Targeting the Immunoregulator SRA/CD204 Potentiates Specific Dendritic Cell Vaccine-Induced T-cell Response and Antitumor Immunity

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Abstract

Although dendritic cell (DC) vaccines offer promise as cancer immunotherapy, further improvements are needed to amplify their clinical therapeutic efficacy. The pattern recognition scavenger receptor SRA/CD204 attenuates the ability of DCs to activate CD8+ T-cell responses. Therefore, we examined the impact of SRA/CD204 on antitumor responses generated by DC vaccines and we also evaluated the feasibility of enhancing DC vaccine potency by SRA/CD204 blockade. DCs from SRA/CD204-deficient mice were more immunogenic in generating antitumor responses to B16 melanoma, compared with DCs from wild-type mice. Similarly, siRNA-mediated knockdown of SRA/CD204 by lentiviral vectors improved the ability of wild-type DCs to stimulate the expansion and activation of CD8+ T cells specific for idealized or established melanoma antigens in mice. Using SRA/CD204-silenced DCs to generate antigen-targeted vaccines, we documented a marked increase in the level of antitumor immunity achieved against established B16 tumors and metastases. This increase was associated with enhanced activation of antigen-specific CTLs, greater tumor infiltration by CD8+ T cells and NK cells, and increased intratumoral ratios of both CD4+ and CD8+ T-effector cells to CD4+CD25+ T-regulatory cells. Our studies establish that downregulating SRA/CD204 strongly enhances DC-mediated antitumor immunity. In addition, they provide a rationale to enhance DC vaccine potency through SRA/CD204-targeting approaches that can improve clinical outcomes in cancer treatment. Cancer Res; 71(21); 6611-20. ©2011 AACR.

Introduction

Cancer immunotherapy aims to achieve immune-mediated control of tumor growth by priming T-cell responses to target tumor-associated antigens. Dendritic cells (DC) are the most potent and highly specialized antigen-presenting cells (APC) capable of inducing immune responses to self- or foreign-antigens (1). DC-based vaccines represent a promising approach for harnessing the patient’s own immune system to eliminate cancer cells (2). Encouraging clinical outcomes have led to approval of the first DC cancer vaccine, Sipuleucel-T (APC8015), by the U.S. Food and Drug Administration for treatment of metastatic prostate cancer (3).

Although active immunization with DCs holds promise for cancer immunotherapy, the limited success thus far in the clinic indicates that improvements are necessary to provide more benefits to cancer patients. One of major efforts to enhance DC vaccine potency has been focused on promoting DC maturation and costimulation as a means of enhancing antitumor immunity (2). Many aspects of positive signaling in the generation of effective immune response have been reported. However, information remains limited on the counterbalancing inhibitory pathways in DCs that can dampen induction of immunity against tumor-associated self-antigens (4, 5). Knowledge of these immunosuppressive regulators could be exploited therapeutically to enhance DC vaccine efficacy.

Scavenger receptor SRA/CD204 is expressed primarily on myeloid cells, including DCs, and functions as an innate pattern recognition receptor (6). In addition to the established roles that SRA/CD204 plays in lipid metabolism, atherosclerosis, and pathogen recognition (7, 8), our recent studies suggest that SRA/CD204 serves as a suppressor of vaccine-induced antitumor immunity (9). In addition, SRA/CD204 attenuates a CD8+ T-cell response elicited by an immunostimulatory adjuvant targeting the toll-like receptor (TLR) 4 signaling (10). The increased CD8+ T-cell activation was attributed to the enhanced DC functions upon inflammatory stimulation [e.g., lipopolysaccharide (LPS)] in the absence of SRA/CD204 (10). Several lines of evidence support the involvement of SRA/CD204 in restricting or limiting inflammatory responses (11–13). Our recent studies uncovered a novel molecular mechanism underlying the SRA/CD204-mediated
downregulation of TLR4 signaling in DCs and activation of transcription factor NF-κB, a master regulator of immunity (14). Given that engagement of TLR signaling pathways in DCs is a promising mechanism for boosting vaccine responses (15), sequestering the suppressors of TLRs could potentially promote the stimulatory capability of DCs in mobilization of CTLs and antitumor immunity.

Self-inactivating lentiviral system has been shown to be a safe and efficient vehicle for genetic modification of DCs and has considerable potential for clinical applications (16). In this study, we sought to examine the impact of SRA/CD204 on DC vaccine-induced immune activation and the feasibility of disabling SRA/CD204 by lentivirus-mediated silencing to improve the antitumor potency of DC vaccine. We show that SRA/CD204-silenced DCs are highly effective in promoting functional activation of CTLs and antitumor immunity against the poorly immunogenic B16 melanoma and metastases. Our studies reveal the profound effects of SRA/CD204 downregulation on DC vaccine-augmented antitumor immune responses and show the principle of silencing SRA/CD204 as a means to break tolerance against tumor-associated self-antigens.

Materials and Methods

Mice and cell lines

C57BL/6 mice were purchased from the NIH (Bethesda, MD). SRA/CD204 knockout mice (SRA−/−), Pmel transgenic mice, and OT-I mice (17) were purchased from the Jackson Laboratory (Bar Harbor, ME). All experimental procedures were conducted according to the protocols approved by the VCU Institutional Animal Care and Use Committee.

Reagents and antibodies

Recombinant gp100 protein was prepared using a BacPAK baculoviral expression system (BD Biosciences) as previously described (18). OVA257–264 (SIINFEKL) and gp10025–33 (KVPRNQDWL) peptides were purchased from AnaSpec Inc. Mouse monoclonal antibodies to CD4 (GK1.5), CD8α (53-6.7), CD3 (HL3), IFN-γ (XMG1.2), CD25 (PC61), CD90.1 (OX-7), isotype control rat IgG2b (RTK4530), and IgG1 (RTK2071) were purchased from BioLegend. SRA/CD204 polyclonal antibodies and mAb (2F8) were purchased from R&D Systems and AbD Serotec, respectively.

Lentivirus-mediated gene silencing

Bone marrow-derived DCs (BM-DCs) were generated from bone marrow cells with mouse GM-CSF (Peprotech Inc.) as previously described (10). Lentiviruses encoding mouse SRA/CD204 short hairpin RNA (shRNA) or scramble shRNA were packaged using Phoenix cells cotransfected with pLKO.1 constructs and pMD.G and pCMVΔR8.91. Viruses were collected and concentrated using ultracentrifugation. Day 3 BM-DCs or DC progenitors were infected with lentiviruses in the presence of 4 µg/mL polybrene and 20 ng/mL GM-CSF. Culture media were replaced with GM-CSF–containing media on days 4 and 6. On day 8, the infected BM-DCs were analyzed for SRA/CD204 knockdown efficiency and used for studies.

In vitro stimulation of T cells

DCs were pulsed with OVA (10 µg/mL) or gp100 protein (25 µg/mL) for 2 hours, followed by stimulation with LPS (500 ng/mL) for additional 2 hours. DCs were washed and incubated with 1 × 105 OT-1 cells or Pmel cells. T-cell proliferation was measured based on 3H-thymidine (3H-TdR) incorporation. Culture supernatant was collected 48 hours later and analyzed for cytokine levels using ELISA.

Immunization and T-cell functional assays

Mice were vaccinated subcutaneously twice at weekly intervals with antigen-loaded DCs with in vivo LPS stimulation for 2 hours before immunization. For intracellular IFN-γ staining, lymphoid cells were stimulated with OVA257–264 or gp10025–33 peptide (1 µg/mL) at 37°C for 72 hours. After treatment with PMA (10 nmol/L) plus ionomycin (1 µmol/L) in the presence of brefeldin A (5 µg/mL) for 5 hours, cells were stained with fluorescein isothiocyanate-conjugated anti-CD8 antibodies and subsequently permeabilized using a Cytofix/Cytoperm kit (BD Biosciences). The cells were then stained with PE-conjugated anti-IFN-γ antibodies and analyzed using fluorescence-activated cell sorting (FACS) by gating on CD8+ T cells. ELISPOT was done 48 hours after cell stimulation with CTL epitopes as previously described (10). In vivo CTL assays were done using peptide-loaded CFSEhigh splenocytes as targets (10). For some experiments, splenocytes were stimulated with OVA257–264 in the presence of IL-2 for 5 days and used as effector cells in a standard chromium release assay.

Adoptive T-cell transfer

A total of 5 × 106 Pmel cells or CFSE-labeled OT-I cells were transferred intravenously into recipient mice, followed by DC immunization subcutaneously next day. Cells from the spleen and draining lymph nodes were harvested 5 days later, and stained with anti-CD8 and anti-CD90.1 antibodies. Cells were gated on CD8+CD90.1− (for Pmel cells) or CD8+CFSE− (for OT-I cells).

Figure 1. Enhanced antitumor response induced by vaccination with SRA/CD204-deficient DCs. C57BL/6 mice (n = 5) were immunized with WT DCs or SRA−/− DCs that had been pulsed with OVA protein and stimulated with LPS. Mice were challenged with B16-OVA cells (⁎, P < 0.005). Results representative of 3 independent experiments with similar results are shown.
Tumor studies

For prophylactic study, mice were immunized with DC vaccines 3 times at weekly intervals. One week later, mice were inoculated s.c. with \(5 \times 10^5\) B16-OVA tumor cells. In therapeutic studies, mice were established with tumors by injecting B16-gp100 cells on day 0. Tumor-bearing mice were treated subcutaneously with DC vaccines on days 4, 7, and 10. CD8\(^+\) or CD4\(^+\) T cells were depleted using 2.43 or GK1.5 monoclonal antibodies, respectively (9). For analysis of tumor-infiltrating lymphocytes (TIL), tumors were digested with collagenase D (10 \(\mu\)g/mL) and DNase I (100 \(\mu\)g/mL) for 1 hour at 37°C. Single cell suspensions were stained with antibodies for CD4, CD25, CD8, and NK1.1.

Treatment of experimental lung metastases

Lung metastases were established in mice by i.v. injecting \(4 \times 10^5\) B16-Luc cells in 200 \(\mu\)L of PBS (without \(Ca^{2+}\) or \(Mg^{2+}\)). Mice were treated with DC vaccines on days 4, 7, 10, and 13. Three weeks after tumor injection, mice were injected with \(\alpha\)-luciferin (150 mg/kg) and examined using a Xenogen IVIS

Figure 2. Silencing of SRA/CD204 results in increased capability of DCs to stimulate OVA-specific OT-I cells. A, screening of shRNAs capable of silencing SRA/CD204 in primary DCs. BM-DCs were transfected with LV-GFP shRNA or with 3 designed LV-SRA shRNA sequences (Seq #1, Seq #2, and Seq #3) at a multiplicity of infection of 10. Cells were subjected to immunoblotting analysis. B, BM-DCs were infected with LV-scrambled shRNA, LV-SRA shRNA or left untreated, and analyzed for SRA/CD204 expression. C, lentiviral infections do not induce type I IFN response. mRNA levels of IFN-\(\beta\) in BM-DCs were examined using RT-PCR. LPS-stimulated DCs serve as a positive control. D, transcriptional upregulation of inflammatory genes enhanced by SRA/CD204-silencing. DC-Scram and DC-SRA shRNA were stimulated with LPS and mRNA levels of inflammatory genes were assessed using qRT-PCR. The results are presented as fold induction over untreated DC-Scram samples (\(\times P < 0.005\)). E, increased proliferation of OT-I cells by SRA/CD204-silenced DCs. Cells were cocultured with OT-I cells at a ratio of 1:20 (\(\times P < 0.005\)). F, increased IFN-\(\gamma\) production of OT-I cells by SRA/CD204-silenced DCs. Levels of IFN-\(\gamma\) in the DC-OT-I cell coculture system was assessed by ELISA (\(\times P < 0.005\)). G, increased expansion of adoptively transferred OVA-specific OT-I cells by vaccination with SRA/CD204-silenced DCs. Mice (\(n = 3\)) received CFSE-labeled OT-I cells and were immunized with OVA protein-loaded, LPS activated DC-Scram or DC-SRA shRNA. Spleen (SP) and lymph nodes (LN) were analyzed using FACS by gating on CD8\(^+\)CFSE\(^+\) cells. Representative histograms of 3 independent experiments with similar results are shown.
Imaging System (Caliper Life Sciences). Tumor tissues were stained with hematoxylin and eosin (H&E), and slides were scanned with the Ariol SL-50 automated scanning system (Genetix Ltd).

Statistical analysis
The data are expressed as the mean values ± SE. Comparisons between 2 groups were done using Student t test. Comparisons between multiple groups were carried out using ANOVA test. A value of P < 0.05 is considered to be statistically significant.

Results
Vaccination with SRA/CD204-deficient DCs induces an enhanced antitumor response
We previously showed that DCs lacking SRA/CD204 upon TLR4 stimulation were more efficient than wild-type (WT) counterparts in activating OVA-specific CD8⁺ T cells (10). To examine whether the enhanced CD8⁺ T-cell activity could lead to improved tumor protective immunity, we initially used the well-defined B16 melanoma model, which expresses the cytotoxic form of the model antigen OVA. Although mice receiving WT DC vaccines showed modest inhibition of tumor growth compared with untreated mice, mice vaccinated with SRA⁻/⁻ DCs developed a robust antitumor response (Fig. 1), suggesting enhanced immunogenicity of DCs in the absence of SRA/CD204 in the context of DC vaccine therapy.

SRA/CD204 silencing enhances the capability of DCs to activate OVA-specific CD8⁺ T cells
Self-inactivating lentiviral vectors (LV) encoding shRNA were used for SRA/CD204 silencing in DCs because of their safety and superior transduction efficiency in primary cells (19). We initially screened several shRNAs targeting different sequences of SRA/CD204 and identified SRA shRNA #3 (hairpin sequence CCGGGAATTCGATATCGAGTTGAGGTTTACGGATTCCAGAACCTGCTTTTG) capable of efficiently downregulating SRA/CD204 (Fig. 2A). We found that lentiviral infection of early BM-DC cultures (i.e., day 3 BM-DC) resulted in a better transduction efficiency and reduced virus-associated toxicity without affecting DC differentiation or functions (data not shown). The protein levels of SRA/CD204 expression in DCs infected with LV-SRA shRNA was reduced by approximately 90% compared with cells untreated or infected with LV-scrambled shRNA (Fig. 2B). The lentiviral infection of DCs did not seem to induce activation of the type I interferon (e.g., IFN-β) response pathway under the conditions tested (Fig. 2C), which permits more reliable study of the immunologic effect of SRA/CD204 silencing in DCs. We next examined whether SRA/CD204 silencing rendered DCs more responsive to LPS stimulation as previously shown in SRA-deficient DCs. As expected, SRA/CD204-silenced DCs displayed higher transcription levels of inflammatory genes, including Ifn-α, Il-6, and ip10, compared with scrambled shRNA-treated cells (Fig. 2D).

DCs transduced with scrambled shRNA (DC-Scram) or LV-SRA/CD204 shRNA (DC-SRA shRNA) were pulsed with OVA

Figure 3. Immunization with SRA/CD204-silenced DCs enhances OVA-specific CD8⁺ T-cell activation and effector functions. A, enhanced IFN-γ production by CD8⁺ T cells in mice immunized with SRA/CD204-silenced DCs. Mice (n = 3) were immunized with OVA-pulsed DC-Scram or DC-SRA shRNA. IFN-γ production by cells from SP and LN was assessed by ELISPOT (*, P < 0.005). B, improved cytolytic activity in mice immunized with SRA/CD204-silenced DCs. After DC vaccinations, mice (n = 3) were injected i.v. with 5 × 10⁵ OVA⁻/⁻, C3-pulsed, CFSE⁺⁺ splenocytes (antigen-positive target), and nonpulsed, CFSE⁺⁺ splenocytes (negative control) mixed at a ratio of 1:1. SP and LN cells were harvested 12 hours later and analyzed using FACS. Percentage of target killing is indicated by the numbers in parentheses. C, increased tumor-killing activity of CD8⁺ T-effector cells. Splenocytes were stimulated with OVA⁻/⁻ and subjected to chromium release assays using B16-OVA tumor cells as targets (*, P < 0.01). Data shown are representative of 2 independent experiments.
protein and stimulated with LPS. The genetically modified DCs were cocultured with naïve OT-I cells that specifically recognize H2Kb-restricted OVA257–264. DC-SRA shRNA induced much stronger proliferation (Fig. 2E) and IFN-γ production (Fig. 2F) of OT-I cells than did DC-Scram. We used adoptive T-cell transfer assays to examine the capability of SRA/CD204-silenced DCs to activate naive OT-I cells in vivo. Expansion of OT-I cells was greatly enhanced in mice immunized with DC-SRA shRNA compared with those immunized with DC-Scram (Fig. 2G).

We further examined the effector function of OVA-specific CD8+ T cells using ELISpot assays. Mice immunized with SRA/CD204-silenced DCs showed a significant increase in IFN-γ-producing CD8+ T cells than those immunized with DC-Scram in the spleen and lymph nodes (Fig. 3A). Compared with DC-Scram immunized mice, mice immunized with DC-SRA shRNA displayed enhanced cytotoxicity against antigen-positive targets (Fig. 3B), as indicated by increased killing of OVA257–264 pulsed, CFSE<sup>hi</sup> splenocytes (39% vs. 60% lysis in the spleen and 40% vs. 71% lysis in the lymph nodes) observed in an in vivo CTL assay. This result was consistent with chromium release assays showing that splenocytes from mice immunized with DC-SRA shRNA exhibited more potent cytolytic activities against B16-OVA cells (Fig. 3C).

**SRA/CD204-silenced DCs promote activation of CD8+ T cells specific for melanoma antigen**

We tested whether SRA/CD204-silenced DCs can enhance an immune response against self-antigen gp100 that is naturally expressed in the B16 tumor (20) and being used as a promising antigen target in clinical trials (21, 22). Similar to the observations made in the OVA model system, SRA/CD204-silenced DCs exhibited enhanced capability to stimulate the proliferation (Fig. 4A) and IFN-γ production (Fig. 4B) of the gp100<sub>25–33</sub>-specific Pmel cells in vitro compared with scrambled shRNA-treated DCs. In addition, adoptively transferred Pmel cells (i.e., CD8+ CD90.1<sup>+</sup> T cells) showed greater expansion in mice vaccinated with SRA/CD204-silenced DCs than in those receiving DC-Scram or left untreated (Fig. 4C). Intracellular cytokine staining also showed increased frequency of IFN-γ-secreting Pmel cells following vaccination with SRA/CD204-silenced DCs (Fig. 4D).

**SRA/CD204-silenced DC vaccine potentiates a CTL response and antitumor efficacy**

To evaluate the endogenous gp100-specific CD8<sup>+</sup> T-cell response, mice were immunized with gp100-pulsed, scramble or SRA shRNA-transfected DCs. SRA/CD204-silenced DCs induced a stronger activation of gp100-specific CD8<sup>+</sup> T cells in the spleen and lymph nodes, as indicated by ELISpot assays for IFN-γ production (Fig. 5A). In vivo CTL assays showed a greater decrease in the percentage of gp100<sub>25–33</sub> peptide-pulsed, CFSE<sup>hi</sup> cells in mice receiving SRA/CD204-silenced DC vaccine than in mice given DC-Scram vaccine (Fig. 5B), indicating enhanced cytolytic activity of gp100-specific CTLs (69% vs. 42% lysis in the spleen and 58% vs. 34% lysis in the lymph nodes).

**Figure 4.** SRA/CD204 silencing in DCs results in enhanced activation of CD8+ T cells recognizing melanoma antigen gp100. A, increased proliferation of gp100-specific CD8+ cells. DC-Scram or DC-SRA shRNA pulsed with gp100 protein was cocultured with naïve Pmel cells, followed by assays for ³H-thymidine uptake (**, P < 0.005). B, increased production of IFN-γ by Pmel cells when stimulated with SRA/CD204-silenced DCs. Supernatant IFN-γ levels were assessed using ELISA (**, P < 0.005). C, increased expansion of adoptively transferred Pmel cells in vivo. Mice (n = 3) transferred with Pmel cells were immunized with gp100 protein-pulsed DC-Scram or DC-SRA shRNA. Lymph node cells were stained with anti-CD8 and CD90.1 antibodies. D, after Pmel cell transfer and DC vaccination, gp100<sub>25–33</sub>-stimulated IFN-γ production by CD90.1<sup>+</sup> CD8<sup>+</sup> T cells was analyzed using intracellular cytokine staining. Data representative of 2 independent experiments with similar results are shown.
We evaluated the therapeutic efficacy of the SRA/CD204-silenced DCs after antigen loading and LPS stimulation in mice with preestablished B16-gp100 tumors. Vaccination with DC-Scram showed little inhibitory effect on tumor growth and had survival rates no better than those untreated mice. In contrast, vaccine therapy using SRA/CD204-silenced DCs led to profound tumor suppression (Fig. 5C). Mice receiving SRA/CD204-silenced DC vaccine did not show apparent toxicity and any overt signs of gross pathology in their major organs. No depigmentation (vitiligo) was observed in these mice (data not shown). To determine the involvement of immune effector cells in the tumor control mediated by SRA/CD204-silenced DCs, we eliminated T-cell subsets in vivo using antibodies (Fig. 5D). Depletion of CD8^+ cells completely abrogated antitumor activities, whereas lack of CD4^+ cells seemed to modestly improve the tumor suppression, suggesting a possible inhibitory role for CD4^+ Treg cells in this model (23).

**Improved antitumor efficacy by SRA/CD204 silencing in DCs is associated with systemic immune activation in B16 tumor-bearing mice**

ELISPOT assays showed that splenocytes from mice treated with SRA/CD204-silenced DCs, upon stimulation with gp10025–33 peptide, produced much more IFN-γ than those from DC-Scram vaccinated or untreated tumor-bearing mice (Fig. 6A). The results were confirmed using intracellular cytokine staining assays (Fig. 6B). The percentage of IFN-γ-secreting CD8^+ T cells in DC-SRA shRNA immunized mice showed 2.6-fold and 4.1-fold increase in the spleen and tumor-draining lymph nodes, respectively, when compared with DC-Scram immunized mice (Fig. 6B). These results are consistent with earlier studies showing immune-mediated suppression of B16 melanoma involving CD4^+ T cells and IFN-γ (24). We also conducted *in vivo* CTL assays by transferring gp10025–33-pulsed CFSE^+ splenocytes into tumor-bearing mice that have received DC vaccine therapy. Mice...
treated with SRA/CD204 silenced DCs developed increased cytotoxicity against gp100-positive targets compared with scramble DC-Scram–treated mice (Fig. 6C, 73% vs. 42% lysis in the spleen and 82% vs. 51% lysis in the lymph nodes).

To provide more insights into the enhanced antitumor response, we examined TILs in mice receiving vaccine therapies. Although there were few CD8^+ T cells in untreated B16 tumors, SRA/CD204 silencing in DCs resulted in a substantial increase in the percentage of CD8^+ T cells and a modest increase of NK cells (Fig. 6D). Treatment with either DC-Scram or DC-SRA shRNA caused expansion and infiltration of CD4^+ T cells, including CD4^+CD25^+ regulatory T cells (Tregs) and CD4^+CD25^+ T cells, when compared with untreated tumor-bearing mice. However, the percentage of Tregs in the TILs was lower after treatment with DC-SRA shRNA than that following treatment with DC-Scram (Fig. 6E). As a result, the ratio of CD4^+CD25^+ Tregs to CD4^+CD25^+ T cells dramatically increased in the tumor compartment after therapy with SRA/CD204-silenced DC vaccine (Fig. 6E). Similar result was obtained when cells were stained with anti-CD4 and Foxp3 antibodies, as indicated by an increase in the ratios of CD4^+Foxp3^+ to CD4^+Foxp3^- cells (Fig. 6F).

Vaccine therapy with SRA/CD204-silenced DCs results in a marked reduction in metastases of B16 melanoma

To evaluate the potential use of the modified DC vaccine against metastatic tumors, we used a well-established model...
of experimental lung metastasis. Mice were injected intravenously with B16-Luc cells 4 days prior to DC vaccine therapy (Fig. 7A). Intense bioluminescence was seen in the chest, abdomen, and head of untreated mice. Vaccine therapy with DC-Scram substantially reduced bioluminescence in the chest and abdomen. Strikingly, bioluminescence was almost undetectable in mice receiving DC-SRA shRNA, especially in the chest (Fig. 7B). The number of lung metastases generally correlated with the bioluminescence intensity (Fig. 7C). The lungs from untreated mice showed numerous pigmented tumor nodules, whereas less lung metastases were seen in DC-Scram vaccine-treated mice. In contrast, only few scattered tumor nodules of much smaller size were evident in the lungs from mice treated with SRA/CD204-silenced DCs (Fig. 7C). Histopathologic analysis confirmed that the lung sections from mice-treated DC-SRA shRNA treatment displayed significantly smaller and fewer metastatic lesions than those from mice untreated or treated with DC-Scram (Fig. 7D).

Discussion

The presence of various intrinsic immune inhibitory molecules in DCs and associated counterbalancing machineries are pivotal for maintaining immune homeostasis and minimizes the likelihood of developing excessive immune responses (25). However, it also creates a formidable barrier to the development of effective immunotherapies and cancer vaccines. In this study, we have provided the first evidence showing that genetic modification of DCs by silencing SRA/CD204, a negative immune regulator, led to greatly improved DC vaccine potency and tumor control.

Emerging evidence suggests that targeting intrinsic immune inhibitory mechanisms in DCs, such as SOCS1 (suppressor of
cytokine signaling 1; ref. 26) and A20 (5), can be exploited for potentiating therapeutic immunity against cancer. These 2 molecules have been shown to play a role in negative regulation of DC functions by controlling the JAK/STAT and TLR/NF-κB pathways (4, 27). The significance of TLR signaling in enhancing antigen presentation and activating adaptive or acquired immune responses has been well established. TLR-mediated DC activation is a crucial step in this process (28). Interestingly, we recently showed that SRA/CD204 was also capable of attenuating TLR4 signaling-induced NF-κB activation in DCs, resulting in enhanced expression of costimulatory molecules, proinflammatory cytokines (e.g., TNF-α, IL-6 and IL-12), and type I interferons (e.g., IFN-β; refs. 10, 14). Using 2 different systems involving a model antigen (i.e., OVA) and a clinically relevant melanoma-associated antigen (i.e., gp100), we have shown that DCs genetically modified with SRA/CD204-silencing lentiviruses exhibit an enhanced capacity to stimulate an antigen-specific CTL response. These results are consistent with our previous observations of enhancement of TLR4 agonist-induced CD8+ T-cell immunity in SRA−/− mice, which has been attributed to the absence of SRA/CD204 in DCs (10). The robust CD8+ T-cell response elicited by the lentivirally engineered DCs targeting SRA/CD204 are required for effective suppression of preestablished B16 melanomas, indicating that the immunogenicity of DCs and resultant antitumor efficacy may be potentially enhanced by downregulation or blockade of SRA/CD204.

In addition to the enhanced systemic CD8+ T-cell response, the improved tumor control by the SRA/CD204-silenced DCs was associated with profoundly increased infiltration of effector cells, including CD8+ T cells and NK cells, both of which play important roles in immune-mediated eradication of tumor cells. The presence of NK cells in the tumor site may not be surprising because there is an increasing body of in vivo evidence showing the activation of NK cells following DC vaccine therapy (29, 30). Intriguingly, although total tumor infiltrating CD4+ T cells increased in DC-Scram and DC-SRA shRNA-treated mice, the levels of Tregs decreased significantly in the tumor compartment after vaccinations with SRA/CD204-silenced DCs. It could be explained by the more significant expansion/recruitment of CD4+CD25+ or CD4+Foxp3+ T-cell population induced by SRA/CD204-silenced DCs. A possible suppressive function of Tregs in the DC vaccine-generated immunity against B16 tumors was implicated in our antibody depletion study, which is also supported by the previous work by Sutmuller and colleagues using the same tumor model (23). As a result, the increased ratios of infiltrating CD8+ and CD4+ T cells to Tregs in the tumor compartment could contribute to the enhanced antitumor efficacy augmented by SRA/CD204-silenced DC vaccine, because the intratumoral balance of Teffs and Tregs has been shown to directly correlate with tumor suppression (31). In addition, increased cytokine (IL-6, TNF-α) production resulted from the engagement of TLRs on SRA/CD204-downregulated DCs may also help overcome Treg-mediated immune suppression (5, 32). It remains to be examined whether the reduction of SRA/CD204 expression promotes the resistance of DCs to Tregs-mediated suppression as described in the study of A20-silenced DCs (5).

The recent approval of DC vaccine therapy has paved a path for the development of next generation of DC-based immunotherapy (33). Our data indicate that silencing of SRA/CD204 results in DCs with enhanced stimulatory capacity in the context of activation of antitumor immunity. Interestingly, blockade of MARCO, another class A scavenger in DCs, was recently shown to promote generation of tumor-reactive T cells (34), suggesting that the action of individual scavenger receptors in immune modulation needs to be defined and may be exploited for provoking therapeutic benefits. Our study not only sheds light on the under-appreciated regulatory functions of SRA/CD204 in DC biology and immunology, but also offers further insights into strategies for achieving enhanced antitumor immunity by targeting DC-intrinsic immunologic inhibitory molecules.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions


Acknowledgments

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