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Research Paper

CCL21 Is an Effective Surgical Neoadjuvant for Treatment of Mammary Tumors

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KEY WORDS

breast tumor, CCL21, chemokine, neoadjuvant

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ABSTRACT

In previous studies, the chemokine CCL21 has shown biological activities that include T cell, natural killer (NK) cell, and dendritic cell (DC) chemoattraction. The goal of this study was to determine the effects of administering CCL21 to orthotopic mammary tumors in terms of impact on tumor growth rate, immune cell infiltration of the primary tumor, and survival. We found that a single intratumoral administration of CCL21 slowed the growth of orthotopic mammary tumors and increased intratumoral infiltration by T cells, NK cells, and DCs. CCL21 intratumoral administration also prolonged the survival of tumor-bearing mice. Furthermore, mice that received intratumoral neoadjuvant CCL21 prior to surgical resection of tumors survived significantly longer than control mice. The surviving neoadjuvant CCL21-treated mice, when challenged again with cl-66, had a significantly slower rate of tumor growth than challenged control mice. Thus, our data indicate that CCL21 treatment prior to mammary tumor resection can significantly prolong survival and increase resistance to subsequent tumor challenge. Overall, our findings suggest that intratumoral administration of CCL21 has potential as a neoadjuvant immunotherapy for breast cancer.

ABBREVIATIONS

DC, dendritic cell; NK, natural killer; MANOVA, multivariate analysis of variance

INTRODUCTION

Adjuvant or neoadjuvant immunotherapies can potentially be used regionally or systemically against tumors, with minimal side effects. Specific immune responses against tumors are initiated by DCs via presentation of antigens from phagocytosed tumor cells to T lymphocytes.[1] NK cells are cellular mediators of innate immunity, and there is evidence suggesting they have activity against some types of tumors, including breast tumors.[2-5] DCs, naïve T cells, and NK cells, due to their expression of the CCR7 receptor, are attracted by the chemokine CCL21 (also known as 6Ckine and secondary lymphoid tissue chemokine).[6-9] CCL21 is naturally expressed by high endothelial venules and in T cell zones of spleen and lymph nodes.[9] In addition to its chemotactic function, CCL21 induces anti-apoptotic signaling in DCs and mesangial cells, and stimulates DC phagocytosis.[10-12] Ectopic expression of CCL21 can induce the formation of lymph node-like structures composed of lymphocytes and DCs.[13] In murine models, CCL21 has been demonstrated to be therapeutic for colon cancer, melanoma, and lung cancer.[14-20] In addition, one report showed that three daily intratumoral CCL21 injections slowed the growth of a subcutaneous mammary tumor.[16]

In our studies, after one administration of CCL21, the numbers of intratumoral T cells, NK cells, and DCs in orthotopic mammary tumors increased and the growth of the treated tumors slowed. Furthermore, one intratumoral administration of CCL21 prior to surgical resection of the tumors significantly prolonged survival compared to controls (e.g., compared to administration of PBS prior to resection or CCL21 with no resection). We also found that cl-66 tumors generated in surviving mice that had received neoadjuvant CCL21 plus tumor resection grew significantly slower than tumors generated in control mice. These results indicate that CCL21 intratumoral treatments may be a clinically useful immunotherapy in the neoadjuvant setting for patients with breast cancer.

MATERIAL AND METHODS

Animals and tumor model. Female BALB/c mice were purchased from the National Cancer Institute (Frederick, MD), and were ten weeks old at the initiation of studies. Cl-66 is a BALB/c cell line from a spontaneously arising mammary adenocarcinoma that produces metastases to the bone marrow and other organs.[21,22] Tumor cells were thawed from low passage stocks and passaged 2–3 times before injection.

Preparation of implants and surgical procedures. Sucralfate (20 μg, sucrose octasulfate aluminum complex, Sigma, St. Louis, MO) was added to CCL21 (PeproTech, Rocky Hill, NJ) dissolved in sterile PBS with 0.05% normal mouse serum (or to PBS alone supplemented with 0.05% mouse serum), and the solution was mixed 1:1 v/v with Hydron® (Interferon Sciences, New Brunswick, NJ), as described previously.[23] The mixtures were air-dried in a laminar flow hood under ultraviolet light, and rehydrated in PBS with 0.05% mouse serum immediately before implantation. Recipient animals were anesthetized with Xylazine and Ketamine, the skin over the tumors was shaved and sterilized with Betadine, and the Hydron implants were inserted into incisions that were then closed with 5-0 Nylon suture. For tumor resections, the mice were anesthetized, and the tumor area was shaved. Sterile incisions were made over the middle of the tumors, blood vessels were cauterized and tumors were removed, and the incisions were closed with 5-0 Nylon suture. Sterile PBS (0.3 ml/mouse) was given intraperitoneally to compensate for any mild blood loss.

Tumor growth inhibition assessment. Orthotopic mammary tumors were generated by the injection of 1 x 10^5 cl-66 tumor cells into the fourth inguinal mammary fatpad of BALB/c mice. At the time of treatment, the tumors were verified not to be significantly different in size between treatment and control groups (mean of 60 mm^3; n = 15–20 mice/group). CCL21 was given as a single 6 μg dose of CCL21-Hydron per mouse. Control PBS-Hydron implantations were given as a single dose per mouse to a separate cohort. Tumor growth rates were monitored twice weekly by measurement of tumors with calipers in two perpendicular orientations, and tumor volumes were calculated using the formula $width^2$ x length/2.

Flow cytometric analysis of tumor-infiltrating cells. Prior to the analysis of tumor-infiltrating immune cells, orthotopic cl-66 mammary tumors were generated in BALB/c mice and treated when they reached 60 mm^3 as described above with one administration of CCL21 or PBS (n = 4 mice per cohort). The mice were sacrificed one day after treatment and tumors were harvested. Non-necrotic tumor tissue from each resected tumor was minced. Following 1 hour of treatment with collagenase (200 U/ml; Sigma-Aldrich, St. Louis, MO) and deoxyribonuclease I (270 Ku/ml; Sigma-Aldrich) at 37°C, mononuclear cells were isolated with Lympholyte®-M (Cedarlane, Hornby, Ontario), and analyzed by flow cytometry for the presence of $CD3^+CD8^+$ T cells and NK cells. The following antibodies were used: anti-CD3 (145-2C11), and anti-CD8a (53-6.7) (BD PharMingen, San Diego, CA), and DX5 (eBioscience, San Diego, CA). Data were acquired with a FACSCalibur (BD, San Jose, CA) after forward and side scatter gating. For each sample, 30,000–100,000 events were collected, and the data were statistically analyzed with CellQuest (BD).

Confocal microscopy of tumor-infiltrating cells. Female BALB/c mice were inoculated with cl-66 tumor cells in the mammary fat pad and treated with CCL21 or PBS as described above when tumors were 60 mm^3 (mean volume). On day 3 after treatment, mice (n = 3 per treatment type) were euthanized. Tumors and marginal tissues were resected and frozen in Tissue Freezing Media (Triangle Biomedical Sciences, Durham, NC). Frozen tumor samples were cryosectioned and fixed in ice-cold 1:1 acetone and methanol. Non-specific binding was blocked with 10% normal goat serum (Vector Laboratories, Burlingame, CA). Rat anti-mouse CD8a (53-6.7) and hamster anti-mouse CD11c (HL3) both from BD PharMingen, San Diego, CA, were used as primary antibodies. CD11c Cy5-conjugated AffiniPure Goat Anti-Armenian hamster IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and Alexa Fluor 488 goat anti-rat IgG (Invitrogen, Carlsbad, CA) were used as secondary antibodies. The antibodies were sequentially applied to the frozen, acetone/methanol fixed sections and incubated according to the manufacturer's instructions. Fluorescence images were obtained with an LSM 410 confocal laser scanning microscope (Zeiss, Goettinger, Germany).

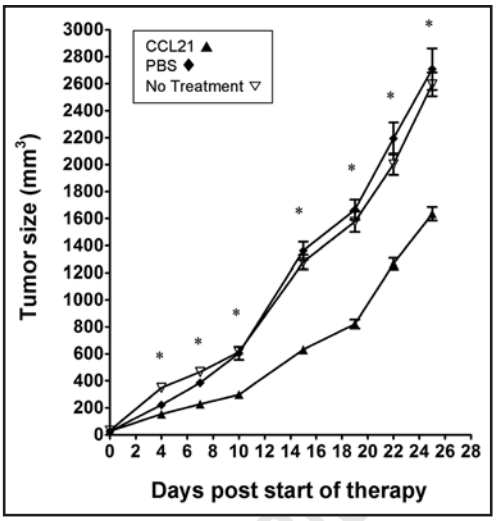

Figure 1. Cl-66 mammary tumors received 6 μg CCL21 in Hydron (▲), or PBS in Hydron without CCL21 (♦), or no treatment (▽) (n = 15–20 mice/group). Repeated measures analysis indicated CCL21 therapeutic activity was significantly greater than PBS alone. Asterisks indicate time points at which tumors treated with CCL21 were significantly smaller than PBS-treated or untreated tumors, as determined by the Student's t test. Bars indicate standard error of the mean.

Survival and tumor challenge studies. For evaluation of CCL21 as a neoadjuvant, once cl-66 tumors were 60 mm^3 (Day 0, at 13 days after cl-66 injection), CCL21-Hydron (with 6 μg of CCL21) or PBS-Hydron was implanted into each tumor. Four days following initial treatment, tumors were surgically resected from a subset of the mouse cohorts, and all of the mouse cohorts (n = 25/group) were monitored for survival. On day 100, surviving mice from the neoadjuvant CCL21-Hydron tumor resection group (17 mice), the neoadjuvant PBS-Hydron tumor resection group (ten mice), and the tumor resection only group (12 mice) were challenged with 1 x 10^5 cl-66 cells in the fourth inguinal mammary fat pad. The mammary fat pad on the side opposite the one previously inoculated was used for the rechallenge injection. On the same day, a group of 20 naïve, age-matched mice were also injected with 1 x 10^5 cl-66 cells in the fourth inguinal mammary fat pad. On day 9, 13 and 16 after cl-66 injection, tumors were measured with calipers as described above.

Statistical analysis. The statistical significance of differences in tumor growth rate was assessed with JMP IN 4.04 (SAS Institute

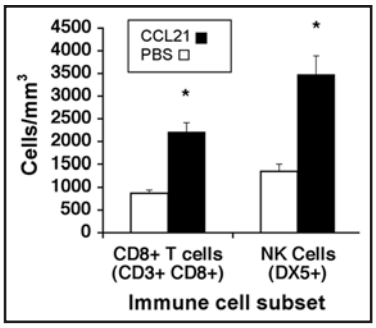

Figure 2. Administration of intratumoral CCL21 significantly increased the absolute number of CD8+ T cells and NK cells in cl-66 mouse mammary tumors relative to administration of PBS. Mice (n = 4 mice per treatment type) received a single treatment of 6 μg CCL21 in Hydron (black bars), or PBS-Hydron without CCL21 (white bars). The mice were sacrificed and the tumors were harvested one day after treatment, and tumor non-parenchymal cells were isolated and analyzed by flow cytometry for the presence of $CD3^+CD8^+$ T cells and NK cells. The average total cells/mm^3 was obtained by determining the mean for each group after calculating the individual cells/mm^3 by the following formula: (total number of tumor-infiltrating cells in a tumor) x (% marker positive cells in a tumor)/individual tumor volume in mm^3. Bars indicate the standard error of the mean. The Student's t test was used to assess statistical significance, and asterisks indicate a significant increase in cells/mm^3 compared to control ($p < 0.05$).

Inc., Cary, NC) using repeated measure multivariate analysis of variance (MANOVA). The Student's t test in JMP IN 4.04 was used to determine the statistical significance of differences in tumor volume at specific time points or in the numbers of tumor-infiltrating immune cells with distinct phenotypes. Differences in time until death were assessed with log-rank statistical analysis and graphed on a Kaplan-Meier survival curve with Graph Pad Prism software. For all experiments, a p value <0.05 was considered significant.

RESULTS

Intratumoral administration of CCL21 slowed cl-66 mammary tumor growth. CCL21 was delivered intratumorally in a slow release matrix (Hydron) to assess the therapeutic activity of CCL21 against mammary tumors, using as a model the metastatic murine mammary tumor cl-66. Hydron is an FDA-approved hydrogel polymer used previously by other investigators to deliver CCL21,[23] and our preliminary results suggested that CCL21's therapeutic activity against mammary tumors was increased by Hydron delivery (data not shown). As shown in (Fig. 1), a single CCL21 administration to the cl-66 orthotopic tumor significantly decreased the tumor growth rate compared to PBS (MANOVA $p < 0.001$) or no treatment (MANOVA $p < 0.001$). The difference in tumor growth between the CCL21-treated cohort versus the untreated and PBS-treated groups at individual time points after Day 0 was also statistically significant ($p < 0.0001$ for all points with the exception of 0.0069 at Day 4 for CCL21 versus PBS).

Immune cell infiltration of mammary tumors following intratumoral CCL21 treatment. Infiltration of immune cells into cl-66 mammary tumors in response to CCL21 was examined. Significantly increased numbers of infiltrating $CD8^+$ T cells and NK cells were detected in cl-66 primary tumors one day after treatment with CCL21 (Fig. 2). Immunofluorescent staining was also used to identify cells expressing CD11c and/or CD8 in sections from CCL21- and PBS-treated tumors. T cells ($CD8^+$), DCs ($CD11^+$),

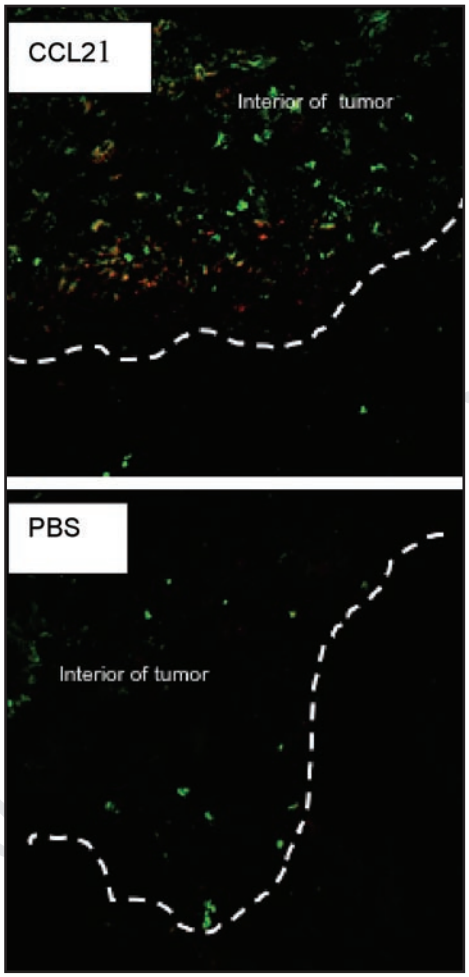

Figure 3. Cytotoxic T lymphocytes ($CD3^+CD8^+$), DCs ($CD11c^+$) and lymphoid DCs ($CD11c^+CD8^+$) were more plentiful in tumors from the CCL21- treated group, relative to the PBS-treated group. Confocal laser scanning images of cells expressing CD8 and CD11c in CCL21-treated (top) and PBS-treated (bottom) tumor samples are shown. The green color represents Alexa Fluor 488-conjugated antibody specific for CD8 and the red color represents Cy5-conjugated antibody specific for CD11c. The confocal laser scanning microscopy was performed with 40X oil objectives. $CD8^+$ T cells (green), $CD11c^+$ DCs (red) and $CD11c^+CD8^+$ lymphoid DCs (yellow) were found to be concentrated along the interior side of the tumor boundary (indicated by the white line). Similar results were obtained with all the mice analyzed (n = 3 mice/treatment group).

and double staining lymphoid DCs ($CD11c^+CD8^+$) were more readily apparent in tumors from the CCL21-treated group compared to those from PBS-treated mice (Fig. 3).

CCL21 is effective as a surgical neoadjuvant. We examined the ability of CCL21 to serve effectively as a surgical neoadjuvant. Four days after CCL21 or PBS treatment, the primary tumors were surgically resected from some of the mice, and all of the animals were subsequently monitored for survival. As shown in Figure 4, CCL21 treatment followed by tumor resection significantly prolonged survival, compared to PBS plus surgery or to tumor resection alone, or to CCL21 without surgery. In the same experiment, among mice that did not have tumors resected, the CCL21 group survived significantly longer than mice that received PBS or no treatment (Fig. 4).

At Day 100, surviving mice used in the experiment from which data are shown in Figure 4 were challenged with cl-66 tumor cells.

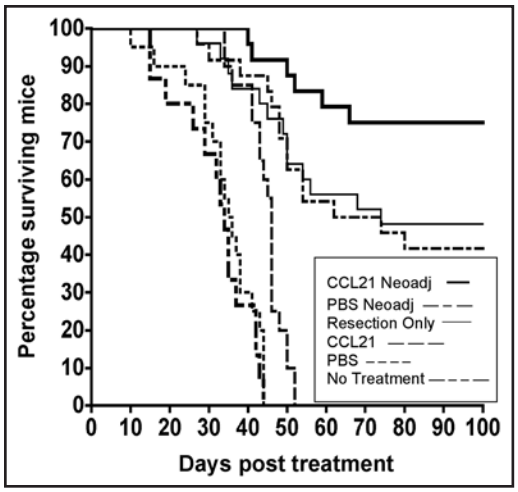

Figure 4. Mice that received neoadjuvant CCL21 plus tumor resection survived significantly longer than mice that received any other treatment tested ($p < 0.05$). Mice that received CCL21 without tumor resection survived significantly longer than mice that received PBS without tumor resection or that received no treatment ($p < 0.05$). Cl-66 tumors in BALB/c mice were treated with PBS or 6 μg CCL21 in Hydron (n = 25 mice per treatment group). After four days, tumors were resected from the CCL21 Neoadjuvant, PBS Neoadjuvant, and Resection Only groups, and then all mice, including the CCL21 (without resection), PBS (without resection), and No Treatment groups, were monitored for survival. Differences in time until death were assessed with log-rank statistics.

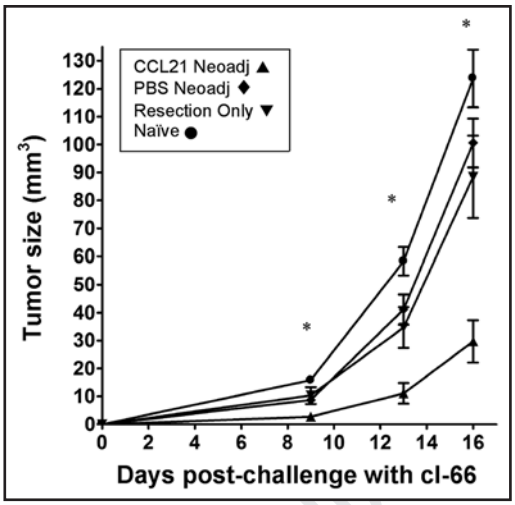

Figure 5. Tumor growth was significantly slower in challenged mice that had previously received neoadjuvant CCL21 followed by tumor resection. On day 100, surviving mice from the neoadjuvant CCL21/resection group (17 mice), neoadjuvant PBS/resection group (ten mice), and tumor resection alone group (12 mice,) were challenged by cl-66 injection. On the same day, as additional controls, 20 naïve, age-matched BALB/c mice were also injected with the same number of cl-66 cells. The significance of differences in tumor growth rate was assessed with MANOVA. Asterisks indicate individual time points at which tumors treated with CCL21 were significantly smaller than PBS-treated or untreated tumors, as determined by the Student's t test.

These surviving mice included mice from the neoadjuvant CCL21/resection group, neoadjuvant PBS/resection group, and tumor resection alone group. For the surviving mice, the challenge injection was made into the mammary fat pad on the side opposite the one earlier inoculated with cl-66. Naïve, age-matched, BALB/c mice were also challenged with cl-66 as controls. The resulting cl-66 mammary tumors grew significantly slower in mice that had originally received neoadjuvant CCL21 plus tumor resection, compared to mice that had originally received PBS plus tumor resection (<0.001 by MANOVA), or resection alone (0.0013 by MANOVA), or naïve mice (<0.001 by MANOVA) (Fig. 5). The difference in tumor growth between the CCL21/resection group and each of the control groups at individual time points after Day 0 (i.e., the day of tumor challenge) was also statistically significant, as calculated by Student's t test (for CCL21/resection versus PBS/resection groups, $p = 0.0008$ at Day 9 and at $p < 0.0001$ thereafter; for CCL21/resection versus resection only, $p = 0.0096$ at Day 9, 0.0042 at Day 13, and 0.0007 at Day 16; for CCL21/resection versus naïve mice at each point after Day 0, $p < 0.0001$).

DISCUSSION

In our study, a single administration of CCL21 into a mammary tumor in the orthotopic site (the mammary fat pad) was examined as the experimental treatment. Cellular infiltrates in the treated tumors were characterized, and found to include increased numbers of T cells, NK cells and DCs. Our work combined intratumoral administration of CCL21 with primary tumor resection, a model that is relevant to a potential treatment protocol for many breast cancer patients. The combination of CCL21 and tumor resection significantly extended survival relative to controls. Furthermore, mice receiving a tumor challenge subsequent to CCL21 neoadjuvant and tumor resection had a significantly slower tumor growth rate (relative to control treated mice or naïve mice), indicating that protective immunity against the cl-66 tumor had been induced. Thus, in many aspects, our study has extended the earlier findings by others that multiple intratumoral injections of CCL21 (3 μg daily for three days) slowed the growth of MT-901 mammary tumors implanted subcutaneously in the flank (16).

The number of infiltrating T cells, NK cells, and DCs was demonstrated in our study to be increased by CCL21 treatment of cl-66 tumors. Consistent with our results, the delivery of CCL21 via a herpes simplex virus-derived vector directly into lymphomas or colon carcinomas was shown to result in tumor eradication or slowed growth (respectively), associated with T cell and DC tumor infiltration.[24] These findings suggest that CCL21 may have anti-tumor effectiveness mediated by DCs and T cells. However, the therapeutic efficacy in our model may be at least partially due to the attraction of large numbers of NK cells to the cl-66 tumors by CCL21. NK cells have previously been suggested to be effective against breast tumors.[2,4] In addition, the chemokine CCL19 (which binds to the same receptor as CCL21) transduced into murine mammary tumor cells has been shown to induce tumor rejection via a mechanism involving NK cells as well as $CD4^+$ cells.[25] Thus, T cells and NK cells may both contribute to the effectiveness of intratumoral CCL21 as a surgical neoadjuvant used against cl-66 mammary tumors.

CCL21 has been demonstrated in various murine models to be therapeutic against cancer,[14-20] but previously the available information about its efficacy against mammary tumors has been very limited.[16] In comparison to many other cancers, primary human breast cancers would be relatively accessible for intratumoral administration of CCL21 and for subsequent resection of the primary tumor. Our results from this study provide support for breast cancer clinical trials that utilize a neoadjuvant approach for CCL21 therapy.

References

1. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392:245-52.
2. Maiche AG, Jekunen A, Rissanen P, Virkkunen P, Halavaara J, Turunen JP. Sudden tumour regression with enhanced natural killer cell accumulation in a patient with stage IV breast cancer. Eur J Cancer 1994; 30A:1642-6.
3. Vujanovic NL, Yasumura S, Hirabayashi H, Lin WC, Watkins S, Herberman RB, Whiteside TL. Antitumor activities of subsets of human IL-2-activated natural killer cells in solid tissues. J Immunol 1995; 154:281-9.
4. Divino CM, Chen SH, Yang W, Thung S, Brower ST, Woo SL. Anti-tumor immunity induced by *interleukin-12* gene therapy in a metastatic model of breast cancer is mediated by natural killer cells. Breast Cancer Res Treat 2000; 60:129-34.
5. Ohashi M, Yoshida K, Kushida M, Miura Y, Ohnami S, Ikarashi Y, Kitade Y, Yoshida T, Aoki K. Adenovirus-mediated interferon alpha gene transfer induces regional direct cytotoxicity and possible systemic immunity against pancreatic cancer. Br J Cancer 2005; 93:441-9.
6. Robertson MJ. Role of chemokines in the biology of natural killer cells. J Leukoc Biol 2002; 71:173-83.
7. Vitale M, Della Chiesa M, Carlomagno S, Romagnani C, Thiel A, Moretta L, Moretta A. The small subset of CD56brightCD16- natural killer cells is selectively responsible for both cell proliferation and interferon-gamma production upon interaction with dendritic cells. Eur J Immunol 2004; 34:1715-22.
8. Cyster JG. Chemokines and cell migration in secondary lymphoid organs. Science 1999; 286:2098-102.
9. Willimann K, Legler DF, Loetscher M, Roos RS, Delgado MB, Clark-Lewis I, Baggiolini M, Moser B. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. Eur J Immunol 1998; 28:2025-34.
10. Sanchez-Sanchez N, Riol-Blanco L, de la Rosa G, Puig-Kroger A, Garcia-Bordas J, Marin D, Longo N, Cuadrado A, Cabanas C, Corbi AL, Sanchez-Mateos P, Rodriguez-Fernandez JL. Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. Blood 2004; 104:619-25.
11. Wornle M, Schmid H, Merkle M, Banas B. Effects of chemokines on proliferation and apoptosis of human mesangial cells. BMC Nephrol 2004; 5:8.
12. Yanagawa Y, Onoe K. CCR7 ligands induce rapid endocytosis in mature dendritic cells with concomitant up-regulation of Cdc42 and Rac activities. Blood 2003; 101:4923-9.
13. Fan L, Reilly CR, Luo Y, Dorf ME, Lo D. Cuttting edge: Ectopic expression of the chemokine TCA4/SLC is sufficient to trigger lymphoid neogenesis. J Immunol 2000; 164:3955-9.
14. Vicari AP, Ait-Yahi S, Chemin K, Mueller A, Zlotnik A, Caux C. Antitumor effects of the mouse chemokine *6Ckine/SLC* through angiostatic and immunological mechanisms. J Immunol 2000; 165:1992-2000.
15. Kirk CJ, Hartigan-O'Connor D, Mule JJ. The dynamics of the T-cell antitumor response: Chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res 2001; 61:8794-802.
16. Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, Chamberlain JS, Giedlin M, Aukerman L, Mule JJ. T cell-dependent antitumor immunity mediated by secondary lymphoid tissue chemokine: Augmentation of dendritic cell-based immunotherapy. Cancer Res 2001; 61:2062-70.
17. Sharma A, Stolina M, Luo J, Strieter RM, Burdick M, Zhu LX, Batra RK, Dubinett SM. Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. J Immunol 2000; 164:4558-63.
18. Sharma S, Stolina M, Zhu L, Lin Y, Batra R, Huang M, Strieter R, Dubinett SM. Secondary lymphoid organ chemokine reduces pulmonary tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 2001; 61:6406-12.
19. Sharma S, Yang SC, Hillinger S, Zhu L, Atianzar K, Strieter RM, Riedl K, Huang M, Dubinett SM. SLC/CCL21-mediated anti-tumor responses require IFNgamma, MIG/CXCL9 and IP-10/CXCL10. Mol Cancer 2003; 2:22.
20. Yang SC, Hillinger S, Riedl K, Zhang L, Zhu L, Huang M, Atianzar K, Kuo BY, Gardner B, Batra RK, Strieter RM, Dubinett SM, Sharma S. Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. Clin Cancer Res 2004; 10:2891-901.
21. Miller FR, Miller BE, Heppner GH. Characterization of metastatic heterogeneity among sub-populations of a single mouse mammary tumor: Heterogeneity in phenotypic stability. Invasion Metastasis 1983; 3:22-31.
22. Murphy BO, Joshi S, Kessinger A, Reed E, Sharp JG. A murine model of bone marrow micrometastasis in breast cancer. Clin Exp Metastasis 2002; 19:561-9.
23. Soto H, Wang W, Strieter RM, Copeland NG, Gilbert DJ, Jenkins NA, Hedrick J, Zlotnik A. The CC chemokine binds the CXC chemokine receptor. PNAS USA 1998; 95:8205-10.
24. Tolba KA, Bowers WJ, Muller J, Houseknecckt V, Giuliano RE, Federoff HJ, Rosenblatt JD. Herpes simplex virus (HSV) amplicon-mediated codelivery of secondary of secondary lymphoid tissue chemokine and CD40L results in augmented antitumor activity. Cancer Res 2002; 62:6545.
25. Braun SE, Chen K, Foster RG, Kim CH, Hromas R, Kaplan MH, Broxmeyer HE, Cornetta K. The CC chemokine CK beta-11, *MIP-3* beta/ELC/Exodus 3 mediates tumor rejection of murine breast cancer cells through NK cells. J Immunol. 2000; 164:4025.