

Immunological treatment of ovarian cancer

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Purpose of review

Development of immunological treatments for ovarian cancer has not been a conspicuous success story over the past few years. Only a handful of clinical trials have reported immunological responses, and correlation with clinical benefit has been elusive. Several recent studies presented in this review, however, point to a revival of optimism for the development of novel immunotherapeutic strategies.

Recent findings

The cloning and sequencing of CA125, coupled with novel structural and functional insights, undoubtedly represent important steps forward. The possibility that CA125 could play a role in evasion of immunity by ovarian tumors may represent a new challenge, but does not detract from its potential as a therapeutic target. Of the recent clinical trial reports, the most intriguing results were seen from immunotherapy with a conventional mouse monoclonal antibody specific for CA125, in which human anti-mouse antibody responses correlated significantly with improved survival of patients with advanced stage ovarian cancer and clinical evidence of recurrent disease at the time of treatment.

Summary

There is little doubt that CA125 will undergo a renaissance as an important target antigen for development of novel immunological treatments, particularly with regard to cellular therapies. Identification of other novel ovarian tumor antigens will also accelerate research focused on stimulation of T-cell immunity. Current research trends suggest a paradigm shift in emphasis from vaccines designed to elicit antibody responses to strategies such as dendritic cell vaccination that are designed to induce broader immunity, including ovarian tumor antigen-specific helper T-lymphocyte and cytotoxic T-lymphocyte responses.

Keywords

ovarian cancer, dendritic cells, vaccination, immunotherapy, CA125

Curr Opin Obstet Gynecol 16:87–92. © 2004 Lippincott Williams & Wilkins.

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Current Opinion in Obstetrics and Gynecology 2004, 16:87–92

Abbreviations

CTL	cytotoxic T lymphocyte
KLH	keyhole limpet hemocyanin
MAb	monoclonal antibody
TADG	tumor-associated differentially expressed gene product
TRAG	taxol-resistance-associated gene
DTH	delayed-type hypersensitivity

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1040-872X

Introduction

The search for effective treatments for ovarian cancer has frustrated gynecologic oncologists for decades, and continues to do so today. Ovarian cancer is the fifth most common malignancy affecting women in the United States, and remains the cancer with the highest mortality rate among gynecological tumors. Although over 70% of patients respond well to surgery and first-line chemotherapy, the majority develop recurrent, chemotherapy-resistant disease. Overall, the 5-year survival rate for the International Federation of Gynecologists and Obstetricians stage III disease is 20–25%, and for stage IV disease it is a dismal 5%. The root of the problem can be attributed to the insidious onset of ovarian cancer, such that as many as two-thirds of patients present with advanced disease at the time of diagnosis. The prevailing situation raises two major issues which need to be addressed. The first is the clear need for techniques that would facilitate earlier diagnosis of ovarian cancer, and the second is the equally pressing need for novel treatments that prevent disease recurrence or progression following first-line treatment. In pursuit of the latter goal, tumor-specific immunological therapy has received increasing attention, and recent progress in this arena is the focus of this review. The identification of novel ovarian tumor antigens, and the recent cloning, sequencing and characterization of CA125, an antigen widely known as the target for the only current diagnostic test for ovarian cancer, has led to intensified research on the development of cellular immunotherapies that stimulate both T-cell and humoral immunity.

Antibody therapy and vaccination

Vaccination with carbohydrate antigens is primarily aimed at induction of antibody responses, and has been the subject of several clinical trials with ovarian cancer patients. Antigen-specific antibody responses have been recorded, but correlation of clinical outcome with immune responses has been elusive. In a variation on this theme, a recent update describes immune responses and clinical outcome from vaccination with a sialyl-Tn-

KLH (keyhole limpet hemocyanin) conjugate (Therapote[®], Biomira, Inc., Edmonton, Canada) following high-dose chemotherapy and autologous stem cell rescue in breast and ovarian cancer patients [1•]. Sialyl-Tn is a carbohydrate associated with MUC1 mucin, which is expressed by up to 90% of ovarian tumors, and has been a popular choice of antigen for several past clinical trials. Vaccination was well tolerated, and elicited humoral and cellular immune responses in a majority of patients. Based on current results, however, it is uncertain that the reported immunogenicity of the sialyl-TN–KLH conjugate vaccine will translate into clinical benefit. This concern is underlined by the recently announced results from a multicenter phase III randomized double-blind clinical trial of Therapote[®], which enrolled over 1000 women with metastatic breast cancer. A press release issued by Biomira, Inc. and Merck KgaA (the sponsors) on 16 June 2003 stated that this pivotal phase III trial did not meet the two predetermined statistical endpoints of time to disease progression and overall survival [2•].

One of the more intriguing recent reports described the outcome of immunotherapy with a murine monoclonal antibody (B43.13) specific for CA125 in a large cohort of ovarian cancer patients, the majority of which had stage III or stage IV disease at the time of diagnosis [3••]. All patients had clinical evidence of recurrent disease at the time of treatment. Forty-four patients were treated with Tc^{99m}-labeled B43.13, and antibody responses against the B43.13 variable region and CA125 itself were measured retrospectively. More than 60% of the patients met predefined criteria for treatment-induced human anti-murine antibody responses, and these responses were associated with improved survival rates. Of the 32 patients who could be evaluated for anti-CA125 antibodies, 28% showed a threefold increase over baseline. Over 56% of patients survived longer than 12 months after initiation of treatment and 34% survived longer than 24 months. Median survival time increased about threefold for human anti-murine antibody responders (22.6 months) versus nonresponders (7.2 months, $P < 0.0016$). The authors suggest that monoclonal antibody (MAb) B43.13 mediates its therapeutic action by forming immune complexes with CA125, thus enhancing processing and presentation to the immune system, promoting generation of both humoral and cellular immune responses. An alternative, but not necessarily exclusive, interpretation is that induction of anti-murine antibodies initiates a classical idio-type network of immune responses. In this scenario, a human antibody response to B43.13 would mimic the CA125 antigen, which would stimulate an anti-idiotypic response that would also have reactivity against CA125. The potential therapeutic benefit of such a sequence of events is illustrated by an earlier clinical trial in which ovarian cancer patients were vaccinated with the murine

monoclonal anti-idiotypic antibody ACA125, which mimics the CA125 antigen [4]. In this trial, anti-anti-idiotypic antibodies were detected in 28 of 42 patients. The survival of patients with an anti-anti-idiotypic response averaged 19.9 months, compared with only 5.3 months for those that failed to mount a response ($P < 0.0001$), suggesting that an antibody response against a CA125 mimic antigen (ACA125 antibody) had a significant impact on clinical outcome.

Another recent report describes treatment of a small cohort ($n = 5$) of ovarian cancer patients with a chimeric MAb (c-MOV18) that mediates antibody-dependent cellular cytotoxicity *in vitro* [5]. The chimeric antibody was constructed by fusion of the variable regions of murine MOV18 (specific for membrane folate receptor) with human IgG1 constant regions. Each patient received a total dose of 200 mg c-MOV18 over a period of 4 weeks. Treatment-related toxicity was mild, and no antibody responses against c-MOV18 were found. Three of the five patients remained stable for 4 months, 9 months and 14 months, respectively. Although this is a strategy that may have therapeutic potential, no conclusions can yet be drawn regarding immune responses or clinical benefit from c-MOV18 treatment.

Peptide vaccination

Clonal analysis of the T-cell response following vaccination of an ovarian cancer patient with peptides from HER2/neu that encompass both helper epitopes and HLA A2-restricted cytotoxic T lymphocyte (CTL) epitopes has revealed considerable T-cell diversity, including peptide-specific CD4⁺ helper T cells, CD8⁺ CTL that could lyse HLA A2-transfected, HER2/neu-expressing tumor cells, and T-cell receptor $\gamma\delta$ expressing clones that were also cytotoxic and HLA A2-restricted [6•]. A more extensive study of HER2/neu peptide vaccination in 64 patients with HER2/neu overexpressing, stage III or IV ovarian, breast, or nonsmall cell lung cancer found that peptide-specific T-cell immunity was consistently induced [7••]. In this phase I trial, patients received one of three peptide vaccine formulations, consisting of three peptides from the intracellular domain of HER2/neu, three peptides from the extracellular domain, or three peptides that each incorporated an HLA A2-binding motif (representing a CTL epitope), respectively. Vaccines (with granulocyte-macrophage colony-stimulating factor adjuvant) were administered intradermally once a month for 6 months. Of those patients who received all six vaccinations, 92% developed T-cell responses to at least one peptide in the vaccine mix. One of the more remarkable findings of this study is that a majority of the vaccinees showed evidence of epitope spreading, that is generation of an immune response to an epitope or portion of the HER2/neu protein that was not a component of the original

vaccination. Epitope spreading is thought to result from endogenous tumor antigen processing and presentation following events initiated by T cells specific for the vaccine antigen or peptide. For example, lysis of tumor cells by vaccine antigen-specific CTL may result in local inflammation and recruitment of antigen-presenting cells which process and present other tumor antigens or epitopes, stimulating a broader repertoire of T-cell specificities.

Epitope spreading may be a particularly significant component of immune responses to vaccines that rely solely on CTL peptide epitopes as the immunizing antigen, as it may offer a mechanism by which CTL peptide vaccination can be augmented by endogenous processing and presentation of tumor antigens for stimulation of CD4⁺ T-cell responses. This reasoning suggests that CTL peptide vaccines might induce broader and more durable immunity than one might expect from responses to the vaccine peptide(s) alone. Clinical experience with breast and ovarian cancer patients, however, has indicated that vaccination with peptides that incorporated both helper sequences and CD8⁺ CTL epitopes more effectively induced durable peptide-specific CD8⁺ CTL with lytic capacity against tumor cells [8]. Induction of antigen-specific helper CD4⁺ T-cell responses by this approach was confirmed by predominant CD4⁺ T-cell infiltrates in biopsies from positive delayed-type hypersensitivity (DTH) sites [7••]. In contrast, vaccination with a peptide representing a CD8⁺ CTL epitope alone induced immunity of limited duration, and with specificity only for the immunizing peptide [9••].

Dendritic cell vaccination

Thus far, exploration of the potential for dendritic cell immunotherapy of ovarian cancer has been relatively limited. Schlienger and colleagues [10•] have recently reported that dendritic cells pulsed with killed autologous primary ovarian tumor cells and matured with CD40 ligand and TRANCE (tumor necrosis factor-related activation-induced cytokine) were capable of inducing antigen-specific T cells that secreted IFN γ upon stimulation with autologous tumor cells [10•]. From these observations, the authors concluded that antigen-pulsed dendritic cells may be a viable option for therapeutic vaccination against ovarian cancer. This opinion is supported by prior studies showing that tumor lysate-pulsed dendritic cells, or dendritic cells pulsed with acid-eluted peptides from ovarian cancer cells, could induce HLA class I-restricted CTL responses against autologous ovarian tumor cells [11–13]. The potential therapeutic benefit of vaccination with autologous tumor antigen-pulsed dendritic cells is illustrated by the clinical results following vaccination of melanoma patients with tumor lysate-pulsed dendritic cells [14]. In

this earlier study, two of four patients with advanced, metastatic melanoma showed at least a partial clinical response, which correlated with positive DTH responses to skin tests with tumor lysate or defined melanoma CTL peptides. A limitation of this approach is that the identity of the tumor antigens recognized by dendritic cell-stimulated CTL is not well defined, and it is not clear that CTL responses are truly tumor-specific. The possible induction of unacceptable autoimmune side effects may impose significant limitations on the clinical utility of tumor-lysate pulsed dendritic cell vaccination as adjuvant therapy for ovarian cancer. A more recent clinical trial of MUC1 and HER2/neu peptide-pulsed dendritic cell vaccination in patients with advanced ovarian or breast cancer reported peptide-specific CTL responses in five of 10 patients [15]. Interestingly, this trial also provided evidence of epitope spreading. In one patient vaccinated with MUC1 peptides, carcinoembryonic antigen (CEA) and MAGE3 peptide-specific T-cell responses were detected, and in a second patient, MUC1-specific T-cell responses were detected after seven vaccinations with HER2/neu peptide-pulsed dendritic cells. Therapeutic vaccination with dendritic cells loaded with defined and specific ovarian tumor antigens or peptides may be a preferred option, both in terms of clinical benefit and limitation of autoimmune toxicity, particularly for long-term control of disease progression.

Emerging ovarian tumor antigens

There has been no shortage of novel tumor antigens reported for ovarian cancer over the past few years, yet relatively few stand out as strong candidates for immunotherapy. Some are highly expressed by a large proportion of ovarian tumors, but may also be expressed by normal tissues (e.g. folate-binding protein), whereas others may represent excellent targets in terms of immunogenicity and lack of expression by normal tissues, but are of limited utility by virtue of a low frequency of expression by ovarian tumors. The most obvious example of this category is HER2/neu, which is the target of a humanized MAb (Herceptin), an FDA-approved treatment that has won widespread plaudits. HER2/neu, however, is expressed at significant levels by only about 10% of ovarian cancers.

One of the more promising groups of novel ovarian tumor antigens identified in recent years encompasses the serine protease antigens [16•]. Serine proteases are thought to play key roles in cancer invasion and metastasis, and by virtue of their frequent overexpression in advanced disease represent logical targets for immunotherapy. The serine protease antigens include hepsin, stratum corneum chymotryptic enzyme, and the tumor-associated differentially expressed gene product-12 (TADG-12), TADG-14 (kallikrein-8, neuropsin),

TADG-15, and TADG-16 (testisin). Many of these antigens are broadly expressed across the major pathological types of ovarian cancer, but have very limited expression in normal ovarian tissue or other normal adult tissues.

Of potentially major clinical significance, a recent study has shown that paclitaxel and doxorubicin-resistant ovarian tumor cell lines overexpress multiple MAGE and GAGE tumor antigens [17••]. Earlier work had identified a taxol-resistance-associated gene (TRAG-3), but it appeared unlikely that TRAG-3 was itself responsible for the drug-resistant phenotype, as TRAG-3 transfection of tumor cell lines did not result in drug resistance. Evaluation of the SKOV-3 ovarian tumor cell line and other breast and ovarian tumor cell lines revealed MAGE and GAGE gene overexpression in the paclitaxel and doxorubicin-resistant subclones and cell lines, whereas gemcitabine-resistant subclones showed no change in MAGE or GAGE gene expression. Furthermore, analysis of MAGE2 and MAGE6 transfectants of the paclitaxel-sensitive OVCAR8 cell line demonstrated a fourfold increase in resistance to paclitaxel and a twofold increase in resistance to doxorubicin, but not to other drugs, notably topotecan and cisplatin. MAGE2 and MAGE6 overexpression also endowed transfected OVCAR8 cells with a growth advantage. Collectively, these observations suggest that MAGE and GAGE antigens may be excellent targets for adjuvant immunotherapy of drug-resistant advanced ovarian cancer. However, no firm conclusions can be drawn until comparative analysis of MAGE and GAGE antigen expression by primary tumor samples from patients with paclitaxel/doxorubicin-sensitive and resistant disease has been completed.

SEREX analysis may also prove valuable for the identification of immunogenic antigens associated with ovarian cancer. Screening of sera from 25 late-stage ovarian cancer patients against independent cDNA libraries identified a set of nine antigens recognized by antibodies produced by the ovarian cancer patients, but not recognized by sera from a large panel of normal female donors [18•]. These antigens included p53, NY-ESO-1, UBQLN1, HOXB6, TOP2A, putative helicase RUVBL, HEXIM1, DDX5 and HDCMA. Forty per cent of serous ovarian cancer patients tested had serum IgG to at least one of these antigens, and 14% had antibodies to two or more of these antigens. Importantly, real-time polymerase chain reaction analysis showed that three of the SEREX-defined antigens, TOP2A, HOXB6 and DDB5, were overexpressed in ovarian tumors relative to normal tissues, suggesting that these antigens may be potential targets for immunotherapy. The key advantage to SEREX analysis is the potential for identification of tumor-associated antigens that are

immunogenic. A possible limitation, though, is the fact that these patients have advanced disease, suggesting that the immune response to these antigens, at least at the antibody level, is ineffective in controlling disease progression. In this context, it may be illuminating to determine whether ovarian cancer patients show any evidence of T-cell responses against tumor antigens identified by SEREX analysis.

CA125: target antigen or tumor defense mechanism?

Although CA125 has long been recognized as the gold standard for monitoring patients with ovarian cancer, and has been used as a target antigen for antibody-based therapy or vaccination, it has not been considered as a target for cellular immunotherapy. The major reason has been the lack of information on the primary sequence of CA125, and the nonavailability of CA125 in recombinant form. The elucidation of the sequence and structure of CA125 thus represents a major advance in the field [19,20,21••]. The CA125 protein consists of a short cytoplasmic tail, a transmembrane domain, an exceptionally large extracellular structure composed of multiple repeats of a 156 amino acid domain, and an N-terminal domain in excess of 11 000 amino acids [21••]. The core protein may have a mass that exceeds 2 million Da. CA125 is also extensively glycosylated, and the total mass may be as high as 3.5 million Da [21••]. Knowledge of the primary sequence and structure of CA125 may allow identification of epitopes recognized by CD8⁺ CTL and helper CD4⁺ T cells, thus paving the way for cell-based therapies, including dendritic cell immunotherapy and vaccination.

In contrast with the renewed interest in CA125 as a therapeutic target, new insights into the glycobiology of CA125 suggest a potential role in modulation of tumor-specific immune responses. Comprehensive characterization of CA125 oligosaccharides has shown the presence of both core type 1 and type 2 O-glycans, a feature of which is the presence of branched core 1 antennae in the core type 2 glycans [22••]. In addition, CA125 also possesses high mannose N-glycans and biantennary, triantennary and tetraantennary bisecting N-glycans [22••]. Bisecting N-glycans have been implicated in modulation of natural killer cell cytotoxicity. Insertion of glycoporphin into the membranes of K562 cells reduces their sensitivity to natural killer cell lysis [23]. The presence of N-linked glycans on glycoporphin was essential for induction of resistance to natural killer cells [23], raising the possibility that bisecting N-glycans present on CA125 may modulate natural killer cell function. Another interesting facet of CA125 glycobiology is that high mannose N-glycans and Lewis^x-terminated glycans are bound by the C-type lectin DC-SIGN expressed by dendritic cells [24,25•]. DC-

SIGN plays important roles in dendritic cell trafficking and T-cell adhesion, through binding to intercellular adhesion molecule type 2 and 3, respectively [26•]. This has led to the suggestion that DC-SIGN binding of high mannose N-glycans present on CA125 may inhibit dendritic cell function [22••], a proposal that is supported by the finding that mycobacterial lipoarabinomannan (which has a mannose-rich core) binds to DC-SIGN and prevents dendritic cell maturation [27••].

In the context of immune modulation, the recent observation that both soluble and membrane-associated forms of CA125 are ligands for galectin-1 may also be significant [28••]. Galectin-1 binding is dependent on β -galactose-terminated O-glycans on CA125, and available evidence suggests that CA125 may act as a chaperone for secretory transport of galectin-1, thus regulating its cell surface expression [28••]. This is important because galectin-1 binds to CD7, CD43 and CD45 on activated T cells, inhibits IL-2 production and induces T-cell apoptosis [29–31]. Collectively, these observations raise the possibility that CA125-dependent tumor cell surface expression of galectin-1 may play an important role in defense against ovarian tumor-specific T cells.

Conclusion

As is necessarily the case in a still nascent field, the more striking results have come from the laboratory rather than from clinical investigation. The most notable recent reports have focused on the sequencing and characterization of CA125, which has opened up new areas of research, both in terms of its utility as a target for ovarian cancer immunotherapy, and also with respect to its biological function, which may itself have a significant impact on immunological intervention. The identification and cloning of other novel ovarian tumor antigens is also important. Thus far, most clinical studies of immunotherapy have targeted only one antigen, which may show varying immunogenicity in different individuals, and may also be expressed at markedly different levels by different pathological types of ovarian cancer. The ability to target multiple antigens, or to select specific tumor antigens according to the molecular profile of a surgical or biopsy sample from an individual patient's tumor, may improve the immunogenicity of therapeutic vaccines and may enhance the prospects for correlation of immune response with clinical benefit. The possibility that certain tumor antigens could be selectively overexpressed by chemotherapy-resistant ovarian tumors, as suggested by the in-vitro studies of Duan and colleagues [17••], may also be an important consideration in the selection of target antigens for immunotherapy.

In the clinical arena, the most intriguing recently reported results come from a clinical trial of immu-

notherapy with the CA125-specific mouse MAb B43.13 in patients with late stage ovarian cancer and clinical evidence of recurrent disease, in which human anti-mouse antibody responses correlated with prolonged survival [3••]. The immunological mechanisms underlying the clinical response to treatment with B43.13 are not well understood, but the clinical results should strongly encourage further studies.

Although there is relatively little to report from current studies, one of the most promising areas for future clinical research lies at the interface of peptide/protein vaccination and dendritic cell immunotherapy. Writing in a recent issue of *Current Opinion in Immunology* on the subject of dendritic cell vaccination for cancer, Schuler and colleagues [32••, pp. 143] concluded that 'In the long run, *ex vivo* DC vaccination may be the method of choice for treating tumor-bearing patients...'. The recent advances in the sophistication of dendritic cell vaccine technology, coupled with the identification of a series of highly promising novel ovarian tumor antigens, lead us to support this conclusion.

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