Secondary Lymphoid Organ Chemokine Reduces Pulmonary Tumor Burden in Spontaneous Murine Bronchoalveolar Cell Carcinoma

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ABSTRACT

The antitumor efficiency of secondary lymphoid organ chemokine (SLC), a CC chemokine that chemotaxtacts both dendritic cells (DCs) and T lymphocytes, was evaluated in SV40 large T-antigen transgenic mice that develop bilateral multifocal pulmonary adenocarcinomas. Injection of recombinant SLC in the axillary lymph node region led to a marked reduction in tumor burden with extensive lymphoctic and DC infiltration of the tumors and enhanced survival. SLC injection led to significant increases in CD4 and CD8 lymphocytes as well as DC at the tumor sites, lymph nodes, and spleen. The cellular infiltrates were accompanied by the enhanced elaboration of Type 1 cytokines and the antiangiogenic chemokines IFN-γ-inducible protein 10, and monokine induced by IFN-γ (MIG). In contrast, lymph node and tumor site production of the immunosuppressive cytokine transforming growth factor β was decreased in response to SLC treatment. In vivo, after stimulation with irradiated autologous tumor, splenocytes from SLC-treated mice secreted significantly more IFN-γ and granulocyte macrophage colony-stimulating factor, but reduced levels of interleukin 10. Significant reduction in tumor burden in a model in which tumors develop in an organ-specific manner provides a strong rationale for additional evaluation of SLC in regulation of tumor immunity and its use in lung cancer immunotherapy.

INTRODUCTION

Effective antitumor responses require both APCs and lymphocyte effectors (1). Because tumor cells often have limited expression of MHC antigens and lack costimulatory molecules, they are ineffective APCs (2). In addition, tumor cells secrete immunosuppressive mediators that contribute to evasion of host immune surveillance (3–5). To circumvent this problem, investigators are using ex vivo generated DCs to stimulate antitumor immune responses in vivo. In experimental murine models, DCs pulsed with tumor-associated antigenic peptides (6) or transfected with tumor RNA have been shown to induce antigen-specific antitumor responses in vivo (7). Similarly, fusion of DCs with tumor cells or intratumoral injection of cytokine-modified DCs has also been shown to enhance antitumor immunity (8–10). Consequently, it has been suggested that effective anticaner immunity may be achieved by recruiting professional host APCs for tumor antigen presentation to promote specific T-cell activation (11). Thus, chemokines that attract both DCs and lymphocyte effectors to lymph nodes and tumor sites could serve as potent agents in cancer immunotherapy.

Chemokines, a group of homologous, yet functionally divergent proteins, directly mediate leukocyte migration and activation and play a role in regulating angiogenesis (12). Chemokines also function in maintaining immune homeostasis and secondary lymphoid organ architecture (13). Several chemokines are known to have antitumor activity. Tumor rejection has been noted in various murine tumor models in which tumor cells have been modified with chemokines including MIP1α, RANTES, lymphotactin, TCA3, JEMCP-1, MCAF, MIP3α, MIP3β, and IP-10 (14–22). In this study, we evaluated the antitumor properties of a CC chemokine, SLC, in a spontaneous murine model of lung cancer. In the SV40 TAg transgenic mice, adenocarcinomas develop in an organ-specific manner and, compared with transplantable tumors, the pulmonary tumors in these mice more closely resemble human lung cancer. SLC, normally expressed in high endothelial venules and in T-cell zones of spleen and lymph nodes, strongly attracts naïve T cells and DCs (23–30). The capacity of SLC to chemotaxtact DCs (16) is a property shared with other chemokines (17–19). However, SLC may be distinctly advantageous because of its capacity to elicit a Type 1 cytokine response in vivo (31). DCs are uniquely potent APCs involved in the initiation of immune responses (22). Serving as immune system sentinels, DCs are responsible for Ag acquisition in the periphery and subsequent transport to T-cell areas in lymphoid organs where they prime specific immune responses. SLC recruits both naïve lymphocytes and antigen-stimulated DCs into T-cell zones of secondary lymphoid organs, colocalizing these early immune response constituents and culminating in cognate T-cell activation (23). In this study, using transgenic mice that develop lung cancer spontaneously, we demonstrate that SLC mediates potent antitumor responses in vivo leading to a significant reduction in tumor burden.

MATERIALS AND METHODS

Cell Culture. Clara cell lung tumor cells (CC-10 Tag and H-2) were derived from freshly excised lung tumors that were propagated in RPMI 1640 (Irving Scientific, Santa Ana, CA) supplemented with 10% FBS (Gemini Bioproducts, Calabasas, CA), penicillin (100 units/ml), streptomycin (0.1 mg/ml), and 2 mM of glutamine (JRH Biosciences, Lenexa, KS) and maintained at 37°C in humidified atmosphere containing 5% CO2 in air. After two in vivo passages, CC-10 Tag tumor clones were isolated. The cell lines were Mycoplasma free, and cells were used up to the tenth passage before thawing frozen stock cells from liquid N2.

CC10Tag Mice. The transgenic CC-10 Tag mice, in which the SV40 large TAg is expressed under control of the murine Clara cell-specific promoter, were used in these studies (33). All of the mice expressing the transgene developed diffuse bilateral bronchoalveolar carcinoma. Tumor was evident in cognate T-cell activation (23). In this study, using transgenic mice that develop lung cancer spontaneously, we demonstrate that SLC mediates potent antitumor responses in vivo leading to a significant reduction in tumor burden.

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genic mice, presence of the transgene was confirmed by PCR of mouse tail biopsies. The 5' primer sequence was SM19-TAG: 5'-TGAGCCTTCTAG-GCTCTGAAAAG-3', and the 3' primer sequence was SM36-TAG: 5'-AG-GCATCCACCTAGTCCCTCAT-3'. The size of the resulting PCR fragment is 650 bp. DNA (1 μg) was amplified in a total volume of 50 μl, which contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM each deoxynucleotide triphosphates, 0.1 μM primers, 2.5 mM MgCl₂, and 2.5 units of Taq polymerase. PCR was performed in a Perkin-Elmer DNA thermal cycler (Norwalk, CT). The amplification profile for the SV40 transgene consisted of 40 cycles, with the first cycle denaturation at 94°C for 3 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min, followed by 39 cycles with denaturation at 94°C for 1 min, and the same annealing and extension conditions. The extension step for the last cycle was 10 min. After amplification, the product was visualized under molecular weight standards on a 1.5% agarose gel stained with ethidium bromide. All of the experiments used pathogen-free CC-10 TAg transgenic mice beginning at 4–5 weeks of age.

The SLC Therapeutic Model in CC-10 TAg Mice. CC-10 TAg transgenic mice were injected in the axillary node region with murine recombinant SLC (0.5 μg/injection; Pepro Tech, Rocky Hill, NJ) or normal saline diluent, which contained equivalent amounts of murine serum albumin (Sigma Chemical Co., St. Louis, MO) as an irrelevant protein for control injections. Beginning at 4–5 weeks of age, SLC or control injections were administered three times per week for 8 weeks. The endotoxin level reported by the manufacturer was <0.1 ng/μg (1 endotoxin unit/μg) of SLC. The dose of SLC (0.5 μg/injection) was chosen based on our previous studies (31) and the in vitro biological activity data provided by the manufacturer. Maximal chemotactic activity of SLC for total murine T cells was found to be 100 ng/ml. For in vivo evaluation of SLC-mediated antitumor properties we used 5-fold more than this amount for each injection. At 4 months, mice were sacrificed, and lungs were isolated for quantification of tumor surface area. Tumor burden was assessed by microscopic examination of H&E-stained sections with a calibrated graticule (a 1-cm² grid subdivided into 100 1-mm² squares). A grid square with tumor occupying >50% of its area was scored as positive, and the total number of positive squares was determined as described previously (4). Ten separate fields from four histological sections of the lungs were examined under high-power (x20 objective). Ten mice from each group were not sacrificed so that survival could be assessed.

Cytokine Determination from Tumor Nodules, Lymph Nodes, and Spleens. The cytokine profiles in tumors, lymph nodes, and spleens were determined in both SLC and diluent-treated mice as described previously (4). Non-neoplastic tumors were harvested and cut into small pieces and passed through a sieve (Bellco, Vineland, NJ). Axillary lymph nodes and spleens were harvested from SLC-treated tumor-bearing, control tumor-bearing, and normal control mice. Lymph nodes and spleens were teased apart, RBC depleted with distilled, deionized H₂O, and brought to tonicity with 1 ddH₂O, and brought to tonicity with 1 PBS. After a 24-h culture period, tumor node supernatants were evaluated for the production of IL-10, IL-12, GM-CSF, IFN-γ, TGF-β, VEGF, MIG, and IP-10 by ELISA and PGE-2 by EIA. Tumor-derived cytokine and PGE-2 concentrations were corrected for total protein by Bradford assay (Sigma Chemical Co.). For cytokine determination, after secondary stimulation with irradiated tumor cells, splenocytes (5 × 10⁶ cells/ml), were cocultured with irradiated (100 Gy, Cs¹³⁷ γ-rays) CC-10 TAg tumor cells (10⁵ cells/ml) at a ratio of 50:1 in a total volume of 5 ml. After a 24-h culture, supernatants were harvested and GM-CSF, IFN-γ, and IL-10 determined by ELISA.

Cytokine ELISA. Cytokine protein concentrations from tumor nodules, lymph nodes, and spleens were determined by ELISA as described previously (34). Briefly, 96-well Costar (Cambridge, MA) plates were coated overnight with 4 μg/ml of the appropriate antimouse mAb to the cytokine being measured. The wells of the plate were blocked with 10% FBS (Gemini Bioproducts) in PBS for 30 min. The plate was then incubated with the antigen for 1 h, and excess antigen was washed off with PBS/Tween 20. The plate was incubated with 2 μg/ml of biotinylated mAb to the appropriate cytokine (PharMingen) for 30 min, and excess antibody was washed off with PBS/Tween 20. The plates were incubated with avidin peroxidase, and after incubation in O-phenylenediamine substrate to the desired extinction, the subsequent change in color was read at 490 nm with a Molecular Devices Microplate Reader (Sunnyvale, CA). The recombiant cytokines used as standards in the assay were obtained from PharMingen, IL-12 (Biosource) and VEGF (Oncogene Research Products, Cambridge, MA) were determined using kits according to the manufacturer’s instructions. MIG and IP-10 were quantified using a modification of a double ligand method as described previously (35). The MIG and IP-10 antibodies and protein were obtained from R&D (Minneapolis, MN). The sensitivities of the IL-10, GM-CSF, IFN-γ, TGF-β, MIG, and IP-10 ELISA were 15 pg/ml. For IL-12 and VEGF the ELISA sensitivities were 5 pg/ml.

PEG2 EIA. PEG2 concentrations were determined using a kit from Cayman Chemical Co. (Ann Arbor, MI) according to the manufacturer’s instructions as described previously (3). The EIA plates were read by a Molecular Devices Microplate reader (Sunnyvale, CA).

Flow Cytometry. For flow cytometric experiments, two or three fluorochromes (PE, FITC, and Tri-color; PharMingen) were used to gate on the CD3 T lymphocyte population of tumor nodule, lymph node, and splenic single cell suspensions. DCs were defined as the CD11c and DEC 205 bright populations within tumor nodules, lymph nodes, and spleens. Cells were identified as lymphocytes or DCs by gating based on forward and side scatter profiles. Flow cytometric analyses were performed on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA) in the University of California, Los Angeles, Jonsson Cancer Center Flow Cytometry Core Facility. Between 5,000 and 15,000 gated events were collected and analyzed using Cell Quest software (Becton Dickinson).

Intracellular Cytokine Analysis. T lymphocytes from single cell suspensions of tumor nodules, lymph nodes, and spleens of SLC-treated and diluent-treated CC-10 TAg transgenic mice were depleted of RBC with distilled, deionized H₂O and were evaluated for the presence of intracytoplasmic GM-CSF and IFN-γ. Cell suspensions were treated with the protein transport inhibitor kit GolgiPlug (PharMingen) according to the manufacturer’s instructions. Cells were harvested and washed twice in 2% FBS/PBS. Cells (5 × 10⁶) were resuspended in 200 μl of 2% FBS/PBS with 0.5 μg of FITC-conjugated mAb specific for cell surface antigens CD3, CD4, and CD8 for 30 min at 4°C. After two washes in 2% FBS/PBS, cells were fixed, permeabilized, and washed using the Cytofix/Cytoperm kit (PharMingen) following the manufacturer’s protocol. The cell pellet was resuspended in 100 μl of Perm/Wash solution and stained with 0.25 μg of PE-conjugated anti-GM-CSF and anti-IFN-γ mAb for intracellular staining. Cells were incubated at room temperature in the dark for 30 min and washed twice, resuspended in 300 μl of PBS/2% paraformaldehyde solution, and analyzed by flow cytometry.

RESULTS

SLC Mediates Potent Antitumor Responses in a Murine Model of Spontaneous Bronchoalveolar Carcinoma. We evaluated the antitumor efficacy of SLC in a spontaneous bronchoalveolar cell carcinoma model in transgenic mice in which the SV40 large TAg is expressed under control of the murine Clara cell-specific promoter, CC-10 (33). Mice expressing the transgene develop diffuse bilateral bronchoalveolar carcinoma and have an average life span of 4 months. SLC (0.5 μg/injection) or the same concentration of murine serum albumin was injected in the axillary lymph node region beginning at 4 weeks of age, three times per week and continuing for 8 weeks. At 4 months when the control mice started to succumb because of progressive lung tumor growth, mice were sacrificed in all of the treatment groups, and lungs were isolated and paraffin embedded. H&E staining of paraffin-embedded lung tumor sections from control-treated mice revealed large tumor masses throughout both lungs with minimal lymphocytic infiltration (Fig. 1 A and C). In contrast, SLC-treated mice had significantly smaller tumor nodules with extensive lymphocytic infiltration (Fig. 1 B and D). Mice treated with SLC had a marked reduction in pulmonary tumor burden as compared with diluent-treated control mice (Fig. 1 E). SLC-treated mice had prolonged survival compared with mice receiving control injections. Median survival was 18 ± 2 weeks for control-treated mice, whereas mice treated with SLC had a median survival of 34 ± 3 weeks (P < 0.001).
Fig. 1. SLC mediates potent antitumor responses in a murine model of spontaneous lung cancer. The antitumor efficacy of SLC was evaluated in the spontaneous bronchioalveolar carcinoma model in transgenic mice in which the SV40 large T Ag is expressed under control of the murine Clara cell-specific promoter, CC-10 (41). Mice expressing the transgene develop diffuse bilateral bronchoalveolar carcinoma and have an average life span of 4 months. SLC (0.5 μg/injection) or the same concentration of murine serum albumin was injected in the axillary lymph node region of 4-week-old transgenic mice three times a week for 8 weeks. At 4 months when the control mice started to succumb because of progressive lung tumor growth, mice in all of the treatment groups were sacrificed, and their lungs were isolated and embedded in paraffin. H&E staining of paraffin-embedded lung tumor sections from control-treated mice evidenced large tumor masses throughout both lungs without detectable lymphocytic infiltration (A and C). In contrast, the SLC therapy group evidenced extensive lymphocytic infiltration with marked reduction in tumor burden (B and D). Arrows in D depict tumor (×1) and infiltrate (×2). (A and B, ×32; C and D, ×320) E, reduced tumor burden in SLC-treated mice. Tumor burden was quantified within the lung by microscopy of H&E-stained paraffin-embedded sections with a calibrated graticule (a 1-cm² grid subdivided into 100 1-mm² squares). A grid square with tumor occupying >50% of its area was scored as positive, and the total number of positive squares was determined. Ten separate fields from four histological sections of the lungs were examined under high-power (∼20 objective). There was reduced tumor burden in SLC-treated CC-10 mice compared with the diluent-treated control group. Median survival was 18 ± 2 weeks for control-treated mice. In contrast, mice treated with SLC had a median survival of 34 ± 3 weeks. (P < 0.001; n = 10 mice/group).
SLC Treatment of CC-10 TAg Mice Leads to Enhanced DC and T Cell Infiltrations of Tumor Sites, Lymph Nodes, and Spleen. To determine the cellular source of GM-CSF and IFN-γ, single cell suspensions of tumors, lymph nodes, and spleens were isolated from SLC and diluent control-treated CC-10 TAg mice. T-lymphocyte infiltration and intracellular cytokine production were assessed by flow cytometry. The cells were also stained to quantify DC infiltration at each site. Compared with the diluent-treated control group, the SLC-treated CC-10 TAg mice showed significant increases in the frequency of cells expressing the DC surface markers CD11c and DEC 205 at the tumor site, lymph nodes, and spleen (Table 2). Similarly, as compared with the diluent-treated control group, there were significant increases in the frequency of CD4 and CD8 cells expressing IFN-γ and GM-CSF at the tumor sites, lymph nodes, and spleen of SLC-treated CC-10 TAg mice (Table 2).

**DISCUSSION**

Host APC are critical for the cross-presentation of tumor antigens (1). However, tumors have the capacity to limit APC maturation, function, and infiltration of the tumor site (38–41). Thus, molecules that attract host APC and T cells could serve as potent agents for cancer immunotherapy. A potentially effective pathway to restore Ag presentation is the establishment of a chemotactic gradient that favors localization of both activated DC and Type 1 cytokine-producing lymphocytes. SLC, a CC chemokine expressed in high endothelial venules and in T-cell zones of spleen and lymph nodes, strongly attracts naïve T cells and DCs (23–30). Because DCs are potent APCs that function as principle activators of T cells, the capacity of SLC to facilitate the colocalization of both DC and T cells may reverse tumor-mediated immune suppression and orchestrate effective cell-mediated immune responses. In addition to its immunotherapeutic potential, SLC has been found to have potent angiostatic effects (11), thus adding additional support for its use in cancer therapy. On the basis of these dual capacities we speculated that SLC would be an important protein for evaluation in cancer immunotherapy. Using two transplantable murine lung cancer models, we have shown previously that the antitumor efficacy of SLC is T cell-dependent. In both models, recombinant SLC administered intratumorally led to complete tumor eradication in 40% of the treated mice. The SLC-mediated antitumor response was dependent on both CD4 and CD8 lymphocyte

**Table 1** SLC treatment of CC-10 TAg mice promotes Type 1 cytokine and antiangiogenic chemokine release and a decline in the immunosuppressive and angiogenic cytokines TGF-β and VEGF.

Following axillary lymph node region injection of SLC, pulmonary, lymph node, and spleen cytokine profiles in CC-10 TAg mice were determined and compared with those in diluent-treated tumor bearing control mice and nontumor bearing syngeneic controls. Lungs were harvested, cut into small pieces, passed through a sieve, and cultured for 24 h. Spleocytes and lymph node-derived lymphocytes (5 × 10^5 cells/ml) were cultured for 24 h. After culture, supernatants were harvested, cytokines quantified by ELISA, and PGE-2 determined by EIA. All determinations from lung were corrected for total protein by Bradford assay, and results are expressed in pg/milligram total protein/24 h. Cytokine and PGE-2 determinations from the spleen and lymph nodes are expressed in pg/ml. Compared with lungs from diluent-treated CC-10 tumor-bearing mice, CC-10 mice treated with SLC had significant reductions in VEGF and TGF-β but a significant increase in GM-CSF (8.3-fold; Table 1). Compared with the diluent-treated control group, the SLC-treated CC-10 TAg mice showed significant increases in the frequency of cells expressing the DC surface markers CD11c and DEC 205 at the tumor site, lymph nodes, and spleen (Table 2). Similarly, as compared with the diluent-treated control group, there were significant increases in the frequency of CD4 and CD8 cells expressing IFN-γ and GM-CSF at the tumor sites, lymph nodes, and spleen of SLC-treated CC-10 TAg mice (Table 2).
Single-cell suspensions of tumor nodules, lymph nodes, and spleens from SLC and diluent-treated tumor-bearing mice were prepared. Intragraftplasmatic staining for GM-CSF and IFN-γ and cell surface staining for CD4 and CD8 T lymphocytes were evaluated by flow cytometry. DCs that stained positive for cell surface markers CD11c and DEC205 in lymph node, tumor nodule, and spleen single-cell suspensions were also evaluated. Cells were identified as lymphocytes or DCs by gating based on the forward and side scatter profiles; 15,000 gated events were collected and analyzed using Cell Quest software. Within the gated T-lymphocyte population from mice treated with SLC, there was an increase in the frequency of CD4+ and CD8+ cells secreting GM-CSF and IFN-γ in the tumor sites, lymph nodes, and spleens compared with those of diluent-treated tumor-bearing control mice. Within the gated DC population, there was a significant increase in the frequency of DCs in the SLC-treated tumor-bearing mice compared with the diluent-treated control tumor-bearing mice.

The cytokine production from tumor sites, lymph nodes, and spleens was evaluated for the following reasons: in the SLC-treated group, there was a significant increase in the frequency of DCs in the SLC-treated tumor-bearing mice compared with the diluent-treated control tumor-bearing mice. The following cytokines were measured: VEGF, IL-10, PGE-2, and TGF-β were determined using transplantable murine or human tumors prop-
nosed by Vicari et al. (42) in the C26 colon cancer model. Using C26 colon carcinoma cells transduced with the SLC cDNA, Vicari et al. (42) demonstrated that the SLC-transduced tumor cells had reduced tumorigenicity that was attributed to both immunological and angiostatic mechanisms (42). In recent studies that directly support the antiangiogenic capacity of this chemokine, Arenberg et al. (43) have reported that SLC inhibits human lung cancer growth and angiogenesis in a SCID mouse model.

In the models reported previously, the antitumor efficacy of SLC was determined using transplantable murine or human tumors propag-
atgated at s.c. sites. We embarked on the current studies to determine the antitumor properties of SLC in a clinically relevant model of lung cancer in which adenocarcinomas develop in an organ-specific manner. Transgenic mice expressing SV40 large TAg transgene under the control of the murine Clara cell-specific promoter, CC-10, develop diffuse bilateral bronchoalveolar carcinoma and have an average life span of 4 months (33). The antitumor activity of SLC was determined in the spontaneous model for lung cancer by injecting recombinant SLC into the axillary lymph node region of the transgenic mice. The efficacy of injecting immune stimulators in the vicinity of the lymph nodes for the treatment of cancer has been demonstrated in recent studies; vaccination with tumor cell-DC hybrids in the lymph node region led to regression of human metastatic renal cell carcinoma (44). Our rationale for injecting SLC in the lymph node region was to colocalize DC to T-cell areas in the lymph nodes where they can prime specific antitumor immune responses. In many clinical situations access to lymph node sites for injection may also be more readily achievable than intratumoral administration. Our results show that this approach is effective in generating systemic antitumor responses. SLC injected in the axillary lymph node regions of the CC-10 TAg mice evidenced potent antitumor responses with reduced tumor burden and a survival benefit as compared with CC-10 TAg mice receiving diluent control injections. The reduced tumor burden in SLC-treated mice was accompanied by extensive lymphocytic as well as DC infiltrates of the tumor sites, lymph nodes, and spleens.

The cytokine production from tumor sites, lymph nodes, and spleens of the CC-10 TAg mice was altered as a result of SLC therapy. The following cytokines were measured: VEGF, IL-10, PGE-2, TGF-β, IFN-γ, GM-CSF, IL-12, MIG, and IP-10 (Table 1). The production of these cytokines was evaluated for the following reasons: the tumor site has been documented to be an abundant source of PGE-2, VEGF, IL-10, and TGF-β, and the presence of these mole-

cules at the tumor site has been shown to suppress immune responses (3, 38, 45). VEGF, PGE-2, and TGF-β have also been documented previously to promote angiogenesis (46–48). Antibodies to VEGF, TGF-β, PGE-2, and IL-10 have the capacity to suppress tumor growth in in vivo model systems. VEGF has also been shown to interfere with DC maturation (38). Both IL-10 and TGF-β are immune inhibitory cytokines that may potentially suppress Ag presentation and antagonize CTL generation and macrophage activation (4, 45). Although at higher pharmacological concentrations IL-10 may cause tumor reduc-
tion, physiological concentrations of this cytokine suppress antitumor responses (4, 49–51). Before SLC treatment in the transgenic tumor-bearing mice, the levels of the immunosuppressive proteins VEGF, PGE-2, and TGF-β were elevated when compared with the levels in normal control mice. There was no such increase with IL-10. Similarly there were not significant alterations in IL-4 and IL-5 after SLC therapy (data not shown). SLC-treated CC-10 TAg mice showed significant reductions in VEGF and TGF-β. The decrease in immuno-

suppressive cytokines was not limited to the lung but was evident systemically. SLC treatment of CC-10 TAg transgenic mice led to a decrease in TGF-β in lymph node-derived cells and reduced levels of PGE-2 and VEGF from splenocytes. Thus, possible benefits of a SLC-mediated decrease in these cytokines include promotion of antigen presentation and CTL generation (4, 45), as well as a limitation of angiogenesis (46–48).

It is well documented that successful immunotherapy shifts tumor-
specific T-cell responses from a type 2 to a type 1 cytokine profile

Table 2  SLC treatment of CC-10 TAg mice leads to enhanced dendritic and T cell infiltrations of tumor sites, lymph nodes, and spleen

<table>
<thead>
<tr>
<th>Groups</th>
<th>IFN-γ</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC-10 + diluent</td>
<td>2 %</td>
<td>5 MCF</td>
</tr>
<tr>
<td>CC-10 + SLC</td>
<td>15 MCF</td>
<td>5 MCF</td>
</tr>
<tr>
<td>Lymph node</td>
<td>15 MCF</td>
<td>3 MCF</td>
</tr>
<tr>
<td>CC-10 + diluent</td>
<td>6 MCF</td>
<td>102</td>
</tr>
<tr>
<td>CC-10 + SLC</td>
<td>16 MCF</td>
<td>65</td>
</tr>
<tr>
<td>Spleen</td>
<td>15 MCF</td>
<td>60</td>
</tr>
<tr>
<td>CC-10 + SLC</td>
<td>16 MCF</td>
<td>168</td>
</tr>
</tbody>
</table>

* P < 0.01, n = six mice/group. For DC staining, MCF (mean channel fluorescence) is noted for DEC205. Experiments were repeated twice.

Table 3  Systemic induction of type 1 cytokines and downregulation of IL-10 after SLC treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>IFN-γ</th>
<th>GM-CSF</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tumor</td>
<td>634</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>Mice without tumor</td>
<td>685</td>
<td>87</td>
<td>147</td>
</tr>
<tr>
<td>Stimulated with CC-10 cells</td>
<td>400</td>
<td>104</td>
<td>78</td>
</tr>
<tr>
<td>SLC-treated</td>
<td>379</td>
<td>132</td>
<td>1000</td>
</tr>
<tr>
<td>SLC-treated</td>
<td>617</td>
<td>185</td>
<td>49</td>
</tr>
<tr>
<td>Stimulated with CC-10 cells</td>
<td>2265</td>
<td>287</td>
<td>200</td>
</tr>
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* P < 0.01 compared with diluent-treated mice as well as SLC-treated constitutive levels. Values given reflect mean ± SE for five mice/group.
leads to the generation of systemic antitumor responses. The antitumor and antiangiogenic effects demonstrated in this model of spontaneous bronchoalveolar carcinoma provide a strong rationale for additional evaluation of SLC regulation of tumor immunity and its use in immunotherapy for lung cancer.

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