European Journal of Obstetrics & Gynecology and Reproductive Biology, 36 (1990) 221–238
Elsevier

EUROBS 01004

Plenary Session I – Gynaecological Cancer

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CA 125 and OA 3 as target antigens for immunodiagnosis and immunotherapy in ovarian cancer

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CA 125; OA 3; Antigen; Immunodiagnosis; Immunotherapy; Ovary; Cancer

Introduction

At their surface, ovarian cancer cells often express or secrete glycoprotein molecules that potentially can be used for immunodiagnosis and immunotherapy [1–3]. At present many of these substances are defined by monoclonal antibodies that are reactive with a distinct antigenic determinant or epitope present on these large molecules, while generally the exact chemical structure of the antigen molecule is not known. Monoclonal antibodies (McAbs) produced by immortalized hybridoma cell clones offer several advantages over polyclonal antibodies. These McAbs can be obtained in unlimited quantities and of a stable quality as the hybridoma product is pure (no mixtures of different antibodies) and therefore consists of antibodies with a uniform specificity. High specificity for a given tumor type or site is only obtained after a process of careful screening and selection. It should be remembered that high tumor specificity never reaches unique tumor specificity, since tumor-associated antigens can also be expressed by other tumor types and as well as by normal tissues. Thus, while McAbs are epitope-specific and therefore often antigen-specific, glycoprotein antigens have not been found to be

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tumor-specific. This should be borne in mind when using McAbs for the diagnosis and therapy in ovarian cancer patients.

Many ovarian cancer-associated McAbs have been described to have clinical applicability in the field of immunopathology, immunoserology or immunotargeting [1,2]. Two antibodies, namely the CA 125 detecting OC 125 and the OA 3 antigen detecting OV-TL 3, are the most promising with regard to clinical applicability in gynecological oncology.

Bast et al. [4] first reported on the monoclonal antibody OC 125 in 1981. The OC 125 McAb was obtained by immunizing BALB/c mice with the OVCA 433 cell line isolated from the ascitic fluid of a patient with a serous papillary cystadenocarcinoma. The OC 125 antibody recognizes the so-called CA 125 antigenic determinant present on a high molecular weight glycoprotein complex.

OV-TL 3, also a mouse monoclonal antibody of the IgG1 subclass, was obtained by fusion of murine myeloma cells with spleen lymphocytes from BALB/c mice immunized with a tumor cell suspension prepared from an ovarian endometrioid carcinoma. The precise nature of the OV-TL 3-defined antigen is not known [5].

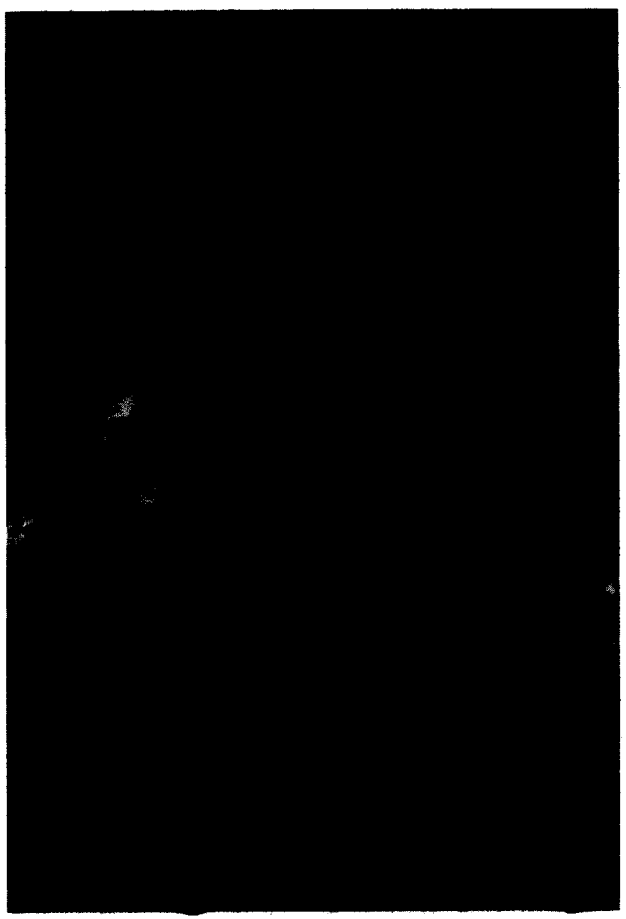
In this survey the present knowledge of these two antibodies is reviewed comparatively.

Immunopathology

Potentially, monoclonal antibodies could be used in immunocytology and histology for more refined subclassification of tumors and differential diagnosis, for staging (detecting of microfoci or occult tumor cells) and for prognostic or therapeutic evaluation (for example see Ref. 6). The OA 3 antigen and the CA 125 are both expressed exclusively in malignancy of epithelial origin, thus providing an instrument for the discrimination of undifferentiated cancers from undifferentiated sarcomas and lymphomas.

In ovarian carcinomas both antigens are frequently expressed, thus rendering a sensitivity for malignant ovarian tumors of 80% (or more) for CA 125 and of 90% or more for OA 3 [5,7,8]. However, the CA 125 antigenic determinant has also been reported to be present in many other adenocarcinomas such as colon and endometrium, and in non-cancerous tissues such as endometriotic lesions and benign ovarian cysts [9].

OV-TL 3 seems to be highly specific for ovarian epithelial tumors and, if this were to be shown as being the case after large scale testing, this antibody could be used for differential diagnostic purposes, especially for the differential diagnosis against colorectal cancer [10]. Where OA reaches a high expression exclusively in ovarian cancers, CA 125 can also be present in a fair amount of endometriosis and the lining or content of benign ovarian cysts. There is a remarkable qualitative difference in antigen expression between both antigens in cancer tissues. CA 125 is non-homogeneously present within the tissue, there being CA 125-positive and CA 125-negative cells and concentrations of CA 125 substances within tumor clefts (Fig. 1). This reflects the original nature of this antigen in normal tissues: that of a secretory product, synthesized by adeno cells and secreted at their apical cell surface into the lumen of the ductal tract.



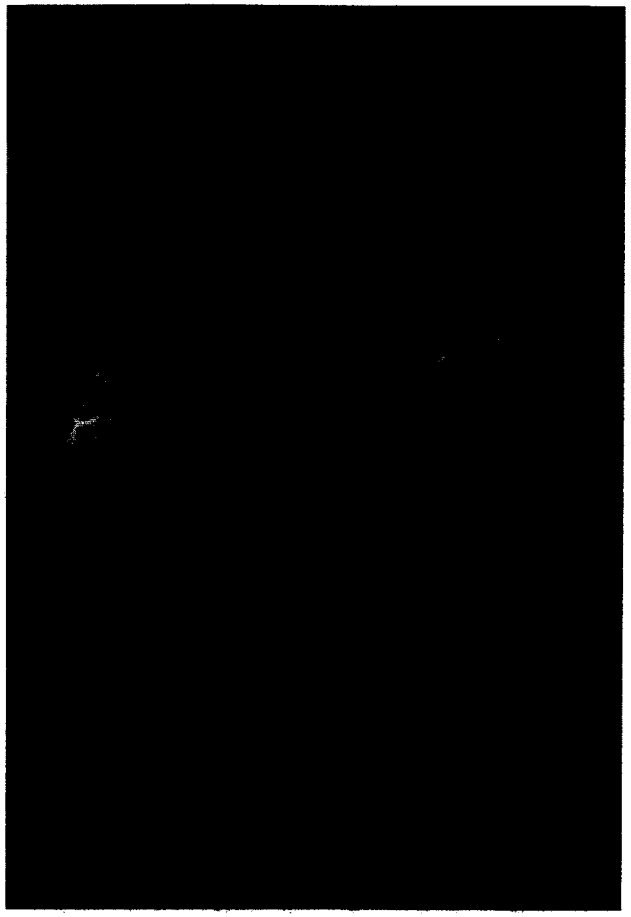


Fig. 1 (left panel). CA 125 antigen pattern in ovarian cancer specimen (OC 125 immunofluorescence).

Fig. 2 (right panel). OA 3 antigen pattern in the same ovarian cancer specimen (OV-TL 3 immunofluorescence).

The OA 3 antigen is not secreted by normal cells in the genital tract, notwithstanding a slight positivity in OV-TL 3 staining of the normal cell lining of the oviduct. OV-TL 3 reacts with an antigen that is membrane-bound and, which, in malignant cells, is present at the total circumference of each and every tumor cell (Fig. 2).

This difference in antigen pattern and in antigen nature as reflected in immunohistology has its consequences for immunoserology and for immunotargeting.

Immunoserology

Tumor detection, tumor identification, staging, treatment monitoring and early detection of tumor recurrence are some of the theoretical possibilities, when the antigen is shed by the tumor cells and subsequently enters the circulation, to which under normal circumstances the antigen, present at the luminal cell side, has no access.

The membrane bound OA 3 antigen is not shed, and thus not found in the circulation. Therefore, although OA 3 has a high specificity and sensitivity in ovarian cancer, there is no OV-TL 3 antibody based serum immunoassay.

The OC 125-based homologous double determinant assay, introduced in 1983 [11], has now a definite place in gynecology, although the discussion with regard to its real clinical value is not yet finished.

In this immunoradiometric serum assay (IRMA), OC 125 levels that remain elevated. In many instances CA 125 McAbs are fixed to a solid substrate, thereafter the patient's serum is added. The serum antigens present are now caught by the substrate-fixed antibody and the serum is washed away. A second group of OC 125 McAbs is now introduced, each Ab being connected to a radioactive tracer isotope. The second antibody binds to the antigen caught by the first, fixed OC 125 Abs. The amount of measurable tracer signal given in arbitrary units per ml of serum is proportional to the amount of CA 125 antigen present.

The cut-off level of the CA 125 IRMA has been arbitrarily set at 35 U/ml. Various studies report positivity in healthy controls ranging from 0 to 5.2% for this cut-off level. Remarkably high levels of CA 125 can be found in the first trimester of pregnancy and during menstruation. Several benign diseases were found to be associated with frequent CA 125 positivity, some of which may cause differential diagnostic problems. Patients with effusions caused by benign diseases such as congestive heart failure, tuberculosis or liver cirrhosis can be highly positive, as can patients with benign gynecological diseases such as uterine myofibroma, benign ovarian tumors, and especially endometriotic cysts. Thus, a CA 125-positive test should be interpreted with caution, since healthy controls and especially patients with benign disease can have elevated levels of CA 125. When monitoring ovarian cancer patients it should be remembered that intercurrent benign diseases as peritonitis or small bowel obstruction could mimic progression of cancer when judged on the basis of CA 125 levels alone.

The CA 125 antigen can be found elevated in over 70% of patients with ovarian cancer, but also in many patients with non-gynecological malignancies (for review see Fig. 9). Therefore, the role of the CA 125 test in differential diagnosis between

various malignancies is limited. Pretreatment CA 125 serum levels in ovarian cancer reflect the amount of circulating tumor-associated antigen in relation to the extent of disease at the moment of staging. In stage I and II disease, almost half of all patients have elevated CA 125 serum levels [12]. Consequently, in more than half of all patients with invasive disease limited to one or both ovaries CA 125 serum values were within the normal range. Therefore, discrimination between malignant and benign ovarian tumors on the basis of the CA 125 IRMA test alone is very difficult [13].

The CA 125 assay has proved its clinical value in the monitoring of disease and detection of recurrence. An important characteristic of this tumor-marker test is its ability to reflect the behavior of a tumor mass during therapy and in the follow-up period after completion of therapy. Most authors consider a doubling or halving of serum values to be of clinical significance. The overall reported correlation between CA 125 levels and the course of disease is high (76% to 95%).

CA 125 elevations have been reported to precede, in 90% of all cases, the clinical detection of tumor progression or tumor recurrence by a period of up to 17 months. Recent data suggest that CA 125 determinations after the first courses of chemotherapy can predict the final clinical outcome. Measurements of the half-life of CA 125 during chemotherapy might be helpful in discriminating between good and poor responders.

Nearly all patients with CA 125 levels above 35 U/ml who appeared clinically free of tumor were shown to have tumor at a second-look operation. Only half of all patients with marker levels within the normal range were surgically and microscopically free of tumor. The other half still had tumor present despite normal CA 125 levels.

Thus, CA 125 tests can be a great help in the management of ovarian cancer patients, especially during treatment monitoring. At the time of second-look, surgical procedures should be cancelled for those patients with CA 125 levels that remain elevated. In many instances CA 125 has proved to be more sensitive than the conventional methods such as physical diagnostic investigation, ultrasound and CT scanning.

Immunotargeting

Monoclonal antibodies, conjugated to γ and β energy radiating nuclids open possibilities for tumor imaging and tumor destruction, respectively [14,15]. Intravenous and intraperitoneal routes of administration of these immunoconjugates allow selective targeting with promising ratios of tumor to normal tissue antibody uptake. Diagnostic tumor targeting in patients with ovarian cancer has been reported both with OC 125 based conjugates [16–19], as well as with OV-TL 3-based conjugates [20–23]. Both antibodies have proven to be of potential value in tumor imaging in these initial studies. However, OV-TL 3-based immunoconjugates are expected to be superior to those that are OC 125-based, on basis of the following theoretical grounds:

1. OV-TL 3 has a better specificity for ovarian cancer than OC 125 [5,10].

2. The OA 3 antigen is more homogeneously present within ovarian cancer tissue (unpublished observations, see Figs. 1 and 2).
3. The OA 3 antigen is membrane-bound and not shed into the circulation [24]. CA 125 is present in the blood of ovarian cancer patients, leading to antibody-antigen complex formation (and therefore rapid clearance) following intravenous administration of OC 125 in patients [25].
4. Characteristics of in vitro binding and of biodistribution in tumor-bearing athymic mice are favorable for OV-TL 3 when studied in comparison with OC 125 [26–28].

In a recent, multicentered prospective tumor-localization study 53 patients, diagnosed or suspected to have ovarian cancer, were injected i.v. with OV-TL 3 F(ab')2 fragments, conjugated to Indium-111. A single i.v. dose was given, containing 1 mg of antibody conjugated to 4 mCi Indium (range 3.5 to 4.4 mCi).

There were no allergic reactions, and the procedure was well-tolerated by all patients.

The immunoscintigraphic results were better when compared to those obtained by CT-scanning, ultrasound or NMR [21–23]. Therefore, radionuclide immunolocalization of ovarian cancer deposits using ^{111}In-OV-TL 3 F(ab')2 fragments seems promising as a highly discriminating method that can be used without severe toxicity.

The results of this imaging study also indicate that therapeutic tumor-targeting using other radio nuclides (e.g., ^{131}I or ^{90}Y) are now within reach.

Conclusion

CA 125 and OV-TL 3 are valuable antibodies for the detection of ovarian cancer-associated antigens. Circulating CA 125 antigen forms the basis for a serum tumor marker test which has shown its clinical applicability already unequivocally. For in vivo diagnostic or therapeutic targeting in ovarian cancer patients, there is a preference for OV-TL 3 antibody-based immunoconjugates that are reactive with the tumor cell membrane-bound target antigen OA 3.

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