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 chemoattracts 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 lymphocytic 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 I 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 vitro, 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. Because tumor cells often have limited expression of MHC antigens and lack costimulatory molecules, they are ineffective APCs. In addition, tumor cells secrete immunosuppressive mediators that contribute to evasion of host immune surveillance. 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 anticancer 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. Chemokines also function in maintaining immune homeostasis and secondary lymphoid organ architecture. 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, JE/MCP-1/ MCAF, MIP3α, MIP3β, and IP-10. 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. The capacity of SLC to chemoattract DCs 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 (21). 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. 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 (Irvine Scientific, Santa Ana, CA) supplemented with 10% FBS (Gemini Bioproducts, Calabasas, CA), penicillin (100 units/ml), streptomycin (0.1 mg/ml), and 2% of the 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 bilaterally by microscopic examination as early as 4 weeks of age. After 3 months of age, the bronchoalveolar pattern of tumor growth coalesced to form multiple bilateral tumor nodules. The CC-10 Tag transgenic mice had an average life span of 4 months. Extrathoracic metastases were not noted. Breeding pairs for these mice were generously provided by Francesco J. DeMayo (Baylor College of Medicine, Houston, TX). Transgenic mice were bred at the West Los Angeles Veteran Affairs vivarium and maintained in the animal research facility. Before each experiment using the CC-10 Tag trans-
genic mice, presence of the transgene was confirmed by PCR of mouse tail biopsies. The 5′ primer sequence was SM19-TAG: 5′-TGGACCTTTCTAG-GTCTTGAAAG-3′, and the 3′ primer sequence was SM36-TAG: 5′-AG-GCATTCACCAGTCCCTCATT-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 run on a 1% agarose gel stained with ethidium bromide. All of the experiments used pathogen-free CC-10 TAg transgenic mice beginning at 4–5 week 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 (×20 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-necrotic tumors were harvested and cut into small pieces and passed through a sieve (Belco, 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 dH2O, and brought to toxicity with 1 × PBS. After a 24-h culture period, tumor nudeus 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 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 week of age.

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 and spleens 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. 1E). 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).

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SLC mediates potent antitumor responses in a murine model of spontaneous lung cancer. The antitumor efficacy of SLC was evaluated in the spontaneous bronchogenic 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 Promotes Type 1 Cytokine and Antiangiogenic Chemokine Release and a Decline in the Immunosuppressive Cytokines TGF-β and VEGF. On the basis of previous reports indicating that tumor progression can be modified by host cytokine profiles (36, 37), we evaluated the cytokine production from tumor sites, lymph nodes, and spleen after SLC therapy. Cytokine profiles in the lungs, spleens, and lymph nodes of CC-10 TAg mice treated with recombinant SLC were compared with those in diluent-treated control mice bearing tumors as well as nontumor bearing controls. SLC treatment of CC-10 TAg mice led to systemic induction of Type 1 cytokines but decreased production of immunosuppressive mediators. Lungs, lymph node, and spleens were harvested, and after a 24-h culture period, supernatants were evaluated for the presence of VEGF, IL-10, IFN-γ, GM-CSF, IL-12, MIG, IP-10, and TGF-β by ELISA and for PGE-2 by EIA. Compared with lungs from the diluent-treated group, CC-10 TAg mice treated with SLC had significant reductions in VEGF (3.5-fold) and TGF-β (1.83-fold) but an increase in IFN-γ (160.5-fold), IP-10 (1.7-fold), IL-12 (2.1-fold), MIG (2.1-fold), and GM-CSF (8.3-fold; Table 1). Compared with the diluent-treated group, splenocytes from SLC-treated CC-10 TAg mice revealed reduced levels of PGE-2 (14.6-fold) and VEGF (20.5-fold) but an increase in GM-CSF (2.4-fold), IL-12 (2-fold), MIG (3.4-fold), and IP-10 (4.1-fold; Table 1). Compared with diluent-treated CC-10 TAg mice, lymph node-derived cells from SLC-treated mice secreted significantly enhanced levels of IFN-γ (2.2-fold), IP-10 (2.3-fold), MIG (2.3-fold), and IL-12 (2.5-fold) but decreased levels of TGF-β (1.8-fold; Table 1). The immunosuppressive mediators PGE-2 and IL-10 were not altered at the tumor sites of SLC-treated mice; however, there was a significant reduction in the level of PGE-2 in the spleen of SLC-treated mice.

To determine whether SLC administration induced significant specific systemic immune responses, splenocytes from SLC and diluent-treated CC-10 TAg mice were cocultured in vitro with irradiated CC-10 TAg tumor cells for 24 h, and GM-CSF, IFN-γ, and IL-10 were determined by ELISA. After secondary stimulation with irradiated tumor cells, splenocytes from SLC-treated tumor-bearing mice secreted significantly increased levels of IFN-γ (5.9-fold) and GM-CSF (2.2-fold). In contrast, IL-10 secretion was reduced 5-fold (Table 3).

### 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

<table>
<thead>
<tr>
<th>Group</th>
<th>PGE-2</th>
<th>VEGF</th>
<th>IL-10</th>
<th>IFN-γ</th>
<th>GM-CSF</th>
<th>IL-12</th>
<th>MIG</th>
<th>IP-10</th>
<th>TGF-β</th>
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<td>Lung</td>
<td></td>
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<tr>
<td>CC-10 + diluent</td>
<td>12419 ± 384</td>
<td>980 ± 38</td>
<td>213 ± 11</td>
<td>26 ± 5</td>
<td>74 ± 5</td>
<td>110 ± 8</td>
<td>47.8 ± 5</td>
<td>108 ± 6</td>
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<td>11945 ± 208</td>
<td>222 ± 53*</td>
<td>239 ± 20</td>
<td>474 ± 26*</td>
<td>611 ± 11*</td>
<td>235 ± 15*</td>
<td>98.4 ± 4</td>
<td>187 ± 2*</td>
<td>154 ± 15*</td>
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<td>FVB control</td>
<td>6023 ± 40</td>
<td>422 ± 53</td>
<td>129 ± 8</td>
<td>122 ± 28</td>
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<td>167 ± 7</td>
<td>72.3 ± 6</td>
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<td>118 ± 9</td>
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<tr>
<td>FVB control mice</td>
<td>72 ± 2</td>
<td>107 ± 36</td>
<td>85 ± 6</td>
<td>101 ± 24</td>
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<td>Diluent-treated CC-10</td>
<td>643 ± 51</td>
<td>267 ± 14</td>
<td>87 ± 11</td>
<td>106 ± 3</td>
<td>45 ± 3</td>
<td>67 ± 6</td>
<td>42</td>
<td>63 ± 3</td>
<td>&lt;15</td>
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<td>SLC-treated CC-10</td>
<td>44 ± 10*</td>
<td>13 ± 1*</td>
<td>84 ± 11</td>
<td>107 ± 9</td>
<td>110 ± 4*</td>
<td>137 ± 5*</td>
<td>142*</td>
<td>261 ± 5*</td>
<td>17 ± 5</td>
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<td>Lymph node</td>
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<tr>
<td>FVB control mice</td>
<td>148 ± 3</td>
<td>204 ± 18</td>
<td>78 ± 6</td>
<td>98 ± 23</td>
<td>65 ± 2</td>
<td>195 ± 5</td>
<td>70</td>
<td>&lt;15</td>
<td>102 ± 4</td>
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<tr>
<td>Diluent-treated CC-10</td>
<td>94 ± 3</td>
<td>142 ± 12</td>
<td>81 ± 4</td>
<td>89 ± 9</td>
<td>42 ± 2</td>
<td>95 ± 10</td>
<td>46</td>
<td>43 ± 3</td>
<td>139 ± 6</td>
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<tr>
<td>SLC-treated CC-10</td>
<td>113 ± 4</td>
<td>221 ± 32</td>
<td>86 ± 20</td>
<td>192 ± 8*</td>
<td>44 ± 3</td>
<td>233 ± 6*</td>
<td>106*</td>
<td>100 ± 2*</td>
<td>86 ± 7*</td>
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a P < 0.01 compared to diluent-treated CC-10 tumor-bearing mice.

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...
Single-cell suspensions of tumor nodules, lymph nodes, and spleens from SLC and diluent-treated tumor-bearing mice were prepared. Intracytoplasmic 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 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. 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IFN-γ (%)</th>
<th>GM-CSF (%)</th>
<th>IFN-γ (%)</th>
<th>GM-CSF (%)</th>
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<td>CC-10 + diluent</td>
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<td>CC-10 + SLC</td>
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<td>187</td>
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<td>Lymph node</td>
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<tr>
<td>CC-10 + diluent</td>
<td>3.2</td>
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<tr>
<td>CC-10 + diluent</td>
<td>3.9</td>
<td>163</td>
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<td>CC-10 + SLC</td>
<td>4.7</td>
<td>206b</td>
<td>4.7</td>
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* P < 0.01, n = six mice/group. For DC staining, MCF (mean channel fluorescence) was noted for DE205. Experiments were repeated twice.

No tumor
Mice without tumor, constitutive 634 ± 45 55 ± 7 32 ± 4
Stimulated with CC-10 cells 685 ± 39 87 ± 5 147 ± 8
Diluent-treated
Diluent-treated, constitutive 400 ± 38 104 ± 11 78 ± 2
Stimulated with CC-10 cells 379 ± 28 132 ± 5 1000 ± 69
SLC-treated
SLC-treated, constitutive 617 ± 42 185 ± 3 49 ± 2
Stimulated with CC-10 cells 2265 ± 107a 287 ± 10a 200 ± 7a

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

<table>
<thead>
<tr>
<th>Group</th>
<th>IFN-γ (%)</th>
<th>GM-CSF (%)</th>
<th>IL-10 (%)</th>
</tr>
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</table>
| No tumor             | Mice without tumor, constitutive 634 ± 45 55 ± 7 32 ± 4
|                      | Stimulated with CC-10 cells 685 ± 39 87 ± 5 147 ± 8
| Diluent-treated      | Diluent-treated, constitutive 400 ± 38 104 ± 11 78 ± 2
|                      | Stimulated with CC-10 cells 379 ± 28 132 ± 5 1000 ± 69
| SLC-treated          | SLC-treated, constitutive 617 ± 42 185 ± 3 49 ± 2
|                      | Stimulated with CC-10 cells 2265 ± 107a 287 ± 10a 200 ± 7a

It is well documented that successful immunotherapy shifts tumor-specific T-cell responses from a type 2 to a type 1 cytokine profile.
(52). Responses depend on IL-12 and IFN-γ to mediate a range of biological effects, which facilitate antitumor immunity. IL-12, a cytokine produced by macrophages (53) and DC (54), plays a key role in the induction of cellular immune responses (55). IL-12 has been found to mediate potent antitumor effects that are the result of several actions involving the induction of CTL. Type 1-mediated immune responses, and natural killer activation (53), as well as the impairment of tumor vascularization (56). IP-10 and MIG are CCX chemokines that chemotactically activate T cells expressing the CXCR3 chemokine receptor (57). Both IP-10 and MIG are known to have potent antitumor and antiangiogenic properties (14, 58–60). The lugs of SLC-treated CC-10 TAg mice revealed significant increases in IFN-γ, IL-12, IL-10, MIG, and GM-CSF. MIG and IL-10 are potent angiostatic factors that are induced by IFN-γ (59, 61, 62) and may be responsible in part for the tumor reduction in CC-10 TAg mice after SLC administration. Because SLC is documented to have direct antiangiogenic effects (11, 63), the tumor reductions observed in this model may be attributable to T cell-dependent immunity as well as participation by T cells secreting IFN-γ in inhibiting angiogenesis (62). Hence, an increase in IFN-γ at the tumor site of SLC-treated mice could explain the relative increases in IP-10 and MIG. Both MIG and IP-10 are chemotactic for stimulated CCXCR3-expressing T lymphocytes that could additionally amplify IFN-γ at the tumor site (64). Flow cytometric determinations revealed that both CD4 and CD8 cells were responsible for the increased secretion of GM-CSF and IFN-γ in SLC-treated mice. An increase in GM-CSF in SLC-treated mice could enhance DC maturation and antigen presentation (32). Additional studies are necessary to precisely define the host cytokines that are critical to the SLC-mediated antitumor response.

The increase in the Type 1 cytokines was not limited to the lung but was evident systemically. SLC treatment of CC-10 TAg transgenic mice led to systemic increases in Type 1 cytokines and antiangiogenic chemokines. Hence, splenocytes from SLC-treated CC-10 TAg mice had an increase in GM-CSF, IL-12, MIG, and IP-10 as compared with diluent-treated CC-10 TAg mice. Similarly, lymph node-derived cells from SLC-treated mice secreted significantly enhanced levels of IFN-γ, IL-10, MIG, and IL-12. Recent studies suggest that the evaluation of type 1 responses at the LN sites may provide insights into antitumor responses in patients receiving immune therapy (65). The increase in GM-CSF and IFN-γ in the spleen and lymph nodes of SLC-treated mice could in part be explained by an increase in the frequency of CD4 and CD8 cells secreting these cytokines. The increase in Type 1 cytokines was in part attributable to an increase in specificity against the autologous tumor; when cocultured with irradiated CC-10 TAg tumor cells, splenocytes from SLC-treated CC-10 TAg mice secreted significantly increased amounts of GM-CSF and IFN-γ but reduced levels of IL-10. Whereas the T cells secrete cytokines in response to stimulation with CC-10 cells, we have not yet confirmed that this cytokine secretion is tumor-specific. Cell surface staining of CC-10 cells followed by flow cytometry did not show detectable levels of MHC class II molecules. Although the tumor did not show MHC class II expression, CD4+ type 1 cytokine production may have occurred because splenic APC were present in the assay. Although in vitro tumor-stimulated splenic T cells from SLC-treated mice showed reduced expression of IL-10, SLC therapy did not lead to a decrease of IL-10 levels in vivo. The in situ microenvironment may provide other important factors from cellular constituents in addition to T cells that determines the overall levels of IL-10. This may explain the discrepancies in the in vitro and in vivo results.

Taken together, the current study indicates that SLC injected in the axillary lymph node region in the spontaneous lung cancer model leads to the generation of systemic antitumor responses. The antitumor properties of SLC may be attributable to its chemotactic capacity in colocalization of DCs and T cells, as well as the induction of cytokines such as IFN-γ, IP-10, MIG, and IL-12. Additional studies will be required to delineate the importance of each of these cytokines in SLC-mediated antitumor responses. The potent antitumor properties 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.

REFERENCES


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

Sherven Sharma, Marina Stolina, Li Zhu, et al.