Tumor Antigens in Ovarian Malignancy

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Treatment of cancer of the ovary remains the major challenge facing gynecologic oncologists today. This disease is the leading cause of death among gynecologic malignancies, killing more women each year in the United States than cancers of the uterus and cervix combined. Seventy percent of these women are not diagnosed until the disease has spread throughout the peritoneal cavity, and, despite recent advances in chemotherapy, most notably the use of platinum-based combination regimens, the 5-year survival in this group is still a dismal 10–15%.

Before this survival rate is significantly improved, several clinical problems must be solved. Foremost among these is our inability to achieve early diagnosis in most women. Although diet as well as other environmental factors have been studied, most women in whom ovarian cancer develops have no identifiable risk factors. We are thus unable to clearly define a group at high risk for having ovarian cancer develop on whom screening and diagnostic efforts could be focused. The disease is totally asymptomatic in its early stages, and early diagnosis by physical examination is mainly a matter of chance. Conventional imaging techniques using x-rays or sound waves also have not been useful in early diagnosis.

Because clinical evaluation and diagnostic imaging techniques often are not useful for the early diagnosis of ovarian cancer, it should not be surprising that it is difficult to monitor the patient’s response to therapy. Progressive disease with development of large masses and ascites will be readily apparent, but small-volume residual disease cannot be detected by current techniques. This situation has appropriately led to the widespread employment of “second-look laparotomy,” obviously a highly invasive diagnostic procedure, for monitoring of response and detection of recurrence. Finally, it is quite apparent that new therapeutic modalities are needed because combination chemotherapy has not been as effective as initially hoped in raising the cure rate.

It has been recognized for many years that tumors can be antigenic and that the immune system can be important in modulating host response to tumors. Recent technologic advances have greatly increased our understanding and knowledge of tumor antigens and have generated enthusiasm that studying these antigens will be helpful in solving the clinical problems.

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early diagnosis, monitoring of response, and effective treatment. This article will
define certain desirable characteristics of
tumor antigens1 and will discuss methods
of study of antigens, and production of
antisera. We will review the more
important of the many antigens associated with
ovarian cancer and will discuss clinical
applications as well.

Tumor Antigens

A basic tenet of tumor immunology is that
human malignancies may have antigenic
differences from normal tissues that can be
detected in tumor tissue or circulating in
serum. The term "antigen" is used, even
though these substances may not provoke
an immune response in the host, because
their primary method of analysis has been
with immunologic techniques.

Perhaps the most sought-after characteristic of tumor antigens is that they be
specific. "Tumor specific" has been defined in
many ways, but a practical definition of a
tumor-specific antigen would be one pro-
duced solely and consistently by a mali-
nant tumor of given type. It was first shown
during the 1950s, using transplantation
experiments in syngeneic mice, that certain
chemically induced tumors produce anti-
gens specific for an individual tumor. This
absolute degree of tumor specificity obviously
would not be clinically useful, be-
cause it would imply that the antigens in
one patient's cancer would be unique and
thus distinguishable from antigens in the
same type of tumor in another individual.
In human beings the evidence for antigens
specific for a given type of tumor has been
elusive. They may not exist, and indeed
such specificity may not be necessary for
most clinical applications. A more realistic
goal may be the identification of tumor-
associated antigens, a term that may in-
clude quantitative antigenic differences
from normal cells, normal antigens newly
acquired during neoplastic transforma-
tion, or reexpression of antigens present
during fetal life (oncofetal antigens). Some
of these antigens have been associated with
several types of malignancies.

An additional desirable characteristic of
tumor antigens is that they be present and
accessible in tumor tissue, an important
requirement for antibody imaging or treat-
ment of tumors. For practical purposes,
this requires that the antigen be a cell sur-
face component, because cytoplasmic and
nuclear antigens are less accessible to circu-
minating antibodies. Certain secretory pro-
ducts of cells also may serve as tumor mark-
ers in serum or effusions, but, unless these
components are present on the cell sur-
face, they could not be useful for imaging
or immunotherapy. Circulation of the tu-
mor antigen in blood would be advan-
tageous for diagnosis and monitoring of
response, and an ideal marker would be
absent (or present in lower levels) in the
blood of normal individuals. To be of most
use, the antigen should be released into the
circulation before the tumor cells spread,
because this would facilitate the diagnosis
being made when the disease is limited to
the ovary. Its absolute and relative levels
should be related to the amount of viable
tumor. It has been suggested that for clini-
cal applications requiring antibody bind-
ing to tumor cells, such as imaging or im-
munotherapy, the presence of circulating
antigen actually may be detrimental be-
cause this may neutralize injected anti-
body.

The measurement of human chorionic
gonadotropin (hCG) in patients with gesta-
tional trophoblastic neoplasia (GTN) has
exemplified some of these desirable char-
acteristics. This glycoprotein consistently is
present in the serum of patients with GTN,
and its relative (though not absolute) levels
parallel disease activity and reflect the re-
sponse to therapy. It lacks specificity in that
hCG may be detected in a variety of condi-
tions, both benign and malignant, but its
detection in tissue or serum can help to
confirm the presence of GTN in cases where the diagnosis is in doubt. Although hCG and other markers may be elevated in certain germ cell tumors of the ovary, no similarly useful markers have been found in the epithelial cancers, which constitute about 90% of ovarian malignancies.

The study of tumor antigens classically has begun with the production of antibodies against the tumor. This may be done by immunization of an animal, generally of another species, with tumor tissue, cells, or extracts to produce an immune response to the antigens contained in the tumor. The animal's serum subsequently will be found to contain antibodies against antigens present in the tumor. Such heterologous (cross species) polyclonal antisera have several technical problems. They are available only in limited amounts from individual animals, and they will contain a wide range of antibodies, some naturally occurring, as well as multiple antibodies directed against various components of the tumor. Many of these will be against normal tissue components, and thus the antisera may require extensive absorption with normal tissue and extensive biochemical separation procedures before anything approaching a "pure" antibody is obtained. Despite these problems, many important tumor antigen systems have been defined by this method.

Among the most important recent advances in the field of immunology has been the development of techniques that allow the in vitro production of large quantities of pure antibody against a single antigenic determinant, produced by a single clone of cells. These monoclonal antibodies are produced by a hybridoma—an immortal cell line derived from the fusion of a normal antibody-secreting B-lymphocyte and a malignant myeloma cell. Certain progeny of these fusions will retain characteristics of both parent cells, namely the specificity of the antibody produced by a single B-cell and the ability of the myeloma cell to grow in culture and produce antibody indefinitely. Of critical importance is the fact that a single B-lymphocyte can produce only a single antibody of defined specificity. The technique generally involves inoculating mice with the desired immunogen and then using the mouse spleen as a source of antibody-producing B-cells. These cells are mixed with a suspension of mouse myeloma cells under conditions that promote cell fusion. Many individual clones of hybridomas will result, which may produce pure antibodies to any of the antigenic specificities on the immunogen. The specificity of the antibody produced by individual clones is determined by reacting the antibody with various tissue sections and cell lines. Antibodies with desired specificity then can be made available in pure form and large quantity, which greatly facilitates their study.

Once antibodies have been produced, various techniques can be used that will identify antibody–antigen interactions to detect and quantitate antigens in fluids, cells, and tissues. Methods in common use include precipitation, agglutination, and detection of radioactivity, enzyme activity, or fluorescence. Further characterization of the antigen identified by the antibody then can be carried out by standard biochemical techniques.

**Antigens Associated with Ovarian Cancer**

Antigens associated with ovarian cancers were detected as early as 1956 by Witebsky et al., who used extracts from human ovarian cancers as their immunogen. They developed several ovarian cancer antisera that reacted with tumor extracts but did not react with human erythrocytes, human serum, or normal human ovaries. They also detected antigenic differences between serous and mucinous tumors, an observation that has been confirmed by many subsequent investigators who used
polyclonal and monoclonal antisera. Witebsky et al. clearly understood the therapeutic implications of this line of research, cautiously stating in 1956 that

The underlying idea in these investigations, of course, is to produce cancer-specific antisera which would not react with the constituents of normal tissues. Cancer-specific antibodies in these sera would then, it is supposed, combine exclusively with cancer cells, and either damage them by direct combination or with the help of radioactive or other substances which might be built into the antibody carrying molecule...

In the late 1960s, Levi4 raised interest in the early immunodiagnosis of ovarian cancer by demonstrating circulating antibody against ovarian cancer in 12 of 14 women with this disease.

These clinical applications and others will be explored in depth after a review of some of the more recently reported antigens related to ovarian cancers. These will be separated into two groups—antigens detected by polyclonal antisera and antigens detected by monoclonal antisera.

**Antigens Detected by Polyclonal Antisera**

Carcinoembryonic antigen (CEA), a glycoprotein with a molecular weight of approximately 200,000 daltons, first was described in 1965 by Gold and Freeman.5 It has multiple antigenic sites and may vary somewhat in carbohydrate and amino acid structure, thus complicating its detection and measurement. Originally thought to be found only in fetal intestinal mucosa and carcinomas of the intestinal tract, further study detected its presence in a wide variety of human tumors, including those of the breast and gynecologic organs. It is also elevated in several nonmalignant conditions, including inflammatory bowel disease, liver disease, pulmonary disease, and pregnancy and in heavy smokers. Production of CEA or other oncoprotein markers by malignant tumors may result from derepression of an embryonal gene product that occurs during neoplastic transformation.

The lack of specificity of CEA has severely limited its usefulness as a diagnostic tool. Plasma CEA concentration in most human studies was related to extent of disease, tumor CEA concentration, and antigen metabolism. In animal studies absolute plasma CEA levels were related to tumor mass and volume and inversely correlated with hepatic function. Although not useful in differential diagnosis, serial measurements of CEA are useful in monitoring the clinical course of patients with colon cancer who have elevated CEA levels before treatment. In human ovarian cancer, the cell type of the tumor strongly influences plasma and tumor CEA levels. Cyst fluid of mucinous tumors contains high levels of CEA, and plasma levels are elevated in approximately 50% of patients who have these tumors. Cyst fluid and plasma levels are generally much lower in patients with serous tumors.

\( \alpha \)-Fetoprotein (AFP) is a glycoprotein with a molecular weight of 70,000 daltons, first identified in 1956. In human fetuses, it is synthesized by the yolk sac and liver and can be detected in fetal serum as early as 4 weeks gestation. It is the predominant serum protein in fetal life, carrying out most of the functions ascribed to albumin in the adult. It is detected in highest concentrations in sera of patients with primary hepatocellular carcinomas and germ cell tumors of the gonad. In areas of the world with a high prevalence of liver cancer, AFP has been useful for screening and early diagnosis, monitoring of response to therapy, and detection of recurrences. The monitoring function is possible because serum levels reflect the amount of viable tumor.

Among ovarian malignancies, AFP is reliably present in tumor tissue and serum in patients with endodermal sinus tumors, embryonal cell carcinomas, and mixed germ cell tumors containing these ele-
ments. Marker production actually can be used diagnostically to distinguish endodermal sinus tumors from embryonal carcinomas, because the former contain only AFP by immunohistochemical staining, while the latter contain both AFP and hCG. Because these germ cell tumors of the ovary are rare, and because serum levels of AFP are elevated in a variety of benign liver disorders as well as in pregnancy, the marker has no role as a screening test.

Ovarian cystadenocarcinoma associated antigen (OCAA) is a high-molecular-weight mucoprotein that is important in several respects. The antigen was defined by Bhattacharya and Barlow, who used antisera raised in rabbits against pooled extracts of human ovarian cancer tissue. After extensive absorption to remove nonspecific antibodies, an antibody persisted that identified an antigen present in each of seven mucinous adenocarcinomas. The antigen was not found in normal ovarian tissue, nor in germ cell or stromal tumors, and was immunologically distinct from CEA, AFP, and normal histocompatibility antigens. Interestingly, although the antigen appeared to be of cytoplasmic origin in immunofluorescent studies, a reliable radioimmunoassay was developed that demonstrated its presence in serum. Using a serum level of 10 ng/ml as the upper limit of normal, elevated antigen levels were detected in approximately 70% of patients who had advanced ovarian adenocarcinomas. Initial serum levels were related to tumor burden, and subsequent levels correlated well with response to therapy. Because sera from patients with advanced malignancies of the colon, breast, or cervix may cross-react with OCAA in the radioimmunoassay, the antigen does not appear useful for diagnosis. In patients with ovarian adenocarcinomas whose antigen levels are initially elevated, however, it may be useful in following response to therapy.

Ovarian Cancer Antigen (OCA) is a high-molecular-weight glycoprotein defined by Knauf and Urbach for which a radioimmunoassay has been developed. The antigen was detected in human ovarian adenocarcinomas but was not found in normal ovarian tissue. Serum OCA levels were elevated in 52% of patients with ovarian cancer and in only 8% of normal patients. Although the specificity of OCA was too low to allow its use as a diagnostic method, in patients whose serum levels were elevated, serial levels did correlate with disease status.

More recently, a fraction of OCA, designated NB/70K, has been identified that appears promising. Serum levels were elevated in about 60% of women with advanced ovarian cancer and in 38% of women with stage I tumors.

Antigens Detected by Monoclonal Antisera

With the use of the hybridoma technique, a number of laboratories have produced antibodies that react with ovarian tumors. Most of the antibodies produced in this manner will detect cell surface antigens that are widely distributed and are of little investigational use. The search is for clones that produce an antibody of restricted distribution that is of interest to the researcher or clinician. Some of these will be described here.

CA125, described by Bast el al. is the designation of an antigenic determinant associated with at least two high-molecular-weight (200,000 daltons) cell surface glycoprotein molecules. Multiple CA125 determinants are present on each of these molecules, and they are detected by the murine monoclonal antibody called OC125. This antibody, an IgG1, was raised against a cell line derived from an ovarian serous papillary adenocarcinoma. Initial results indicated a fairly high degree of specificity, with the antibody reacting with 6 of 6 epithelial ovarian carcinoma cell lines and with cryostat sections of 12 of 20 ovarian cancers. There was reactivity with
only 1 of 14 cell lines from nonovarian tumors and no reactivity with sections of 12 nonovarian carcinomas.

Further studies indicated a wider tissue distribution of the antigen, with small amounts being found in embryonic celomic epithelium and amnion, as well as in normal adult tissues derived from celomic epithelium. The antibody also reacted with several malignancies derived from these tissues. Subsequent work on the tissue distribution of CA125 in ovarian cancers has shown that about 80% of nonmucinous ovarian carcinomas contain the antigen, while most mucinous tumors do not.

A radioimmunoassay has been developed to measure CA125 levels in serum, with antigen activity being defined in arbitrary units relative to a reference standard. Considerable experience has been gained in the measurement of this antigen in the serum of patients with ovarian cancer, patients with other malignancies, patients with benign gynecologic disorders, and healthy people. There is the potential for a clinical role in monitoring of response to therapy and diagnosis.

Several additional monoclonal antibodies reacting against ovarian cancers have been described by other researchers. Most of these have not been studied as extensively as CA125. These antibody-antigen systems will be described using the designation applied by the original authors; some of these refer to the antibody and some to the antigen itself.

In 1982, Bhattacharya et al. described two IgG1 monoclonal antibodies, designated ID3 and ID5, that were relatively specific for mucinous ovarian cancers. Twelve of 14 mucinous carcinomas contained the antigen, while the antigen was not found in any other gynecologic or nongynecologic tumor tested, nor in any normal adult tissue tested. Of fetal tissues tested, only an intestinal extract contained the antigen. The restricted distribution of this antigen may make it useful as a marker for mucinous ovarian cancers. The same investigators later described two additional monoclonal antibodies, designated 4F4 and 7A10, raised against an ovarian adenocarcinoma extract. These IgG1 antibodies both identified a 48,000-dalton peptide that appeared similar to the OCAA antigen previously identified by these authors. They reacted with approximately 90% of ovarian tumors and 60% of other tumors tested but not with normal adult tissues or sera. Of importance is the fact that this antigen, unlike many others described in ovarian tumors, can be detected not only in frozen sections but in formalin-fixed, paraffin-embedded sections. This allows its study in a wider range of patients and tumors because the sections can be evaluated retrospectively.

An antigen with wider distribution is detected by the IgG1 monoclonal antibody OC133, which has been described by Berkowitz et al. They compared OC133 with a conventional polyclonal heteroantiserum raised against the same cell line and found the monoclonal reagent to be somewhat more specific, although it reacted with nonovarian tumor cell lines as well as normal endometrium and endocervix.

A number of interesting monoclonal antibodies reacting with ovarian cancers have been produced by Mattes et al. at the Memorial Sloan-Kettering Cancer Center. Several of these have sufficient specificity to be of potential use in imaging or immunotherapy. One antibody, MD144, is of interest because thus far it has only reacted with the immunizing cell line and has failed to react with 153 other cell lines and 27 different normal adult tissues.

A promising monoclonal antibody, designated F36/22, recently has been described by Croghan et al. This antibody detects the so-called ductal carcinoma antigen, which was present in all primary ovarian cancers, metastatic tumors, and ascitic tumor cells tested. The antigen was not present on reactive mesothelial cells.
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and thus may be of value in distinguishing benign from malignant effusions. Serum levels may reflect the clinical course of the disease. Because this antibody is cytotoxic to tumor cells in vitro and in vivo, it has interesting potential therapeutic applications.

In addition to the antibodies discussed here, many others have been described, both in this country and abroad. Space restrictions preclude an exhaustive catalog of these antibodies. Interested readers are referred to the primary literature.

Clinical Applications

Although multiple cell surface and secretory antigens associated with ovarian cancers have been identified and characterized, none of these has yet been shown to fulfill the criteria for the "ideal" antigen as described earlier. Clinical research using these antigens is underway, but the proper clinical use has not yet been clearly defined. In this section we will discuss four broad areas of potential clinical application: diagnosis, monitoring of response, tumor imaging, and therapy.

Diagnosis

Development of sensitive enzyme and radioimmunoassays has allowed the accurate detection of antigen in serum and tissue and has tantalized researchers and clinicians alike with the possibility of a "blood test" for the early diagnosis of ovarian cancer. Such a test would, of course, depend upon the presence of circulating antigen in early disease, and its overall usefulness would depend on its sensitivity, specificity, and cost and the incidence of the disease in population. The difficulty involved in using such a test for screening a general population can be appreciated from the following example. Approximately 30 cases of ovarian cancer would develop in 100,000 American women ages 40–70, who were followed for 1 year. If we screen this population with a blood test for ovarian cancer that has a sensitivity of 100%, all 30 cases would be detected. Even with a specificity of 99.9% (0.1% false-positive rate), however, this screening would result in 100 false-positive results (0.1% of 100,000). Thus, our population of 100,000 would yield a total of 150 positive results, of which 100 would be false-positive results and only 30 would be true-positive results. This would not necessarily negate the value of the test, but one would have to be sure that the next step taken in evaluating women with a positive result, be it a repeat blood test, an imaging technique, or even laparoscopy or laparotomy, would have a high enough accuracy and a low enough morbidity to justify the entire sequence. Screening of high-risk populations, such as women with a family history of ovarian cancer or those with an adnexal mass, would yield a more favorable ratio of true-positive to false-positive cases, but similar problems would remain.

Statistical considerations aside, radioimmunoassays for serum levels have been developed for several of the antigens described above, and some information is available on antigen levels in various population groups. The best studied of these is CA125, which has been found by Niloff et al.17 to be elevated above an arbitrarily defined normal level in the serum of 1% of nonpregnant patients with benign gynecologic disorders. The antigen was elevated in the sera of 16% of women in the first trimester of pregnancy and in about one-fourth of patients with nongynecologic malignancies. It is also elevated in a small proportion of patients with hepatic and renal disease and in 0.2% of apparently healthy blood donors. Although it is elevated in the serum of about 80% of women with advanced nonmucinous epithelial ovarian carcinomas, it is probably present much less frequently in early ovarian cancers. The results of studies evaluating the usefulness of CA125 in the early diagnosis of
ovarian cancer are not yet conclusive; however, given the apparent limitations imposed by its sensitivity and specificity, it does not appear promising for this use. The fact that CA125 levels are normal in many women with small amounts of recurrent tumor found at second-look laparotomy also suggests that it will not be useful in early diagnosis.

Levels of CEA are elevated in the plasma of about 50% of women with advanced ovarian cancer but only about 30% of women with early ovarian cancer, obviously not a percentage that makes it useful for diagnosis. According to Dembo et al., serum levels of NB/70K were elevated in 60% of patients with advanced ovarian cancer and in about 5% of control subjects, as well as in patients with benign gynecologic neoplasms, nongynecologic malignancies, and hepatic and renal disease.

Unfortunately, there is no marker yet that is useful in the early diagnosis of ovarian cancer.

**Monitoring of Response**

In patients undergoing chemotherapy for ovarian cancer it is often difficult to assess their response to treatment. Most patients undergo an exploratory second-look laparotomy if there is no clinical evidence of disease after completion of their planned chemotherapy. In at least half of these patients occult tumor is found. Clinical diagnosis of recurrence often cannot be made until bulky tumor masses and malignant effusions are present. There are several tumor antigens discussed in this article whose serum levels appear to correlate with disease activity and that may therefore be useful in monitoring patients' responses to therapy.

hCG and AFP have been mentioned as reliable markers of disease activity in advanced cases of germ cell malignancies containing choriocarcinoma and endodermal sinus tumor, respectively. Levels will fall as the disease responds to chemo-therapy. Rising levels indicate recurrence. It is as yet uncertain whether "negative" levels of these markers can obviate the need for second-look surgery in patients who have been treated with chemotherapy, because it may be that small amounts of tumor do not produce measurable levels of the marker.

Results of evaluation of CEA as a monitor of response have been mixed. In some studies, among patients with ovarian cancer who have initially elevated levels of CEA, subsequent levels seem to parallel disease activity. Other studies have been unable to document this relationship. Part of the confusion may lie in the fact that clinical assessment of disease activity is quite difficult. Additionally, variations in assay methods, chemotherapy-induced liver dysfunction, and elevation of antigen levels in benign conditions can confound results. For these reasons CEA has not gained clinical acceptance as a monitoring tool.

CA125 may be useful in monitoring response to therapy. Bast et al. have found that antigen levels in serum were elevated in more than 80% of patients with non-mucinous epithelial cancers, and increases or decreases in antigen level paralleled disease status in about 90% of patients. In several subsequent studies, antigen levels were reported to correlate with disease status with about the same degree of accuracy. In a number of instances elevations of CA125 preceded the clinical diagnosis of recurrence by 1–7 months. Unfortunately, in patients with small tumor volumes there may be no measurable antigen in the circulation, so a normal CA125 level does not rule out the need for second-look laparotomy. In a recent study, Niloff et al. examine this issue. In 56 patients whose CA125 levels had been elevated previously, 36 patients had normal levels at the time of second-look surgery. Tumor was found at surgery in twenty-two of these 36 patients (61%). On the other hand, of 20 patients
with elevated CA125 levels at the time of their second-look surgery, 18 were found to have tumor, 1 subsequently had recurrence, and 1 remains clinically free of disease at 3 months follow-up. Thus, while a normal CA125 level was not accurate in predicting disease status, an elevated level reliably predicted the presence of tumor or future recurrence. As further experience is gained it is possible that an elevated CA125 level could be considered sufficient evidence of disease progression to allow modification of therapy before clinical evidence of progression or without the need for second-look surgery.

**Imaging**

The ability to accurately image ovarian tumors potentially could lead to a broad range of clinical applications, including early diagnosis, differential diagnosis, and monitoring of response. Thus far, conventional imaging techniques, including computerized tomography (CT) and ultrasound, have been unrewarding for this application. The ability to develop antibodies to antigens contained in ovarian tumors has stimulated interest in the possibility of conjugating these antibodies to radioisotopes to allow radiographic imaging of primary, metastatic, or recurrent disease. The degree of specificity of the antibody may be less important for imaging than for other clinical uses, but antigen must be present on the cell surface to allow binding. It has been suggested that the presence of circulating antigen actually may interfere with imaging, because antibody–isotope conjugates may bind to antigen in the serum and thus fail to localize in tumor tissue.

Several different isotopes have been used for labeling, including iodine 131, iodine 125, iodine 128, and indium 111. The use of the iodine-labeled antibodies requires blocking the thyroid with iodine, usually in the form of potassium iodide, in order to prevent selective uptake of the radioactive iodine in the thyroid. The higher the specificity of the antibody itself, the more label that will localize in the tumor and the less that will be present as background interference. Antibody–antigen binding must persist long enough to allow gammacamera imaging, which is done at an interval after injection appropriate for the isotope being used.

Using I-131–labeled polyclonal heteroantisera to CEA, Goldenberg was able to image 10 of 10 primary and 11 of 14 metastatic ovarian cancer sites. The smallest tumors detectable by this technique were about 2 cm in diameter. Tumors were successfully imaged in patients with both normal and elevated serum CEA levels. Some of the tumors detected were missed by conventional radiography, including CT and ultrasound. Using the same technique, van Nagell was able to image 13 of 13 primary ovarian tumors and 6 of 9 metastatic sites. Interestingly, most of the tumors imaged were serous carcinomas, and, although serum levels were normal in most of these patients, the tumor tissue itself contained significant amounts of CEA in all patients tested. Tumors smaller than 2 cm in size were not detected.

In England, Epenetos et al. have used monoclonal antibodies directed against a breast tissue component for radioimmunolocalization of ovarian cancer. These antibodies react with a variety of epithelial neoplasms. Using I-123–labeled antibodies, they were able to image ovarian cancers in 11 of 12 patients. Studying the kinetics of their technique, they found that iodination of the antibodies did not interfere with antigen binding. Imaging was achieved despite the fact that the mean tumor uptake of radioisotope was less than 1% of the injected amount. In an animal model that used nude mice bearing human cancers, this same group was able to image tumors as small as 1 mm. Intraperitoneal tumors were also detectable in this system.

With further refinements in immuno-
logic and imaging techniques, radioimmunolocalization of ovarian tumors offers great potential for clinical application.

**Treatment**

Immunotherapy of human tumors has been attempted in one form or another for approximately a century. The basic assumption underlying any form of immunotherapy is that tumor cells have at least quantitative and perhaps qualitative antigenic differences from normal cells that can be exploited in a way to interfere with tumor growth. Most attempts at immunotherapy have involved nonspecific immunostimulation with bacterial toxins or other biologic substances. Various vaccines using tumor cells or cell preparations, often treated to increase antigenicity, also have been used. This section will consider the use of antitumor antibodies in the treatment of tumors.

This approach is still highly experimental and raises a number of theoretic considerations. The relative lack of specificity of most antibodies has caused concern about damage to normal tissues. Because the search for specific antigens found only in a given tumor has not been successful, this hazard may have to be accepted. Even with this lack of specificity, it is still likely that most antibody-directed therapy will be more "specific" than conventional chemotherapy, which causes considerable toxicity to normal tissues. In addition to damage to normal tissues containing antigens that cross-react with the tumor antigen, it is possible that there will be other forms of toxicity resulting from the use of these antibodies. These include allergic reactions and renal damage from circulating immune complexes. An immune response against injected antibody also could decrease therapeutic effects. In both animal and human systems, administration of antitumor antibodies has caused so-called antigenic modulation—loss of antigen sites from tumor cell surfaces and selection of non-antigen-bearing clones. Another potential problem of antibody therapy is that the presence of circulating antigen may interfere with free antibody reaching the target cells. The development of antibodies against antigens that do not circulate in the blood offers one approach to this problem. It also may be possible to decrease circulating antigen levels by conventional treatment with surgery or chemotherapy before antibody administration. An additional consideration is that many antibodies, although they may bind to tumor cell surface antigens, are not cytotoxic, and may therefore need to be coupled to a radioisotope or chemotherapeutic agent to effect cell kill. Lastly, recognizing that only a tiny fraction of intravenously injected antibody may localize in tumor, alternative routes of administration may need to be explored.

Antibody-directed chemotherapy has given mixed results when studied in vitro and in animal models when such agents as methotrexate, chlorambucil, and vindesine are used. In an in vitro system, Embleton et al. showed that conjugation of vindesine to a monoclonal antibody partially inactivated the drug. However, it was shown that the antibody–drug complex would bind to antigen-positive tumor cells and was selectively cytotoxic for these cells as compared with nonreactive cells. In another study, polyclonal heteroantiserum against a murine tumor was bound to chlorambucil, which was found not to lose alkylating activity. Both in vitro and in vivo it was a more effective tumor inhibitor than either free chlorambucil or the antibody alone. In humans, some favorable responses have been seen when monoclonal antibodies have been used in small studies to treat leukemia, lymphoma, melanoma, and gastrointestinal tumors.

These preliminary results indicate that antibodies, either alone or coupled to isotopes or chemotherapeutic agents, may be useful in treating human malignancies.
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Conclusion

A great deal of promising work has been done in the identification of antigens associated with ovarian cancer. Although no truly specific tumor antigen has been identified, this deficiency should not prevent successful clinical application. The development of techniques for the production of monoclonal antibodies has accelerated the pace of this research and raised our hopes for exciting developments in the field of tumor immunology.

References

