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Combinatorial strategies for alleviation of tumor-associated immune suppression and therapeutic vaccination against ovarian cancer

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The prospects for development of therapeutic cancer vaccines have enjoyed a renaissance in recent years, with further encouragement accruing from the US FDA's approval in 2010 of Provenge® (Dendreon Corporation, Seattle, WA, USA), an autologous dendritic cell (DC) vaccine for prostate cancer. Provenge was the first active cellular therapy to reach the market, breaking through what was widely perceived to be a glass ceiling for cellular therapeutics and cancer vaccines. This renewed optimism has also been fuelled by advances in our knowledge of the mechanisms of immune regulation in the tumor microenvironment. This is particularly true for ovarian cancer, which represents a paradigm for self-defense against immunological attack. Although ovarian cancer recruits multiple mechanisms of immune suppression, thus presenting a formidable barrier to effective tumor vaccination, an understanding of these mechanisms may yield opportunities for adjuvant treatments that counterbalance immune suppression and promote vaccine-induced antitumor immunity.

The various mechanisms of immune suppression employed by ovarian cancer are of more than academic interest to tumor immunologists, since many parameters of immune function have a direct impact on the course of disease. A groundbreaking study from Curiel and colleagues showed that ovarian tumors recruit immuno suppressive Tregs, thus conferring immune privilege, and that Treg infiltration was associated with poor prognosis and

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increased mortality [1]. These observations demonstrated that immune regulation has a direct and profound relationship with the pathogenesis of disease. Other parameters of immune regulation in ovarian cancer also influence patient outcomes, including expression of B7-H1 (PD-L1) by ovarian tumor cells and infiltrating macrophages or DCs, resulting in T-cell anergy and apoptosis through the engagement of PD-1 expressed by effector T cells [2,3]. Expression of B7-H1 has been associated with differentiation and recruitment of Treg [4], and clinical studies have shown that B7-H1 expression correlates with increased morbidity and mortality in ovarian cancer patients [5]. Expression of B7-H4 (a related member of the family of immunomodulatory accessory receptors) by ovarian tumor-associated macrophages has also been correlated with Treg infiltration and adverse patient outcomes [6]. Two independent studies have further shown that ovarian tumor expression of indoleamine 2,3-dioxygenase (IDO) is associated with poor prognosis [7,8]. IDO first came to prominence for its role in immune suppression and fetal protection at the maternal–fetal interface, and is now widely acknowledged to be an important mediator of T-cell anergy through tryptophan catabolism. IDO is known to be expressed by ovarian tumor cells, and most probably by infiltrating myeloid-derived suppressor cells, including DC and macrophages. Of particular note, IDO expression drives the differentiation and recruitment of Treg by DCs [9], and blocks the conversion of Treg to p roinflammatory Th17 cells [10,11].

In contrast to the evidence that Treg infiltration is associated with poor outcomes in ovarian cancer, a counterpoint is furnished by the recent observation that Th17 cell infiltration correlates with more favorable clinical outcomes [12]. Tumor-infiltrating Th17 cells were positively associated with effector cells and negatively associated with Treg infiltration, leading to the question of whether Th17 cells could be induced or expanded to therapeutic advantage, either by tumor vaccines or adoptive immunotherapy [13]. There is a substantial body of evidence suggesting that Th17 responses can be induced *in vitro* with proinflammatory cytokines, notably IL-1β. Such an approach may be viable for adoptive T-cell immunotherapy of ovarian cancer, but the adjuvant use of cytokines in support of tumor vaccination is likely to carry a significant risk of off-target toxicities. Of the alternatives, DC vaccination designed to drive ovarian tumor antigen-specific Th17 responses appears to be the strongest option [14]. In comparison with other approaches to tumor vaccination, the plasticity of DC function allows greater scope for fine-tuning T-cell responses, and currently available evidence suggests that modulation of MAPK signaling in DCs may provide the cornerstone for stimulation of Th17 immunity [14].

If we accept the premise that tumor antigen-specific Th17 responses will be of therapeutic benefit for ovarian cancer patients, either through DC vaccination or alternative approaches to tumor vaccination, we still have to confront the myriad mechanisms of immune suppression in the ovarian tumor microenvironment, which remain the greatest barrier to effective antitumor immunity. The history of tumor vaccination has shown that stimulation of tumor antigen-specific T-cell responses is relatively facile, yet translation to clinical efficacy has remained stubbornly elusive. The judicious use of adjuvants designed to alleviate tumor-associated immunosuppression may furnish the key to successful therapeutic vaccination. Several chemotherapeutic agents have shown evidence of activity against Treg, including fludarabine, imatinib mesylate (Gleevec®, Novartis, Basel, Switzerland) and, most notably, cyclophosphamide, which has long been known for its ability to inhibit Treg suppression and potentiate cellular immune responses against tumors [15]. Cyclophosphamide is gaining popularity as an adjuvant in clinical trials of tumor vaccines, and has the advantages of being cheap, well tolerated and easy to administer orally. In a remarkable twist to the story, a recent report has shown that cyclophosphamide may also promote Th17 cell differentiation in cancer patients [16], further adding to its appeal as an adjuvant for clinical trials of ovarian tumor vaccines.

We should also consider the root causes of Treg recruitment in the ovarian tumor microenvironment. Treg infiltration is driven by expression of CCL22 [1], of which the most prominent sources are likely to be tumor-associated macrophages and DCs. Treg differentiation and expansion may be promoted by multiple mechanisms, including local expression B7-H1 and B7-4, both of which correlate with Treg infiltration in ovarian tumors, and expression of IDO, which may also antagonize Th17 differentiation. A notable feature is that all of these mechanisms are independent prognostic indicators of poor outcomes, and all may be mediated by tumor-associated macrophages or DCs. Accordingly, adjuvant treatments that modify the function of tumor-infiltrating myeloid suppressor cells may have a profound impact on Treg recruitment, local immunity and clinical responses to vaccination. There is some evidence from animal studies that gemcitabine, used as a single agent [17] or in combination with a cyclooxygenase inhibitor [18] has activity against myeloid suppressor cells, preferentially depleting this population in tumor-bearing mice. However, absolute depletion of myeloid cells may not be the most favorable course of action, since tumor-associated macrophages and, to a lesser extent, myeloid DCs also have the ability to stimulate Th17 responses [12]. An alternative course of action may reside with the use of agents that modulate myeloid suppressor cell activity, a function that could be fulfilled by adjuvant chemotherapy with aminobisphosphonates. This class of drugs, which includes zoledronate and pamidronate, inhibits metastatic spread to the bone, and can also impair angiogenesis by blocking VEGF signaling and MMP-9 expression by tumor-associated macrophages. Zoledronate can block myeloid-derived suppressor cell expansion and proangiogenic activity [19], and recent evidence points to a proinflammatory shift in myeloid cell function, favoring polarization from a protumor M2 phenotype to an antitumor M1 phenotype with reduced expression of VEGF and IL-10 and increased production of type I cytokines such as IFN-γ [20,21].

Although the available clinical evidence provides a strong rationale for the design of tumor vaccines that promote Th17 immunity in ovarian cancer patients [12,13], this is likely to be a contentious issue, since the various studies that show therapeutic benefit from Th17 immunity are counterbalanced by reports that indicate a role for IL-17 in tumor progression [14]. We believe that Th17-based therapeutic vaccines for ovarian cancer should be tested in clinical trials, but there remains the question of whether vaccination alone would be sufficient to provide clinical benefit in the face of continuing local immune suppression in the tumor microenvironment. The long history of clinical disappointment from tumor vaccination has clearly shown that stimulation of antitumor immunity is not in itself sufficient, and that there is a clear need for adjuvant treatments that create a more favorable environment for vaccine efficacy. Cyclophosphamide has obvious appeal, since it may help to support vaccine-driven Th17 responses in addition to abrogating Treg activity. We also feel that there is a strong case for targeting tumor-associated macrophages/myeloid suppressor cells, since these populations are likely the drivers that fuel Treg infiltration, differentiation and expansion in ovarian tumors.

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Biographies

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