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Dendritic cell vaccination against ovarian cancer – tipping the Treg/Th17 balance to therapeutic advantage?

Martin J Cannon, PhD[1,2], **Hannah Goyne, MD**[1], **Pamela JB Stone, MD**[2], and **Maurizio Chiriva-Internati, PhD**[3]

[1]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

[2]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA

[3]Department of Internal Medicine, Division of Hematology and Oncology, Texas Tech University Health Sciences Center, Lubbock, Texas, USA

Abstract

The pathology of ovarian cancer is characterized by profound immunosuppression in the tumor microenvironment. Mechanisms that contribute to the immunosuppressed state include tumor infiltration by regulatory T cells (Treg), expression of B7-H1 (PDL-1), which can promote T cell anergy and apoptosis through engagement of PD-1 expressed by effector T cells, and expression of indoleamine 2,3-dioxygenase (IDO), which can also contribute to effector T cell anergy. Expression of both B7-H1 and IDO has been associated with differentiation and recruitment of Treg, and clinical studies have shown that each of these mechanisms correlates independently with increased morbidity and mortality in patients with ovarian cancer. In a remarkable counterpoint to these observations, ovarian tumor infiltration with Th17 cells correlates with markedly improved clinical outcomes. In this Future Perspectives review, we argue that dendritic cell (DC) vaccination designed to drive tumor antigen-specific Th17 T cell responses, combined with adjuvant treatments that abrogate immunosuppressive mechanisms operative in the tumor microenvironment, offers the potential for clinical benefit in the treatment of ovarian cancer. We also discuss pharmacological approaches to modulation of MAP kinase signaling for manipulation of the functional plasticity of DC, such that they may be directed to promote Th17 responses following DC vaccination.

Keywords

Th17 T cells; Regulatory T cells; ovarian cancer; dendritic cells; p38 MAPK

1. Introduction

In recent years, it has become increasingly apparent that ovarian tumors avail themselves of multiple mechanisms of immune evasion, the most prominent of which is recruitment and

Corresponding author: Dr. Martin J. Cannon, Tel 501-296-1254, Fax 501-686-5359, mjcannon@uams.edu.

Declaration of interest

The other authors declare no conflict of interest.

MJC is founder of DCV Technologies Inc, a biotechnology company dedicated to the clinical developement of dendritic cell vaccines for the treatment of cancer.

M Chiriva-Internati is founder of Kiromic Inc, a biotechnology company that seeks to develop therapeutic cancer vaccines.

infiltration of regulatory T cells that suppress anti-tumor immunity. Landmark studies from Curiel and colleagues showed that regulatory T cells (Treg) are recruited to ovarian tumors by the chemokine CCL22 (predominantly expressed by ovarian tumors), and that the presence of Treg confers immune privilege and is associated with a poor prognosis and increased mortality [1]. Other investigators have corroborated these observations, showing that high expression of the forkhead box transcription factor foxp3, which is preferentially expressed by $CD4^+$ Treg, is an independent prognostic factor for reduced overall survival in ovarian cancer [2], and that a high $CD8^+$ T cell/Treg ratio is associated with a more favorable prognosis for this disease [3]. These observations support the idea that depletion of tumor-associated Treg, or inhibition of Treg function, may be beneficial, particularly in conjunction with active tumor-specific immunotherapy.

In contrast with the strong evidence that Treg infiltration is associated with poor outcomes in ovarian cancer (and other malignancies), the recent observation that Th17 T cell infiltration in ovarian cancer correlates with markedly more favorable clinical outcomes provides a striking counterpoint [4]. Tumor-infiltrating Th17 cells were positively associated with effector cells and negatively associated with Treg infiltration, with the latter relationship arguably being founded on the known reciprocal regulation of Treg and Th17 differentiation [5,6]. Tumor-associated macrophages were shown to be efficient inducers of T cell IL-17 production, through an IL-1β-dependent mechanism [4], an observation that is consistent with evidence pointing to a critical role for IL-1β in the induction of human Th17 responses [7–9]. Furthermore, Kryczek and colleagues found a positive correlation between ascites IL-17 and the Th1-associated chemokines CXCL9 and CXCL10, and provided evidence that Th17 T cell production of IL-17 and IFNγ induced expression of CXCL10. In turn, the levels of CXCL9 and CXCL10 in tumor ascites positively correlated with tumor-infiltrating $CD8^+$ T cells [4].

2. The pros and cons of Th17-based immunotherapy

These observations have inevitably led to the question of whether Th17 cells could be therapeutically induced or expanded, either by tumor vaccines or adoptive immunotherapy [10]. Although the current evidence in ovarian cancer appears to present a strong case in favor of Th17-based anti-tumor immunotherapy, this is a controversial issue, since a number of studies have pointed to a role for IL-17 in promoting tumor growth and invasion [11–16]. On the other hand, several recent reports have supported the view that Th17 responses may have therapeutic benefit in promoting anti-tumor immunity and survival. In the B16 mouse model of melanoma, adoptive T cell therapy with tumor-specific Th17 cells prompted strong activation of tumor-specific $CD8^+$ T cells (which were required for the antitumor effect), thus indicating that Th17-driven inflammation can play a pivotal role in antitumor immunity [17]. Induction of Th17 responses in a mouse model of pancreatic cancer has also been shown to delay tumor growth and improve survival [18]. In similar vein, tumor growth and pulmonary metastasis was enhanced following injection of the MC38 colon cancer cell line in IL-17-deficient mice [19], again suggesting a protective role for IL-17-expressing T cells. Most notably, the pretreatment frequency of $CD4^+$ Th17 cells in prostate cancer patients was found to correlate with the clinical response to a whole cell vaccine [20], suggesting that the association of Th17 cells with improved survival may not be unique to ovarian cancer.

Furthermore, and in marked contrast with the prevailing opinion that $CD4^+$ Th1 T cell responses and $CD8^+$ CTL responses represent an optimal line of attack for antitumor immunotherapy, recent evidence has suggested that Th17-based cellular immunotherapy may offer the potential for greater therapeutic efficacy. Groundbreaking studies from the National Cancer Institute have clearly shown that adoptively transferred $CD4^+$ Th17 cells were markedly more effective than $CD4^+$ Th1 cells in eradication of advanced B16

melanoma in a mouse model [21]. These investigators further showed that, compared with Th1 cells, Th17 cells enjoy a survival advantage in vivo, suggesting that their improved persistence may be a key reason for their greater ability to control disease.

3. Can dendritic cells be educated to drive Th17 responses against ovarian cancer?

This section is based on the premise that active immunotherapy, and particularly dendritic cell (DC) vaccination, designed to drive a tumor antigen-specific Th17 T cell response holds the potential to be of clinical benefit for patients with ovarian cancer. Various studies have shown that Th17 T cell differentiation *in vitro* can readily be driven by cytokines, notably IL-1β (see above), suggesting that tumor antigen-specific Th17-based adoptive T cell immunotherapy may be a viable approach for treatment of ovarian cancer. However, such procedures are cumbersome and complex, and are not readily translated to clinical practice. A more practical and efficient alternative may be found with DC vaccination. DC are remarkable for their plasticity in directing T cell differentiation and effector function, and thus the key to success may reside in our ability to educate DC to drive ovarian tumor antigen-specific Th17 responses. How could this be achieved? Several recent studies have indicated that regulation of the p38 and ERK MAPK signal transduction pathways in DC plays a central role in direction of T cell differentiation. Inhibition of MEK 1/2 and ERK MAPK signaling promotes IL-12 production and Th1 T cell responses, whereas inhibition of p38 MAPK increases signal transduction through ERK 1/2 and blocks IL-12 production [22]. At face value, these observations suggest that inhibition of p38 MAPK signaling would be disadvantageous for DC-driven anti-tumor T cell responses, since this would abrogate Th1 responses, which are widely held to be important for effective anti-tumor immunity. However, p38 inhibition promotes differentiation and survival of monocyte-derived DC [23], and p38 inhibition or MEK/ERK MAPK activation restores deficiencies in DC function in myeloma patients [24], suggesting that treatment of DC with pharmacological inhibitors of p38 signaling may confer some benefit. Furthermore, p38 MAPK signaling in DC is associated with increased expression of IL-10 and the induction of tolerance in a mouse model of melanoma, thus contributing to the suppression of anti-tumor T cell responses [25]. Inhibition of p38 signaling in DC from tumor-bearing mice markedly suppressed expression of IL-10 and restored the capacity of DC to stimulate T cells.

Of particular significance, blockade of the p38 pathway can attenuate regulatory T cell induction by DC [26], whereas blockade of the ERK pathway suppresses DC-driven Th17 responses [27], suggesting that p38 blockade (which enhances ERK phosphorylation) may favor a switch from Treg induction to Th17 differentiation and expansion. These observations could have major implications for the rational design of DC vaccines against ovarian cancer.

4. Expert opinion

The proposal that tumor antigen-specific $CD4^+$ Th17 immune responses may benefit cancer patients is a challenging position to adopt. Based on experimental evidence, there is little doubt that Th17 responses can drive tumor progression, invasion, and angiogenesis. On the other hand, it is equally evident from experimental models and clinical studies that Th17 responses can support robust anti-tumor immunity and favor patient survival. How can these apparently opposing observations be reconciled? First, it is probable that Th17 responses are not homogeneous, and that differing effector functions under that broad umbrella are likely to have different outcomes. The ultimate challenge for tumor immunologists will be to dissect the nuances of Th17 function, and to determine how to drive a response that favors anti-tumor immunity rather than disease progression [16].

In the case of ovarian cancer, clinical evidence presents a strong rationale for basing active immunotherapy on strategies that drive a Th17 response [4]. We propose that manipulation of DC function to drive ovarian tumor antigen-specific Th17 responses may afford the best opportunity for immunological treatment of ovarian cancer through DC vaccination, and we have discussed experimental evidence that inhibition of the p38 MAPK signaling pathway in DC may be an appropriate line of investigation to achieve this goal.

Assuming that such a strategy is viable, there remain numerous barriers to successful DC vaccination for ovarian cancer. Immunosuppressive mechanisms operative in the ovarian tumor microenvironment include infiltrating Treg (discussed above), and expression of B7-H1 (PDL-1) by tumor cells cells and infiltrating macrophages, resulting in apoptosis and anergy [28,29]. Of particular clinical interest, a retrospective analysis of human ovarian cancers revealed that patients with higher B7-H1 expression had a significantly poorer prognosis than those for whom the tumors had lower B7-H1 expression [30]. Expression of indoleamine 2,3-dioxygenase (IDO), which can contribute to recruitment of Tregs [31,32], has also been associated with poor clinical outcomes in ovarian cancer [33,34]. Tumor expression of endothelin-1, which can inhibit effector T cell migration across vascular endothelium into the tumor microenvironment, may also reduce the efficacy of immunotherapy or vaccination [35]. The optimal strategy for DC vaccination may thus combine adjuvant treatments designed to abrogate immunosuppression in the tumor microenvironment. B7-H1 may be blocked with specific antibodies, and IDO function can be blocked with 1-methyl-tryptophan, a competitive inhibitor of enzyme function that is currently being tested in clinical trials. Small molecule antagonists of endothelin receptors are also undergoing clinical tests [36]. Last, but not least, various strategies can be applied to abrogation of tumor-associated Treg activity, notably treatment with denileukin diftitox (ONTAK) or low-dose cyclophosphamide [37]. Paclitaxel, which is commonly used for treatment of ovarian cancer, may also have activity against Treg [38]. Given the current weight of evidence, we would advocate further studies on the potential for treatment of ovarian cancer with DC vaccination formulated to drive Th17 responses, in combination with adjuvant treatments designed to blockade immunosuppressive mechanisms that prevail in the ovarian tumor microenvironment.

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