

· Original Article ·

Antitumor immunity by a dendritic cell vaccine encoding secondary lymphoid chemokine and tumor lysate on murine prostate cancer

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Abstract

Aim: To investigate the antitumor immunity by a dendritic cell (DC) vaccine encoding secondary lymphoid chemokine gene and tumor lysate on murine prostate cancer. **Methods:** DC from bone marrow of C57BL/6 were transfected with a plasmid vector expressing secondary lymphoid chemokine (SLC) cDNA by Lipofectamine2000 liposome and tumor lysate. Total RNA extracted from SLC+lysate-DC was used to verify the expression of SLC by reverse transcriptase-polymerase chain reaction (RT-PCR). The immunotherapeutic effect of DC vaccine on murine prostate cancer was assessed. **Results:** We found that in the prostate tumor model of C57BL/6 mice, the administration of SLC+lysate-DC inhibited tumor growth most significantly when compared with SLC-DC, lysate-DC, DC or phosphate buffer solution (PBS) counterparts ($P < 0.01$). Immunohistochemical fluorescent staining analysis showed the infiltration of more CD4⁺, CD8⁺ T cell and CD11c⁺ DC within established tumor treated by SLC+lysate-DC vaccine than other DC vaccines ($P < 0.01$). **Conclusion:** DC vaccine encoding secondary lymphoid chemokine and tumor lysate can elicit significant antitumor immunity by infiltration of CD4⁺, CD8⁺ T cell and DC, which might provide a potential immunotherapy method for prostate cancer. (*Asian J Androl* 2008 Nov; 10: 883–889)

Keywords: dendritic cell; secondary lymphoid chemokine; prostate cancer; tumor lysate

1 Introduction

Prostate cancer is the second leading cause of cancer death in men. Despite the effectiveness of hormone therapy, most of these patients will eventually develop

hormone-refractory disease. Therefore, new investigational therapies are essential. Immunotherapy could present a novel strategy for hormone refractory prostate cancer (HRPC) therapy. Dendritic cells (DC) are derived from hematopoietic cells of the bone marrow and are central to the stimulation of an antitumor T cell response through presentation of tumor antigens. DC present processed peptides in the context of major histocompatibility complex (MHC) Class I and Class II molecules to cytotoxic T lymphocyte (CTL) and express high levels of adhesion signals and costimulatory molecules [1]. Studies have been initiated to assess the therapeutic potential of a DC-based vaccine with pros-

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tate tumor antigen-derived peptides or gene products. DC loaded with the whole antigenic pool of the tumor in the form of protein lysate or total mRNA or even whole tumor cells themselves in either an allogeneic or a syngeneic situation is used to strengthen the host antitumor immune response. The genetic modification of DC, particularly with chemokine genes, represents a rational approach to modify the tumor microenvironment that favors innate or adaptive immunity to prevent or reverse transcriptase polymerase chain reaction. Chemokine gene transfer offers the possibility to trigger the recruitment of initiators and/or effectors of the immune response to the tumor microenvironment, which is demonstrated in previous studies on chemokines, such as macrophage-derived chemokine, macrophage inflammatory protein and fractalkine [2–5].

Secondary lymphoid chemokine (SLC), a CC chemokine expressed in high endothelial venules and in T-cell zones of the spleen and lymph nodes, strongly attracts naive T cells and DC. Co-localization of these cells within the local tumor environment could enhance the induction of tumor-specific T cells. High levels of SLC expression regulates the co-localization of CCR7 expressing antigen presenting DC and naive T cells, thus facilitating activation and priming of immune responses [6–8]. In addition to its immunotherapeutic potential, SLC has potent angiostatic effects [9].

In the present study we evaluated the determinants and efficacy of the antitumor responses in a murine prostate cancer model after intratumoral administration of DC encoding secondary lymphoid chemokine and tumor lysate. We utilized tumor lysate's multiple antigen epitope, which was recognized by DC to induce extensive T cell reaction, and the capacity of SLC of attracting T cells, NK cells and DC to the tumor site to generate systemic antitumor responses.

2 Materials and methods

2.1 Cell line and mice

Murine prostate cancer line RM-1(H-2K^b) was purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Pathogen-free C57BL/6(H-2K^b,I-Ab) male mice (6–8 weeks of age and 16–18 g in weight) were purchased from the Animal Maintenance Facility of the Shanghai Medical College at Fudan University (Shanghai,

China).

2.2 Generation of bone marrow dendritic cells and recombinant pAAV-IRES-hrGFP/SLC plasmid transfection

Bone marrow cells flushed from tibias and femurs were depleted of erythrocytes by incubating in 0.9% ammonium chloride for 3 min at 37°C. The cells were washed in HBSS and cultured in complete culturing medium containing 10% FBS (GIBCO-BRL, Gaithersburg, MD, USA), with 10 ng/mL recombinant mouse GM-CSF and 20 ng/mL recombinant mouse IL-4 (PeproTech, Rocky Hill, NJ, USA) at 2×10^6 cells/mL. On day 4, non-adherent cells were harvested by gentle pipetting and stained with anti-CD11c antibody-conjugated microbeads (Miltenyi Biotec, Auburn, CA, USA) to magnetically sort CD11c⁺ immature bone marrow DC. The purity of the sorted immature bone marrow DC was consistently greater than 90%, as analyzed by immunofluorescence staining.

The recombinant pAAV-IRES-hrGFP/SLC plasmid was obtained from the Department of Anatomy, Histology and Embryology at Medical College, Fudan University (Shanghai, China). To optimize the multiplicity of infection for SLC, every 4×10^5 DC were transduced with 1 µg recombinant pAAV-IRES-hrGFP/SLC plasmid. The SLC plasmid was first mixed with Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA) for 15 min and then added to the resuspended DC. After incubation for 1 h at 37°C, 7% CO₂, the mixture was washed twice and then cultured in complete RPMI medium containing recombinant mouse GM-CSF (20 ng/mL), IL-4 (10 ng/mL) until further analyses were performed.

2.3 Dendritic cells loaded with RM-1 tumor cell lysate

After adding the supernatant of tumor cell lysate to DC transduced with SLC and incubated for 18 h at 37°C, 7% CO₂, the DC were harvested. Thus, the construction of DC vaccine encoding secondary lymphoid chemokine and tumor lysate was completed.

2.4 Polymerase chain reaction (PCR) analysis of SLC

Total RNA extracted from SLC+lysate–CDC, SLC–CDC, Lysate–CDC and DC was used to verify the expression of SLC by reverse transcriptase polymerase chain reaction (RT-PCR). According to the sequence of SLCcDNA and multiple clone site of the pAAV-IRES-hrGFP vector, the primer used for amplification was as follows: Upper 5'-A TTC TAC AGC TCT GGT CTC ATC

CTC A-3', Lower 5'-CG CTC GAG GTC TCT TTT CTA
GCT CCC TCT TTG 3'.

2.5 Establishment and treatment of subcutaneous tumors

Tumors were established by s.c. injection of 2×10^6 RM-1 cells into the right suprascapular of C57BL/6 male mice. On days 5 and 12 after the tumor challenge, DC vaccine was injected through foot pads and intratumorally, respectively. Tumors were evaluated by caliber every 2 days by measuring length, width and height (mm), and the volume of tumors was calculated using the formula: $V(\text{mm}^3) = 0.52 (\text{length} \times \text{width} \times \text{height})$. Survival was also monitored. On day 24 after tumor establishment, all mice were killed and the tumors were extracted and imbedded in paraffin, then sliced and stained by hematoxylin and eosin.

2.6 Immunohistochemical fluorescent analysis of tumors

Tumors were imbedded in OTC and processed into $5 \mu\text{m}$ sections for immunohistochemical staining. The sections were washed twice for 5 min by PBS. Non-specific proteins were blocked in 0.1 mg/mL BSA. Sections were then incubated with a monoclonal Biotin-labeled anti-mouse CD8 Ab, anti-mouse CD4 Ab and a monoclonal Biotin-labeled anti-mouse CD11 Ab for 24 h at 4°C . Then the sections were washed three times by PBS and labeled with phycoerythrin-labeled anti-mouse IgG (CD8) and StreptAvidin-FITC (CD4, CD11).

2.7 Statistical analysis

Statistical analyses of the data were performed using the Kruskal-Wallis one-way analysis of variance on ranks, followed by multiple pairwise comparisons according to Dunn's method. Survival curves were compared using the log-rank test. Significance at the $P < 0.05$ level is denoted.

3 Results

3.1 Characterization of transfected dendritic cell by RT-PCR

The SLCcDNA was successfully transfected into DC by verification of RT-PCR. The SLCmRNA was detected in group SLC-DC and SLC+lysate-CDC, whereas other groups had no SLCmRNA expression (Figure 1).

3.2 Intratumoral injection of SLC+lysate-CDC inhibits tumor growth and prolongs the survival of mice

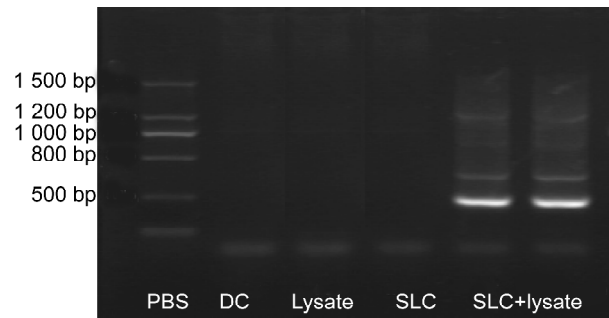


Figure 1. Reverse transcriptase-polymerase chain reaction (RT-PCR) confirmed the expression of secondary lymphoid chemokine mRNA in transferred dendritic cell. PBS, phosphate buffer solution; DC, dendritic cells; SLC, secondary lymphoid chemokine.

The antitumor efficacy of SLC+Lysate-CDC was evaluated in C57BL/6 male mice with established RM-1 tumors, comparing with SLC-CDC, Lysate-CDC, DC and PBS. On days 5 and 12 after the tumor challenge, DC vaccine was injected through foot pads and intratumorally, respectively. On day 24 after tumor establishment, all mice were killed and the tumors were extracted. The growth rate of tumors treated with SLC+lysate-CDC declined significantly (Figure 2A). The average volume of the tumors in each of the five groups were $266 \pm 255 \text{ mm}^3$ (SLC+lysate-CDC), $1204 \pm 392 \text{ mm}^3$ (lysate-CDC), $1430 \pm 117 \text{ mm}^3$ (SLC-CDC), $2934 \pm 286 \text{ mm}^3$ (DC), $4331 \pm 2108 \text{ mm}^3$ (phosphate buffer solution [PBS]) and $4331 \pm 2108 \text{ mm}^3$ (PBS) (Figure 2B). Survival was also improved in SLC+lysate-CDC treated tumors. On day 65 after RM-1 tumor cell inoculation, there were still three mice treated with SLC+lysate-CDC alive (Figure 2C). From this experiment, we presumed that intratumoral injection of SLC+lysate-CDC inhibited tumor growth and prolonged the survival of the mice.

3.3 Intratumoral injection of SLC+lysate-CDC promotes the infiltration of CD4^+ , CD8^+ T cells and $\text{CD11}^+\text{DC}$

The tumors were sliced into sections and stained by hematoxylin and eosin (HE). The sections were first observed under microscope ($\times 100$). Both vaccines induced focal areas of inflammatory cell infiltration. However SLC+lysate-CDC treated tumors contained a higher infiltration of inflammatory cells than other tumors (Figure 3).

To better quantitate the degree of T cells and DC infiltration into the tumors, tumors were imbedded in OTC

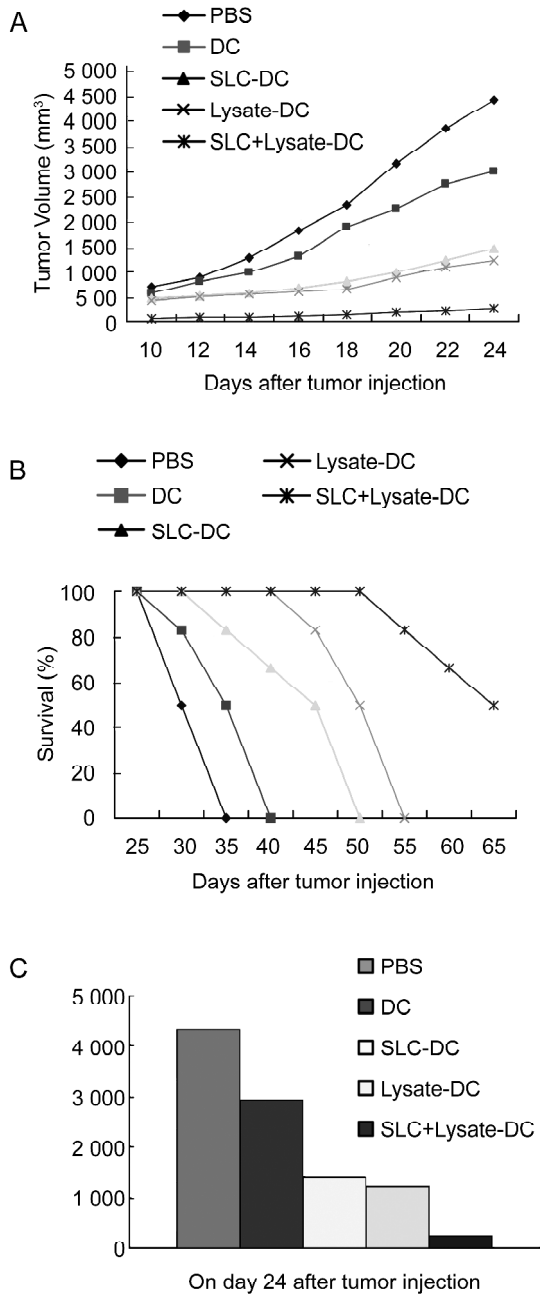


Figure 2. Induction of the therapeutic antitumor immunity. (A): Tumor volume (mm³) in mice treated with secondary lymphoid chemokine (SLC)+lysate-dendritic cells (DC) and other counterparts was demonstrated. The growth rate of tumors treated with SLC+lysate-DC declined significantly. (B): On day 24 the average volume of the tumor treated with SLC+lysate-DC was 266 mm³, which had a statistical difference compared with other counterparts ($P < 0.01$). (C): Percentage survival of mice treated. The survival rate of the SLC+lysate-DC treated mice was 50% at day 65, which had a statistical difference compared with other counterparts ($P < 0.01$). PBS, phosphate buffered saline.

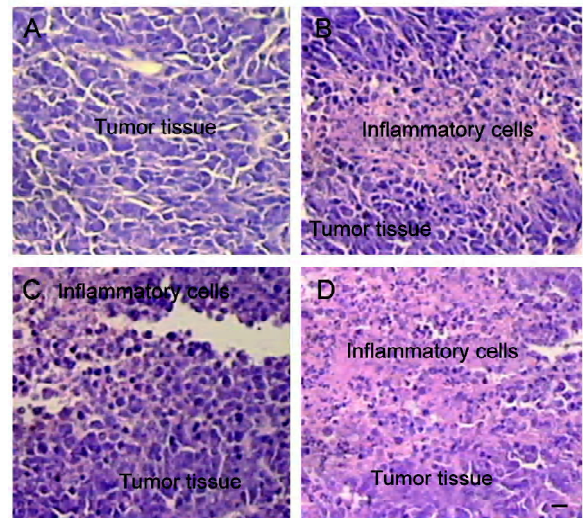


Figure 3. Tumors treated with dendritic cells (DC) vaccines were stained by haematoxylin and eosin (HE). (A): Tumors treated with just DC showed almost no inflammatory cell infiltration. Normal tumor tissue existed. (B): In tumors treated with secondary lymphoid chemokine (SLC)-DC, approximately one-third of the tumor tissue was infiltrated with inflammatory cells. (C): Tumors treated with Lysate-DC also demonstrated a lot of inflammatory cell infiltration. (D): There was a significant infiltration of inflammatory cells in tumors treated with SLC+Lysate-DC. Almost all the tumor tissue was destroyed. Bar = 100 μ m.

and processed into 5 μ m sections for immunohistochemical fluorescent staining. There was a more significant infiltration ratio of CD4⁺, CD8⁺ T cells and CD11+DC in the SLC+lysate-DC group than in any other group ($P < 0.01$; Figures 4, Table 1).

4 Discussion

Host APC are critical for the cross-presentation of tumor antigens [10]. However, tumors have the capacity to limit APC maturation, function, and the infiltration of the tumor site [11–13]. DC generation and maturation are inhibited by prostate cancer cells [14] and DC are eliminated by apoptosis [15] in the prostate tumor mass [16]. To break this tolerance, the transplantation of functional DC engineered to overexpress and present peptides from specific prostate tumor antigen may be effective. However, as prostate cancer cells are genetically unstable and represent shifting targets [17], single-agent vaccines might result in selection of genetic variants that escape the immune attack. Therefore, in spite

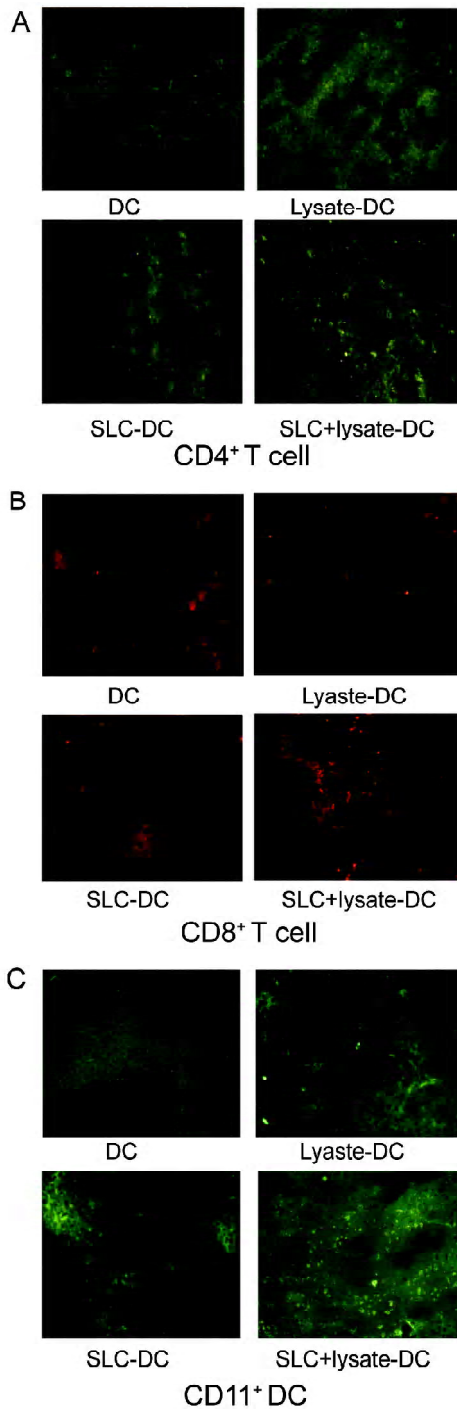


Figure 4. Immunohistochemical fluorescent analysis of tumors. (A): The infiltration of CD4⁺ T cells in tumors treated with secondary lymphoid chemokine (SLC)+lysate–dendritic cells (DC) was more significant than in other tumors. (B): There was more infiltration of CD8⁺T cells in tumors treated with SLC+lysate–CDC than in other tumors. (C): The infiltration of CD11⁺DC was more significant in tumors treated with SLC+lysate–CDC than in other tumors.

Table 1. Infiltration ratio of immune cells. The data show that the tumor treated with secondary lymphoid chemokine (SLC)+lysate–dendritic cells (DC) have more significant immune cell infiltration than any other tumors ($P < 0.01$). The infiltration ratio of CD4⁺, CD8⁺ T cells and CD11⁺ DC in tumors treated with SLC+lysate–DC was 32.24%, 18.58% and 21.52%, respectively.

	CD4 ⁺ T	CD8 ⁺ T	CD11 ⁺ DC
DC	11.38 ± 1.46	5.46 ± 0.55	10.28 ± 0.63
SLC–DC	15.24 ± 0.67	11.02 ± 0.73	14.50 ± 0.51
Lysate–DC	21.00 ± 1.08	7.20 ± 0.41	16.16 ± 0.59
SLC+lysate–DC	32.24 ± 0.91	18.58 ± 0.48	21.52 ± 0.53

of the fact that clinical benefit has been demonstrated in patients vaccinated with DC loaded with peptides as well as a single antigen in the form of a gene or protein, the general view is that optimal antitumor response requires polyclonal effector populations directed against a wide range of tumor epitopes rather than a response restricted to a single tumor antigen. The use of vaccines containing multiple tumor-derived antigens might elicit a broader antitumor response than single antigen vaccines and circumvent, to some extent, the problem of immune escape [18]. This approach, termed polyepitope vaccination, has already been evaluated as a DNA vaccine [19]. An alternative to this approach is the use of DC loaded with the whole antigenic pool of the tumor in the form of protein lysate or total mRNA, or even whole tumor cells themselves, in either an allogeneic or a syngeneic situation [20–22]. Another alternative described, both in mice and humans, is the hybridization of tumor cells with DC.

However, the application of tumor antigen-pulsed DC-based vaccines still has limitations and human clinical trials utilizing this strategy for the treatment of advanced malignancies have had only modest results. A limitation is that subcutaneously/intradermally injected DC vaccines fail to migrate efficiently to secondary lymphoid organs where they encounter naive T cells, which is a critical step for the initiation of a primary immune response. Almost all transferred DC remained at the immunization site 24 h after transfer. Inefficient migration of exogenous DC to lymphoid organs might lower the frequency of their encounter with T cells. Therefore, if transferred DC produce chemokines to intensively attract T cells, they might prime immune response efficiently, even though the DC do not migrate to lymphoid organs.

Chemokines are being used in tumor immunotherapy in animal models with evidence that local chemokine delivery can increase the number of infiltrating T cells and mediate delayed tumor growth. Because DC are potent APC that function as principal activators of T cells, the capacity of SLC to facilitate the co-localization of both DC and T cells might reverse tumor-mediated immune suppression and orchestrate effective cell-mediated immune responses. Tumor therapies using SLC have proven successful. The ability to induce migration of naive CD4⁺, CD8⁺ T cells, and to expand such cells at sites of tumor antigen expression provides a powerful tool for priming T cells responses in potentially immunosuppressed hosts [23]. Liang *et al.* [24] found that SLC induced a significant delay in tumor progression, which was paralleled by a profound infiltration of DC and activated CD4⁺ T cells and CD8⁺ T cells (CD3⁺ CD69⁺ cells) into the tumor site. Yang *et al.* [25] demonstrate that recombinant SLC administered intratumorally leads to complete tumor eradication, increases in the CD4⁺, CD8⁺ T cells, as well as DC expressing CD11c⁺.

Above all, our experiment showed that intratumoral administration of SLC+lysate-DC vaccine has strong anti-tumor effects. First, tumor lysate has larger repertoires of TAAs, which can lessen the possibility of tumor escape and increase the probability of CTL cross-priming with antitumor activity. Second, tumor injection of transferred DC was not only favorable to tumor antigen presentation by DC, but also resulted in greater CD4⁺ and CD8⁺ T lymphocyte infiltration into tumors through the chemotactic property of SLC secreted by modified DC. Finally, the capacity of SLC to facilitate the co-localization of both DC and T cells might reverse tumor-mediated immune suppression and orchestrate cell-mediated immune responses. Our study could provide a new vaccine strategy for the treatment of prostate cancer and assist in the ultimate development of a new therapeutic vaccine for prostate cancer patients.

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