Depletion of Dendritic Cells Delays Ovarian Cancer Progression by Boosting Antitumor Immunity

Eduardo Huarte,1 Juan R. Cubillos-Ruiz,1 Yolanda C. Nesbeth,1 Uciane K. Scarlett,1 Diana G. Martinez,2 Ronald J. Buckanovich,3 Fabian Benencia,4 Radu V. Stan,5,6 Tibor Keler,7 Pablo Sarobe,8 Charles L. Sentman,9 and Jose R. Conejo-Garcia10,11

Departments of Microbiology and Immunology, Pathology, and Medicine, Dartmouth Medical School, Lebanon, New Hampshire; Departments of Internal Medicine and Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan; Department of Biomedical Sciences, Ohio University, Athens, Ohio; Celldex Therapeutics, Phillipsburg, New Jersey; and Center for Applied Medical Research, University of Navarra, Pamplona, Navarra, Spain

Abstract

Dendritic cells (DC) and cytokines that expand myeloid progenitors are widely used to treat cancer. Here, we show that CD11c+DEC205+ DCs coexpress α-smooth muscle actin and VE-cadherin home to perivascular areas in the ovarian cancer microenvironment and are required for the maintenance of tumor vasculature. Consequently, depletion of DCs in mice bearing established ovarian cancer by targeting different specific markers significantly delays tumor growth and enhances the effect of standard chemotherapies. Tumor growth restriction was associated with vascular apoptosis after DC ablation followed by necrosis, which triggered an antitumor immunogenic boost. Our findings provide a mechanistic rationale for selectively eliminating tumor-associated leukocytes to promote antitumor immunity while impeding tumor vascularization and to develop more effective DC vaccines based on a better understanding of the tumor microenvironment. [Cancer Res 2008;68(18):7684–91]

Introduction

Despite significant advances in our understanding of the tumor cell cycle, the 5-year survival rate for ovarian carcinoma patients is still lower than 40%. As ovarian cancer cells express immunogenetic determinants (1–4), immunotherapy offers great promise to complement standard treatments. However, therapies that induce protective immunity against melanoma have failed against ovarian carcinoma (5) due to an incomplete understanding of its peculiar microenvironment. Therefore, to develop effective immunotherapies against ovarian cancer, better knowledge of the immunosuppressive networks that operate in its atypical milieu is required.

Our previous work unveiled a mechanism whereby immature dendritic cells (DCs) contribute to ovarian cancer progression by acquiring a proangiogenic phenotype in response to vascular endothelial growth factor (VEGF; refs. 6–8). VE-cadherin+ DCs are the most abundant leukocyte subset (>30% of total cells) in the microenvironment of ID8-Defb29/Vegf-a mouse ovarian tumor (6) as well as in human solid ovarian carcinoma samples but not in human ascites (7). Unlike classic myeloid-derived suppressor cells (9) in other epithelial tumors (10, 11), most CD11b+ leukocytes in mouse ovarian cancer coexpress CD11c and DEC205, supporting their DC lineage (6). Most importantly, although most ovarian tumor-infiltrating DEC205+CD11c+ are negative for costimulatory CD80 and exhibit low levels of MHC-II and CD86, their capacity to efficiently present antigens can be exposed under inflammatory conditions (6).

DCs are primarily viewed as orchestrators of peripheral tolerance under steady-state conditions but inducers of immunity during inflammation (12). Consequently, DCs are largely viewed as being crucial for antitumor immune responses in the context of cancer (13), and numerous DC-based vaccine trials have been attempted as a novel intervention against many cancer types. Moreover, cytokines are widely used to expand myeloid progenitors, including those that give rise to DCs, in cancer patients (14). In contrast, emerging studies suggest that DCs can also function as inducers of T-cell tolerance under certain inflammatory conditions (11, 15–21).

Because of the abundance and immature phenotype of ovarian cancer DCs, we hypothesized that these cells could represent important contributors to tumor growth and therefore novel therapeutic targets. Here, we show how the elimination of DCs dramatically delayed ovarian cancer progression. These effects were mediated by an antitumor immunogenic boost preceded by tumor necrosis. Our findings provide a rationale for manipulation of critical elements of the tumor microenvironment as antitumor therapy and to develop more effective DC-based vaccines based on a better understanding of their potential to support tumor development.

Materials and Methods

Animals, tissues, and treatments. Mice were purchased from the National Cancer Institute or The Jackson Laboratory. Animal experiments were approved by our Institutional Animal Care and Use Committee. Stage III to IV human ovarian carcinoma specimens were procured through Research Pathology Services at Dartmouth under an approved protocol. Single-cell suspensions were generated as we described (7). We generated ID8-Defb29/Vegf-a flank or i.p. tumors as described (6). Tumor volumes were calculated by the formula $V = 0.5(L \times W^2)$, where $L$ is length and $W$ is width. To generate ID8-luciferase, parental ID8 cells were transduced thrice with pFB-neo-Luciferase retroviruses and selected with 0.8 mg/mL G418. For visualization of tumor burden, mice were injected with 0.2 mL of 15 mg/mL luciferin (Promega). After 10 min, animals were anesthetized with isoflurane and imaged using the IVIS 200 system (Xenogen Corp.). ID8-OVA cells were described (22). The anti-DEC205 NLDC145 antibody, conjugated or not to ribosome-inactivating saporin, was provided by Celldex Therapeutics. Treatments started 1 d after tumor challenge.

To generate chimeric mice, we lethally irradiated congenic (CD45.1) mice (two doses of 500 rads, 24 h apart). Four hours later, $2 \times 10^6$ cells from...
Depleted administration eliminates tumor CD11c+ DCs

A, fluorescence-activated cell sorting (FACS) analysis of a mechanically dissociated metastatic stage III ovarian carcinoma specimen. These data are representative of three primary and five metastatic stage III to IV samples (see also Supplementary Fig. S1).

**Figure 1.** DEC205+CD11b+ DCs, but not monocyte/macrophages, represent the most abundant leukocyte subset in the microenvironment of solid ovarian carcinoma specimens (gated on CD45+ cells). A, fluorescence-activated cell sorting (FACS) analysis of a mechanically dissociated metastatic stage III ovarian carcinoma specimen. These data are representative of three primary and five metastatic stage III to IV samples (see also Supplementary Fig. S1). B, FACS analysis of the same tumor and its matching tumor ascites.

The bone marrow of healthy (CD45.2) ITGAX-diphtheria toxin receptor (DTR)-green fluorescent protein (GFP) were iv. injected. After confirming that >95% of circulating cells were of donor origin, mice were ip. challenged with 2 × 10^6 ID8-luciferase tumor cells 8 wk after reconstitution. Treatments (150 ng of ip. DT every 3 d, or PBS) started 3 d later.

**Figure 2.** Elimination of CD11c+ DCs abrogates tumor growth. A, ITGAX-DTR-GFP mice (n = 6 per group; two independent experiments) were challenged with a s.c. injection of 10^7 ID8-Defb29/Vegf-a cells in 200 μL Matrigel and immediately received 4 ng/g body weight DT in PBS (top) or PBS (bottom). Tumors were removed at 2 mo. B, administration of DT did not result in reduced tumor growth in WT mice. C, ITGAX-DTR-GFP mice were s.c. inoculated with 10^7 ID8-Defb29/Vegf-a cells (in 200 μL Matrigel). After 10 d, mice received 2 ng/g DT in PBS (ITGAX + DT) or a similar volume of PBS (ITGAX + PBS). D, DT (Depleted), but not PBS (Control), administration eliminates tumor CD11c+ DCs (GFP+, arrows) within 48 h. Magnification is included. Data are representative of three independent experiments.

**Histology, flow cytometry, and Bio-Plex.** Biotinylated tomato lectin (150 μg in 150 μL of PBS; Vector) was injected through the left ventricle and allowed to circulate. The vasculature was then perfused with 1% paraformaldehyde in PBS for 3 min. Cryosections of OCT-embedded tumors (8 μm thick) were immunostained as described (6). Biotinylated lectin was detected by rhodamine-streptavidin (Leinco) or Alexa Fluor 350 (Molecular Probes). Green fluorescent protein (GFP) signal was enhanced with a rabbit anti-GFP Alexa Fluor 488 antibody (Molecular Probes).

Flow cytometry was performed on a FACSCanto (BD Biosciences). (CD45+DEC205+VE-cadherin+) DCs were sorted from mechanically dissociated human ovarian carcinoma specimens using a FACSAria sorter (BD Biosciences).
An allophycocyanin-labeled tetramer consisting of Kβ folded with GQKMNAQAI peptide was provided by the NIH Tetramer Core Facility (Atlanta, GA).

Anti-mouse antibodies were specific for CD31 (MEC13.3), CD45 (30-F11), CD45.1 (A20), CD25 (PC61), CD69 (H1.2F3), and CD11c (HL3; all from BD Biosciences); VE-cadherin (rabbit; MedSystems Diagnostics); NG2 (rabbit; Millipore); DEC205 (NLDC145; Serotec); MHC-II (NIMR-4; eBioscience); FoxP3 (staining buffer set; eBioscience); and anti-actin and α-smooth muscle-Cy3 (1A4; Sigma). Anti-human antibodies were specific for CD45 (HI30), DEC205 (MG38), CD3 (UCHT1), CD4 (OKT4), CD8 (RPA-T8), CD31 (M89D3), CD11b (ICRF44), and CD14 (M5E2), all from BD Biosciences.

Human VEGF-A, basic fibroblast growth factor (FGFb), and interleukin-8 (IL-8) and mouse Vegf-a, FGFb, and CXCL1 were detected using a Human-27-Plex or a Mouse-9-Plex panel cytokine assays, respectively (Bio-Rad), with supernatants from human CD45+DEC205+ leukocytes sorted from dispersed ovarian carcinoma samples, or with mouse CD11c+DEC205+ cells sorted from tumor ascites, following the manufacturer’s instructions.

**Results**

**Elimination of CD11c+ DCs decreases ovarian cancer growth.** To investigate the contribution of CD11c+ DCs to ovarian tumor progression, we used CD11c-DTR-GFP transgenic mice (ITGAX-DTR-GFP). In this strain, CD11c+ DCs express the receptor of DT as well as traceable green fluorescence and can be transiently depleted (>92%) by DT administration, whereas DT administration has no effect on CD11c+ cells in wild-type (WT) mice (25, 26). WT or ITGAX-DTR-GFP mice (n = 6 per group; two independent experiments) were treated with either i.p. DT or PBS and immediately challenged with s.c. ID8-Defb29/Vegf-a cells, an aggressive epithelial ovarian cancer line (6). As we have previously reported, these tumors are heavily infiltrated by DEC205+CD11c+MHC-IIlow DCs (6, 8). Supporting the DC lineage of their human counterpart, most DEC205+ leukocytes infiltrating unselected human solid primary (n = 3) or metastatic (n = 5) ovarian carcinoma specimens were CD11b+/C0, whereas expression of CD14, although more variable, was found on a minority of DEC205+ cells in most specimens analyzed (Fig. 1A; Supplementary Fig. S1A). In contrast, and in agreement with previous reports (27), we found that most leukocytes in tumor ascites (n = 3) were monocyte/macrophage cells (CD14+/CD11b−), underscoring the importance of microenvironmental factors (Fig. 1B; Supplementary Fig. S1B). Most importantly, the temporary ablation of mouse CD11c+ DCs at the time of tumor injection resulted in 3-fold lower tumor size after 2 months (441.4 ± 59 mm<sup>3</sup> versus 146.8 ± 33.4 mm<sup>3</sup>; P < 0.05, Wilcoxon test; Fig. 2A). Reduced tumor growth was not caused by an unknown antitumor effect of DT because tumor growth was not affected in WT littermates (Fig. 2B).

To determine the involvement of DCs in the progression of established tumors, we performed a temporary depletion of CD11c+ cells in mice bearing 10-day-old flank ovarian tumors (n = 10 per group). This resulted in a 3-fold reduction in tumor size for 5 additional weeks of growth (713.2 ± 65.1 mm<sup>3</sup> versus 254.1 ± 137 mm<sup>3</sup>; P = 0.03, Wilcoxon test; Fig. 2C). Thus, CD11c+ cells were also involved in the growth of established tumors.
Elimination of CD11c+ DCs results in tumor necrosis and increased survival. Histologic analyses after DT administration showed substantial necrosis in central areas of the tumor, compared with mice receiving PBS, and confirmed the elimination of CD11c+ (GFP+) cells from viable tumor locations (Fig. 2D). Tumor necrosis became obvious after ~36 h of treatment and reached a maximum at 48 h (Fig. 3A). As antitumor T cells require longer than 36 h to become reactivated (28), it is unlikely that these initial antitumor effects were caused by an adaptive immune response. Because of the role of CD11c+ cells in ovarian cancer vascularization (6, 7), we hypothesized that impeded tumor vascularization accounted for the reduction in tumor growth. Correspondingly, preceding tumor cell death (24 h after DT administration), we found expression of active caspase-3 (indicative of apoptosis) on ~40% of (CD31+) blood vessels in all tumors examined, whereas tumor samples from control mice only showed scattered caspase-3 staining on CD31+ cells (Fig. 3B). Together, these data indicate that CD11c+ DCs acquire a proangiogenic phenotype in the tumor microenvironment that is important for the maintenance of tumor vasculature and therefore the progression of ovarian cancer.

However, although the ID8-Defb29/Vegf-a cell line induces aggressive tumors that mimic the physiopathology of human ovarian cancer (6), it is maximally engineered to attract immature DCs. To establish the therapeutic potential of constantly depleting CD11c+ DCs in hosts bearing orthopic (i.p.) ovarian tumors that do not ectopically express Defb29 and Vegf-a, we reconstituted lethally irradiated congenic (CD45.1+) mice with bone marrow from (CD45.2+) ITGAX-DTR-GFP animals, which allows multiple injections of DT without compromising survival (26). Eight weeks after reconstitution, >95% of blood leukocytes were of donor origin in all mice (data not shown). Animals (n = 6 per group; two independent experiments) were then challenged with parental ID8 cells, expressing luciferase for intravital monitoring of tumor progression. In treated mice, DCs were then depleted every 4 days with i.p. DT, whereas control animals were injected with PBS. Depletion of CD11c+ cells resulted in a reduction of tumor burden (Fig. 3C; Supplementary Fig. S2A) and induced a significant increase in survival (P < 0.01, log-rank test; Fig. 3D).

A novel anti-CD11c IT eliminates tumor-infiltrating DCs in nontransgenic hosts. To define the therapeutic potential of depleting DCs in nontransgenic tumor-bearing hosts, we generated an anti-CD11c IT by cloning the variable regions of the anti-mouse CD11c N418 hybridoma, fused to a Pseudomonas exotoxin A lacking the cell-binding domain (29, 30). The IT was expressed and purified, along with the control truncated toxin lacking the single-chain antibody, which is harmless because it cannot get internalized.

Incubation of CD11c+ DCs sorted from tumor ascites with 15 mg/mL of IT resulted in the death of ~70% of cells within 40 h. Interestingly, the same dose of IT caused the death of only ~30% of bone marrow–derived DCs (Supplementary Fig. S2B), whereas the...
i.v. administration of 20 μg of IT (n = 3) eliminated <40% of splenic CD11c+ cells after the same period (Supplementary Fig. S2C). To test the effects of our IT in tumor-bearing hosts, we collected ascites from mice with ID8-Defb29/Vegf-a tumors (n = 5), which contained ~30% of CD11c+ cells. Tumor ascites cells were carboxyfluorescein diacetate succinimidyl ester (CFSE) labeled and i.p. injected (2 × 10^6 cells per mouse) into healthy C57BL/6 animals (n = 5 per group). After 5 h, mice received an i.p. injection of IT (0.33 mg/kg body weight) or PBS (control). Two days later, CD11c+CFSE+ cells were found in peritoneal samples, but not in any other locations, in both groups, although the total was 5-fold lower in mice treated with the anti-CD11c IT compared with control animals (Supplementary Fig. S2D). In contrast, CD11c+ CFSE+ cells were not decreased. Importantly, comparable proportions of CD11c+CFSE+ DCs were found in spleen and draining (mediastinal) lymph node samples from both groups. These data indicate that the anti-CD11c IT can predominantly deplete peritoneal ovarian cancer–infiltrating DCs with little effect on DCs from lymphatic compartments that may potentially elicit antitumor immunity.

**Depletion of CD11c+ DCs delays tumor progression and enhances the effect of standard chemotherapies.** Weekly i.v. injections of anti-CD11c IT induced a dramatic decrease in the growth of established ID8-Defb29/Vegf-a flank ovarian tumors (n = 5 per group) when treatment started at day 7 (6-fold; 109.8 ± 65.1 mm^3), or as late as day 21 (3.5-fold; 184.60 ± 75.1 mm^3), compared with mice treated with a truncated toxin (646.1 ± 163.2 mm^3; P < 0.001 for both, Wilcoxon test; Fig. 4A, left). As observed in transgenic mice, the depletion of CD11c+ DCs induced central necrosis within 48 h (Fig. 4A, right).

As a model for aggressive human ovarian carcinoma, we i.p. injected ID8-Defb29/Vegf-a cells into WT mice (n ≥ 6 mice per group; refs. 6, 31). Mice with established tumors received the chemotherapeutic drug topotecan along with various doses of the anti-CD11c IT or truncated control. Topotecan or IT treatment alone induced a similar and significant 20% increase in the median life span (P < 0.05, log-rank test; Fig. 4B). No weight loss or animal death was detected throughout the treatments. Furthermore, the combination of topotecan and a daily treatment with IT for 2 weeks resulted in a further 18% increase in survival (P < 0.05). Importantly, a continuous administration of IT following the administration of topotecan extended life span by 53% compared with individual treatments (P < 0.01). Corresponding results were observed at lower doses of IT (Supplementary Fig. S2F).

Even in the absence of antitumor adaptive immunity, i.v. administration of anti-CD11c IT also impaired the growth of severe combined immunodeficient (SCID) mice (2,267 ± 398 mm^3 versus 832 ± 250 mm^3; n = 5 per group; P = 0.03, Wilcoxon test; Supplementary Fig. S2F). Although these effects can be only attributable to the impairment of tumor vasculature, central necrosis after DC depletion was observed, although less dramatic than in ID8-Defb29/Vegf-a tumors (Supplementary Fig. S2G).

Suggesting the potential of using multiple targeting strategies, the beneficial effect of DC depletion was confirmed by administering an anti-CD11b saporin-based IT, which also induced a significant increase in survival compared with saporin alone (P < 0.01; Fig. 4C).

**Depletion of DEC205+ DCs induces comparable antitumor effects.** To confirm that the impairment of tumor progression is only attributable to the elimination of the predominant CD11c+DEC205+ DC population, we used a different IT consisting of the DEC205-specific NLDC145 antibody, conjugated to ribosome-inactivating saporin. Although DEC205 is expressed on other cells, this antibody primarily targets CD11c+MHC-II+ DCs in vivo (32). We confirmed that the i.p. administration of 0.25 mg/kg body weight of anti-DEC205-saporin resulted in the elimination of >50% of splenic DCs, but not other leukocyte subsets, in healthy mice (Supplementary Fig. S2H). The same dose of anti-DEC205-saporin every 4 days in mice bearing i.p. ID8-Defb29/Vegf-a ovarian cancer resulted in a >30% increase in the median life span compared with mice injected with PBS (P < 0.01, log-rank test; Fig. 4D). In addition, control mice treated with the same regimen of unconjugated anti-DEC205 antibody also exhibited a significant (P < 0.05, log-rank test) increase in survival compared with mice receiving PBS, although it was lower than that induced by anti-DEC205-saporin (P < 0.05, log-rank test; Fig. 4D). Together, these data confirm that the elimination of DCs and not other leukocytes from tumor-bearing hosts accounts for the antitumor effects.

**Elimination of tumor DCs induces a boost in specific antitumor immunity.** Although at the latest stage of cancer progression virtually all T lymphocytes in tumor ascites were not activated (CD69+/CD11c-) in either group, up to 40% of cytotoxic T cells from animals depleted of DEC205+ DCs were antigen experienced (CD44+) compared with 4% in control mice (Fig. 5A). We surmised that the release of tumor antigens induced by the elimination of most DCs and the subsequent tumor disruption may enhance, rather than impair, the endogenous antitumor immune response. To test this hypothesis, C57BL/6 WT mice (n = 4) were challenged with ID8-Defb29/Vegf-a tumors, and after 5 days of tumor progression, mice were depleted of DCs every 2 days with i.p. injections of anti-CD11c IT (0.2 mg/kg) for 2 weeks. One month after tumor challenge, before the occurrence of massive ascites, the elimination of DCs resulted in an increase in the proportion of activated (CD69+) CD8+ T cells in peritoneal wash samples. Most importantly, the peritoneal cavity of mice depleted of CD11c+ DCs contained a ~6-fold higher proportion of cytotoxic T cells specifically recognizing an H-2D^b–restricted mesothelin epitope expressed by ID8 tumor cells, ranging from 2% to 5% of CD8+ T cells (Fig. 5B; ref. 33).

To confirm the antigen specificity of the immunogenic boost against a different antigen, we challenged CD8+ transgenic ovalbumin (OVA)-specific (OT-I) mice with OVA-expressing ID8 cells admixed with tumor ascites and injected them with IT or the truncated toxin 1 day later. As expected, the spleens of mice depleted of DCs exhibited a much higher frequency of T cells producing IFN-γ in response to OVA (Fig. 5C) and irradiated tumor cells (Fig. 5D) in enzyme-linked immunospot (ELISPOT) analysis compared with spleens from control mice. Together, these data confirm that the elimination of CD11c+DEC205+ DCs from the tumor microenvironment, likely followed by the possible generation of new waves of DCs to a less suppressive milieu, dramatically enhanced, rather than abrogated, the specific antitumor immune response.

**Tumor-infiltrating DCs acquire pericyte-like attributes.** Vascular apoptosis and ensuing central necrosis resulting from

---

*Unpublished observations.*
Figure 5. Elimination of DCs boosts antitumor immune responses. A, FACS analysis of CD44 and CD69 in tumor ascites from tumor-bearing mice injected with the anti-DEC205 IT (Depleted) or PBS (Control). B, FACS analysis of peritoneal wash samples from tumor-bearing mice injected with the anti-CD11c IT (Depleted) or PBS (Control) 1 mo after tumor challenge. C, OT-I mice (n = 4) were i.p. injected with 10^6 OVA-expressing ID8 cells mixed with 10^7 ID8-Defb29/Vegf-a tumor ascites cells (containing 30% CD45^+CD11c^+VE-cadherin^+ DCs) and received 0.5 mg/kg of IT or truncated toxin (Ctrl) 1 d later. After 7 d, mice received again 10^7 ID8-Defb29/Vegf-a tumor ascites cells i.p. followed by a new DC depletion or control treatment at day 8. Splenocytes were collected at day 11 and stimulated for 7 d with OVA (1 μg/mL, Sigma). IFN-γ ELISPOT was performed against bone marrow–derived DCs pulsed with OVA (1 μg/mL, 10:1, splenocyte to DC ratio). D, depletion of CD11c^+ DCs in OT-I transgenic mice also enhanced the amount of IFN-γ-producing splenocytes responding to DCs pulsed for 4 h with γ-irradiated and UV-irradiated OVA-ID8 tumor cells (10:1, DC to ID8 ratio).

the elimination of CD11c^+ DCs supports our previous finding that tumor-infiltrating DCs are important for tumor vascularization (6). Confirming the massive recruitment of DCs by ovarian tumors, 85% of CD11c^+ cells banding in the viable interface after density gradient centrifugation of mechanically dissociated ID8-Defb29/Vegf-a solid tumors coexpressed DEC205 (Supplementary Fig. S3A). To further characterize their role in tumor blood vessel formation, we perfused the vasculature of flank tumor-bearing ITGAX-DTR-GFP mice (n = 6 per group) by intracardiac injection of biotinylated tomato lectin. All specimens contained structures assembled by GFP^+ (CD11c^+) cells, which primarily clustered around the tumor periphery, suggesting a permanent recruitment of these cells to the area of growth. Although these also represent areas of active angiogenesis, most peripheral CD11c^+ structures were not perfused by lectin (Fig. 6A, left, and B, left) nor contained erythrocytes (data not shown), indicating that they are assembled before being perfused by blood. In contrast, in central areas of the tumor, >50% of perfusable blood vessels were surrounded by irregularly scattered GFP^+ (CD11c^+) leukocytes (Fig. 6A, right). Although GFP fluorescence colocalized with intravascular lectin in selected structures inside the tumor (Fig. 6B, middle), most GFP^+ cells in central areas were distributed in a pericyte-like pattern (near endothelial cells but in outer layers) or a perivascular pattern (near the vessel wall but not in contact with vessels; Fig. 6B, right). Surprisingly, virtually all CD45^+CD11c^+DEC205^+ leukocytes in dispersed solid tumors or tumor ascites expressed α-smooth muscle actin (SMA; Fig. 6D; Supplementary Fig. S3B and C) and NG2 (Supplementary Fig. S3D, left) but not platelet-derived growth factor receptor (Supplementary Fig. S3D, right). As reported (6, 7), most DCs also coexpressed VE-cadherin, which was confirmed by PCR (Supplementary Fig. S3B) and further supported by the presence of numerous tumor-infiltrating MHC-II^+GFP^+ leukocytes in mice transferred with transgenic VE-cadherin-GFP bone marrow cells (Supplementary Fig. S3E). More importantly, CD45^+CD11c^+ DCs sorted from mouse tumor ascites secreted Vegf-a and CXCL1, whereas CD45^+DEC205^+ cells procured from dispersed stage III to IV human ovarian carcinoma specimens, but not CD4^+ lymphocytes, secreted FGFb, VEGF-A, and high levels of CXCL8 (Fig. 6E). These factors are known to induce the recruitment and promote the survival of sprouted endothelial cells (34).

Discussion

Here, we show that the depletion of DCs in mice bearing ovarian tumors with four different methods that target one of three DC markers (CD11c, DEC205, or CD11b) significantly delayed cancer progression. DC depletion activates multiple antitumor mechanisms. On the one hand, it causes vascular apoptosis followed by tumor necrosis, supporting the concept of a critical contribution of DCs to ovarian cancer vascularization (6). On the other hand, transient elimination of DCs paradoxically boosted the antitumor immune response, likely because the early disruption of tumor tissue makes available massive amounts of tumor antigens to undepleted or newly generated DCs. Therefore, our findings provide a mechanistic foundation to enable more effective biological therapy for cancer patients targeting tumor-associated leukocytes and other critical elements of the tumor microenvironment to complement the standard "surgical debulking/chemotherapy" approach.

DCs are primarily viewed as professional antigen-presenting cells specialized in the stimulation of naive T lymphocytes. Because our system only allows the depletion of most but not all DC subsets, and only in a temporary manner, our results do not challenge this dogma. Because at least 85% of CD11c^+ cells in the solid tumor microenvironment coexpresses DEC205, these effects should be attributed to the elimination of DCs and not other non-DC leukocytes. It is likely that by "resetting the system," newly generated DCs at lymphatic locations engulf these antigens and present them to naive T cells in a less tolerogenic milieu.
Ovarian tumor cell death takes place more rapidly after DC depletion than with standard antiangiogenic strategies based on VEGF signaling neutralization and is preceded by vascular cell apoptosis, suggesting that tumor-infiltrating DCs are important for maintaining tumor blood vessels. The crucial role of different leukocyte subsets on neoangiogenesis has been previously recognized (35–37). In ovarian cancer, CD11c+ cells home to the wall of most blood vessels within the tumor, where they express SMA and NG2 and secrete VEGF-A, FGF, and IL-8, which are critical factors for the recruitment and support of sprouted endothelial cells. CD11c+ cells also assembled unperfused structures and express VE-cadherin in areas of active angiogenesis, suggesting that they could be also important for initiating nascent tumor vessels. Although tumor-infiltrating DCs acquire endothelial-like and pericyte-like attributes in different tumor areas, we cannot claim with our current data that they turn into bona fide endothelial cells or canonical pericytes, as they retain leukocyte markers. Our results rather point to an alternative “scaffold” model, whereby CD11c+ phagocytes (8) generate tubular structures in the extracellular matrix, which are permeated by blood due to the leaky nature of tumor vasculature. Sprouted endothelial cells brought by the blood flow would then line these channels, displacing leukocytes to outer layers as the vessel matures. DCs could therefore provide paracrine as well as direct structural support for the survival, stabilization, and branching of new blood vessels, thus becoming crucial players in ovarian cancer vascularization.

Figure 6. DCs support tumor vascularization. Tumor-bearing ITGAX-DTR-GFP mice were perfused with an intracardiac injection of biotinylated tomato lectin at different times after tumor inoculation. A, left, unperfused (white arrows) and blood transporting (yellow arrows) structures assembled by CD11c+ (GFP+) cells mixed in the growing edge of all specimens analyzed. Magnification, ×200. Right, in central areas of the tumor, CD11c+ (GFP+) leukocytes are irregularly scattered on the wall of most neovessels. Magnification, ×400. B, confocal microscopy analysis of the different kinds of structures assembled by CD11c+ (GFP+) DCs. Magnification, ×600. Left, another example of a different unperfused GFP+ structure; middle, colocalization of tomato lectin and GFP+ cells in selected blood vessels; right, projection of stack images. CD11c+ cells in big vascular arrangements were predominantly found in an abluminal second layer. C, FACS analysis of SMA and VE-cadherin expression on CD11c+ DCs from tumor ascites. D, CD45+DEC205+ DCs (hDEC205+) or CD3+CD4+ lymphocytes (hCD3+D4+) were FACS sorted from eight unselected human ovarian carcinoma suspensions. CD45+CD11c+ DCs (mCD11c+) were sorted from the ascites of ID8-Defb29/Vegf-a tumor-bearing mice. Cells (10⁶/mL) were stimulated for 4 h with phorbol 12-myristate 13-acetate/ionomycin (50 ng/1 μg/mL). Cytokines were determined by Bio-Plex analysis.
In summary, our results show how the elimination of proangiogenic DCs by targeting different DC-specific markers impairs ovarian cancer progression. Our findings provide a rationale for the design of more effective cancer interventions that incorporate the targeting of critical contributors to cancer progression different from tumor cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 4/2/2008; revised 5/19/2008; accepted 6/11/2008.

Grant support: 2006 Liz-Tilberis Award, National Cancer Institute grants R01CA124515 and R01CA101748, American Cancer Society award IBG-82-003-22, and National Center for Research Resources grant 2P20RR016437-06. E. Huarte was supported by the "Ramon y Cajal" program.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the Immunology Laboratory and the NIH Tetramer Core Facility for performing the Bio-Plex experiments and providing the tetramer, respectively.

References


4. Hasegawa K, Koizumi F, Noguchi Y, et al. SSX and LAGE-1 cancer-testis antigens are potential targets for performing the Bio-Plex experiments and providing the tetramer, respectively.


Depletion of Dendritic Cells Delays Ovarian Cancer Progression by Boosting Antitumor Immunity

Eduardo Huarte, Juan R. Cubillos-Ruiz, Yolanda C. Nesbeth, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-08-1167

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2014/09/16/0008-5472.CAN-08-1167.DC1

Cited articles
This article cites 37 articles, 17 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/18/7684.full.html#ref-list-1

Citing articles
This article has been cited by 16 HighWire-hosted articles. Access the articles at:
/content/68/18/7684.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.