Phenotype, distribution, generation, and functional and clinical relevance of Th17 cells in the human tumor environments

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Th17 cells play an active role in autoimmune diseases. However, the nature of Th17 cells is poorly understood in cancer patients. We studied Th17 cells, the associated mechanisms, and clinical significance in 201 ovarian cancer patients. Tumor-infiltrating Th17 cells exhibit a polyfunctional effector T-cell phenotype, are positively associated with effector cells, and are negatively associated with tumor-infiltrating regulatory T cells. Tumor-associated macrophages promote Th17 cells through interleukin-1β (IL-1β), whereas tumor-infiltrating regulatory T cells inhibit Th17 cells through an adenosinergic pathway. Furthermore, through synergistic action between IL-17 and interferon-γ, Th17 cells stimulate CXCL9 and CXCL10 production to recruit effector T cells to the tumor microenvironment. The levels of CXCL9 and CXCL10 are associated with tumor-infiltrating effector T cells. The levels of tumor-infiltrating Th17 cells and the levels of ascites IL-17 are reduced in more advanced diseases and positively predict patient outcome. Altogether, Th17 cells may contribute to protective human tumor immunity through inducing Th1-type chemokines and recruiting effector cells to the tumor microenvironment. Inhibition of Th17 cells represents a novel immune evasion mechanism. This study thus provides scientific and clinical rationale for developing novel immunomodulating strategies based on promoting the Th17 cell population in cancer patients. (Blood. 2009;114:1141-1149)

Introduction

Adaptive immunity plays a crucial role in tumor immunosurveillance.1-3 It has been shown that tumor-infiltrating effector T cells are associated with improved prognoses in multiple human cancers,4,6 whereas tumor-infiltrating regulatory T (Treg) cells are negatively associated with patient outcome.5-7 Th17 cells are newly identified effector CD4+ T cells. Th17 cells and interleukin-17 (IL-17) play an active role in inflammation and autoimmune diseases.5-15 Th17 cells are found in both mouse and human tumors.16,17 However, the biologic role of Th17 cells is poorly understood in the tumor microenvironment. In this report, we examined the phenotype, cytokine profile, generation, functional relevance, and immunologic and clinical predictive values of Th17 cells in 201 patients with ovarian cancers. We provide novel insight into the nature of Th17 cells in the tumor microenvironment in patients with cancer. This information may be useful for designing more effective cancer immunotherapies.

Methods

Human subjects

We studied previously untreated patients with 201 ovarian carcinomas. Survival data were available for 85 patients (supplemental Table 1, available on the Blood website; see the Supplemental Materials link at the top of the online article). Patients gave written, informed consent in accordance with the Declaration of Helsinki. The study was approved by the University of Michigan Institutional Review Board.


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Th17 cells were polarized from tumor-associated T cells (10^6/mL) for R&D Systems). CD4^+CD25^{hi}^ T cells were sorted from peripheral blood or ovarian cancer tissues.7 Different concentrations of tumor-associated Treg cells were added into the coculture. In some cases, ARL67156 (50 μM; Sigma-Aldrich) was added into the culture as described. T-cell phenotype and cytokine profile were determined by fluorescence-activated cell sorter (FACS) or enzyme-linked immunosorbent assay (ELISA; R&D Systems) as we described.16,18,19

siRNA knockdown of human IL-23 gene expression

HEK293 cells were transfected with a Flag-tagged IL-23 expression plasmid and either a nonfunctional scrambled control siRNA or IL-23–specific siRNA using Lipofectamine 2000 (Invitrogen). After the siRNA treatment, the hIL-23 silencing efficiency was measured by Western blot using an anti-Flag tag (not shown). Blood or tumor-associated macrophages were transfected with the siRNA or pmaxGFP vector using Nucleofector technology (Macrophages Nucleofector Kit; Amaxa Biosystems) as we described.20 The transfection efficiency reached 60% to 80% as confirmed by pmaxGFP vector transfection.

Cytokine and chemokine detection

The mRNA levels of cytokines and chemokines were detected by real-time reverse-transcriptase polymerase chain reaction (PCR). All experiments were performed using gene-specific primer pairs and SYBR green I (Invitrogen) fluorescence detection in a Multiplex instrument (Eppendorf). Data analysis is based on the Ct method with normalization of the raw data to housekeeping gene.3,19 The protein levels of cytokines and chemokines were detected by either intracellular staining or ELISA kits (all from R&D Systems).

Induction of CXCL9 and CXCL10

Th17 cells were polarized from tumor-associated T cells (10^6/mL) for 3 days with tumor-associated macrophages (0.5 × 10^6/mL) in the presence of Th17-inducing cytokine cocktail as we described.20 The polarized cells were extensively washed with fresh medium and cultured for additional 40 hours. The polarized Th17 cell supernatants were collected. Primary ovarian cancer cells (OC8) or macrophages (10^5/mL)9 were cultured with IL-17 (10 ng/mL), IFN-γ (0-50 ng/mL), IL-17 plus IFN-γ, or 100% Th17 cell-polarized culture supernatants for 2 to 3 days. In some cases, the neutralizing anti–human IFN-γ (2 μg/mL, clone 25723, IgG2b) and anti–IL-17 receptor (2 μg/mL, clone 13617, IgG) were added into the culture. The supernatants were subjected to measuring CXCL9 and CXCL10 with ELISA kits (R&D Systems).

Migration assay

CD8^+ T-cell migration was assessed as we described21 using ovarian cancer–associated CD8^+ T cells (5-20 × 10^5). T cells were induced to migrate with tumor ascites. In some cases, mouse anti–human CXCR3 (57226.11, IgG2b, 500 ng/mL) were added 2 hours before migration assay. Experiments were performed in triplicate. Migration was expressed as a percentage of migrated cells after subtracting the spontaneous migration (Migration index).

Tissue immunofluorescence staining

Immunofluorescence analysis was performed as described.22 Tissues were stained with monoclonal mouse anti–human CD8 (1/40 dilution,clone HIT8a, IgG2b, BD Biosciences), and mouse anti–human EpCam (1/40 dilution, clone 5E11, IgG1; StemCell Technologies) followed by AlexaFluor 568–conjugated goat anti–mouse IgG2b and AlexaFluor 488–conjugated goat anti–mouse IgG1 (all 2 μg/mL; Invitrogen). Positive cells were quantified by ImagePro Plus software and expressed as the mean number of the positive cells per mm^2 tissue section.

Statistical calculations

Pearson coefficient was computed to assess relationships between proteins and immune cell subsets in the tumor environments. Student t tests were used to compare IL-17 expressions across stage (II/III vs IV), grade (0-2 vs 3), histology type (serous, mucinous, endometroid vs clear cells and undifferentiated), and debulking (optimal residual disease vs suboptimal residual disease) categories, with P values less than .05 considered significant. Overall patient survival was defined as the interval between date of diagnosis and date of death or last follow-up, whichever occurred earlier. The known tumor-unrelated deaths (eg, intercurrent disease and accidental death) were excluded from the death record for this study. Data were censored at the last follow-up for patients who were disease-free or alive at the time of last follow-up. Univariate association between IL-17, other factors, and overall survival was assessed using log-rank test, and survival function estimates were computed using the Kaplan-Meier method. Cox proportional hazards model was used to assess the effect of IL-17 on survival, after adjusting for surgical debulking. All analyses were performed using SAS 9.1 (SAS Institute Inc) and STATISTIC (StatSoft Inc) software.

Results

Distribution, phenotype, and cytokine profile of Th17 cells

IL-17^+CD4^+ (Th17) cells are found in patients with cancer.16,17 However, the distribution, phenotype, and cytokine profile of Th17 cells remain poorly defined in human tumors. We first evaluated the tissue distribution of Th17 cells in ovarian cancer patients. The prevalence of Th17 cells was comparable in tumor-draining lymph nodes, cancer patient peripheral blood, and normal donor peripheral blood (Figure 1A). However, the proportion of Th17 cells was higher in tumors than these compartments (Figure 1A). This suggests that Th17 cells may be induced or/migrate into the tumor microenvironment.16

We next examined the phenotype of IL-17^+ cells in the tumor microenvironment. We found that IL-17 was exclusively expressed by T cells. Less than 1% tumor-infiltrating CD8^+ T cells expressed IL-17, whereas 99% of the tumor-infiltrating IL-17^+ T cells were IL-17^+CD4^+ (Th17) cells (Figure 1B). Tumor-infiltrating Th17 cells expressed high levels of CXCR4, CCR6, and CD161 (Figure 1C) and multiple CD49 integrins (Figure 1D), but not CCR2, CCR5, and CCR7 (supplemental Figure 1). The expressed homing molecules may be associated with Th17 cell migration and retention within tumor.20

We also analyzed the markers for T-cell activation/effector function and immune suppression. Tumor-infiltrating Th17 cells expressed little HLA-DR, CD25, and granzyme B (Figure 1E). This suggests that Th17 cells may not be conventional effector T cells and may not mediate effector function through the granzyme B pathway. The B7-H1 receptor, PD-1, may be expressed in functionally exhausted T cells. The B7-H1/PD-1 pathway22 and FOXP3^+ Treg cells23,24 contribute to immune suppression in the tumor microenvironment. We found that Th17 cells expressed minimal PD-1 and FOXP3 (Figure 1F). This indicates that Th17 cells are distinct from Treg cells and functionally exhausted PD-1^+ T cells.

We further analyzed the cytokine profile of human tumor-infiltrating Th17 cells. IL-10^+ and IL-10^− Th17 cells have been observed in mice.15,16,18,23,27,28 We found that Th17 cells expressed minimal IL-10 (Figure 1F) and high levels of polyfunctional effector cytokines, including tumor necrosis factor-α, IL-2, and IFN-γ (Figure 1G). Tumor-infiltrating T cells, including Th17 cells, did not express IL-4 (not shown). Similar cytokine profiles were
observed in 5 other human tumor types studied, including colon carcinomas, hepatocellular carcinomas, melanoma, pancreatic cancers, and renal cell carcinomas (not shown). These data indicate that Th17 cells exhibit an effector T-cell cytokine profile with polyfunctionality as described in infectious diseases.27,28

Th17 cells and their associations with immune cell subsets in the tumor microenvironment

Multiple immune cell populations, including T-cell subsets and antigen-presenting cell (APC) subsets, infiltrated the tumor microenvironment. We evaluated the relationships between Th17 cells and immune cell subsets in the same ovarian cancer environment. We first analyzed the correlation between Th17 cells and T-cell subsets. We quantified Th17, IFN-γ+ IL-17+ T cells, IFN-γ+ CD8+, and IFN-γ+ CD4+ T cells, and Treg cells in the same tumors. Flow cytometry analysis revealed that Th17 cells were positively correlated with IFN-γ-expressing T-cell subsets, including IFN-γ+ CD4+ T cells (Figure 2A), IFN-γ+ CD8+ (Figure 2B), and IFN-γ+ IL-17+ T cells (Figure 2C) in the same tumor microenvironment. However, the proportion of Th17 and Treg cells was inversely correlated in the same tumors (Figure 2D).

We further analyzed the relationship between Th17 cells and innate immune cells in the same ovarian cancer ascites. Eosinophils were rarely observed (supplemental Figure 2A). Moderate levels of mast cells (supplemental Figure 2B), neutrophils (supplemental Figure 2C), and NK cells (Figure 2E) were detected. However, Th17 cells had no correlation with eosinophils, mast cells, and neutrophils (supplemental Figure 2). We found that the levels of NK cells were higher in patients with high levels of Th17 cells than in patients with low levels of Th17 cells in the same tumor microenvironment (Figure 2E).

Finally, we analyzed the relationship between Th17 cells and APC subsets. Plasmacytoid dendritic cells,21 myeloid dendritic cells, and macrophages are the main APC populations in ovarian cancer19 (supplemental Figure 3A). These 3 APC subsets were found in the tumor ascites and tumor (supplemental Figure 3A). However, there were no quantitative correlations between Th17 cells, and myeloid DCs (supplemental Figure 3B), plasmacytoid DCs (supplemental Figure 3C), and macrophages (supplemental Figure 3D). We further investigated the functional association between Th17 cells and APC subsets in the subsequent studies.

Altogether, the data demonstrate that Th17 cells are quantitatively and positively correlated with NK cell–mediated innate immunity and adaptive T-cell immunity.

Induction and suppression of Th17 cell development in the tumor microenvironment

Th17 cells are basically found in the tumor microenvironment in patients with cancer.18 APCs contribute to T-cell polarization. We investigated the role of tumor-associated macrophages (TAMs), plasmacytoid DCs, and myeloid DCs in Th17 cell induction in ovarian cancer. We found that tumor-associated plasmacytoid DCs had minimal effects on Th17 cell induction (supplemental Figure 4). TAMs and myeloid DCs isolated from ovarian cancers stimulated Th17 cell induction from memory T cells, and not from naive T cells (supplemental Figure 4, Figure 3A). TAMs were more efficient than normal macrophages (M0s) in eliciting T-cell IL-17 production, and the induction was dose dependent (Figure 3B). Macrophages outnumbered myeloid DCs in ovarian cancer19,21 (supplemental Figure 3) and were superior to inducing Th17 cells than myeloid DCs (supplemental Figure 4, Figure 3B).29 Our subsequent studies focused on tumor-associated macrophages.

We investigated the mechanism by which TAMs induce Th17 cells. We found that TAMs expressed higher levels of IL-1β and IL-23p19 mRNA, compared with normal macrophages (Figure 3C). Blockade of IL-1, but not IL-6 and transforming growth factor-β (TGF-β), consistently and largely reduced TAM-mediated...
induction of Th17 cells (Figure 3D, data not shown). Blocking IL-23 with specific siRNA further helped reduce Th17 cell induction (Figure 3D). Our data suggest that IL-1/H9252 plays a predominant role in TAM-mediated Th17 cell induction in patients with ovarian cancer. Because TAMs are potent Th17 cell inducers (Figure 3A-B,D), we examined why there were limited numbers of Th17 cells in the tumor microenvironment (Figure 1). We hypothesized that tumor-associated Treg cells might suppress Th17 cell development. To test this hypothesis, we first stimulated T cells with TAMs in the presence of tumor-associated Treg cells. Treg cells suppressed Th17 cells and T-cell IL-17 production in a dose-dependent manner (Figure 3E-F).

We further studied the mechanism by which Tregs suppressed Th17 induction. Tumor-associated Treg cells highly expressed CD39 (supplemental Figure 5A-B), an ectonucleotidase that converts ATP into adenosine. Mouse Treg cells may mediate T-cell suppression through adenosine induction.30,31 We found that ARL67156, a structural analog of ATP and an ectonucleotidase inhibitor, partially but significantly recovered T-cell IL-17 production suppressed by tumor-associated Treg cells (Figure 3G). These data indicate that Th17 cell development is partially suppressed by tumor-associated Treg cells through the adenosinergic pathway.

Th17, Th1, Th2-type cytokines and chemokines

To further examine the relationships between Th17 cells and the types of immune responses in the ovarian cancer microenvironment, we quantified numerous representative cytokines and chemokines associated with Th17, Th1, and Th2-type responses in the ovarian cancer ascites.

Th17 cells were the only cell type expressing IL-17 in the ovarian cancer ascites. We detected variable levels of IL-17 in ascites fluid. Interestingly, the levels of IL-17 were positively correlated with IL-1β and IL-1α (supplemental Figure 6A-B), but not with TGF-β, IL-6 (supplemental Figure 6C; and data not shown), IL-21 (supplemental Figure 6D). IL-23 (supplemental Figure 6E), and PGE2 (supplemental Figure 6F). IL-23 protein was barely detectable in most of the samples tested (supplemental Figure 6E). All these molecules have been reported to be associated with Th17 cell development.30-32 Given that the levels of IL-1α were less than 5 pg/mL (supplemental Figure 6B), the data further support that IL-1β plays a selective and crucial role in Th17 cell induction in the ovarian cancer microenvironment (Figure 3C-D).

Cytokines associated with Th1 and Th2-type responses, including IL-12, IL-2, and IL-4, were less than 10 pg/mL in ovarian cancer ascites. IL-17 has been reported to induce tumor angiogenesis.33,34 Consistent with previous reports, high levels of angiogenic factors, including IL-8 and vascular endothelial growth factor,
were detected in the ascites. However, IL-17 was not correlated with these angiogenic molecules (supplemental Figure 7).

In addition to cytokines, we further evaluated the relationship between IL-17 and chemokines associated with Th1-type response, including CXCL9, CXCL10, and with Th2-type response, including CXCL12 and CCL22. Interestingly, we observed a significant positive correlation between IL-17, CXCL9, and CXCL10 (Figure 2A-B). Although we detected high levels of CXCL12 and CCL22, IL-17 had no association with these chemokines (supplemental Figure 8). The data indicate that, in addition to Th1-type effector T cells and NK cells (Figure 2), Th17 cells and IL-17 are correlated with Th1-type chemokines in the ovarian cancer microenvironment.

In addition, we examined the mechanistic relationship between Th17 cells and tumor immunity. Th17 cells or IL-17 had no direct effects on primary ovarian cancer cell proliferation and apoptosis (supplemental Figure 9). As Th17 cells are positively correlated with Th1-type chemokines and effector T cells, we hypothesized that Th17 cells induce Th1-type chemokines, and in turn recruit Th1-type effector T cells into tumor microenvironment. To test this hypothesis, we initially studied the effects of Th17 cells on Th1-type chemokine production. We found that IFN-γ and IL-17 synergistically induced the production of CXCL9 and CXCL10 by primary ovarian cancer cells and macrophages (Figure 4C-D; and data not shown). Consistent with this observation, real-time PCR revealed that the levels of IL-17 were positively correlated with that of CXCL9 and CXCL10 in the same tumor tissues (supplemental Figure 10). In further support, the supernatants derived from Th17 cells induced high levels of CXCL10 production. This production was blocked by neutralizing anti–human IFN-γ and anti–IL-17 (Figure 4E). These data indicate that Th17 cells induce Th1-type chemokine production.

Th17, Th1-type chemokines, and effector T-cell trafficking

Tumor-associated effector CD8+ T cells highly expressed CXCX3, the receptor for CXCL9 and CXCL10 (Figure 5A). Tumor-associated effector CD8+ T cells efficiently migrated toward tumor ascites in a dose-dependent manner. The migration was reduced by neutralizing anti-CXCR3 (Figure 5B). We also quantified the number of tumor-infiltrating CD8+ T cells by immunofluorescence staining. The mRNA levels of CXCL9 and CXCL10 were positively correlated with tumor-infiltrating CD8+ T cells in the same tumor (Figure 5C-D). Furthermore, when we divided tumor tissues into 2 groups based on the median levels of IL-17, we observed that the levels of tumor ascites IL-17 were positively associated with tumor-infiltrating CD8+ T cells (Figure 5E-F). Altogether, the data support the notion that Th17 cells induce Th1-type chemokines through IL-17 and IFN-γ, and in turn recruit Th1-type effector T cells and NK cells into tumor microenvironment.
Increased tumor-associated IL-17 predicts improved patient survival

Our current data suggest that Th17 cells may contribute to protective tumor immunity in ovarian cancers. IL-17 is released into the tumor environment consisting of the abdominal cavity. IL-17 was detectable in all the ovarian cancer ascites we evaluated (Figure 4A-B). We analyzed the impact of IL-17 levels in the ascites on patient survival.

There was a significant association between ascites IL-17 levels and survival in the group as a whole (n = 1100, P = .001), and also for patients in stage II/III (n = 57, P = .01) and stage IV (n = 28, P = .005). Tumor ascites IL-17 was a significant predictor of death hazard (95% confidence interval, P = .001) even after controlling for surgical debulking and other parameters using a Cox proportional hazards model (Figure 6; Table 1, supplemental Table 1).

As an alternative analysis, patients were divided into 2 groups based on the median values of IL-17 (220 pg/mL). Survival functions were significantly different for the 2 groups (Figure 5A; P = .001). The median survival in the high IL-17 group was 78 months, compared with 27 months in the low IL-17 group. Tumor ascites IL-17 was a significant predictor of death, even after controlling for surgical debulking. Patients in the high IL-17 group had a significantly lower death hazard compared with those in the low IL-17 group (hazard ratio = 0.08; 95% confidence interval, 0.03-0.20, P < .001).

Furthermore, when the analyses were stratified by stage, we found significant association between ascites IL-17 and survival for patients in stage III (n = 52, P = .01; Figure 6B) as well as stage IV disease.
We additionally found that patients in stage IV had significantly reduced IL-17 in ascites compared with those in stage III (Figure 6D; \( P = 0.03 \)). Th17 cells are the IL-17 producers in the tumor. Therefore, decreased tumor ascites IL-17 or/and Th17 cells are a significant predictor of increased risk for reduced survival in ovarian cancer.

Discussion

In this study, we have applied multiple complementary strategies to map out the phenotype, mechanisms of induction, biologic function, and clinical relevance of Th17 cells in the tumor microenvironment of patients with ovarian cancer. We have shown that tumor-infiltrating Th17 cells highly express effector cytokines, but little in the way of molecules associated with immune suppression. This cytokine profile reveals a phenotype for polyfunctional effector T cells similar to that observed in patients with infectious diseases.\(^27,28\) This phenotype was universally found in 6 different human cancer types that we examined. It suggests that tumor-associated Th17 cells may be functional effector T cells. In line with this possibility, we found that Th17 cells are negatively associated with the presence of Treg cells\(^7\) and are positively associated with effector immune cells, including IFN-\(\gamma\) effector T cells, CD8\(^+\) T cells, and NK cells in the same tumor microenvironment. The data are consistent with several lines of evidence. (1) Transgenic T cells polarized with TGF-\(\beta\) and IL-6 can induce tumor eradication in mice.\(^35\) (2) Forced expression of IL-17 ectopically in tumor cells can suppress tumor progression through enhanced antitumor immunity in immune-competent mice.\(^36,37\) (3) IL-17-deficient mice exhibit accelerated tumor growth and lung metastasis.\(^38\) (4) Both blocking indoleamine 2,3-dioxygenase\(^39\) and adjuvant IL-7 treatment\(^40\) result in improved antitumor immunity, which is associated with marked CD8\(^+\) T-cell activation and Th17 cell enhancement. (5) In patients with prostate cancer, a significant inverse correlation is found between Th17 skewing and tumor grade.\(^17\) Along this line, we have detected IL-17 in tumor-associated ascites, and the levels of IL-17 positively predict patient survival. Th17 cells are the sole cellular source for IL-17 in the human tumor microenvironment. Hence, the data provide evidence that Th17 cells may contribute to protective tumor immunity in humans with advanced tumors. In addition to CD8\(^+\) effector T cells,\(^4-6\) our data indicate that Th17 cells are an important immune component in tumor immunosurveillance.\(^1,2\)

The next question is how Th17 cells mediate antitumor immunity in patients with cancer. Th17 cells do not express granzyme B and perforin, and have no direct effects on primary ovarian cancer cell proliferation and apoptosis. Th17 cells may not mediate a direct tumor cytotoxic activity against tumor cells. Recent compelling evidence demonstrates that trafficking properties and location of effector T cells play a central role in the control of tumor growth and recurrence.\(^5-6\) In line with this notion, we found that IL-17 was positively associated with tumor-infiltrating IFN-\(\gamma\)-effector T cells and with Th1-type chemokines CXCL9 and CXCL10, but not with Th2-type chemokines CXCL12 and CCL22. Mechanistically, Th17 cell-derived IL-17 and IFN-\(\gamma\) synergistically induced the production of Th17 cells.

Table 1. Relationship between IL-17 and clinical pathologic characteristics in ovarian cancer patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age*</td>
<td>1.003 (0.98-1.03)</td>
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<tr>
<td>Stage, II/III vs IV</td>
<td>1.84 (0.97-3.48)</td>
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<td>Grade, 0-2 vs 3</td>
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<td>Histology type, serous/ mucinous/endometrial versus clear cells and undifferentiated</td>
<td>1.36 (0.69-2.69)</td>
<td>NA</td>
</tr>
<tr>
<td>Debulking, optimal versus suboptimal residual disease</td>
<td>0.17 (0.07-0.42)</td>
<td>0.189 (0.078-0.458)</td>
</tr>
<tr>
<td>IL-17*</td>
<td>0.994 (0.992-0.997)</td>
<td>0.994 (0.991-0.997)</td>
</tr>
</tbody>
</table>

*Age (in years) and IL-17 (in pg/mL) are continuous variables. Other variables are binary, as noted.
†Adjusted HRs are based on a multivariable Cox proportional hazards model with debulking (binary: optimal vs suboptimal) and IL-17 (continuous) as covariates.
synergistically induce environmental Th17 cells express both IL-17 and IFN-γ. In support of this notion, human psoriatic
migration toward tumor. The levels of CXCL9 and CXCL10 of CXCL9 and CXCL10 are 2 potent antiangiogenic
cytokines.44 IL-17 is also not associated with IL-23, and IL-23 plays a minor role, if any, in Th17 cell development in
human ovarian cancer. However, these potential effects may possibly be overweighted by the antitumor immunity and
angiogenic and proinflammatory roles of IL-17 derived from Th17 cells in human tumors. However, these potential effects
may possibly be overweighted by the antitumor immunity and antiangiogenic activities mediated by Th17 cell–induced CXCL9
and CXCL10 in patients with cancer.

We have further demonstrated that tumor-associated macrophages are capable of inducing Th17 cell development in vitro.
IL-1β, but not IL-1α, IL-6, TGF-β, and IL-23, is crucial for Th17 cell induction and is positively associated with IL-17 in
ovarian cancer ascites. Consistent with this observation, the levels of IL-1α and IL-23 are negligible in ovarian cancer
ascites. It suggests that IL-1α and IL-23 play a minor role in Th17 cell development in human ovarian cancer. However,
IL-1α, IL-1β, and IL-23 are involved in memory Th17 cell expansion in patients with psoriasis.20,29 It is possible that the
molecular mechanisms are distinct in inducing Th17 cells in patients with tumors versus autoimmune diseases. The role of
IL-6 and TGF-β in Th17 cell development remains controversial in humans.45-48 High levels of IL-6 and TGF-β are often detected
in the tumor microenvironment.49 If IL-6 and TGF-β have played potent roles in promoting Th17 cells, one may expect
substantial numbers of Th17 cells in human tumors. However, it is evident that the numbers of Th17 cells are limited, compared
with Treg cells and other T-cell subsets in the tumor microenvironment.56 Blockade of IL-1, rather than IL-6 and TGF-β,
reduced Th17 cell induction. Furthermore, IL-17 and Th17 cells are not quantitatively associated with IL-6 and TGF-β.
Therefore, at least these 2 cytokines are not crucial for Th17 cell development in the ovarian cancer microenvironment.

We have also investigated the underlying mechanisms by which Th17 cells are limited in the tumor microenvironment. Interest-
ingly, the levels of Treg cells and Th17 cells are inversely associated in the same tumors. Tumor-associated Treg cells highly
express CD39, an ectonucleotidase that converts ATP into adenosine, and suppress Th17 cell development through the adenosinergic
pathway. Although it has been reported that mouse Treg cells may apply this pathway to suppress T-cell activation,30,31 we
demonstrated, for the first time, that human tumor-associated Treg cells inhibit Th17 cells with a similar molecular mechanism.
In addition to multiple modes of immune-suppressive mechanisms demonstrated in the tumor microenvironment,49-53 as human Th17
cells probably mediate protective tumor immunity, inhibition of Th17 cell development may be a novel immunoediting mechanism
for tumor to escape tumor immunity.

In conclusion, we have extensively defined the nature of Th17 in the human tumor microenvironment. Our data provide
immunologic and clinical evidence linking Th17 cells to immune protection in human cancer and suggest that inhibition of
Th17 cell development is a novel immune evasion mechanism. This study thus provides the rationale for developing
novel immune-boosting strategies based on promoting the Th17 cell population in cancer patients.

Acknowledgments

This work was supported in part by the National Cancer Institute (CA123088, CA099985; W.Z.) and the Marsha Rivkin Center for
Ovarian Cancer Research (I.K.).

Authorship

Contribution: I.K., R.L., and W.Z. designed research, analyzed data, and wrote the paper; M.B. analyzed data; P.C., E.H., E.F.,
D.S., T.H.W., A.C., G.C., and R.L. provided specimen and clinical information and reviewed the paper; and I.K., L.V., W.S., and S.W.
performed research.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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