Virchows Arch A (1987) 410:481–486

Virchows Archiv A

Immunohistological characterization of a monoclonal antibody (OV632) against epithelial ovarian carcinomas

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Summary. A hybridoma cell line (OV632) producing monoclonal antibody against ovarian carcinomas was developed from the spleen cells of a mouse immunized with cystic fluid from a serous cystadenocarcinoma. Immunohistological studies in frozen sections showed that 22 out of 28 nonmucinous ovarian carcinomas, which included serous, endometrioid, clear cell, and undifferentiated tumours, reacted with this antibody. Three out of 7 mucinous ovarian carcinomas were positive, whereas only 7 out of 122 extra-genital malignant lesions, predominantly adenocarcinomas, were positive. The negative cases included 38 breast carcinomas and 24 colon carcinomas, tumours which are responsible for most of metastatic disease in the ovary. On the basis of these findings, the antibody OV632 is considered appropriate for histodiagnostic purposes as an aid in the distinction between primary and secondary ovarian cancer.

Key words: Adenocarcinoma of the ovary – Immunohistochemistry – Monoclonal antibody

Introduction

Ovarian cancer is responsible for approximately 50% of all deaths due to malignancies of the gynaecological tract (Cutler and Young 1975; Silverberg and Lubera 1983). Since the current therapeutic approach to disseminated ovarian carcinoma differs from that used for other malignancies (Vogl et al. 1979), it is important to distinguish primary ovarian tumours from metastases to the ovary. Approximately 5–10% of tumours in the ovary are of metastatic origin (Kempson and Hendrickson 1978) and are mainly metastases of intestinal or breast carcinomas. However, primary ovarian carcinoma and metastatic tumour to the ovary often present the same clinical picture (Johansson 1960; Kempson and Hendrickson 1978; Ulbright et al. 1984), and histological distinction between these may be difficult or impossible (Scully 1980; Ulbright et al. 1984).

Immunohistochemistry is a useful tool in surgical pathology, but none of the currently available monoclonal antibodies against epithelial ovarian cancer are sufficiently specific to be suitable for this purpose. In 1981, Bast et al. developed a monoclonal antibody (OC125) that reacts with a large proportion of the serous, endometrioid, and clear cell ovarian cancers and not with normal adult tissues, except for those of the fallopian tubes, endocervix, and endometrium (Kabawat et al. 1983a). However, OC125 reacts with approximately 40% of adenocarcinomas of the breast (Koelma et al. 1986) and with 4 out of 11 adenocarcinomas of the lung (Kabawat et al. 1983b) and is therefore less suitable for use in surgical pathology.

In this report we describe a monoclonal antibody (OV632) against nonmucinous ovarian carcinomas that is sufficiently specific to be used with respect to the differential diagnosis of primary ovarian carcinoma versus adenocarcinomas metastatic to the ovary.

Material and methods

Tissues and cyst fluids. Fresh tissue specimens and cyst fluids from ovarian carcinomas were obtained as soon as possible after surgery. Tissues were snap-frozen and stored at −70° until use.

The 72 primary ovarian tumours (Table 1) used in this study were classified according to Scully (1980). In addition, 13 tissue specimens of metastatic lesions from patients with metastatic ovarian cancer were tested; this material represented serous ($n=9$), endometrioid ($n=2$), clear cell ($n=1$), and undif-

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Table 1. Reactivity of OV632 with ovarian tumours

Tumour	Positive total
Serous carcinoma	15/16
Serous borderline	1/ 3
Serous benign	5/ 9
Mucinous carcinoma	3/ 7
Mucinous borderline	2/ 6
Mucinous benign	4/17
Endometrioid carcinoma	3/ 4
Clear cell carcinoma	2/ 5
Undifferentiated carcinoma	2/ 3
Brenner tumour	0/ 2

Table 2. Reactivity of OV632 with non-gynaecological tumours

Tumour	Positive total
Epithelial carcinomas	
Kidney	1/ 18
Pancreas	0/ 1
Thyroid	2/ 4
Bladder	1/ 4
Breast	0/ 38
Lung	0/ 17
Stomach	1/ 8
Colon	0/ 24
Seminoma testis	0/ 2
Melanoma	1/ 4
Mesothelioma	0/ 1
Carcinoid	1/ 1
	7/122

ferentiated ($n=1$) tumours. Use was also made of 122 tumour tissue specimens from non-gynaecological lesions (Table 2).

Normal adult tissues ($n=69$) were obtained from surgical specimens or at autopsy and represented the ureter, urinary bladder, kidney, stomach, jejunum, appendix, colon, uterine cervix and corpus, pancreas, testis, prostate, lung, liver, breast, muscle, brain, lymph nodes, thyroid gland, and skin. Up to five specimens of each type of normal tissue obtained from different patients were used. Fetal tissues were obtained from legal abortions and stillborns.

Cyst fluid of a serous cystadenocarcinoma was used for intraperitoneal immunization, because such fluids are known to contain large amounts of tumour antigens (van Nagell et al. 1981; Ockhuizen et al. 1985; de Bruijn et al. 1986). Each animal was injected with 0.5 ml of a mixture of cyst fluid diluted 1:10 with PBS mixed with an equal volume of Freund's complete adjuvant (H37 RA, Sigma, St. Louis, MO, USA). Booster injections were performed at two-week intervals with cyst fluid and Freund's incomplete adjuvant (1:1) (Sigma). Three days after the last injection the mice were killed and the spleen cells were used for fusion.

The spleen cells of immunized mice were fused with SP2/O cells essentially according to Kohler and Milstein (1975). After fusion the cells were plated on soft agar in 20 dishes (diameter 5 cm) and cultured at 37° C for 10 days.

Nitrocellulose filters saturated with cyst fluid or proteins from a homogenate made from a normal adult human liver were used as first screen to discriminate between relevant and irrelevant antibody-producing colonies. The filters were prepared according to Sharon et al. (1979) and placed on top of the agar for 24 hours. This procedure is described in detail elsewhere (Oosterwijk et al. 1986). Colonies producing antibodies that reacted to the filter coated with the cyst fluid but not to the filter coated with liver cell extract were selected and grown in suspension on microtiter plates. Undiluted culture media used for these cells were tested on thin sections of a variety of frozen tissues. Colonies producing antibodies giving positive results on ovarian carcinoma sections and negative results on liver and lung tissue sections were subcloned and further analysed on frozen sections of other tissues.

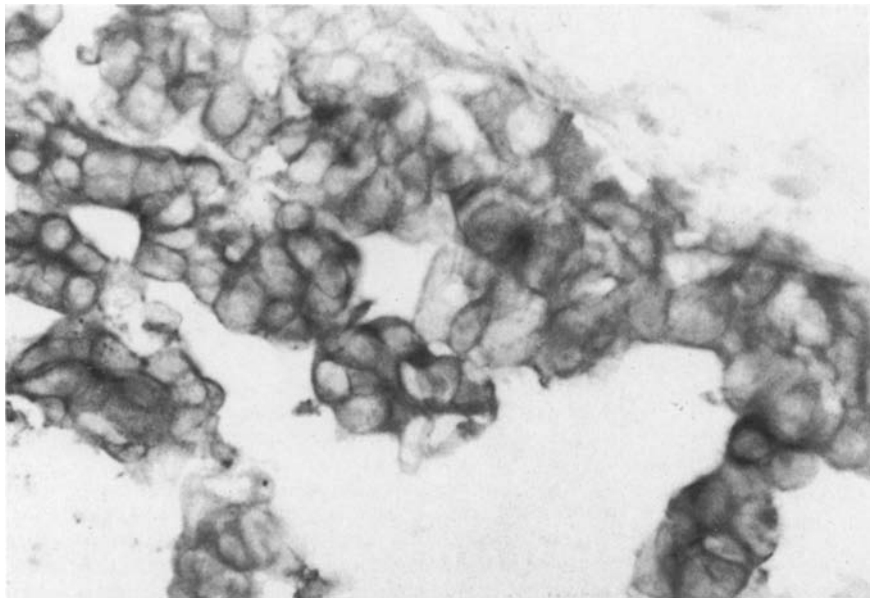

Fig. 1. Immunohistologic staining with monoclonal antibody OV632 applied to a frozen tissue section of a clear cell carcinoma of the ovary. Counterstained with haematoxylin. ×200

Immunohistochemical staining. For immunohistochemical staining of frozen tissues use was made of the indirect peroxidase method described elsewhere (de Bruijn et al. 1986). Sections 4 microns thick were fixed in acetone for 4 min and then washed in PBS. Endogenous peroxidase activity was not inhibited. The sections were incubated at room temperature for 2 h with undiluted culture medium from the clone(s) to be tested, washed with PBS, and incubated for 30 min with a peroxidase-conjugated rabbit-anti-mouse-Ig-antibody (Dakopatts, Denmark). After a wash, the staining reaction was performed with a freshly prepared solution of 3-amino-9-ethylcarbazole in 0.1 M acetate buffer (pH 5.0) containing 0.03% H_2O_2, which gives a red reaction product. The sections were then counterstained with haematoxylin and mounted for microscopical examination. Sections were scored as negative when no cells showed staining.

Sections of formalin-fixed and paraffin-embedded tissues of ovarian carcinomas were used to establish the influence of routine formalin fixation and embedding on the staining with OV632. For comparison of the overlapping of staining by our monoclonal OV632 and monoclonal OC125 (Oris Lapam, France), use was made of the fact that these two antibodies have different heavy chains: IgG2b and IgG1, respectively. Antibodies OV632 and OC125 were applied simultaneously to tissue sections. After incubation and washing, goat anti-mouse second antibodies specific for each of the heavy chains (Nordic, Tilburg, The Netherlands) and labeled with TRITC (anti-IgG1) and FITC (anti-Ig2b) were applied. The sections were then washed in PBS, mounted in Fluoromount, and examined in a fluorescence microscope at the respective specific wavelengths.

Results

The normal ciliated epithelium lining the fallopian tube reacted positive. A small proportion of the superficial endometrial cells in 2 out of 7 uteri and endocervical cells in 1 out of 7 uteri stained positive for OV632. In the ovary, positive staining occurred in follicular cells lining follicular cysts. The superficial germinal epithelium of the ovary and inclusion cysts did not stain. Focal positivity was observed in epithelial cells from the appendix, pancreas, and prostate gland. Ganglion cells in the myenteric plexus of the intestine were also positive. The cellular distribution pattern of the antigen determined by OV632 was cytoplasmic in all cases. All other normal adult tissues were negative. Fetal coelomic epithelium was negative.

Immunohistology of frozen sections showed that antibody OV632 stained approximately 80% of the nonmucinous carcinomas, including serous, endometrioid, clear cell, and undifferentiated carcinomas (Table 1). The majority of these positive cases showed staining throughout the tumours, although negative cells were present (Figs. 1, 2). The staining occurred in the cytoplasm in a pattern comparable to that seen in normal tissues (Figs. 1–4). Nine out of 30 mucinous tumours were positive, although only focally (Table 1). Twelve out of 13 metastases of ovarian carcinoma showed positivity. The negative case concerned a metastasis of an endometrioid ovarian cancer. Of the uterine endometrial carcinomas, 5 out of 9 were positive.

Two-wavelength indirect immunofluorescence microscopy showed an unequivocal difference between the cytoplasmic staining patterns of OV632 and the membranous staining of OC125 (Fig. 3). Antibody OV632 did not react with ovarian carcinoma tissue after routine formalin fixation and paraffin embedding; even a short incubation of frozen tissue sections with 2% formalin blocked the staining completely.

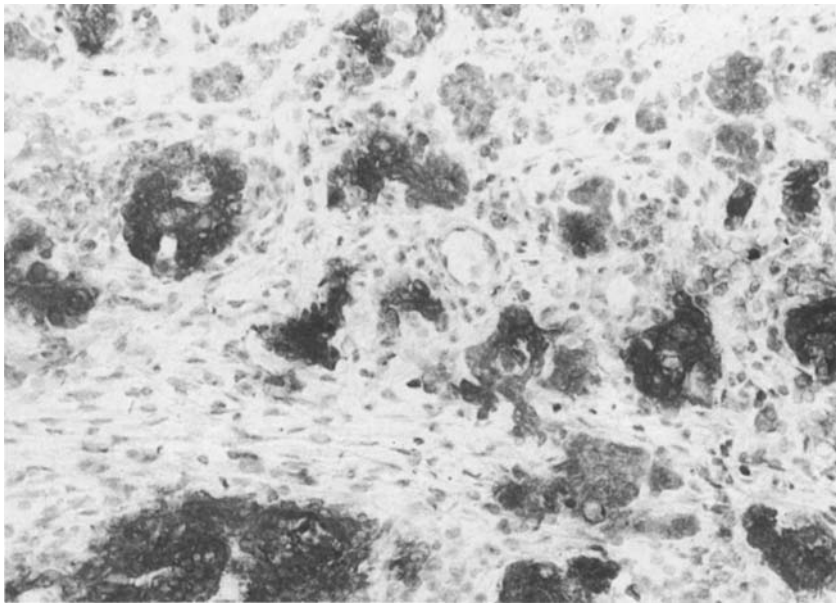

Fig. 2. Immmunoperoxidase staining with monoclonal antibody OV632 applied to an omental metastasis of a serous cystadenocarcinoma grade III. Note the heterogeneity of antigen expression within the same metastatic lesion. Counterstained with haematoxylin. ×100

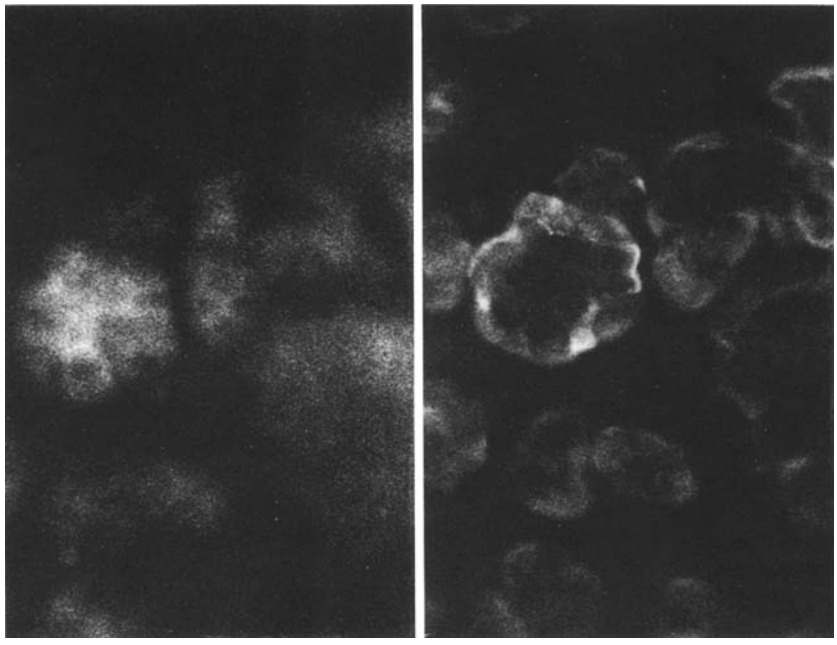

Fig. 3. Two wavelength indirect-immunofluorescence staining of frozen tissue sections of a serous cystadenocarcinoma, obtained with OV632 (*left*) and OC125 (*right*). Note the cytoplasmic staining pattern of OV632 versus the membranous positivity of OC125. ×600

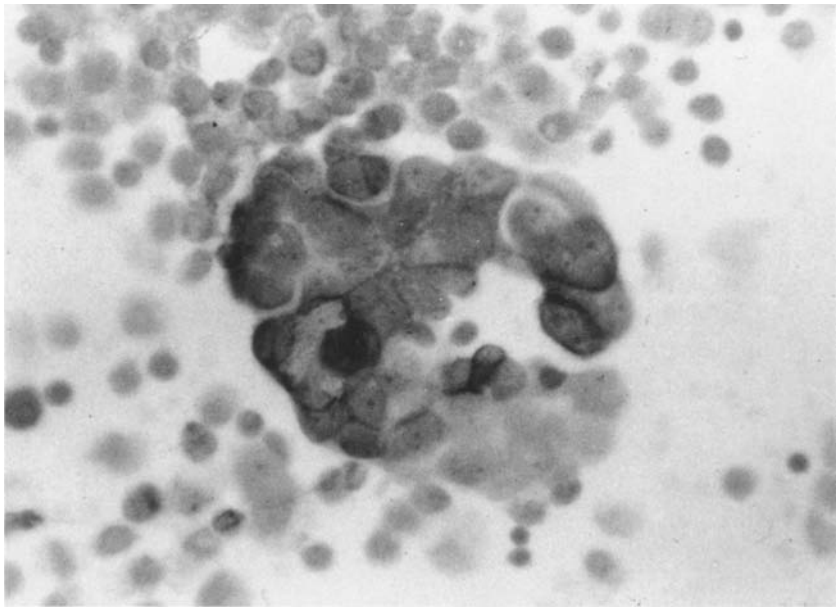

Fig. 4. Staining of tumour cells in malignant ascites fluid of a patient with serous cystadenocarcinoma stage IV. Indirect immunoperoxidase reaction for OV632. ×400

Non-gynaecological tumours

The staining results for 122 malignant lesions in 14 different extra-genital organs, including adenocarcinomas of the breast, colon, lung, and kidney, are summarized in Table 2. In this group all breast carcinomas ($n=38$) and colon carcinomas ($n=24$) were completely negative. Seven out of 122 malignancies of non-gynaecological sites showed cytoplasmic positivity (Table 2), five of them focal staining of tumour cells and the other two, which were thyroid carcinomas, diffuse staining. The cytoplasmic pattern and the staining intensity in these tumours did not differ from those observed in ovarian carcinomas.

Discussion

The purpose of this study was to produce murine monoclonal antibodies capable of distinguishing human ovarian adenocarcinoma cells from tumour cells of other origins. One monoclonal antibody, OV632, was selected because of its limited range of reactivity against non-ovarian carcinoma tis-

sues. The present tissue-distribution results obtained by use of the indirect immunoperoxidase method in frozen sections showed that the antigen recognized by antibody OV632 occurred in approximately 80% of the epithelial ovarian carcinomas examined, but only in a small percentage of the non-ovarian malignancies.

Ovarian epithelial tumours are thought to derive from the germinal epithelium lining the ovary and the subcapsular inclusion cysts (Scully 1980). This germinal epithelial lining of the ovary and of the fallopian tube, the endometrium, and endocervix are of mullerian origin. The observation that epithelial cells of the fallopian tube, the endometrium, epithelial ovarian tumours, and endometrial carcinomas of the uterus, are positive with OV632 may reflect their shared relationship to the mullerian duct. However, no detectable immunoperoxidase staining of the ovarian surface epithelium or germinal inclusion cysts was observed. Because fetal and adult coelomic epithelia are also negative for OV632, it is conceivable that OV632 reacts with a differentiation antigen that is expressed mainly in the fallopian tube. The antigen recognized by OV632 might possibly be an early mullerian antigen retained in the fallopian tube in the adult and reappearing in tumours originating from mullerian-derived tissues.

The pattern of staining shown by antibody OV632 in the present study and the data in the literature show that this antibody is distinct from antibodies reactive to ovarian cancer antigens reported by other workers (Bast et al. 1981; Kabawat et al. 1983a, b; Mattes et al. 1984; Bhattacharya et al. 1982; Gangopadhyay et al. 1985; Croghan et al. 1984; Tagliabue et al. 1985). Bast et al. (1981) have described the production of a monoclonal antibody (OC125) against serous cystadenocarcinomas. The antigenic determinant detected by OC125 was associated with coelomic and amnionic epithelium during embryonic development (Kabawat et al. 1983a, b) and was found in adult tissues derived from coelomic epithelium (Kabawat et al. 1983a). OC125 reacted with over 80% of the nonmucinous epithelial ovarian carcinomas tested and with a limited number of mucinous carcinomas. Furthermore, OC125 recognized epitopes present in 40% of breast carcinomas (Kabawat et al. 1983b; Koelma et al. 1986) and some adenocarcinomas of the lung (Kabawat et al. 1983b). Besides different staining patterns of normal tissues and tumours, our immunoperoxidase studies have shown that antibody OV632 recognized a cytoplasmic determinant, whereas OC125 stained mainly cell membranes. In two-wavelength indirect immunofluorescence experiments with OC125 and OV632 we also found that these two antibodies stained different regions of ovarian tumours.

Other antibodies to nonmucinous tumours were described by Mattes et al. (1984), who reported five distinct monoclonal antibodies to several cell-surface antigens of human ovarian and endometrial carcinoma. One of them, the MH55 antigen, was weakly expressed on ovarian and uterine cancer cell lines but not on other cell lines and tissues tested. Additional histological studies on carcinoma specimens are needed before any diagnostic usefulness can be appraised.

Several monoclonal antibodies have been described that recognize antigenic determinants present in mucinous cystadenocarcinomas, representing 6–10% of malignant epithelial tumours of the ovary (Bhattacharya et al. 1982; Gangopadhyay et al. 1985). Monoclonal antibody 1D5 was stated to be highly specific (Bhattacharya et al. 1982). Less specific monoclonal antibodies against both mucinous and nonmucinous ovarian carcinomas have been reported, for example F36/22 (Croghan et al. 1984), and MOv1 and MOv2 (Tagliabue et al. 1985).

The data presented here indicate that OV632 can distinguish ovarian carcinoma from a variety of other tumours. Over 80% of primary nonmucinous ovarian adenocarcinomas stained positively with OV632, whereas colon carcinomas and breast carcinomas, together representing the majority of metastatic tumours in the ovary, do not react with OV632. On the basis of these findings, we conclude that antibody OV632 can be a useful adjunct in routine surgical pathology in the discrimination between ovarian and nonovarian carcinomas both in the ovary itself and in cases of adenocarcinoma of unknown origin in the abdominal cavity.

Acknowledgements. The authors thank Coby Huiskens-van der Meij and Trudy van Leeuwen-Herberts for technical assistance, Marian Kantebeen-Zoetemelk for secretarial help, and Ingeborg Koelma for critical comments. Monoclonal antibody OC125 was a gift from Oris Lapam Industries, France.

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Accepted October 24, 1986