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Soluble Expression of Recombinant Human Secondary Lymphoid Chemokine (SLC) in *E. coli* and Research on Its *In Vitro* and *In Vivo* Bioactivity

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Secondary lymphoid tissue chemokine (SLC) is a CC chemokine that plays an important role in leukocytes homing to lymphoid tissues. The ability of SLC to co-localize both T cells and dendritic cells formed the rationale to evaluate its utility in cancer immunotherapy. The *in vivo* antitumor effect of murine SLC (mSLC) has been well documented, but little is known about that of human SLC (hSLC). To investigate the antitumor efficiency *in vivo* of hSLC, the hSLC gene was artificially synthesized and induced to express as a soluble form in *Escherichia coli*. After purification, the purity of the recombinant human SLC (rhSLC) protein was above 95% by SDS-PAGE analysis. The K_d of rhSLC binding to peripheral blood lymphocytes (PBLs) was 0.2186 ± 0.02675 μM as assessed by FACS, and the maximal chemotactic index of rhSLC was 9.49 at 100 nM as assessed by *in vitro* chemotaxis assay. Then genomic sequences of hSLC and mSLC, and of human CCR7 (hCCR7) and murine CCR7 (mCCR7), the receptor for SLC, were aligned. It was found that hSLC and mSLC share 70.72% identity and hCCR7 and mCCR7share 86.77% identity. Furthermore, we found that rhSLC could chemoattract murine peripheral blood mononuclear cells (PBMCs) *in vitro*. On the basis of these facts, immune competent mice inoculated with S180 sarcoma cells were chosen as an *in vivo* model. Intratumoral injections of rhSLC inhibited tumor growth and increased survival. These findings suggest that, despite its incapability to bind to either human or murine CXCR3, which is related to angiostasis, rhSLC can induce an antitumor response *in vivo* by another route. This report proves that rhSLC has a potent tumor-inhibition ability that makes it a promising candidate agent in cancer immunotherapy.

Key words: antitumor response, chemokine, *Escherichia coli*, recombinant human secondary lymphoid chemokine.

Chemokines constitute a superfamily of small secreted proteins with common structural features that share the ability to chemoattract leukocytes (*1*, *2*). Chemokines also function in regulating angiogenesis (*3*, *4*), and in maintaining immune homeostasis and secondary lymphoid organ architecture (*5*). Secondary lymphoid tissue chemokine (SLC) is a CC chemokine localized in high endothelial venules and T cell zones of spleen and lymph nodes (*6*, *7*). SLC has potent chemotactic activity for T cells, B cells (*7*, *8*) and mature dendritic cells (DCs) (*9*), and plays an important role in T cell priming by co-localization of antigen-presenting DCs and T cells (*10*). The capacity to facilitate the co-localization of both DCs and T cells forms a strong rationale for the use of SLC in cancer therapy.

Effective antitumor responses require both antigen-presenting cells (APCs) and lymphocyte effectors (*11*). However, tumor cells themselves are ineffective APCs because most of them have limited expression of MHC antigens and lack costimulatory molecules (*12*). Furthermore, tumor cells produce immune inhibitory factors to promote escape from immune surveillance (*13*, *14*). Therefore, it has been suggested that effective antitumor immunity may be achieved by recruiting professional host APCs for tumor antigen presentation to promote specific T cells activation (*15*). Thus, chemokines that attract both DCs and lymphocyte effectors to tumor sites could serve as potent agents in cancer immunotherapy (*16*).

The *in vivo* antitumor effects of murine SLC (mSLC) have been well documented (*16–25*). mSLC is a ligand for two specific G protein–coupled seven-transmembrane-domain chemokine receptors, CCR7 (*26*) and CXCR3 (*27*). Whereas CCR7 is expressed on naïve T cells and mature DCs (*28*), CXCR3 is expressed preferentially on Th1 cytokine–producing lymphocytes with a memory phenotype (*29*) and that are related to angiostasis (*23*). Thus, mSLC can exert its antitumor effects both by inducing angiostasis (*23*) and by facilitating the co-localization of naïve T cells and mature DCs, which serve as professional host APCs at the site of tumorgenesis (*16*).

However, human SLC (hSLC), unlike mSLC, is not a ligand for human or murine CXCR3 (*29*) and does not

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inhibit angiogenesis or tumor growth in the SCID mouse model (*23*). The *in vivo* antitumor effects of hSLC in immune competent models have not been reported to date. Given the ability of hSLC to attract activated DCs (*9*) and T cells (*7*, *8*) through CCR7, it was hypothesized that, even though incapable of inhibiting angiogenesis, hSLC can also mediate an antitumor response *in vivo*.

To test this hypothesis, *Escherichia coli* was chosen to express hSLC considering that hSLC is a small molecule with no glycosylation site reported (*6*). Moreover, it is reported that several chemokines have been expressed successfully in *E. coli* (*30–32*) with their bioactivities intact (*33*).

Further experiments were restricted by the difficulty in obtaining an optimal *in vivo* model. According to the results of *in vitro* chemotaxis assays, it was found that hSLC can also chemoattract murine peripheral blood mononuclear cells (PBMCs). So, immune competent mice were chosen as the *in vivo* model in which to assess the antitumor ability of hSLC. This study provides some data by which the role of rhSLC in the regulation of tumor immunity and its use in cancer immunotherapy can be evaluated.

MATERIALS AND METHODS

Cells and Mice—The murine cell line sarcoma S180 was used to assess antitumor response *in vivo*. The cells were routinely cultured in 150-cm^3 tissue culture flasks containing RPMI 1640 supplemented with 10% FBS and maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Human PBMCs were isolated from sodium citrate-treated venous blood obtained from healthy adult donors using Ficoll-Paque (Shanghai Hengxin Chemical Reagent, Ltd., China), and residual erythrocytes were lysed by a hypotonic solution. Human peripheral blood lymphocytes (PBLs) were expanded from PBMC by stimulation with phytohemagglutinin (PHA) (Sigma, USA) for two days, and cultivation for another week in the presence of 400 units/ml IL-2. Female CD-1 mice, the F1 mice from ICR× BALB/c, were purchased from Beijing laboratory animal center and used at 4 to 6 weeks of age.

Construction of Expression Vectors Encoding Recombinant Human SLC—The full-length *slc* sequence was synthesized based on the human *slc* gene searched from GenBank (Locus: BC027918) using SOE-PCR with *E. coli* preferential codons. The following synthesized fragments were used to obtain full-length *slc*:

FR1: 5′-AAAAAGAATATCGCATTTCTTCTTGCATC-TATGTTCGTTTTTTC-3′, FR2: 5′-GCATCTATGTTCGT-TTTTTCTATTGCTACAAACGCGTACGCTAGCGATGGC-GGCGCG-3′, FR3: 5′-AGCGATGGCGGCGCGCAGG-ATTGCTGCCTGAAATATAGCCAGCGTAAAATTCCG-3′, FR4: 5′-CAGGCTCGGTTCCTGTTTACGGTAGCTGCGA-ACAACTTTCGCCGGAATTTTACGCTGGCT-3′, FR5: 5′-AAACAGGAACCGAGCCTGGGCTGCAGCATCCCGGCG-ATCCTGTTTCTGCCGCGTAAACGT-3′, FR6: 5′-TGCAC-CCACAGTTCTTTCGGATCGGCGCACAGTTCCGCCTG-GCTACGTTTACGCGGCAGA-3′, FR7: 5′-CGAAAGAAC-TGTGGGTGCAGCAGCTGATGCAGCATCTGGATAAAA-CCCCGAGCCCGCAG-3′, FR8: 5′-TTTTGCTCGCGCCA-CGATCTTTACGGCAGCCCTGCGCCGGTTTCTGCGGG-CTCGGGGTTT-3′, FR9: 5′-CGTGGCGCGAGCAAAACC-GGCAAAAAAGGCAAAGGCAGCAAAGGCTGCAAACGT-ACCG-3′, FR10: 5′-CTACGGGCCTTTCGGGGTCTGGC-TACGTTCGGTACGTTTGCAGCCTT-3′, PR1: 5′-GGCTC-GAGATGGCGCAGTCTCTGGC-3′; PR2: 5′-CAGGCTCG-GTTCCTGTTTACG-3′, PR3: 5′-AAACAGGAACCGAGC-CTGG-3′, PR4: 5′-TTTTGCTCGCGCCACG-3′ and PR5: 5′-CCGAATTCCTACGGGCCTTTCGGGG-3′.

After confirming the entire nucleotide sequence, the human *slc* gene fragment was amplified with the primers 5′-AGCGATGGCGGCGCGCAGGATTGC-3′ (forward) and 5′-AAAGAATTCCGGGCCTTTCGGGGT-3′ (reverse). The PCR product was digested with *Eco*RI (TaKaRa, Japan) and then cloned into the expression vector pTCM2 between the *Nco*I site, which was end-filled to give a blunt end, and *Eco*RI site. The whole recombinant human *slc* gene was sequenced by Bioasia Biotechnology, Ltd. (Shanghai, China). pTCM2 is a T7-based *E. coli* expression vector coming from altered pET28a. It was constructed in our laboratory. The start codon ATG lies in the inactivity restriction site *Nco*I*, and the human *slc* sequence localizes immediately to ATG so that rhSLC has methionine as the first amino acid at the N-terminus. Because there are a $(His)_6$ tag and *C-myc* sequence prior to the stop codon TAA in pTCM2, the rhSLC has both a $(His)_6$ and *C-myc* tag at C-terminus.

Expression and Purification of rhSLC—The expression vector pTCM2 containing *slc* was transformed into *E. coli* BL21(DE3) star strain and induced to expression by 0.4 mM IPTG at 30°C for 6 h. The cells was harvested and lysed by sonication, and the whole cell lysate was centrifugated at 12,000 rpm for 45 min. Then the supernatant containing the recombinant proteins was purified in two steps using a CM Sepharose column and a Ni-NTA affinity chromatography column (Amersham Biosciences AB. Uppsala, Sweden). All buffers for purification were prepared with pyrogen-free water. The purification product was identified by western blotting analysis using mouse anti–*C-myc* monoclonal antibody (SantaCruz, USA) as the prime antibody and HRP-conjugated goat-anti-mouse IgG antibody (Beijing Zhongshan Biotechnology Co. Ltd., China) as the second antibody. The protein concentration was determined using a BCA kit (Sigma), and the purity was analyzed by SDS-PAGE. The endotoxin level was determined by tachypleus amebocyte lysate (Marin organisms products Factory of Zhanjiang, China) according to the requirements for bacterial endotoxin test biologics.

Flow Cytometry—3×10^5 PBL were incubated with rhSLC at various concentrations (3, 2, 1, 0.5, 0.1, 0.05, 0.01, 0.001 μM) for 40 minutes on ice. Then the cells were washed once with PBSA (PBS containing 1% BSA), incubated with mouse anti–*C-myc* monoclonal antibody (1: 1,000 dilution) for 40 min on ice, washed with PBSA and incubated with FITC-labeled goat-anti-mouse IgG antibody (PharMingen, San Diego, CA) for 40 min on ice. The cells were once washed with PBSA, resuspended in PBSA, and prepared for testing by FACScan flow cytometer (Becton Dickinson, San Jose, CA). Cells were identified as lymphocytes by gating based on forward and side scatter profiles. Ten thousand gated events were collected and analyzed using Cell Quest software (Becton Dickinson). The mean fluorescent intensity was recorded according to Histogram statistics. The K_d was calculated by the method of Scatchard using software Prism version

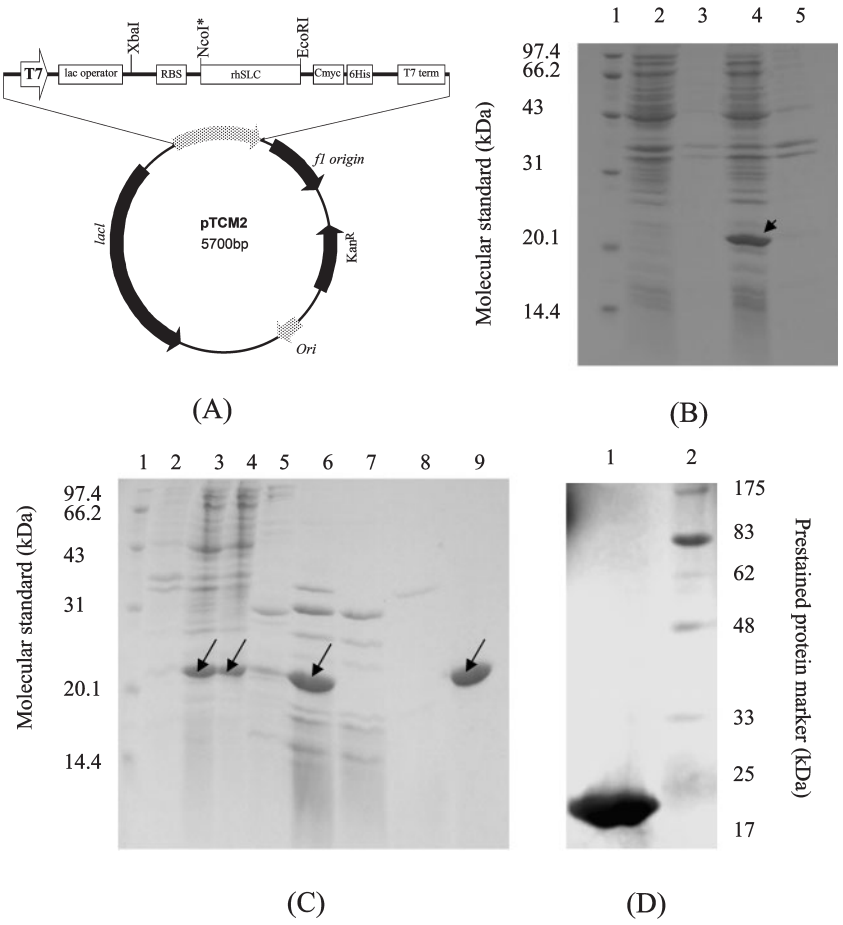


Fig. 1. **Expression and purification of rhSLC.** (A) Schematic diagram showing the expression vector pTCM2 containing the human *slc* gene. The rhSLC protein has methionine as the first amino acid at the N-terminus and both $(His)_6$ and a *C-myc* tag at the C-terminus. (B) SDS-PAGE analysis of rhSLC expression. The expression vector pTCM2 with or without *slc* was transformed into *E. coli* BL21 star strain and induced to expression by IPTG. The bacteria were harvested, lysed and centrifuged. The supernatant and pellet were analyzed by 15% SDS-PAGE. Lane 1: protein molecular size standards; lane 2: supernatant of control cells; 3: pellet of control cells; lane 4: supernatant of cells containing rhSLC; lane 5: pellet of cells containing rhSLC. The band marked with an arrow is rhSLC. (C) SDS-PAGE analysis of rhSLC purification. The supernatant containing rhSLC was purified in two steps on a CM Sepharose column and a Ni-NTA affinity chromatography column. Lane 1: protein molecular size standards; lane 2: pellet of the whole cell lysate; lane 3: supernatant of the whole cell lysate; lane 4: flow-through of CM Sepharose; lane 5: eluate by 0.25 M NaCl from CM Sepharose; lane 6: elute by 0.5 M NaCl from CM Sepharose; lane 7: flow-through from Ni-NTA; lane 8: wash with 40 mM imidazole from Ni-NTA; lane 9: elute by 200 mM imidazole from Ni-NTA. The purity of rhSLC was above 95%. The bands marked by arrows are rhSLC. (D) Western blotting analysis of rhSLC. The purification product was identified by western blotting using mouse anti–*C-myc* monoclonal antibody as the prime antibody and HRP-conjugated goat-anti-mouse IgG antibody as the second antibody. Lane 1: purified rhSLC; lane 2: prestained protein marker (kDa).

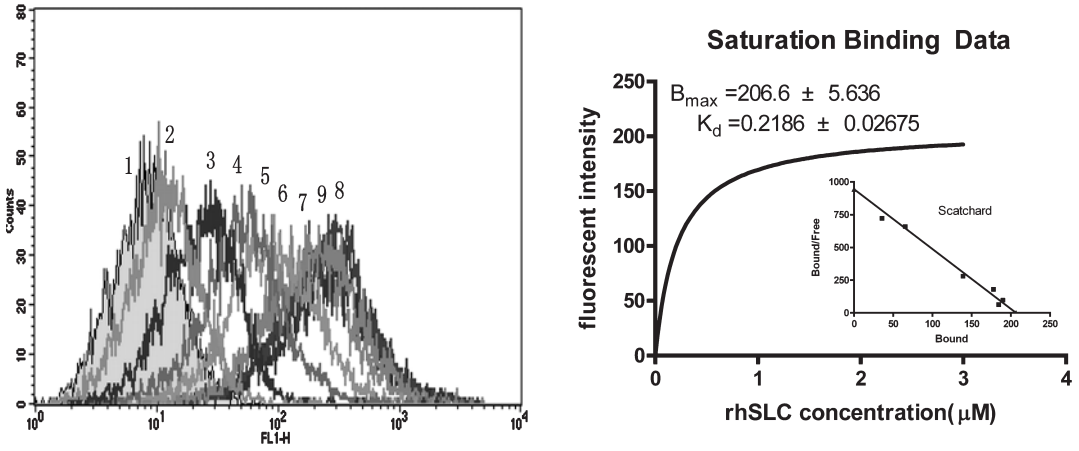
4.0 on the basis of various rhSLC concentrations and the corresponding mean fluorescent intensities.

Chemotaxis Assay—Cell migration was assessed in a 12-well chemotaxis chamber (Neuroprobe, USA) according to the protocol provided by the manufacturer. In brief, PBLs were suspended in RPMI 1640 containing 10% FBS at 5×10^5/ml. The lower wells were filled with 160 μl of assay buffer with or without rhSLC, while the upper wells were filled with 130 μl of cell suspension. The lower and upper wells were separated by a polyvinylpyrrolidone-free polycarbonate filter (Neuroprobe, USA) with a 5 μm pore size. Before use, the surface of the filter facing the lower wells was precoated with 5 μg/ml collagen type IV (Sigma) for 2 h at room temperature and extensively washed with distilled water. Assays were carried out at 37°C for 4 h in a humidified atmosphere of 5% CO_2 in air. Then the filter was washed, fixed and stained with a Liu solution kit (Baso Diagnostic Inc. Taiwan). The number of migrated cells in five randomly selected high power (×160) fields was counted. All assays were done in triplicate. The chemotactic index (CI) is the ratio of the number of cells that migrated toward a chemotactic factor divided by the number of cells that migrated toward control medium.

Tumorigenesis In Vivo—About 10^5 murine sarcoma S180 cells with 1 μg or 0.5 μg rhSLC or PBS were inoculated by s.c. injection in the left axillary region of CD-1 mice. The tumor-inoculating day was set as day 0. From the second day, SLC or PBS was administered in the tumor-inoculated region or intratumorally every other day for a total of twelve treatments. Tumor volume was monitored every other day. Two bisecting diameters of each tumor were measured with a vernier caliper. The volume was calculated using the formula $0.4ab^2$ with a as the larger diameter and b as the smaller diameter. There were 3 mice in each group.

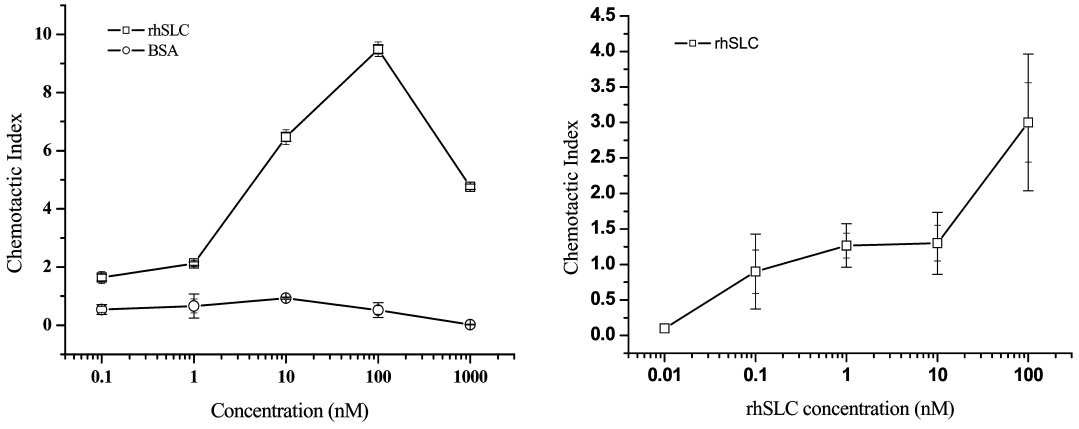
RESULTS

Expression and Purification of rhSLC—The synthesized human *slc* gene was cloned into the expression vector pTCM2. Figure 1A shows a schematic diagram of pTCM2 containing the human *slc* gene. After expression in *E. coli*, it was found that the expression products were mainly in the supernatant (Fig. 1B), indicating that rhSLC was expressed in *E. coli* in a soluble form. The rhSLC migrated as a band of about 20 kDa on SDS-PAGE. Because hSLC has a unique extended C-terminus of 32 amino acids with a positive charge, cation exchange chromatography was chosen as the first purification step. A CM-Sepharose column was used and pH 7.5 was set as the working pH. Some contaminating proteins were eluted by 0.25 M NaCl; rhSLC was mainly eluted by 0.5 M NaCl. Then the coarse purification products were fur-



(A) (B)

Fig. 2. **Specific binding of rhSLC to PBL.** (A) FACS analysis of rhSLC binding to its surface receptor on PBL. PBL were incubated step by step with rhSLC at various concentrations, mouse anti–*C-myc* monoclonal antibodies and FITC-labeled goat-anti-mouse IgG antibodies. Then the cells were analysed by FACS. Cells not incubated with rhSLC were used as a negative control. 1: negative control; 2–9: 0.001, 0.01, 0.05, 0.1, 0.5, 1, 2, 3 μM rhSLC, respectively. (B) Saturation binding of rhSLC to PBL. The K_d was calculated by the method of Scatchard on the basis of various rhSLC concentrations and corresponding mean fluorescent intensities.



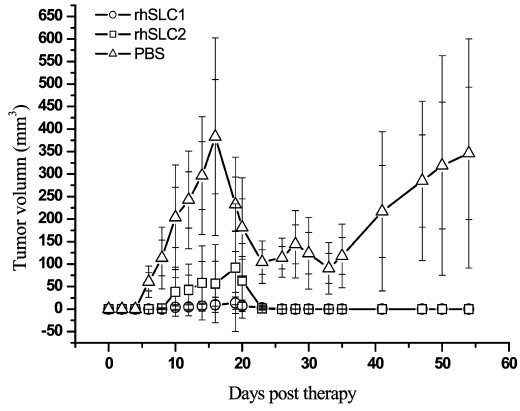
(A) (B)

Fig. 3. **Chemotactic activity of rhSLC *in vitro*.** (A) Chemotactic response of human PBL to rhSLC and BSA. PBL were stimulated with BSA (circles) or rhSLC (squares) at the indicated concentrations. BSA was used as a control. (B) Chemotactic response of freshly isolated murine PBMC to rhSLC. PBMC were stimulated with rhSLC (squares) at the indicated concentrations. All assays were done in triplicate. Each point represents mean ± SE of three separate experiments.

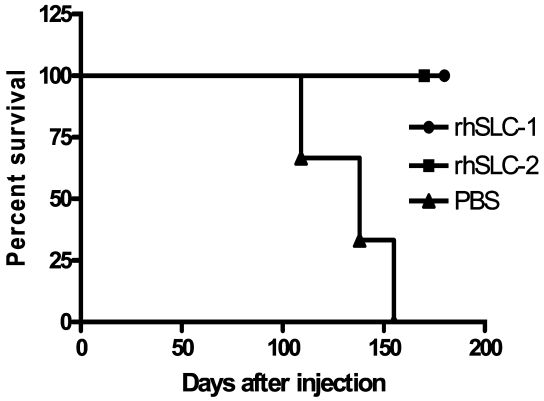
ther purified through a Ni-NTA affinity column based on the $(His)_6$ tag at the C-terminal of rhSLC (Fig. 1C). The purity was above 95% by SDS-PAGE analysis, and the purified protein was identified by western blotting to be rhSLC (Fig 1D). The concentration of pure rhSLC was 362.1 μg/ml and the endotoxin level of rhSLC was below 0.4 EU/μg.

Specific Binding of rhSLC to PBL—It has been reported that naïve T lymphoid cells express CCR7, which is the receptor of hSLC, and that the expression of CCR7 can be upregulated by PHA. So cultured human PBL expanded with PHA+ IL-2 were chosen to test the binding activity of rhSLC to its receptor by flow cytometry. As shown in Fig. 2A, rhSLC could bind potently to PBL, and the binding reached saturation at 1 μM. The K_d was 0.2186 ± 0.02675 μM as calculated by the method of Scatchard. (Fig. 2B)

Chemotactic Activity of rhSLC In Vitro—The chemotactic activity of rhSLC was tested using cultured human PBL expanded with PHA+ IL-2. As shown in Fig. 3A, rhSLC induced vigorous cell migration with chemotactic indexes above 2 at 1–1,000 nM, and a maximal chemotactic index of 9.49 at 100 nM. The chemotactic index of BSA, which served as a control, was below 1 at 0.1–1,000 nM. Then freshly isolated murine PBMCs were used to test whether rhSLC can chemoattract murine PBMC. It was found that rhSLC induced mPBL migration with a maximal chemotactic index of 3 at 100 nM (Fig. 3B). The



(A)



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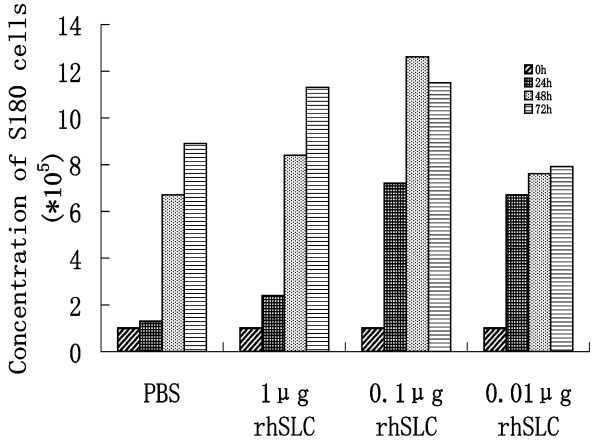

(C)

Fig. 4. **rhSLC mediates antitumor responses in immune competent mice.** Ten thousand mouse sarcoma S180 cells were inoculated s.c. into the left axillary region of CD-1 mice. From tumor establishment day, 0.5 μg (squares) or 1 μg (circles) rhSLC per injection or PBS (triangles) was administrated intratumorally every other day for twenty days. Tumor volume was monitored every other day (n = 3 mice/group). (A) Intratumoral rhSLC administration led to a significant reduction in tumor volume compared with PBS-treated tumor-bearing mice. In the rhSLC treatment group, 100% of mice showed tumor eradication. (B) Survival of mice treated with 0.5 μg (squares), 1 μg rhSLC (circles) or PBS (triangles) after inoculation of mouse sarcoma S180 cells. Mice were observed until day 175 or death. All experiments were done with groups of 3 mice. (C) Proliferation of mouse sarcoma S180 cells with or without rhSLC for 72 h. rhSLC at various concentrations was added to 10^5 S180 cells plated in 96-well Costar plates, and cell numbers were monitored daily for 3 days. Cell proliferation was measured by cell counting. S180 cells cultured with PBS were used as a control.

ability of rhSLC to chemoattract murine PBMCs became the rationale for using immune competent mice as an animal model in which to test the antitumor effect of human SLC *in vivo*.

Inhibition of Tumor Growth and Increase in Survival by Intratumoral Injection of rhSLC—The administration of rhSLC began on the day when murine sarcoma S180 cells were inoculated and continued every other day till the twentieth day. Intratumoral injection of rhSLC significantly inhibited tumor growth as compared with PBS-treated tumor-bearing control mice. The inhibitory effect induced by 1 μg rhSLC was more potent than the effect induced by 0.5 μg rhSLC (Fig. 4A). All mice treated with 1 or 0.5 μg rhSLC showed complete tumor eradication, whereas the mice treated with PBS showed a temporal decrease in tumor volume and then progression that continued until death. Meanwhile, survival was increased by the intratumoral injection of rhSLC as compared with the injection of PBS (Fig. 4B).

Direct Effect of rhSLC on Murine Sarcoma S180 Cells In Vitro—To determine whether the decrease in tumor volume resulted from a direct effect of rhSLC on S180 cells, the *in vitro* proliferation of tumor cells was assessed in the presence of rhSLC. rhSLC at various concentrations was added to 10^5 S180 cells plated in 96-well Costar plates, and cell numbers were monitored daily for 3 days. Cell proliferation was measured by cell counting. As shown in Fig. 4C, rhSLC did not alter the *in vitro* proliferation rate of S180 cells.

DISCUSSION

In this study we have described the *in vitro* and *in vivo* activity of rhSLC. *E. coli* was used for the expression of the human protein. In our hands, the chemokines were expressed uniquely as insoluble proteins in inclusion bodies, but they can be expressed as refolded soluble proteins in the periplasm (*33*). In this study, however, rhSLC with $(His)_6$ and *C-myc* at the C terminus was expressed in a soluble form in the cytoplasm of *E. coli*, which might be a specificity of rhSLC varying from other chemokines. The rhSLC migrated as a band of about 20 kDa on SDS-

PAGE, which was greater than the calculated molecular mass of 15.15 kDa for rhSLC. This difference might be caused by that the abundant number of cysteines in rhSLC, which would cause the protein to assume a complicated three dimensional structure so that the apparent molecular weight is increased in electroporesis (*30*).

In vitro assays showed that rhSLC could bind specifically to and chemoattract human PBLs potently (K_d was 0.2186 ± 0.02675 μM and maximal migration of PBLs was induced by 100 nM rhSLC). The published K_d of hSLC binding to PBLs is in the nM range (*28*, *34–36*), and it has been reported that maximal PBL migration is induced by 100 nM of chemically synthesized hSLC (*34*). It seemes that the rhSLC expressed in a prokaryotic expression system retains bioactivity well. The feasibility of expressing hSLC in *E. coli* would facilitate the mass production and application of hSLC in cancer immunotherapy.

The *in vivo* antitumor activity of mSLC has been well documented (*16–25*), but up to now it has not been reported whether hSLC has antitumor activity in immune competent animals. To assess this ability, the key is to choose an optimal animal model. Chung-Her Jenh and colleagues have illustrated the species specificity of hSLC and mSLC (*29*). They found mSLC is a ligand for both human and mouse CCR7. It seems that there is no species specificity of hSLC and mSLC as the ligand for the receptor CCR7. We analyzed the primary sequences of human and murine SLC and their receptors. According to the alignment analysis made by software DNAMAN, the mature human and murine SLC proteins share 70.27% identity, while the human and murine CCR7 proteins share 86.88% identity overall. More significantly, the N terminal 7 amino acids preceding the conserved cysteines and the following 11 amino acids show 88.88% identity, with variation in only the fifth and fifteenth amino acids. huSLC has Ala^5 and Arg^{15}, whereas mSLC has Gly^5 and Lys^{15}. It has been reported that the N-terminus and the loop region immediately behind of chemokines are important for receptor binding and activation (*37*, *38*). Therefore, the conservation of such regions made it likely that hSLC was also a ligand for murine CCR7. As shown in our results, rhSLC indeed potently chemoattracted freshly isolated murine PBMCs *in vitro*. So immune competent mice could be used as an animal model in which to assess the antitumor activity of hSLC *in vivo*.

mSLC mediates its antitumor effects through both angiostatic and immunological mechanisms (*17*). mSLC can be used alone or serve as adjuvant in cancer immunotherapy. The following approaches have been applied using mSLC as an antitumor agent: injection of the recombinant protein into tumor sites or local lymph node region (*16*, *19*, *23*, *24*); transfection of tumor cells with the mouse *slc* gene (*17*); transfection of the mouse *slc* gene into DCs (*20*, *21*); transfection of tumor cells with the mouse *slc* gene in combination with IL2 and GM-CSF (*18*, *22*); transfection of tumor cells with the fusion protein of mouse SLC and IL2 (*25*). The dual antitumor effects of mSLC are related to its activation of two receptors: CCR7 and CXCR3. Whereas CCR7 is expressed on naïve T cells and mature DC, CXCR3 is expressed preferentially on Th1 cytokine–producing lymphocytes with a memory phenotype and is related to angiostasis. The capacity of mSLC to facilitate the co-localization of both DCs and T cells through CCR7 could reverse tumor-mediated immune suppression and orchestrate effective cell-mediated immune responses. Meanwhile, mSLC could inhibit angiogenesis in the tumor site through CXCR3. According to the above-mentioned reports, chemokines acting alone seem to have limited anti-tumor activity; however, chemokines produce more potent anti-tumor activity when used as adjuvants (*18*).

However, hSLC, unlike mSLC, is not a ligand for the human or mouse CXCR3 receptor (*29*). Douglas and colleagues have reported that hSLC does not inhibit angiogenesis or tumor growth in the SCID mouse model (*23*). Nevertheless, in this study, we found that the intratumoral injection of rhSLC could significantly inhibit tumor growth and increase survival in immune competent mice. Furthermore, the *in vitro* proliferation assay of S180 cells in the presence of rhSLC illustrated that rhSLC has no direct effect on tumor cells. So we can conclude that the lack of angiostatic activity of hSLC does not preclude its antitumor effect, given its ability to chemoattract DCs and T cells through CCR7. Further studies will be required to delineate the immune mechanism in hSLC-mediated tumor reduction and to develop a more optimal manner to use rhSLC in cancer immunotherapy.

Interestingly, whether treated with rhSLC or not, the mice bearing tumors all showed a temporal decrease in tumor volume, which can be explained as the inoculation of tumor cells induces a self antitumor response in the mouse body. But simply depending on self antitumor response, the tumor thereafter progresses continuously until death. On the contrary, the intratumoral injection of rhSLC induced antitumor immunity and enhanced the self antitumor response so that the tumor was totally eradicated.

Collectively, we successfully expressed rhSLC in a soluble form in *E. coli*. Using the purified rhSLC, we studied its *in vitro* bioactivity and found that rhSLC could bind specifically to and chemoattract human PBLs potently. Furthermore, with immune competent mice inoculated with S180 cells as an *in vivo* model, we found that intratumoral injection of rhSLC could significantly inhibit tumor growth and increase survival. All these findings suggest that hSLC is a promising candidate agent in cancer immunotherapy.

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