, then radioiodi-
nated by the lactoperoxidase method (15), and lysed in a lysis buffer
composed of 0.5% Nonidet P-40 plus 1 mm phenylmethylsulfonyl fluoride
in PBS without Ca2+ and Mg2+. Lysates were precleared with Protein
A-Sepharose CL-4B (Pharmacia Fine Chemicals, Piscataway, NJ), and
then, precipitation was carried out by reaction of the cleared lysates with
antibody-coated beads at room temperature. After 5 washings, material
from the beads was eluted with SDS sample buffer (2.3% SDS:10%
glycerol:5% 2-mercaptoethanol:0.065 M Tris-HCl, pH 6.8) and boiled for
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%
gradient slab gels according to Laemmli (10). Slab gels were dried on
3 min. The eluted material was characterized by SDS-PAGE on 5 to 20%

Table 1

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Immunoglobulin type</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIF6</td>
<td>IgM</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>IIE5</td>
<td>IgM</td>
<td>Filamentous</td>
</tr>
<tr>
<td>XIID8</td>
<td>IgG</td>
<td>Perinuclear</td>
</tr>
<tr>
<td>VIE4</td>
<td>IgG</td>
<td>Filamentous</td>
</tr>
<tr>
<td>VIF3</td>
<td>IgG</td>
<td>Filamentous</td>
</tr>
<tr>
<td>IXG11</td>
<td>IgG</td>
<td>Filamentous</td>
</tr>
<tr>
<td>XVE6</td>
<td>IgG</td>
<td>Filamentous</td>
</tr>
</tbody>
</table>

RESULTS

Of 1440 wells into which cells were placed after fusion, 1184
yielded one or more hybridoma clones (82.2%). Three hundred
eighty wells which appeared to contain only single clones were
selected for further study. The supernatants of these wells were
screened by the indirect immunofluorescence method, using a
sarcoma cell line (ALMS) and a carcinoma cell line (ALAB).
Twenty-one wells contained monoclonal antibodies interacting
only with ALMS, and 18 clones were found to secrete antibodies
reacting only with the ALAB cell line. Another 9 monoclonal
antibodies were able to recognize cells of both lines.

With repeated cloning, 7 of the 21 hybridoma clones secreting
sarcoma-associated antibodies were successfully isolated and
expanded. Table 1 shows the morphology of the fluorescence
reaction seen when these 7 antibodies reacted with ALMS. Also
given is their immunoglobulin class. Five antibodies gave fibrillar
reaction (Fig. 1A). One produced a punctate cytoplasmic pattern
(Fig. 1B), and one yielded a diffuse cytoplasmic reaction. All
antibodies were of the IgG class except IIF6 and IIE5, which
were IgM. Supematant titers ranged between 1:10 and 1:640.
In ascites fluid, titers as high as 1:10,000 were observed.

Further analysis carried out with additional sarcoma and car-
cinoma cell lines showed that 2 of the 7 antibodies originally
found to react only with ALMS reacted specifically with sarcoma
and not with carcinoma lines. When tested, these antibodies,
VIE4 and VIF3, reacted, respectively, with 85% (17 of 20) and
90% (18 of 20) of sarcoma lines tested but with none of 8
 carcinoma cell line substrates. The remaining monoclonal anti-
bodies were found to interact with 1 to 4 of 6 carcinoma cell
lines. All the antibodies reacted with 3 or more of the fibroblastic
cell lines tested (Table 2).

Table 3 describes the results of using the ELISA viable cell
assay to determine the specificity of the same ALMS-positive
monoclonal antibody cell assay. VIE4 reacted with 4 sarcoma
and one fibroblast cell lines, but not with 3 carcinoma cell lines.
Monoclonal antibodies IIE5, IXG11, XV6E, and XIID8 interacted
with cells of both sarcoma and carcinoma origin. IIF6 was found
to recognize an antigen on ANS and ALAB breast only. VIF3
was negative against all cell lines, with this assay.

Fig. 2 shows the SDS-PAGE pattern obtained by immunopre-
cipitating the antigen detected by 2 of the sarcoma-associated
monoclonal antibodies. A molecular weight band of 70,000 was
identified by monoclonal antibodies VIF3 and XV6E. The remain-
ing antibodies (Table 1) yielded no detectable precipitation by
the methods used, thus far.

When tested directly against pathological sections of 11 sar-
comas (Table 4), VIF3 reacted with a chondrosarcoma, osteo-
genic sarcoma, fibrosarcoma, and a leiomyosarcoma. VIE4
reacted with the same chondrosarcoma and osteogenic sarcoma
HUMAN SARCOMA AND CONNECTIVE TISSUE ANTIGENS

Table 2
Characterization of monoclonal antibodies by immunofluorescence using sarcoma, fibroblast, and carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Type</th>
<th>VIF3</th>
<th>VIE4</th>
<th>IIIF</th>
<th>IIIE5</th>
<th>IXG11</th>
<th>XVE6</th>
<th>XIID8</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOS-189</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRMS-186</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANS-177</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me-180</td>
<td>Cervical carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlAb</td>
<td>Breast carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fru</td>
<td>Testicular carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LuCa</td>
<td>Lung carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-LU-1</td>
<td>Lung carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-Mee-1</td>
<td>Bladder carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT-29</td>
<td>Breast carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MOS-229</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS-28</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KOS-203</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROS-23</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS-190</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOS-194</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WOS-189</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AOS-177</td>
<td>Osteosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRMS-186</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRMS-226</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRMS-198</td>
<td>Rhabdomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLS-99</td>
<td>Liposarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLS-164</td>
<td>Liposarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WL-190</td>
<td>Liposarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALMS-231</td>
<td>Leiomyosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSS-222</td>
<td>Synovial sarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCC-171</td>
<td>Giant cell sarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSOS-183</td>
<td>Spindle cell sarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFHS-175</td>
<td>Fibrohistiocytoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BFS-166</td>
<td>Fibrosarcoma</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WNS-199</td>
<td>Fibroblast</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANS-177</td>
<td>Fibroblast</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNS-222</td>
<td>Fibroblast</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MNS-229</td>
<td>Fibroblast</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNS-175</td>
<td>Fibroblast</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNS-203</td>
<td>Fibroblast</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me-180</td>
<td>Cervical carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlAb</td>
<td>Breast carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fru</td>
<td>Testicular carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LuCa</td>
<td>Lung carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-LU-1</td>
<td>Lung carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK-Mee-1</td>
<td>Bladder carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT-29</td>
<td>Breast carcinoma</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ND, not determined.

Table 3
ELISA performed using cell lines to determine the expression of connective tissue antigens linked with sarcomas

<table>
<thead>
<tr>
<th>Monoclonal antibodies</th>
<th>VIE4</th>
<th>VIF3</th>
<th>IIIF</th>
<th>IIIE5</th>
<th>IXG11</th>
<th>XVE6</th>
<th>XIID8</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOS 189</td>
<td>±</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRMS 186</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANS 177</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Me 180 cervix</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AlAb breast</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LuCa lung</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

but not against the other 2 tumors. In addition, it reacted with a second chondrosarcoma. No reactions were seen with 10 carcinomas tested (Table 4).

Tests were also completed against normal adult tissues. Findings are shown in Table 5. Epithelial cells of skin, lung, breast, colon, and prostate did not react with VIF3 or VIE4. However, VIF3 did bind to connective tissue cells in adult skin, lung, breast, and prostate. No reactivity was seen with VIE4.

DISCUSSION

Efforts to identify human tumor-specific antigens in humans have been hampered by a lack of well-defined reagents. It is now possible to develop large quantities of relatively purified, high-titer, specific antibodies to well-defined antigens. Unfortunately, human tumor antigens are so far poorly defined, and their only sources are crude tumor extracts. Tumor specificity, unique to tumors alone, therefore, remains difficult to establish even with monoclonal antibodies available. Our approach in this initial stage of developing such antibodies to sarcomas has been not to insist on absolute specificity. Instead, antibodies have been selected...
HUMAN SARCOMA AND CONNECTIVE TISSUE ANTIGENS

<table>
<thead>
<tr>
<th>Tissue</th>
<th>VIF3</th>
<th>VIE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pneumocytes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Breast</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duct cells</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Skin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Epidermis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Melanocytes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dermis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Colon</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prostate</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Epithelium</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stroma</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Tissue sections were from postmortem specimens obtained less than 9 hr after death.

which are specific only in the relative sense of their ability to distinguish sarcoma from other cells, particularly those derived from carcinomas. No assumption is made that these differences are characteristic only of tumors. It is more likely that differentiation markers are being detected.

The 2 monoclonal antibodies described, VIF3 and VIE4, distinguish fixed sarcoma from carcinoma cell lines. One of these antibodies, VIE4, is also able to distinguish between viable sarcoma and carcinoma cell lines. Consistent with the assumption that the antigens being detected are related to cell differentiation is the finding that the same antibodies react with cultured normal fibroblasts.

Studies with more than 20 sarcoma cell lines indicate that monoclonal antibodies VIF3 and VIE4 recognize most forms of sarcoma in culture. The isolation of antibodies with such broad reactivity may in part be due to the use of an allogeneic sarcoma in culture. The isolation of antibodies with such broad reactivities is, therefore, that this provides additional insight into the complex setting of whole tissues. It is, therefore, reassuring that both VIF3 and VIE4 remain specific for connective tissues when tested against pathological sections. The reactivity of VIF3 with a subset of fibroblasts within normal adult connective tissues confirms that this antibody detects a connective tissue differentiation antigen. VIE4 is more selective in its reactivity, since it reacts only with normal connective tissues after they have been placed in culture, a setting in which some differentiation may occur. This greater selectivity of VIE4 may ultimately make it a more useful reagent for the detection of sarcomas. VIF3 and VIE4 react only with 4 of 11 and 3 of 11 human sarcoma tissue sections, respectively, and with none of 10 carcinoma tissues tested. Antibodies to vimentin have also been demonstrated to be capable of distinguishing carcinomas from sarcomas (14). The currently reported monoclonal antibodies are expected to serve as probes in the immunopathological diagnosis of human sarcomas. As the number of such antibodies increases, more definitive classification of human sarcomas should become possible. This in turn can be expected to lead to more effective therapies tailored to specific subclassifications of sarcomas.

REFERENCES

Fig. 1. Photomicrographs of indirect immunofluorescence produced by monoclonal antibodies on a human osteogenic sarcoma cell line (MOS-229). A, monoclonal antibody VIE4, followed by fluorescein isothiocyanate goat anti-mouse IgG, counterstained in Evan's blue. × 250. B, monoclonal antibody VIF3, same procedure, × 250.

Fig. 2. SDS-PAGE pattern of 125I-labeled antigens precipitated by monoclonal antibodies from MOS-229 cells. Immunoprecipitation was done as described in "Materials and Methods." Goat anti-human β2-microglobulin heterologous antibodies precipitated 2 Mr bands: one of 12,000 (β2-microglobulin) and another one of 43,000, presumably HLA antigens. Two antibodies, XVE6 and VIF3, but not VIE4, precipitated Mr 70,000 material.
Monoclonal Antibodies to Human Sarcoma and Connective Tissue Differentiation Antigens

Carl Feit, Arie H. Bartal, Barbara Fass, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/12_Part_1/5752

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.