Scientific Open Access SOAJ Journals

Journal of Gynecology & Obstetrics

Research Article

Open Access

Expression and Significance of Interleukin-17D and CD163 in Epithelial Ovarian Cancer

Robert Saddawi-Konefka[1], Xi Kang[2], Yunying Li[2], Hui Zhang[2] and Jack D. Bui[2]*

[1]Department of Pathology, University of California, San Diego, USA

[2]Fourth Hospital of HeBei Medical University, Shijiazhuang, China

***Corresponding author: Jack Bui,** Email: jbui@ucsd.edu

Received: 05 Dec 2017; **Accepted:** 25 Aug 2018; **Published:** 31 Aug 2018

Abstract

Background and aims: Interleukin-17D (IL-17D) is a novel cytokine belonging to the IL-17 family of cytokines that mediate the recruitment of inflammatory cells during infection. Just recently, IL-17D has been shown to play a key role in mediating anti-tumor immune responses in a murine model of sarcoma. The purpose of this study is to investigate the role of IL-17D and macrophages in the progression of human ovarian cancer.

Methods: In this study, we analyzed 50 cases of malignant epithelial ovarian tumors, 10 cases of benign epithelial ovarian tumors, and 10 normal ovarian tissue samples for the expression of IL-17D and CD163. To examine the role of IL-17D in ovarian cancer progression, we overexpressed IL-17D in two human ovarian cancer cell lines and examined the growth kinetics and infiltrating immune cells in these tumors transplanted into immune deficient mice.

Results: We found robust staining for IL-17D and CD163 in the malignant ovarian cancer group and little staining in benign cancer and normal ovarian tissue. Similarly, we found that IL-17D expressing ovarian cancer cell lines progress more rapidly in vivo than do control transfected cell lines and that the profile of tumor infiltrating immune cell lines differs between IL-17D expressing and control cell lines; IL-17D expressing cells have fewer infiltrating (dendritic cells) DCs and a reduced M1/M2 macrophage ratio.

Conclusions: These results suggest that malignant transformation of ovarian tissue is accompanied by IL-17D expression, which influences tumor progression by directing immune cell infiltration into tumors.

Keywords: Interleukins; Macrophages; Ovarian cancer; Tumor immunology

Introduction

Unlike other solid tumors, ovarian cancer has benefited little from advancement in clinical treatment over the past few decades. In fact, the 1-, 3-, and 5-year survival rates for women diagnosed with ovarian cancer is no better today than in 1980 [1]. About 90% of ovarian cancer is epithelial ovarian cancer. Because of its occurrence site and growth pattern, the early diagnosis of epithelial ovarian cancer is difficult, and metastatic cancer at diagnosis is common, leading to a 5-year survival rate of less than 40%. Despite recent advances underpinning both genetic and environmental factors in contributing to ovarian cancer risk, the pathogenesis of ovarian cancer remains unclear.

Recent studies have shown that the immune system can promote or inhibit tumor formation such that cancers that arise in immune competent individuals have been "edited" to escape immune recognition and/or co-opt immune signals for survival [2,3]. Human ovarian cancer follows this stereotyped pattern of tumorigenesis and tumor immune escape [4]. Recent literature shows that in ovarian cancer tumor infiltrating $CD8^+$ T cells [5,6] and Th17 cells [7] are associated with better prognosis, whereas tumor-associated immune suppressive T-regulatory [8] cells harbor a poor prognosis. The immune response can inhibit ovarian tumor progression by targeting specific antigens expressed by the tumor cells, leading to the expansion of autologous tumor-specific cells [9,10]. In contrast, other components of the immune system can promote tumor formation [11]. Specifically, the cytokine tumor necrosis factor (TNF) is known to promote the growth of epithelial ovarian cancer cells in part via the actions of interleukin-17 (IL-17) [12]. Thus, targeting the tumor microenvironment was considered a major priority in the treatment of ovarian cancer as summarized in a review of a recent meeting of leading ovarian cancer basic researchers and clinicians [1,4].

The IL-17 family of cytokines consists of six members (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F) [13,14]. The best-studied members are IL-17A and IL-17F, which are produced by Th17 $CD4^+$ T cells and mediate immunity to tissue infection. Interestingly, IL-17A transcripts [15] and Th17 cells [16] can also be found in ovarian cancer, although the function of IL-17A in human immune responses to ovarian cancer cells is not clear. IL-17D is a novel member of the IL-17 family whose function is not known. It is expressed outside of the immune system and may promote inflammation by inducing inflammatory cytokines [17]. Using a mouse model, we found that IL-17D can recruit innate immune cells into tumors [18,19] via activation of tumor cell intrinsic responses to oxidative, cellular stress [20]. But, it remains unclear whether or not IL-17D is expressed in human cancers.

Macrophages comprise a significant portion (i.e., almost 50%) of certain tumors and can promote or inhibit tumor progression [21-24]. One paradigm to study the function of tumor associated macrophages (TAMs) classifies TAMs into M1-type, which resemble classically activated macrophages and inhibit tumor progression and M2-type, which are analogous to alternatively activated macrophages and promote tumor growth [21-25]. Macrophages can be found in ovarian cancer specimens, and a recent study indicated that ovarian TAMs inhibit antigen-specific antitumor immune responses [26].

In this study, we examined ovarian tumor samples by immunohistochemistry for the expression of the novel cytokine IL-17D and the macrophage marker CD163 [27,28] to show that ovarian cancer progression is associated with IL-17D expression and/or macrophage inflammatory processes. We found significant expression of both markers in malignant ovarian cancer specimens but not in benign cancer and normal ovarian tissue. To model ovarian cancer cells' IL-17D expression, we generated IL-17D expressing human ovarian cancer cell lines. Growth of IL-17D expressing tumor cells is accelerated in immune deficient mice and is associated with a distinct tumor infiltrating leukocyte profile.

Materials and Methods

Patients

Ovarian gynecologic surgical specimens were collected in the Fourth Affiliated Hospital of Hebei Medical University from 2010 June to 2011 June. Specimens were fixed in 10% formaldehyde for immunohistochemical studies. Among the 70 cases, 50 cases were confirmed epithelial ovarian cancer by pathologists, 10 cases were epithelial ovarian benign tumors (ovarian mucinous cystadenoma), and 10 cases were normal ovarian tissue from patients with uterine fibroids. Of the 50 cases, postoperative pathology showed that 12 were serous ovarian cancer, 4 were mucinous adenocarcinoma, 9 were endometrial carcinoma, 5 were clear cell carcinoma, and 20 were unclassified. The cases included 11 stage I, 3 stage II, 30 stage III, and 6 stage IV

Copyright *© 2018 The Authors. Published by Scientific Open Access Journals LLC.*

(according to the FIGO staging criteria). Twenty-one patients had ascites fluid ≥ 500 mL. All patient samples were collected within six months before surgery from patients who were not treated with radiation, chemotherapy or hormone therapy. Informed consent was obtained from each patient according to the institutional guidelines.

Immunohistochemical staining

Immunohistochemical staining of 10% formaldehyde-fixed, paraffin-embedded, 4 μm serial sections was conducted. Mouse anti-human IL-17D antibody was obtained from R&D Systems (MAB15041); Mouse anti-human CD163 was obtained from (Novocastra Laboratories, New Castle upon Tyne, UK); Stained slides were developed with DAB; and, subsequently, counterstained with hematoxylin and eosin. Staining was quantitated using the histogram function in adobe photoshop to provide a net average luminance for the signals with background subtracted.

Cell lines and mice

Two human ovarian cancer cell lines (HTB-161 and HTB-77) were obtained from ATCC. As described in the ATCC database, the cell lines were generated from extended passaged in vitro and do not have any contaminating human leukocytes. Cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine, nonessential amino acids, sodium pyruvate, sodium bicarbonate, penicillin/ streptomycin, and β-mercaptoethanol. C57BL/6 RAG2$^{-/-}$ x γc$^{-/-}$ (Taconic model #4111) and SCID mice (Charles Rivers strain 561) used were used for tumor transplantation experiments. The RAG2$^{-/-}$ x γc$^{-/-}$ mice lack T, B, natural killer (NK)-T, and NK cells but have an intact myeloid compartment. SCID mice which lack T, B, and NK-T cells but retain NK cells and a myeloid compartment. Therefore, the former mice lack all lymphocyte-mediated cancer immunosurveillance and thus display higher engraftment of human cells.

Virus transfection

Daughter ctrl and over-expressing IL-17D ovarian cancer cell lines were generated by either transducing parental human ovarian cancer cell lines with an empty vector lentivirus (ctrl) or a lentivirus-expressing IL-17D cDNA and selected on blastocydin supplemented media for 10 days.

Tumor transplantation

Tumor cell lines were harvested by trypsinization, washed 3 times with PBS, and injected at 6*10^6/mouse intraperitoneally into RAG2$^{-/-}$ x γc$^{-/-}$ strain mice or SCID mice. Mice were monitored for tumor growth by measurement of tumor size weekly. Mice were defined as tumor free when the palpable tumor size is smaller than 5mm*5mm.

Quantitative PCR (qPCR)

Total RNA was isolated with Trizol reagent (Ambion). Each sample was normalized to 1ug of total RNA, and reverse transcription was preformed by High Capacity cDNA Reverse Transcription Kit (Applied biosystems). qPCR reactions were performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories). Primer/ Probe Mix for mouse IL-17D was purchased from Applied Biosystems.

Intracellular staining FACS

Cells were incubated with 1ug/ml Brefeldin A (BD biosciences, San Jose, CA) and then harvested by trypsinization, washed with PBS, incubated with Cytofix (BD biosciences, San Jose, CA) for 15 mins at 4°C, washed weice with Perm wash (BD biosciences, San Diego) solution, and anti-IL17D (R&D Systems, Minneapolis, MN) or rat IgG2a isotype control (eBioscience, San Diego, CA) monoclonal antibodies were added. Staining was conducted for 30 mins at 4°C in FACS tubes containing 1ul of antibody and 50ml of FACS buffer. Cells were washed twice with Perm Wash and then stained by 2nd antibody (eBioscience, San Diego, CA) on APC channel. Cell were washed twice again with Perm wash and resuspended in FACS stain buffer. All analyses were done on live cells identified by forward and side scatter properties with a FACScanto II (BD Bidsciences, San Jose, CA).

Antibodies and FACS analysis

Analysis of tumor infiltrating immune cells was done as previously described [19]. On various days after the mice are no longer tumor free, tumors were excised from mice, minced, and treated with 1 mg/ml type IA collagenase (Sigma-Aldrich) as previously described (Weinberg et al., 1978). Cells were vigorously resuspended, washed in FACS buffer (PBS + 1% FCS+0.05%NaN3; Sigma-Aldrich) and filtered before staining. Antibodies to CD45, CD206, Ly6G, Ly6C, MHC class 2, F4/80 and CD11c were obtained from BD. Staining was conducted for 15-20 mins at 4°C in FACS tubes containing 0.2-0.5ul of each antibodies, 0.2ul of FC block and 100ml of FACS buffer. 7AAD (Calbiochem) was added at 1mg/ml immediately prior to FACS analysis. All analyses were done on live cell identified by forward and side scatter properties and 7AAD staining with a FACScanto II (BD Biosciences, San Jose, CA). Macrophages were defined as Ly6C-negative F4/80-positive cells. M1-like macrophages were MHC class II-high and CD206-low. M2-like macrophages were MHC class II-low and CD206-high. Neutrophils were Ly6G-positive, CD11c-negative, and MHC class II-negative granulocytic cells. DCs were F4/80-negative CD11c-positive cells. A sample of the gating is shown in Fig. S2.

Statistical Analysis

The χ^2 test, the Kruskal-Wallis H Test, the two-sided test, and the Spearman rank correlation analysis were performed using SPSS 13.0 software.

Results

Robust expression of IL-17D and CD163 in epithelial ovarian cancer but not in epithelial ovarian benign tumor and normal ovarian tissue

Figure 1 shows representative images of IL-17D and CD163 expression in normal tissue (Figures 1A, 1B, 1C), a benign epithelial tumor (Figures 1D, 1E, 1F), and a malignant epithelial tumor (Figures 1G, 1H, 1I). We found significant staining of both IL-17D and CD163 in malignant tissues compared to normal tissue ($p < 0.05$). There was no significant difference in the expression of IL-17D and CD163 between benign tumors and normal tissue, although there was a trend for benign tumors to display more IL-17D and CD163 staining than normal tissue. Notably, IL-17D and CD163 staining in some malignant cancers involved close to 80% of the tumor, suggesting diffuse and widespread infiltration of ovarian cancers by macrophages and cells expressing IL-17D. The IL-17D staining co-localized with macrophage-like cells but was also found in other cells types, suggesting that IL-17D could be produced by multiple cell types. The staining of IL-17D and CD163 was found at both the cell membrane and in the cytoplasm. Since the staining of both IL-17D and CD163 was >80% in some tumors, our data suggest that IL-17D is expressed in some but not all macrophages and some but not all tumor cells or stromal cells.

Expression of IL-17D and CD163 is increased with increased grade and decreased differentiation

Figure 2A shows that IL-17D and CD163 staining was increased in patients with ovarian cancer at stage III and IV compared to stage I and II ($p < 0.05$). On average there was a 1.5-fold increase in IL-17D and CD163 staining in advanced stage (III and IV) compared to early stage (I and II) ovarian cancer. Moreover, the positive expression rate was increased in poorly differentiated versus moderate-high differentiated epithelial ovarian cancer (Figure 2B, $p < 0.05$). Figure 2C shows that among the tumors that showed positive expression of IL-17D and

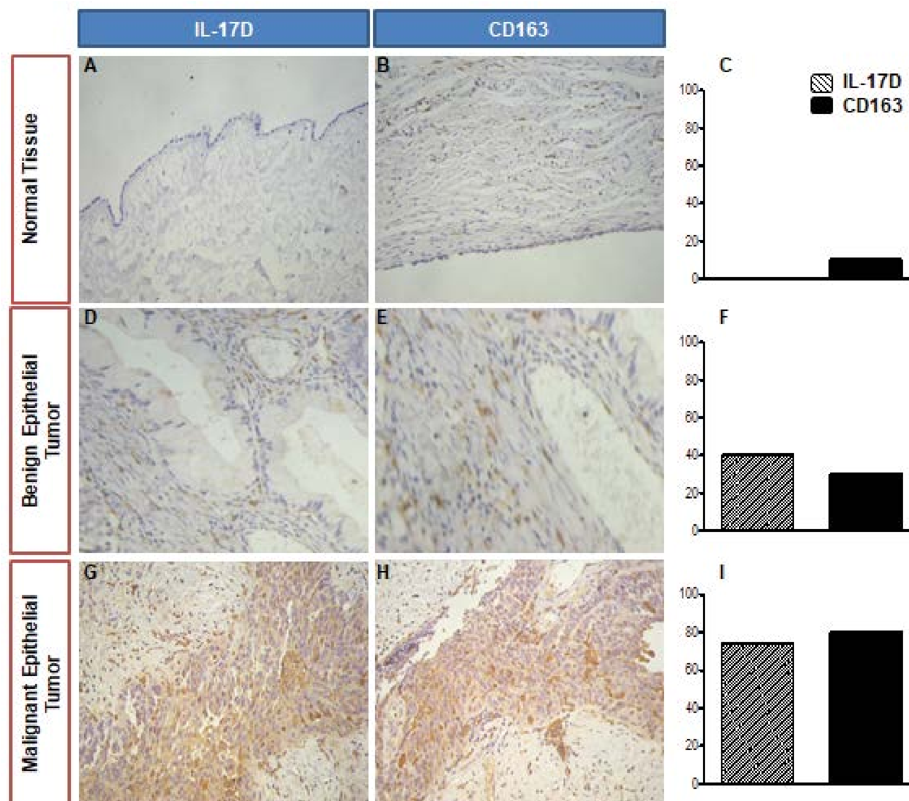


Figure 1: Macrophages and IL-17D are present in malignant but not healthy human ovarian tissues.
IL-17D and CD163 are highly expressed in epithelial ovarian tumors. Example images with quantification of (A-C) normal ovarian tissue, (D-F) benign ovarian epithelial tumor tissue, and (G-I) malignant ovarian epithelial tumor tissue; images at 200x, immunoexpression scored semi-quantitatively for number and intensity of cell staining.

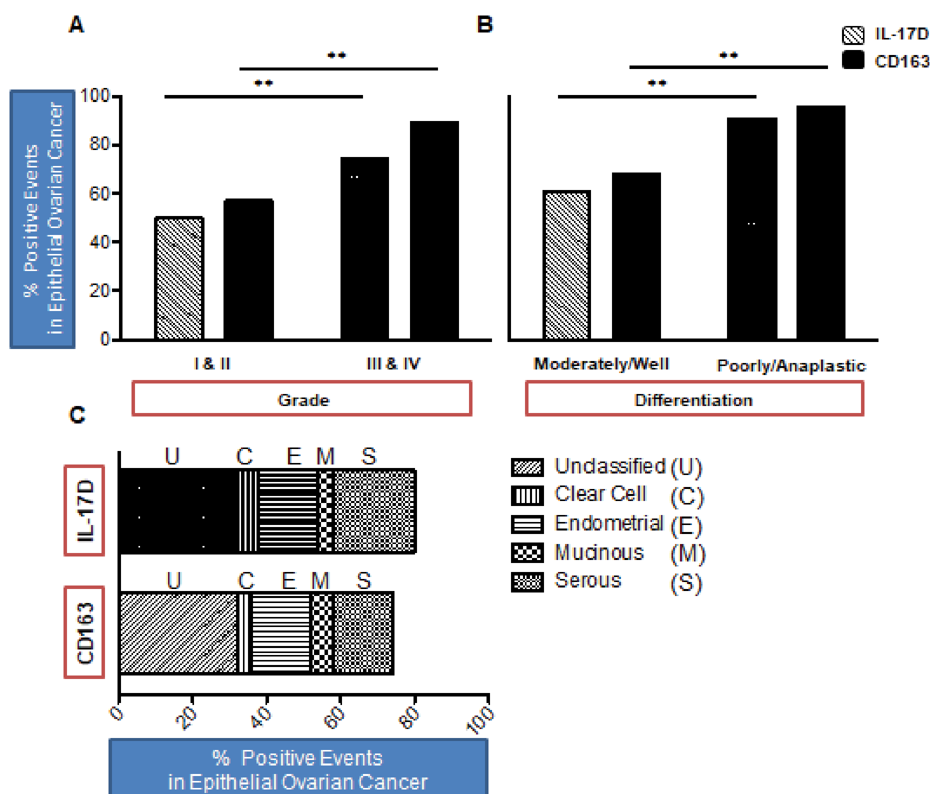


Figure 2: Macrophage accumulation and IL-17D expression directly correlate with malignancy.
Changes in the expression of IL-17D and CD163 directly correlate with the malignant transformation of ovarian tissue. Quantification of IL-17D and CD163 expression in epithelial ovarian tumor tissues categorized by (A) grade or (B) differentiation. (C) Expression of IL-17D and CD163 co-vary among the histology of epithelial ovarian tissues.

CD163, the more prevalent histologies were unclassified and serous, although this could be biased due to our sample demographics. The expression of IL-17D and CD163 was not correlated with the different age groups or the amount of ascites (Table 1). Overall, there was a positive correlation between the expression of IL-17D and CD163 protein as detected by immunohistochemical analysis (Table 2) ($r = 0.388$, $p < 0.05$).

IL-17D expression in ovarian tumor cell lines accelerated tumor development in immune deficient mice in vivo

To examine the role of IL-17D in influencing the growth of ovarian cancer cell lines, we generated a model system in which human ovarian cancer cell lines – HTB-77 and HTB-161 – overexpress IL-17D (Figure 3A), recognizing the caveat that in tumor biopsies, IL-17D could be expressed in macrophages and stromal cells as well. When

Table 1: The relationship between IL-17D and CD163 immunoexpression in tissues and patient clinical parameters.

		IL-17D			CD163		
		Positive	Negative	% (p value)	Positive	Negative	% (p value)
Patient age	<60 Yrs	20	10	66.70%	22	8	73.30%
	≥ 60 Yrs	17	3	85% (0.148)	18	2	90% (0.279)
Ascites volume	≤ 500ml	14	7	66.70%	16	5	76.20%
	> 500ml	23	6	79.3% (0.314)	24	5	82.8% (0.83)

Tumors positive and negative for IL-17D or CD163 were identified by standard pathologic analysis in which a minimum threshold of staining was assessed by a practicing pathologist blinded to the demographic information of the tumor sample. The positive and negative tumors for each antigen were then grouped according to age and ascites volume to determine if there was any correlation. No significant correlation was found.

Table 2: Correlation of IL-17D and CD163 protein in malignant epithelial ovarian tumor samples.

	IL-17D	CD163			
	Positive	Negative	n	R-value	P-value
Positive	33	7	40		
Negative	4	6	10		
n	37	13	50	0.388	0.005

Tumors positive and negative for IL-17D or CD163 were identified as in Table 1. The correlation between positive staining for IL-17D and CD163 was compared.

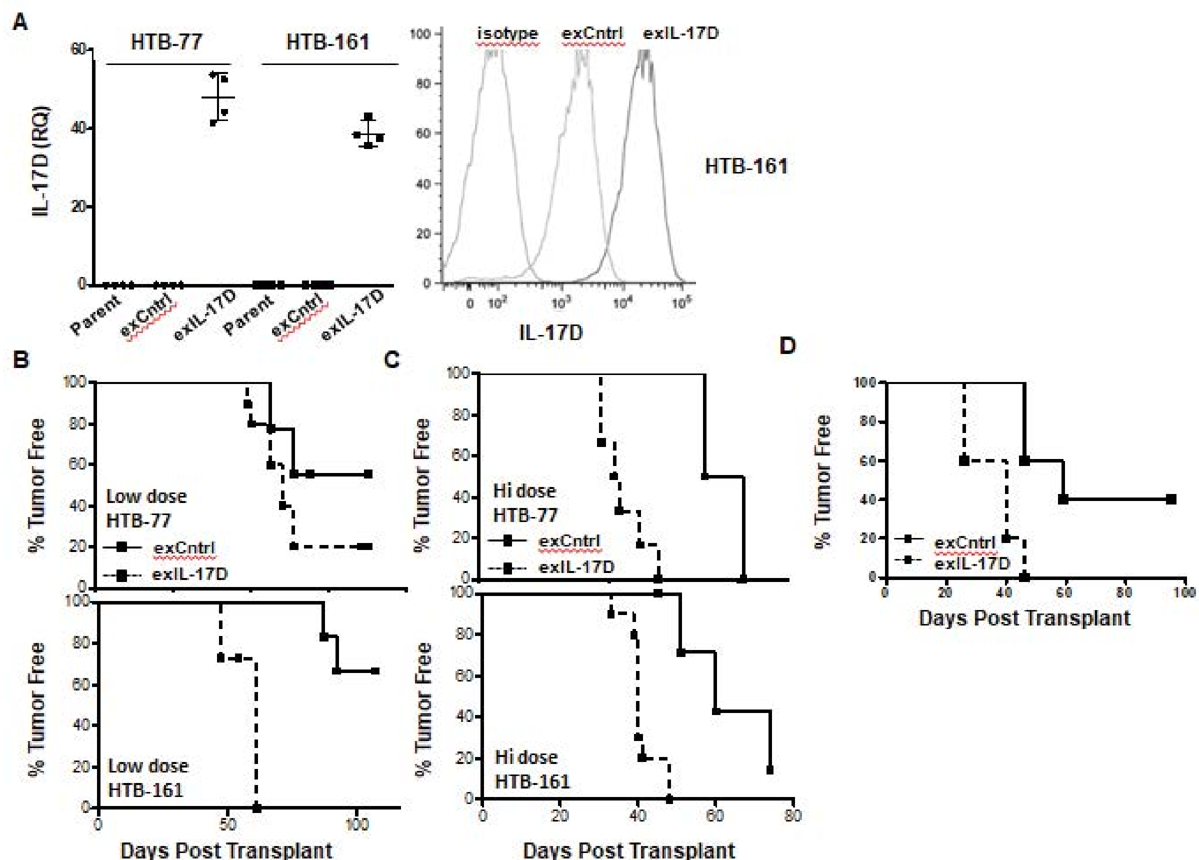


Figure 3: Expression of IL-17D in human ovarian cancer cell lines increases tumorigenesis in vivo.
(A) The human ovarian cancer cell lines HTB-77 and HTB-161 were transfected with either control or IL-17D expressing vectors and IL-17D transcript (left) and protein was examined (right). (B & C) Subsequently, $Rag^{-/-}$x $\gamma c^{-/-}$ or (D) SCID nude mice were transplanted with IL-17D or control-expressing human ovarian cancer cell lines [HTB-77: top panels ; HTB-161: bottom panels]. Cell lines were transplanted at both a high dose (B) and low dose (C).

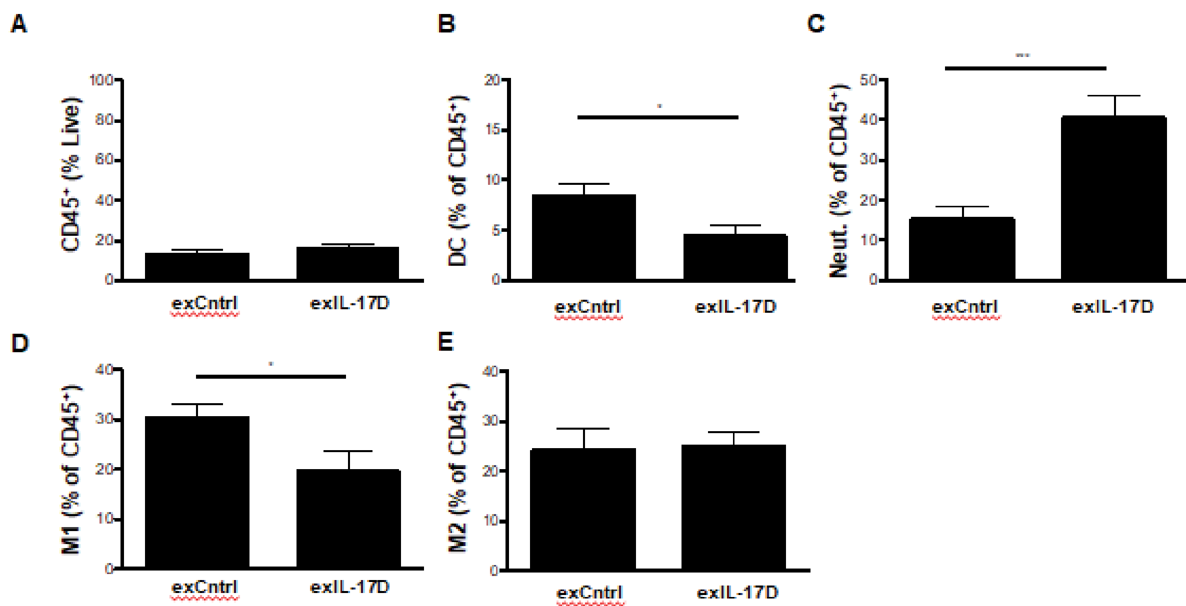


Figure 4: Innate immune cells differentially infiltrate IL-17D expressing tumor xenografts.
IL-17D or control-transduced ovarian cancer cell lines were transplanted immune deficient mice, and the indicated cell types were analyzed by flow cytometry. (A) No differences were observed in the infiltration of total CD45+ cells. (B & D) However, the total number of infiltrating DCs and M1 macrophages were significantly lower in IL-17D expressing cell lines; (C) and, the percent of infiltrating neutrophils was higher in IL-17D expressing cell lines. (E) M2 macrophages did not differentially infiltrate in control versus IL-17D expressing cells in vivo.

transplanted into immune deficient mice, IL-17D-overexpressing cell lines developed more quickly than control cell lines (Figure 3B-D). We observed tumors develop sooner in overexpressing cell lines when transplanted at both a lower (Figure 3B) and higher dose (Figure 3C) in $Rag2^{-/-}$ x $\gamma c^{-/-}$ mice and in scid mice (Figure 3D). We observed no difference between the kinetics of IL-17D expressing and control tumor cell lines grown in vitro (Supplemental Figure 1).

Intratumoral immune cell recruitment was different between IL-17D over expressing and control ovarian tumor tissue in RAG-γ mice

To determine whether IL-17D influences tumor development by directing tumor immunity, we performed a tumor infiltrating leukocyte analysis of control and IL-17D expressing ovarian cancer cell lines (Figure 4; gating strategy Supplemental Figure 2). Total immune infiltration was similar for control and IL-17D expressing cells (Figure 4A), but the percent of infiltrating DCs and M1 macrophages was significantly lower in IL-17D expressing cells (Figure 4B and 4D), while the level of M2 macrophages was remained unchanged (Figure 4E). IL-17D expressing cells had significantly more infiltrating neutrophils than control cell lines.

Discussion

Cancer cells can be characterized functionally by several major hallmarks, including evasion of apoptosis, growth-factor independent proliferation, and anchorage independent growth [29]. Recently, the impact of the immune system on tumor formation has been incorporated into the hallmarks of cancer [29]. It is now appreciated that while cancer cells must evade immune surveillance, they may also take advantage of immune inflammation to augment proliferation via increased angiogenesis [2,3,29]. This dual role of the immune system is especially evident in human ovarian cancer, where tumor-specific $CD8^+$ T cells can eliminate tumor cells while M2 macrophages and T-regulatory cells can promote tumor growth [1,10,26]. Consistent with these findings, our work demonstrates that ovarian cancers contain abundant CD163 staining, a marker for IL-10 activated macrophages [27], supporting the idea that advanced ovarian cancer is associated with a microenvironment that can be considered as “tumor promoting inflammation” [11]. The detection of IL-17D in these sections suggests that this novel cytokine may be involved in this process as well. We speculate that IL-17D leads to the recruitment and/or differentiation of $CD163^+$ macrophages since we found a significant correlation between these stainings. In support of this, we found that overexpression of IL-17D in ovarian cancer cell lines lead to accelerated tumor development in vivo and a reduced M1/M2 macrophage ratio. IL-17A is known to recruit neutrophils and also induce signals that can recruit monocytes, and our finding that IL-17D expressing ovarian cancer cell lines have a higher percent of infiltrating neutrophils suggests that IL-17D may have overlapping roles as IL-17A.

As part of the IL-17 family of cytokines, [13,14] IL-17D possesses a putative cysteine knot structure and is predicted to form dimers. Unlike IL-17A, which is expressed by Th17 helper T cells, IL-17D has been shown to be expressed outside the immune system in normal skeletal muscle, heart, and brain [17]. Interestingly, the expression of IL-17D is increased in local inflammatory lesions such as myocarditis, [30] restrictive cardiomyopathy, [30] and Wegener granulomatosis inflammation, [31] and is decreased in psoriatic skin [32]. These results suggest that IL-17D is a pro-inflammatory cytokine. Indeed, IL-17D has been shown to induce IL-6, IL-8, and GM-CSF from human endothelial cells [17]. Moreover, studies in oysters [33] and chickens [34] have found increased levels of an IL-17D-like transcript in response to bacterial challenge. Our finding that IL-17D is abundantly expressed in human malignant ovarian cancer is consistent with the putative pro-inflammatory role of IL-17D in infection and autoimmune disease. The limitation of our study is that we have not been able to define the cell type that expresses IL-17D and how it is induced in malignancy, although our recent studies indicate that the transcription factor Nrf2, responding to oxidative stress, can directly induce IL-17D [20].

The macrophage is one of the first cells to arrive at sites of inflammation. Through the production of cytokines, chemokines, growth factors, angiogenic factors, and proteases, macrophages are known to promote tissue repair. Macrophages also are part of the innate

immune system and can instruct adaptive immune responses against pathogens and tumors. Recently, tumor-associated macrophages (TAMs) have been shown to play dual roles in immunity to tumors [21,23,24]. At one end of the spectrum, macrophages recognize the tumor as a "wound that does not heal" [35] and hence increase angiogenesis and matrix remodeling, thereby promoting tumor development, invasion, and metastasis. These tumor-promoting macrophages are classified as M2 macrophages [21,23] and resemble alternatively activated macrophages [25] that can be identified by their expression of CD163 [27]. Macrophages can also function to promote anti-tumor responses by promoting the development of antitumor Th-1 T cells. These classically activated M1 macrophages are rare in progressively growing tumors and are abundant in highly immunogenic tumors [36]. However, it is not known what tumor factors determine whether M1 or M2 macrophage activities predominate. Our findings suggest that IL-17D, in the context of advanced ovarian cancers, may promote the recruitment and/or differentiation of M2 macrophages. If so, then a potential therapy for ovarian cancer would be in the blockade of IL-17D activity. Other studies have also found a correlation between CD163 expression and disease progression in serous and mucinous ovarian tumors, [37] endometriod carciniomas, [38] breast cancer, [39] rectal cancer, [40] and leiomyosarcomas [41]. Thus, the correlation between IL-17D and CD163 expression could be tested in other tumor models, and therapy aimed at modulating IL-17D and/or $CD163^+$ M2 macrophage function can be widely applicable to multiple human cancers.

Acknowledgment

JDB is supported by a grant from The Hartwell Foundation, NIH-CA128893, and NIH-CA157885.

References

1. Vaughan S, Coward JI, Bast RC Jr, Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nature Reviews Cancer. 2011; 11:719–725.
2. Schreiber RD, Old LJ, Smyth MJ. Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion. Science. 2011; 331:1565–1570.
3. Bui J, Schreiber R. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? Current opinion in immunology. 2007; 19:203–208.
4. Yigit R, Massuger LFAG, Figdor CG, Torensma R. Ovarian cancer creates a suppressive microenvironment to escape immune elimination. Gynecologic Oncology. 2010; 117:366–372.
5. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proceedings of the National Academy of Sciences. 2005; 102:18538.
6. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. New England Journal of Medicine. 2003; 348:203–213.
7. Kryczek I, Banerjee M, Cheng P, Vatan L, Szeliga W, Wei S, et al. Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments. Blood. 2009; 114:1141–1149.
8. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004; 10:942–949.
9. Goodell V, Salazar LG, Urban N, Drescher CW, Gray H, Swensen RE, et al. Antibody Immunity to the p53 Oncogenic Protein Is a Prognostic Indicator in Ovarian Cancer. Journal of Clinical Oncology. 2006; 24:762–768.
10. Schlienger K, Chu CS, Woo EY, Rivers PM, Toll AJ, Hudson B, et al. TRANCE- and CD40 ligand-matured dendritic cells reveal MHC class I-restricted T cells specific for autologous tumor in late-stage ovarian cancer patients. Clinical Cancer Research. 2003; 9:1517–1527.
11. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454:436–444.
12. Charles KA, Kulbe H, Soper R, Escorcio-Correia M, Lawrence T, Schultheis A, et al. The tumor-promoting actions of TNF-α involve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest. 2009; 119:3011–3023.
13. Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional Specialization of Interleukin-17 Family Members. Immunity. 2011; 34:149–162.
14. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity. 2004; 21:467–476.
15. Kato T, Furumoto H, Ogura T, Onishi Y, Irahara M, Yamano S, et al. Expression of IL-17 mRNA in Ovarian Cancer. Biochemical and Biophysical Research Communications. 2001; 282:735–738.
16. Miyahara Y, Odunsi K, Chen W, Peng G, Matsuzaki J, Wang RF. Generation and regulation of human CD4+ IL-17-producing T cells in ovarian cancer. Proceedings of the National Academy of Sciences. 2008; 105:15505–15510.
17. Starnes T, Broxmeyer HE, Robertson MJ, Hromas R. Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. Journal of immunology. 2002; 169:642–646.
18. Saddawi-Konefka R, O'Sullivan T, Gross ET, Washington A, Bui JD. Tumor-expressed IL-17D recruits NK cells to reject tumors. Oncoimmunology. 2014; 3:e954853.
19. O'Sullivan T, Saddawi-Konefka R, Gross E, Tran M, Mayfield SP, Ikeda H, et al. Interleukin-17D Mediates Tumor Rejection through Recruitment of Natural Killer Cells. CellReports. 2014; 7:989–998.
20. Saddawi-Konefka R, Seelige R, Gross ET, Levy E, Searles SC, Washington A Jr, et al. Nrf2 Induces IL-17D to Mediate Tumor and Virus Surveillance. CellReports. 2016; 16:2348–2358.
21. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, et al. Macrophage polarization in tumour progression. Seminars in Cancer Biology. 2008; 18:349–355.
22. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol. 2002; 196:254–265.
23. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends in immunology. 2002; 23:549–555.
24. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nature Reviews Cancer. 2004; 4:71–78.
25. Gordon S. Alternative activation of macrophages. Nature Reviews Immunology. 2003; 3:23–35.
26. Kryczek I, Wei S, Zhu G, Myers L, Mottram P, Cheng P, et al. Relationship between B7-H4, Regulatory T Cells, and Patient Outcome in Human Ovarian Carcinoma. Cancer Research. 2007; 67:8900–8905.
27. Ambarus CA1, Krausz S, van Eijk M, Hamann J, Radstake TR, Reedquist KA, et al. Systematic validation of specific phenotypic markers for in vitro polarized human macrophages. Journal of immunological methods. 2012; 375:196–206.
28. Lau SK, Chu PG, Weiss LM. CD163: A Specific Marker of Macrophages in Paraffin-Embedded Tissue Samples. American Journal of Clinical Pathology. 2004; 122:794–801.
29. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144:646–674.
30. Wittchen F, Suckau L, Witt H, Skurk C, Lassner D, Fechner H, et al. Genomic expression profiling of human inflammatory cardiomyopathy (DCMi) suggests novel therapeutic targets. J Mol Med. 2006; 85:257–271.
31. Müller A, Lamprecht P. Interleukin-17 in chronic inflammatory and autoimmune diseases: rheumatoid arthritis, Crohn"s disease and Wegener"s granulomatosis. Z Rheumatol. 2007; 67:72–74.
32. Johansen C, Usher PA, Kjellerup RB, Lundsgaard D, Iversen L, Kragballe K, et al. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. British Journal of Dermatology. 2009; 160:319–324.

33. Roberts S, Gueguen Y, de Lorgeril J, Goetz F. Rapid accumulation of an interleukin 17 homolog transcript in Crassostrea gigas hemocytes following bacterial exposure. Developmental & Comparative Immunology. 2008; 32:1099–1104.

34. Lillehoj HS, Park DW, Lee SH, Han JY, Shin JH, Park MS, et al. Cloning and functional characterization of chicken interleukin-17D. Veterinary Immunology and Immunopathology. 2008; 126:1–8.

35. Flier JS, Underhill LH, Dvorak HF. Tumors: wounds that do not heal. The New England journal of medicine. 1986; 315:1650–1659.

36. O'Sullivan T, Saddawi-Konefka R, Vermi W, Koebel CM, Arthur C, White JM, et al. Cancer immunoediting by the innate immune system in the absence of adaptive immunity. Journal of Experimental Medicine. 2012; 209:1869–1882.

37. Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. Pathology International. 2009; 59:300–305.

38. Espinosa I, José Carnicer M, Catasus L, Canet B, D'angelo E, Zannoni GF, et al. Myometrial Invasion and Lymph Node Metastasis in Endometrioid Carcinomas: Tumor-associated Macrophages, Microvessel Density, and HIF1A Have a Crucial Role. The American Journal of Surgical Pathology. 2010; 34:1708–1714.

39. Shabo I, Stål O, Olsson H, Doré S, Svanvik J. Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. International Journal of Cancer. 2008; 123:780–786.

40. Shabo I, Olsson H, Sun X-F, Svanvik J. Expression of the macrophage antigen CD163 in rectal cancer cells is associated with early local recurrence and reduced survival time. International Journal of Cancer. 2009; 125:1826–1831.

41. Lee CH, Espinosa I, Vrijaldenhoven S, Subramanian S, Montgomery KD, Zhu S, et al. Prognostic Significance of Macrophage Infiltration in Leiomyosarcomas. Clinical Cancer Research. 2008; 14:1423–1430.

Supplementary Figures

Figure S1: *in vitro* growth of control and IL-17D expressing human ovarian cancer cell lines.Control transfected and IL-17D transfected cell lines expand at an equal rate compared to parent cell lines in vitro.

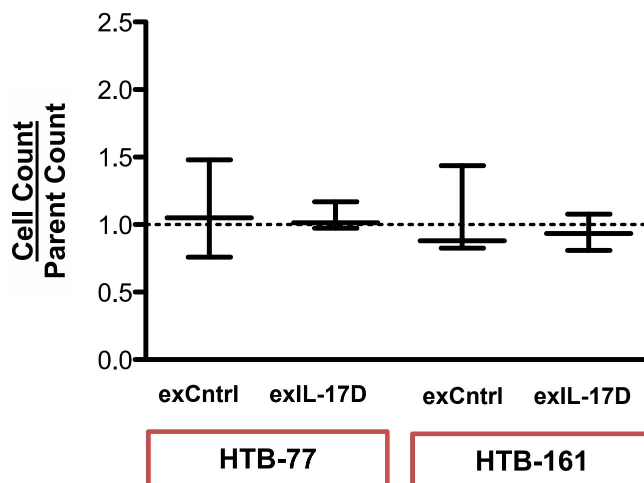
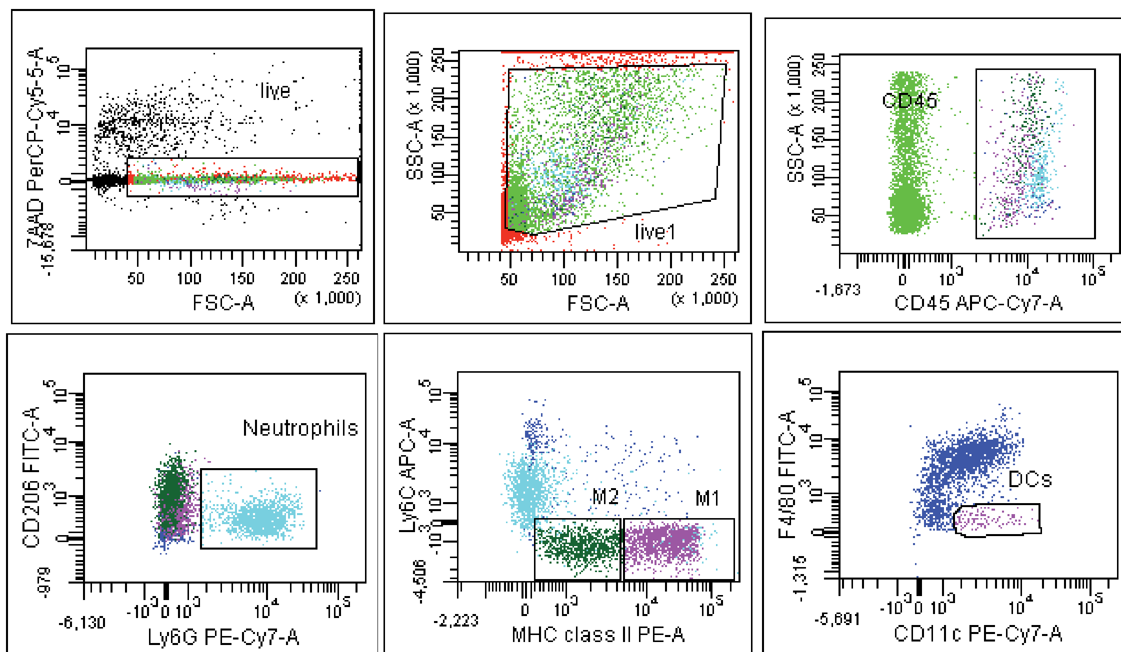


Figure S2: Gating strategy for tumor infiltrating leukocytes in vivo.



Gating strategy showing identification of neutrophils, dendritic cells, and M1 versus M2 macrophages