Scavenger Receptor-A–Targeted Leukocyte Depletion Inhibits Peritoneal Ovarian Tumor Progression

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Abstract

Immunosuppressive leukocytes are emerging as a critical factor in facilitating tumor progression. These leukocytes are converted by the tumor microenvironment to become tolerogenic, facilitate metastasis, and to aid in neovascularization. The predominant variety of suppressive leukocytes found in human and murine ovarian cancer are called vascular leukocytes (VLC), due to sharing functions and cell surface markers of both dendritic cells and endothelial cells. Using the ID8 murine model of ovarian cancer, the aim of this study was to test the efficacy of VLC elimination as an ovarian tumor therapy. We show that carrageenan-mediated depletion of peritoneal tumor-associated leukocytes inhibits ovarian tumor progression. We then identified scavenger receptor-A (SR-A) as a cell surface receptor that is robustly and specifically expressed within human and murine ovarian tumor ascites upon VLCs. Administration of anti–SR-A immunotoxin to mice challenged with peritoneal ID8 tumors eliminated tumor-associated VLCs and, importantly, substantially inhibited peritoneal tumor burden and ascites accumulation. Moreover, the toxin required targeting to SR-A because mice that received untargeted toxin did not exhibit inhibition of tumor progression. We conclude that SR-A constitutes a novel and specific target for efficacious immunotherapeutic treatment of peritoneal ovarian cancer. [Cancer Res 2007; 67(10):4783–9]

Introduction

Leukocytes play an important role in the initiation and progression of tumors. It is becoming increasingly evident that tumors recruit leukocytes and co-opt them to promote a microenvironment that facilitates tumor growth and metastasis (1–4). The leukocytes create a tolerogenic, proinflammatory environment, suppress antitumor T-cell immune responses, assist in tissue remodeling and neovascularization, and potentiate metastasis. Importantly, a number of studies have shown that leukocytes are obligate partners in tumor growth, including the finding that ablation of F4/80⁺ macrophages in solid tumor models inhibits angiogenesis (5). Thus, depletion of tumor-infiltrating leukocytes has become a promising strategy for therapeutic treatment of cancer. However, this presents the challenge of identifying a method that specifically targets these cells, that efficiently inhibits or eliminates them, and that is clinically applicable.

A variety of leukocyte populations have been identified that promote tumor growth, including myeloid suppressor cells, macrophages, and granulocytes (6–9). Indeed, the tumor infiltration by macrophages or dendritic cells correlates inversely with clinical outcome (10–12). In ovarian cancer, the predominant (>30% of the total nucleated cells including tumor cells and >80% of the CD45⁺ cells, in both murine and human ovarian ascites) tumor-recruited leukocyte population is the vascular leukocytes (VLC; ref. 13). Eponymous, these cells exhibit cell surface markers of both dendritic cells (CD11c, DEC205, and CCR6) and endothelial cells (P1H12 and VE-cadherin; refs. 13, 14). Importantly, they also function in both capacities: they can present antigen and they colocalize with, and promote, nascent functional vasculature (13). Moreover, neutralization of VLCs via CCR6 in a solid tumor model impeded tumor growth (13). Therefore, we hypothesized that identification of an effective method to deplete VLCs from ovarian cancer, the clinical manifestation of which is peritoneally localized, would therapeutically inhibit cancer progression.

We used the ID8 murine model of ovarian cancer to assess the requirement for VLCs in peritoneal tumor progression and, significantly, to test the therapeutic benefit of VLC elimination. The ID8 ovarian tumor cell line is derived from murine ovarian epithelial cells, and its progression after peritoneal injection mimics that of human ovarian carcinomas (15). Peritoneal ID8 expansion recruits VLCs into the ascites, and this recruitment can be enhanced with the use of ID8 cells transduced to express the proangiogenic and proinflammatory molecules Vegf-A and β-defensin-29 (herein called ID8), respectively (13). There, the VLCs are thought to contribute to a tolerogenic environment that consequently promotes tumor progression.

Here, we show that scavenger receptor-A (SR-A) is a specific and effective cell surface receptor by which to deplete peritoneal tumor-recruited VLCs. SR-A is robustly expressed by both human and murine VLCs, and its expression within ovarian tumor ascites is limited to VLCs. Importantly, immunotherapy with an anti–SR-A immunotoxin depleted VLCs, inhibited tumor progression, and blocked ascites accumulation. These data identify SR-A as a novel and effective target for ovarian tumor immunotherapy and, moreover, support that leukocyte depletion from the tumor microenvironment is a viable and efficacious treatment for ovarian cancer.

Materials and Methods

Mice. Female C6B6F1 mice (4–6 weeks) were purchased from the National Cancer Institute (Fredricksburg, MD). Animal experiments were approved by the Dartmouth Medical School Institutional Animal Care and Use Committee.

Cells and antibodies. ID8 cells transduced with Defb29 and Vegf (referred to as ID8 cells) and ID8-Vegf cells transduced with green
fluorescent protein (GFP; ID8-C3) were generated and maintained as previously described (13). Human ovarian carcinoma samples were obtained as in previous studies (14). Lewis lung carcinoma (LL/2) cells were obtained from the American Type Culture Collection. Bone marrow–derived dendritic cells were generated as previously described (16, 17). Anti–mouse Fc Block was purchased from BD Biosciences; biotin anti-DEC205 (clone MG38), streptavidin-allophycocyanin (APC), and APC anti-CD45 (50-F11) antibodies from eBiosciences; anti–SR-A (2F8) antibodies from Serotec; APC anti-CD11c (N481) antibodies from Biolegend; anticalreticulin antibodies from Abcam; anti–VE-cadherin antibodies from Bender Medsystems; and anti–lipoprotein receptor-1 (LOX-1) antibodies (23C11) from Cell Sciences. Antihuman monoclonal SR-A (SRA-C6) antibodies were generated as previously described (18). Goat anti-rat IgG conjugated to saporin (Rat-ZAP) was purchased from Advanced Targeting Systems. The Zenon reagent was a generous gift of Dr. C. Sentman (Dartmouth Medical School, Hanover, NH).

**Generation of tumors and harvest of tumor-associated leukocytes.**

Ovarian tumors were generated as previously described (13). Briefly, mice were injected i.p. with 5 × 10^6 ID8 cells. Approximately 6 to 7 weeks later, peritoneal ascites were harvested. The cellular fraction was treated with ACK lysis buffer (0.15 mol/L NH_4Cl, 1.0 mmol/L KHCO_3, 0.1 mmol/L EDTA) to remove RBC, and the remaining cells were resuspended in 0.5% bovine serum albumin (BSA) in PBS or medium for analysis. Alternatively, mice were injected with 1 × 10^7 LL/2 cells s.c. in the right flank. After 9 days, palpable tumors were excised, passed through a 70-μm filter, resuspended in HBSS (Mediatech) with collagenase, and incubated for 2 h at 37°C. The cells were then resuspended in 0.5% BSA in PBS or medium for analysis.

**Flow cytometry and Western blotting.** Cells from murine or human ascites were resuspended at 1 × 10^6/mL in 0.5% BSA in PBS with Fc-blocking antibody and subsequently stained with the indicated antibodies. The antihuman LOX-1 and SR-A antibodies were labeled with the Zenon reagent, according to the manufacturer’s protocol, before staining fluorescence-activated cell sorting (FACS) samples. Flow cytometry was done at the Norris Cotton Cancer Center Englert Cell Analysis Laboratory using a FACS Calibur or FACS Aria, and were subsequently analyzed using CellQuest. For Western blot analysis, FACS-sorted (CD45+VE-cadherin+) VLCs, ID8, or bone marrow–derived dendritic cells were suspended in sample buffer with β-mercaptoethanol, heated for 5 min at 90°C, then run on a 12% SDS-PAGE gel. After transfer to a polyvinylidene difluoride membrane and blocking, membranes were incubated overnight with

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**Figure 1.** Carrageenan-mediated depletion of leukocytes inhibits peritoneal ID8 ovarian cancer progression. A and B, to test VLCs for phagocytic capability, CD45+VE-cadherin+ VLCs were sorted by FACS and allowed to take up 1 μm fluorescent beads (FloSpheres) for 1 h at 37°C. A, cells given FloSpheres were washed and analyzed by flow cytometry. Greater than 87% (open histogram) accumulated beads. B, representative confocal microscopy image of bead uptake by VLCs (×63 magnification). Sorted VLCs given FloSpheres were fixed and subsequently stained with wheat germ agglutinin-488 (green) to decorate the plasma membrane, and with DAPI (blue). C, to test the effectiveness of carrageenan to deplete peritoneal macrophages, carrageenan in PBS, or PBS alone, was injected i.p. into mice. After 72 h, peritoneal exudates from the PBS-treated (top) and carrageenan-treated (bottom) mice were stained for SR-A and F4/80, and analyzed by flow cytometry. D, ablation of peritoneal phagocytic cells inhibits ascites development in murine ID8 ovarian cancer. Mice were injected i.p. with 1 mg type II carrageenan (carrageenan-treated, ◊) or vehicle alone (untreated, □) on days –4 and –2 before tumor challenge. On day 0, mice were injected i.p. with 5 × 10^6 ID8 cells. Subsequent injections of carrageenan or carrier were given twice weekly. Mice were weighed weekly to assess weight gain due to peritoneal ascites accumulation. Bars, SD. Statistical significance (*P < 0.05, **P < 0.01) was determined with the paired Student’s t test.
primary antibody, washed, blocked, and incubated with an horseradish peroxidase secondary antibody. Bands were detected with ECL Plus Western blotting detection reagent (Amersham Biosciences).

**Phagocytic assays.** Sorted VLCs (CD45\(^+\)VE-cadherin\(^+\)) were resuspended in 1 mL at a concentration of \(1 \times 10^6\) cells/mL. One microliter of 1 \(\mu\)m green/yellow or crimson latex FluoSpheres (Molecular Probes) was added to the cells, and cells were allowed to phagocytose for 1 h at 37°C. Samples were then washed twice with PBS. Cellular FluoSphere uptake was analyzed by FACS. For microscopy, cells incubated with crimson Fluosphere were then stained with Alexa-488–labeled wheat-germ-agglutinin (Molecular Probes) for 10 min on ice, washed with PBS, and fixed for 20 min on ice with 2% paraformaldehyde. Cells were then washed, stained with 4',6-diamidino-2-phenylindole (DAPI; Molecular Probes) for 10 min on ice, washed with PBS, and fixed for 20 min on ice with 2% paraformaldehyde. Cells were then washed, stained with 4',6-diamidino-2-phénylindole (DAPI; Molecular Probes) for 10 min on ice, resuspended in mounting medium (50% glycerol, 50% DABCO), and affixed to slides. Microscopy was performed with a Zeiss LSM510 meta microscope (Carl Zeiss Microimaging), taking single optical sections using a \(\times 63\) lens, which in some cases were reassembled as Z-series movies or were flattened into single images using LSM5 Image Browser.

**Carrageenan treatment of ID8 ovarian tumors.** Mice were injected i.p. with \(5 \times 10^6\) ID8 cells (day 0). To ablate phagocytic tumor-infiltrating cells, mice were given 1 mg of type II carrageenan (Sigma) in 500 \(\mu\)L PBS, or 500 \(\mu\)L of PBS alone, on days −4 and −2. Mice were subsequently given additional administrations of carrageenan or sham treatment two weekly. Mouse body weight was monitored to assess tumor progression. Data is derived from at least four mice per treatment group.

**Anti-SR-A targeting of VLCs.** Preparation of anti-SR-A immunotoxin: 4.8 \(\mu\)g of Rat-ZAP in the absence (ZAP) or presence of 4 \(\mu\)g of clone 2F8 rat anti-mouse SR-A antibody (SRA-ZAP) was incubated on ice for 30 min. C576F1 mice were injected i.p. with \(5 \times 10^6\) ID8-C3 cells, in conjunction with SRA-ZAP or ZAP, on day 0. Additional administrations of SRA-ZAP or ZAP were then given every 7 days. Mice were weighed weekly to monitor tumor progression. All mice were sacrificed, ascites volume was measured, and ascites cellularity was assessed after ACK lysis of RBC; in some cases, peritoneal lavage was necessary for mice with ascites <5 mL. Cells were stained with anti-CD11c antibody, and numbers of CD11c\(^+\) (CD11c\(^+\)GFP\(^+\)) and tumor cells (CD11c\(^+\)GFP\(^+\)) in the peritoneum of the mice were calculated by flow cytometry. Data are derived from three separate experiments, with a total of at least six mice in each treatment group.

**Results**

**Depletion of peritoneal leukocytes inhibits ID8 ovarian tumor progression.** Targeted depletion of tumor-associated cells of myeloid origin has proved efficacious in a number of tumor models (5, 19). To test the effect of leukocyte depletion on ovarian cancer, we used the murine ID8 tumor model that causes peritoneal ascites, and exploited the phagocytic abilities of the tumor-associated leukocytes. VLCs, which comprise the majority of the leukocyte population derived from peritoneal ovarian tumors, were efficiently phagocytosed by latex beads. FACS-sorted CD45\(^+\)VE-cadherin\(^+\) VLCs were incubated with fluorescently labeled beads, washed extensively, and analyzed for the presence of internalized beads by FACS analysis and confocal microscopy. The majority of VLCs (>87%) from the ascites of tumor-bearing mice phagocytosed at least one fluorescent bead (Fig. 1A). To confirm that the FACS analysis was reflective of phagocytic uptake rather than merely cell surface binding of the beads, the cells were inspected by microscopy. Fluorescent beads were observed within the VLCs, as seen in a single optical section using confocal microscopy (Fig. 1B) and with reconstruction of a confocal Z-series (Supplementary Data 1).
The phagocytic capability of VLCs provided a mechanism we could use to deplete tumor-associated leukocytes. Carrageenan is a polysaccharide that depletes phagocytic cells when delivered to the peritoneum (20). F4/80<sup>+</sup> peritoneal macrophages in naïve mice were observed to be eliminated 72 h after an i.p. injection of carrageenan (Fig. 1C). To test the effects of carrageenan-mediated leukocyte depletion on ovarian tumor progression, CB6F1 mice were injected with carrageenan on days −4 and −2 prior to subsequent tumor challenge with 5 × 10<sup>6</sup> ID8 cells. Additional carrageenan administrations were given twice weekly. Peritoneal ovarian tumor progression, both clinically and in the ID8 murine model, results in vascular leakage and cellular infiltration characterized by peritoneal ascites (15, 21). Therefore, mouse weight, as a function of ascites accumulation, was used to assess tumor progression. Carrageenan-treated mice were protected from the rapid expansion of ascites beginning around day 30 that the untreated mice exhibited (Fig. 1D). These data indicate that the depletion of tumor-associated leukocytes with the use of carrageenan slows tumor growth and reduces ascites burden. However, carrageenan is not a viable clinical method for ablation of tumor-associated leukocytes; therefore, we sought a specific cell surface receptor on VLCs that could be exploited for targeted depletion of these cells.

**Identification of scavenger receptors on VLCs.** VLCs are proposed to be obligate partners for human and murine ovarian cancer progression. These cells express both endothelial cell surface markers (VE-cadherin; Fig. 2A; refs. 13, 14) and the leukocyte markers CD45 and CD11c (Fig. 2A and B2). Our criteria for cell surface receptors by which to target VLCs were that the receptor expression should be specific to VLCs, the receptor should be expressed on both murine and human VLCs, and the receptor has characterized endocytic function with which to efficiently internalize a targeted ligand. Microarray data indicated that a pair of scavenger receptors, LOX-1 and SR-A, were expressed on VLCs. LOX-1 and SR-A are endocytic receptors that are expressed on both murine and human leukocytes (22, 23). To assess the specificity of expression of scavenger receptors to VLCs, we assayed the ascites derived from ID8 peritoneal tumors. LOX-1 and SR-A were both expressed on CD11c<sup>+</sup> leukocytes within the ascites (Fig. 2B1 and B2, respectively). Importantly, SR-A was robustly expressed within the ascites (>45% of the CD45<sup>+</sup> cells, Fig. 2B3) and was specifically expressed on the CD11c<sup>+</sup> cells (Fig. 2B2 and B4). Western analysis confirmed the FACS analysis, with FACS-sorted CD45<sup>+</sup>VE-cadherin<sup>+</sup> VLCs exhibiting SR-A expression (Fig. 2C). Interestingly, SR-A<sup>+</sup>CD11c<sup>+</sup> cells were also observed to infiltrate solid tumors, with nearly all CD45<sup>+</sup> cells derived from LL/2 (Fig. 2D) and EL4 (data not shown) flank tumors expressing both markers robustly and specifically. Of note is that the ID8 ovarian tumor cells do not express measurable SR-A (Fig. 2C).

To determine if murine expression of LOX-1 and SR-A on ovarian cancer VLCs was reflective of expression patterns on human VLCs, we obtained ascites derived from human ovarian carcinomas. Analysis of human ascites revealed that DEC205<sup>+</sup> leukocytes specifically and robustly expressed SR-A and LOX-1 (Fig. 3A and C). Moreover, SR-A or LOX-1 expression on FACS-sorted CD45<sup>+</sup>VE-cadherin<sup>+</sup> human VLCs correlated with that of DEC205 (Fig. 3B and D).

**SR-A-targeted depletion of VLCs blocks ovarian tumor progression.** Expression of SR-A on VLCs in the ascites of ID8 ovarian carcinoma presented a target by which to deplete VLCs with an anti-SRA immunotoxin. Importantly, nearly all CD11c<sup>+</sup> cells in the peritoneum expressed SR-A and this expression is limited to the CD11c<sup>+</sup> cells (Fig. 2B2). To specifically deplete VLCs from the peritoneum, we used saporin toxin (ZAP) conjugated to the 2F8 anti–SR-A monoclonal antibody (24). To confirm that SR-A<sup>+</sup> cells were targeted and depleted by the toxin, naïve mice were injected i.p. with either anti–SR-A antibody–conjugated saporin toxin (SRA-ZAP) or unconjugated saporin toxin (ZAP). The percentage of SR-A<sup>+</sup> macrophages found in the peritoneal exudates 24 h postinjection was then determined by FACS analysis (Fig. 4A). SR-A<sup>+</sup>-targeted immunotoxin depleted >80% of the SRA<sup>+</sup>F4/80<sup>+</sup> cells in the peritoneum (Fig. 4A and B). Concomitantly, we also determined the percentages of SR-A<sup>+</sup> macrophages found in the spleen from SRA-ZAP and ZAP-treated mice. Although the administration of SRA-ZAP showed a significant depletion of SR-A<sup>+</sup> macrophages in the peritoneum, splenic macrophages were unaffected (Fig. 4B). These data indicate that i.p. injection of the anti–SR-A immunotoxin locally depleted the macrophages.

To then test the effect of VLC depletion on peritoneal ovarian cancer, mice were injected i.p. with 5 × 10<sup>6</sup> ID8-C3 cells (ID8-Vegf cell line that expresses GFP). Mice were treated with weekly injections of SRA-ZAP or ZAP. In vivo tumor progression was assessed as a function of weight gain due to peritoneal ascites. Although mice treated with untargeted ZAP all exhibited tumor-induced ascites (body weight exceeded 25 g), administration of SRA-ZAP significantly (P < 0.01) reduced ascites occurrence (Fig. 5A). To more rigorously measure ascites progression, mice were weighed weekly after tumor challenge. ZAP-treated mice more than doubled in weight in the 7 weeks after tumor.
challenge (Fig. 5B), and typically exhibited discernable abdominal distension ~30 days posttumor challenge. Conversely, SRA-ZAP–treated mice exhibited significantly less (\(P < 0.01\)) weight gain over the same time span, with those increases largely attributable to normal growth.

To directly and quantitatively measure the effects of SRA-ZAP treatment on VLC depletion and tumor burden, mice were sacrificed 7 weeks after ID8-C3 tumor challenge and the peritoneal ascites were examined. SRA-ZAP treatment dramatically and significantly reduced the number of GFP+CD11c+ peritoneal VLCs compared with mice treated with untargeted ZAP (Fig. 6A). Treatment with untargeted ZAP did not significantly reduce the number of peritoneal VLCs compared with untreated mice, indicating minimal nonspecific cellular killing by the unconjugated toxin (data not shown). The ascites volume was reduced >90% in mice treated with SRA-ZAP compared with those given untargeted ZAP (Fig. 6B) and, consistent with this, total cell numbers (after RBC lysis) from peritoneal exudates were significantly reduced through the treatment with SRA-ZAP (Fig. 6C). These results are consistent with the in vivo analyses of ascites progression shown in Fig. 5. Importantly, peritoneal ID8 tumor cell (GFP+CD11c+ SRA+) burden was significantly inhibited through the elimination of the SR-A+ VLCs (Fig. 6D). These data indicate that immunotoxin targeted against SR-A is an effective method to selectively eliminate peritoneal leukocytes. Additionally, these data support that leukocytes are necessary for the progression of ovarian cancer and, accordingly, that elimination of the tumor-associated leukocytes from the tumor microenvironment is a rational and efficacious therapeutic strategy.

**Discussion**

Despite being regarded as cells that mediate and elicit beneficial immune responses, accumulating data indicates that leukocytes are recruited by tumors to propagate tumor growth, angiogenesis, and metastasis (10, 25, 26). Moreover, mice that either do not have various subsets of leukocytes or have had them depleted within the tumor exhibit reduced tumor growth and metastasis (5, 13, 19, 27–30). Thus, tumor therapy directed at depletion of tumor-promoting leukocytes has become a promising strategy that complements methods that directly target tumor cells. However, specific, efficient, and clinically applicable methods to locally deplete tumor-associated leukocytes are needed to effectively...
implement this strategy. This is particularly evident in both human and murine ovarian tumors where >30% of cells found in the peritoneal ascites are CD11c+ leukocytes that are called VLCs. Similar to various types of leukocytes described in solid tumors, VLCs are thought to promote growth of neovasculature and provide an immunosuppressive environment. Here, we report that SR-A provides a specific and feasible target by which to target the depletion of ovarian tumor-associated leukocytes and that, importantly, local leukocyte depletion blocks tumor progression and inhibits the development of ascites.

Current ovarian cancer therapy involves surgical debulking followed by chemotherapy. However, the 5-year survival rate is <50%, and thus the need for alternative approaches (31, 32). One such novel immunotherapy focuses on the tumor-promoting leukocytes found in the tumor ascites. As a proof-of-principle that leukocyte depletion can block tumor progression, we show that carrageenan-mediated leukocyte depletion inhibits progression of ovarian tumor peritoneal ascites. Interestingly, carrageenan is reported to be less effective in solid tumor models (19), indicating that tumor type, tumor location, or route of therapy may affect the therapeutic efficacy of leukocyte depletion. To advance these promising results, we sought a cell surface receptor on ovarian tumor-associated leukocytes by which to target an immunotoxin, because use of carrageenan is not a clinically feasible treatment (33). Our criteria for such a cell surface receptor were that (a) the receptor is specific within the peritoneum to the leukocytes, (b) it is an endocytic receptor by which to expedite immunotoxin uptake, and (c) the receptor is also expressed by human leukocytes. Under these criteria, we have identified SR-A as an ideal target by which to deplete VLCs. The specific expression of SR-A on VLCs within the tumor microenvironment allowed for directed delivery of immunotoxin to these cells. I.p. injection of anti–SR-A–conjugated toxin rendered effective and localized leukocyte depletion. We note that i.p. treatments have previously been shown to be more effective than i.v. treatments for ovarian cancer (34) and, indeed, the National Cancer Institute now encourages i.p. delivery of therapy for clinical treatment of ovarian cancer (35). Weekly injections of anti–SR-A immunotoxin were sufficient to deplete tumor-recruited peritoneal VLCs, to inhibit ascites accumulation, and, most importantly, this resulted in decreased tumor burden. Because the ID8 tumor cells do not express SR-A, we thereby infer that depletion of the SR-A+ leukocytes was sufficient to block tumor progression. Thus, these data support the use of leukocyte depletion as a potential facile and efficacious treatment for ovarian cancer.

Our findings highlight the critical role that leukocytes play in the progression of ovarian cancer. Specifically, this is the first report of the beneficial effects of localized leukocyte depletion upon a peritoneal model of ovarian cancer. Ovarian cancer is particularly amenable to this strategy of treatment because it is contained within the peritoneum until the very late stages (36). Additionally, compared with current T cell–mediated immunotherapies, the strategy of localized leukocyte depletion is advantageous because it does not need to be patient specific and it is not hindered by immunoediting (37, 38). Moreover, we show that SR-A expression is both robust and specific on human ovarian cancer leukocytes. Therefore, we propose that targeted depletion of leukocytes via SR-A may present a rational and viable clinical therapeutic strategy. To optimize the therapeutic efficacy, we anticipate that targeted elimination of leukocytes can be used to complement current and emerging strategies that directly target the tumor cells.

The importance of leukocytes toward the progression of many tumor types, including ovarian cancer, continues to escalate. Despite this, the origin and progenitor population of ovarian cancer VLCs is currently not well understood, although they are likely of myeloid lineage (13, 26). Current efforts focus on identification of the origin of these cells, whereby strategies to impair the expansion and recruitment of these cells can be implemented. Moreover, within ovarian carcinomas, macrophages and dendritic cells have altered cytotoxic capabilities and can

**Figure 6.** Depletion of SR-A+ cells blocks ID8 ovarian tumor progression. As described in Fig. 5, mice were challenged with 5 × 10⁶ ID8-C3 cells and treated with weekly injections of either SRA-ZAP or with untargeted toxin (Zap). Four variables of tumor progression were assessed for each treatment group. Peritoneal exudates were measured for the quantity of CD11c+ VLCs (A), the ascites volume (B), total numbers of cells within the ascites (after RBC lysis; C), and the quantity GFP+ ID8-C3 tumor cells (D). CD11c+ VLCs and GFP+ tumor cells were quantified by FACS analysis. For each variable, SRA-ZAP–treated mice exhibited significant (Student’s t test, * P < 0.05; **P < 0.01) and substantial (>80%) inhibition of peritoneal tumor progression relative to Zap-treated mice.


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