CCL22 Recruits CD4-positive CD25-positive Regulatory T Cells into Malignant Pleural Effusion

Xue-Jun Qin, Huan-Zhong Shi, Jing-Min Deng, Qiu-Li Liang, Jing Jiang, and Zhi-Jian Ye

Abstract

Purpose: The aim of this study was to explore the presence of the chemokines CCL22 and CCL17 in malignant pleural effusion, and the chemoattractant activity of these chemokines on CD4-positive CD25-positive Foxp3-positive regulatory T cells infiltrating into the pleural space.

Experimental Design: The concentrations of CCL22 and CCL17 in both pleural effusions and sera from 33 patients with lung cancer were determined. Flow cytometry was done to determine T lymphocyte subsets in cell pellets of pleural effusion. Pleural cells were analyzed for the expression of CCL22 and CCL17. The chemoattractant activity of CCL22 for regulatory T cells *in vitro* and *in vivo* was also observed.

Results: The concentration of CCL22 in malignant pleural effusion was significantly higher than that in the corresponding serum. Pleural fluid from lung cancer patients was chemotactic for regulatory T cells, and this activity was partly blocked by an anti-CCL22, but not by an anti-CCL17 antibody. Intrapleural administration of CCL22 of patients produced a marked progressive influx of regulatory T cells into pleural space.

Conclusions: Compared with serum, CCL22 seemed to be increased in malignant pleural effusion, and could directly induce regulatory T cell infiltration into the pleural space in patients with malignant effusion.

The development of inflammatory processes in the pleural space may result in increased pleural vascular permeability leading to the accumulation of fluid enriched in proteins, and the recruitment of cells into the pleural space (1). Although malignant pleural effusion is more and more common, very little information is available on the immune mechanisms that are involved in its development. An accumulation of lymphocytes, especially CD4-positive T lymphocytes, frequently occurs in malignant pleural effusion secondary to direct pleural involvement and/or metastases from malignancies (2, 3).

Studies ongoing for more than a decade have provided firm evidence for the existence of a unique CD4-positive CD25-positive T-cell population of "professional" regulatory/suppressor T cells that actively and dominantly prevent both the activation and the effector function of autoreactive T cells that have escaped other mechanisms of tolerance (4–6). CD4-positive CD25-positive Foxp3-positive regulatory T cells are believed to be involved in the control of the local immune response and in the growth of human lung cancer (7–9). Our previous studies have shown that regulatory T cells infiltrating into human malignant pleural effusion behave as regulatory T cells (10). However, the mechanism by which regulatory T cells infiltrate into malignant pleural effusion is unknown so far. It has been reported that the chemokines CCL22 and CCL17 within the microenvironment of gastric cancer were related to the high frequency of regulatory T cells in tumor-infiltrating lymphocytes, with such an observation occurring in the early stage of gastric cancer (11). In addition, patients with malignant pleural effusion have higher percentages of CCR4-positive CD4-positive T cells than patients with nonmalignant effusions (12). In the present study, we were prompted to evaluate whether chemokine signals, especially the CCL22/CCR4 axis, might be responsible for the influx of regulatory T cells into the pleural space.

Materials and Methods

Subjects. The study protocol was approved by our institutional review board for human studies, and informed consent was obtained from all subjects. Pleural fluid samples were collected from 33 patients (age range, 34-82 y) with newly diagnosed lung cancer with malignant pleural effusion. Histologically, 22 cases were adenocarcinoma and 11 were squamous cell carcinoma. A diagnosis of malignant pleural effusion was established by the showing of malignant cells in pleural fluid or/and on closed pleural biopsy specimen. The patients were excluded if they had received any invasive procedures directed into the pleural cavity or if they had suffered chest trauma within 3 mo prior to hospitalization or had a pleural effusion of undiagnosed cause. At

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Received 10/12/08; revised 12/5/08; accepted 12/30/08; published OnlineFirst 3/24/09.

Grant support: 30660064 and 30872343 from the National Natural Science Foundation of China, and in part by research grants 0639044 and 0728137 from the Natural Science Foundation of Guangxi Zhuang Autonomous Zone, China.

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doi:10.1158/1078-0432.CCR-08-2641

Translational Relevance

Regulatory T cells have been found to be increased in malignant pleural effusion, but the mechanism by which regulatory T cells infiltrate into pleural cavity is unknown. We provide evidence for the first time that the chemokine CCL22 is capable of directly inducing regulatory T cell infiltration into the pleural space of patients with malignant pleural effusion. Because regulatory T cells have an inhibitory effect on the surrounding effector T cells, the elimination of the local regulatory T cells might be an effective therapeutic approach against malignant pleural effusion.

the time of sample collection, none of the patients had received any antituberculosis therapy, anticancer treatment, corticosteroids, or other nonsteroid anti-inflammatory drugs.

Sample collection and processing. The pleural fluid samples were collected in heparin-treated tubes from each subject, using a standard thoracocentesis technique within 24 h after hospitalization. Ten milliliters of venous blood were drawn simultaneously. Malignant pleural effusion specimens were immersed in ice immediately and were then centrifuged at 1,200 *g* for 5 min. The cell-free supernatants of malignant pleural effusion and serum were frozen at -80°C immediately after centrifuge for later determining concentrations of CCL22 and CCL17. The cell pellets of malignant pleural effusion were resuspended in HBSS, and mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation (Pharmacia) to determine the T cell subsets within 1 h. A pleural biopsy was done when the results of the pleural fluid analysis were suggestive of malignancy.

Flow cytometry. Four-color flow cytometry was done to determine phenotypes in T cells in malignant pleural effusion and blood. Foxp3, CCR4, or CCR8 expression by CD3-positive CD4-positive CD25-positive T cells was analyzed after staining with anti-CD3-allophycocyanin (UCHT1), anti-CD4-fluorescein isothiocyanate (RPA-T4), anti-CD25-Cy-chrome (M-A251), as well as anti-Foxp3-phycoerythrin, anti-CCR4-phycoerythrin, or anti-CCR8-phycoerythrin monoclonal antibody (mAb). All mAbs and controls were purchased from BD PharMingen, except for the anti-Foxp3 mAb (eBioscience) and were used according to the manufacturers' instructions. Briefly, cells were incubated in the dark at room temperature for 30 min with mAbs at the concentrations recommended by the manufacturers, washed once in fluorescence-activated cell sorter buffer (calcium/magnesium-free HBSS containing 1 mg/mL bovine serum albumin and 0.1 mg/mL sodium azide), and fixed with 2% formaldehyde. In addition, intracytoplasmic staining for human Foxp3 was done using the anti-Foxp3 staining kit (eBioscience), according to the manufacturer's instructions. Flow cytometry was carried out on a Coulter Epics XL-MCL flowcytometer using System II software (Beckman Coulter).

Measurement of CCL22 and CCL17. The concentrations of CCL22 and CCL17 in both pleural fluids and sera were measured by a sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's protocol (R & D Systems Inc.). All samples were assayed in duplicate. The lower detection limits of CCL22 and CCL17 were 62.5 ng/L and 1.0 ng/L, respectively.

Identification of pleural cells able to express CCL22. Double immunofluorescence staining was done on cell pellets of malignant pleural effusion to identify which cell types express CCL22. The cell pellets were fixed in freshly made 4% paraformaldehyde/PBS for 2 h, and washed three times in PBS. The cell pellets were first embedded in 8% agarose gel (Richard-Allan Scientific) and then in paraffin according to standard pathology protocols. The paraffin-embedded cell lines were cut into 4- to 5-μm-thick sections. After permeabilizing with 0.1% triton X-100 in PBS for 15 min at room temperature and washing with PBS, slides were incubated with 10% goat serum (ScyTek) in PBS at 4°C overnight. The primary antibodies were as follows: rabbit polyclonal antibody targeted against human CCL22 (Abcam), mouse antihuman CD3 mAb (Biolegend), specific for T cells; mouse anti-CD163 mAb (Thermo Fisher Scientific Anatomical Pathology), specific for mononuclear phagocytes; and mouse antihuman epithelial membrane antigen (EMA) mAb (Thermo Fisher Scientific Anatomical Pathology) to identify malignant cells. Appropriate species-matched antibodies were used as isotype controls. As secondary antibodies, rhodamine-labeled affinity-purified goat antirabbit IgG was used for labeling the rabbit CCL22 antibody, and fluorescein-labeled affinity-purified goat antimouse IgG was used for labeling the mouse anti-CD3, anti-CD163, anti-EMA mAbs. After nonspecific binding sites were blocked with goat serum, the slides were incubated at 4°C overnight with 1:50 concentrations of primary antibodies as recommended by the manufacturer. After washing, the slides were incubated with selected secondary antibodies for 40 min at room temperature in the dark, correctly matched to the appropriate species, and viewed under imaging fluorescence microscope (Olympus BX51).

Cell isolation. Human regulatory T cells were isolated from healthy control blood donors by Ficoll separation and magnetic bead sorting

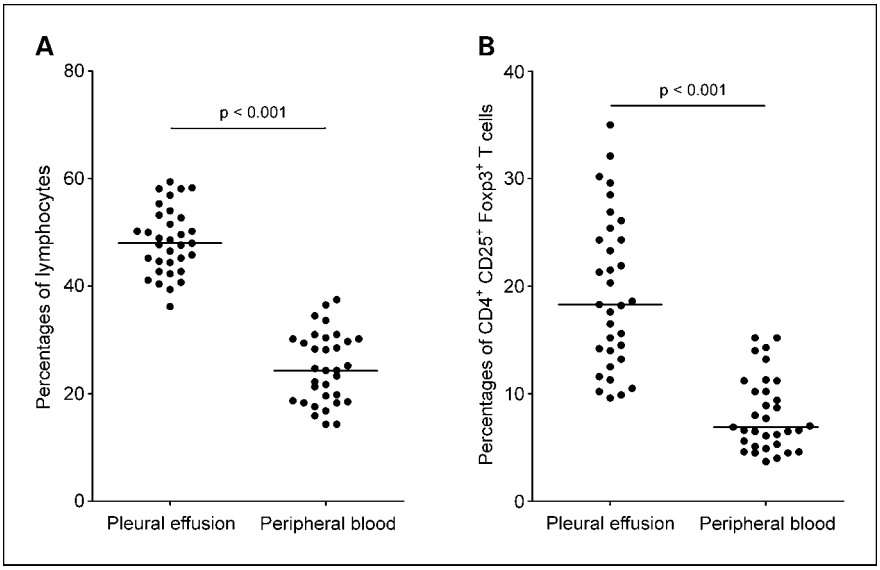

Fig. 1. Percentages of lymphocytes in total nucleated cells (*A*) and frequency of CD4-positive CD25-positive Foxp3-positive T cells in total CD4-positive cells (*B*) in malignant pleural effusion and peripheral blood from patients with lung cancer (n = 33). The percentage of CD4-positive CD25-positive Foxp3-positive T cells present in total CD4-positive T cells was determined by flow cytometry. Horizontal bars, medians. Comparison was made using a Wilcoxon signed-rank test.

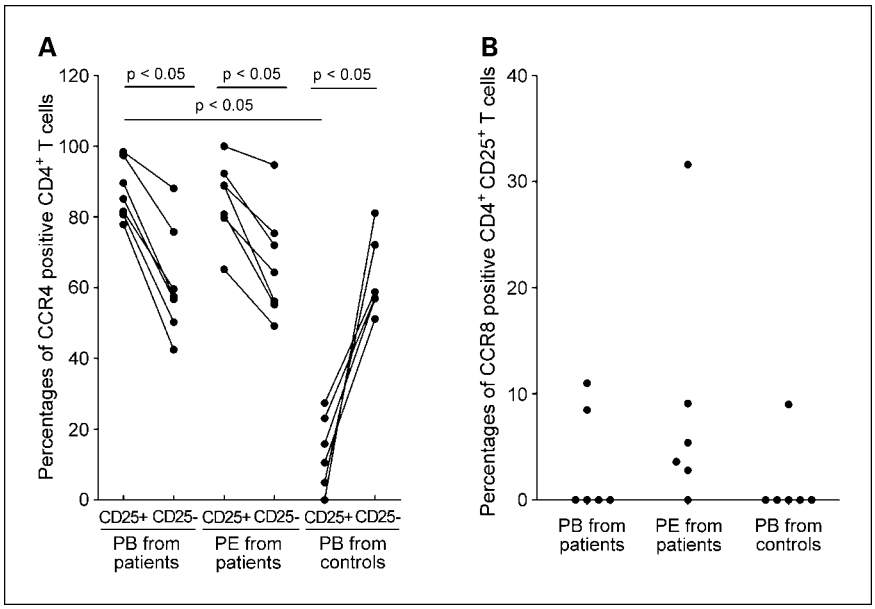

Fig. 2. The expression of CCR4 (*A*) and CCR8 (*B*) on CD4-positive CD25-positive T cells and/or CD4-positive CD25-negative T cells in peripheral blood (PB) and in pleural effusion (PE) from patients with malignant PE ($n = 7$ for CCR4, $n = 6$ for CCR8) and healthy controls ($n = 6$). Comparisons of CCR4 or CCR8 expression between CD4-positive CD25-positive T cells and CD4-positive CD25-negative T cells from the same specimens were made using a Wilcoxon signed-rank test. Comparisons of data of PB between patients with malignant PE and controls were made using a Mann-Whitney U-test.

(CD4-positive CD25-positive regulatory T cell isolation kit, 130-091-301; Miltenyi Biotec). In brief, CD4-positive cells were negatively selected by incubation with a mixture of mAbs against CD16, CD14, CD8, CD19, CD36, CD56, CD123, γδTCR, and CD235a, followed by positive selection with anti-CD25 microbeads to select CD25-positive cells. Only cells of the highest CD25 expression (CD25-high) were selected through incubation with a limiting quantity of anti-CD25 antibody beads, as described (2 μL of anti-CD25 beads/10^7 cells; ref. 13). Seventy-five percent of the freshly isolated CD4-positive CD25-high cells and <4% of the CD4-positive CD25-negative cells were Foxp3-positive, as measured by flow cytometry.

Regulatory T cell chemotaxis assay. Chemotaxis assays were done using 8-μm pore polycarbonate filters in 24-well Transwell chambers (Corning; Costar). Briefly, transwell membranes were coated with fibronectin (5 μg/mL; Chemicon) for 30 min at 37°C. Regulatory T cells isolated from peripheral blood were added to the top chamber resuspended in RPMI medium plus 0.5% bovine serum albumin at 1×10^6 cells/mL in a final volume of 100 μL. Malignant pleural effusion from five lung cancer patients were placed in the bottom chamber of the transwell in a volume of 600 μL, and the chamber was incubated at 37°C in 5% CO_2 atmosphere for 3 h. At the end of incubation, the filter was washed with HBSS lightly, fixed, stained, and mounted on a glass microscope slide. To correct for donor-to-donor variation, migration data of test samples were compared with their corresponding control values (HBSS alone) and expressed as percentages above the control value. To show that CCL22 or CCL17 was responsible for regulatory T cell migration, blocking experiments were done by mixing the malignant pleural effusion with 100 ng/mL of anti-CCL22, anti-CCL17 mAb or mouse IgG irrelevant isotype control (R&D Systems Inc.).

Effects of intrapleural-injected CCL22 on regulatory T cell recruitment. After an additional study protocol had been approved by our institutional review board and informed consent had been obtained from the subjects studied, a total of 10 patients with malignant pleural effusion (6 men) were included in this section of study. Right after collection of malignant pleural effusion samples, 10 μg of recombinant human CCL22 (R&D Systems Inc.) in vehicle (0.1% human serum albumin in 0.9% saline) were injected into the pleural space of five

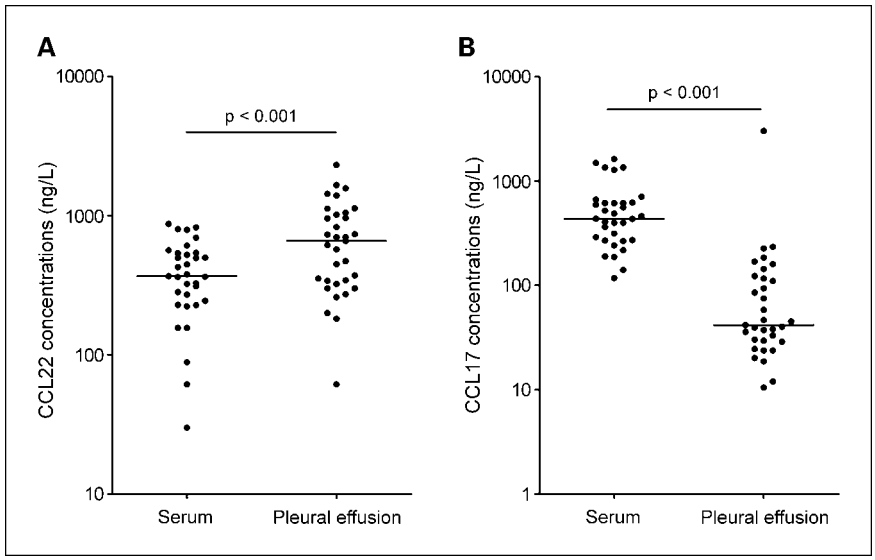

Fig. 3. CCL22 (*A*) and CCL17 (*B*) are present in malignant pleural effusions and sera from patient with lung cancer ($n = 33$). CCL22 and CCL17 concentrations were measured with ELISA. Horizontal bars, medians. Comparisons of CCL22 and CCL17 levels between malignant pleural effusion and sera were made using a Wilcoxon signed-rank test.

patients, and vehicle only was injected into the pleural space of the other five patients. The intrapleural injection of CCL22 or vehicle was randomized. The dose of CCL22 was based upon a preliminary study involving two malignant pleural effusion patients. Malignant pleural effusion collection for determining regulatory T cell numbers by flow cytometry was repeated 6, 12, 24, and 48 h after the injection of CCL22 or vehicle.

Statistics. Data are expressed as mean ± SE. Comparisons of the data between different groups were done using a Mann-Whitney U-test or Kruskal-Wallis one-way ANOVA on ranks. For data on malignant pleural effusion and corresponding serum, paired data comparisons were made using a Wilcoxon signed-rank test. The effects of intrapleural-injected CCL22 or vehicle on regulatory T cell recruitment were compared through one-way repeated-measures ANOVA. Analysis was completed with SPSS version 14.0 Statistical Software, and $P < 0.05$ was considered to indicate statistical significance.

Results

Regulatory T cells in malignant pleural effusion. The percentages of lymphocytes in total nucleated cells in malignant pleural effusion (48.3 ± 1.1%) and the corresponding peripheral blood (24.8 ± 1.1%) are illustrated in Fig. 1A. The percentages of lymphocytes represented the higher values in malignant pleural effusion, showing a significant increase in comparison with those in blood (Wilcoxon signed-rank test, $Z = -5.012$; $P < 0.001$). Consistent with our previous findings (10), a significant increase in regulatory T cells was observed in malignant pleural effusion (19.5 ± 1.2%) compared with blood (8.1 ± 0.6%; Wilcoxon signed-rank test, $Z = -4.869$; $P < 0.001$; Fig. 1B). The vast majority of pleural CD4-positive CD25-positive T cells (87.8 ± 4.5%) expressed CCR4 on their surface, whereas less infiltrating CD4-positive CD25-negative T cells were CCR4-positive cells (66.2 ± 5.8%; $P = 0.018$). Similar results with CCR4 expression were also observed in blood CD4-positive CD25-positive T cells from patients with malignant pleural effusion. Interestingly, in normal control subjects, CCR4 expression in blood CD4-positive CD25-positive T cells was lower uniformly than that in CD4-positive CD25-negative T cells (Fig. 2A). In addition, only a quite low expression of CCR8 could be detected in pleural CD4-positive CD25-positive T cells in some cases of patients with malignant pleural effusion (Fig. 2B), and no CCR8 expression could be observed in CD4-positive CD25-negative T cells.

CCL22 and CCL17 were detected in malignant pleural effusion. Our data showed that CCL22 concentration in malignant pleural effusion (740.6 ± 89.4 ng/L) was much higher than that in serum (410.6 ± 38.9 ng/L; $P < 0.001$;

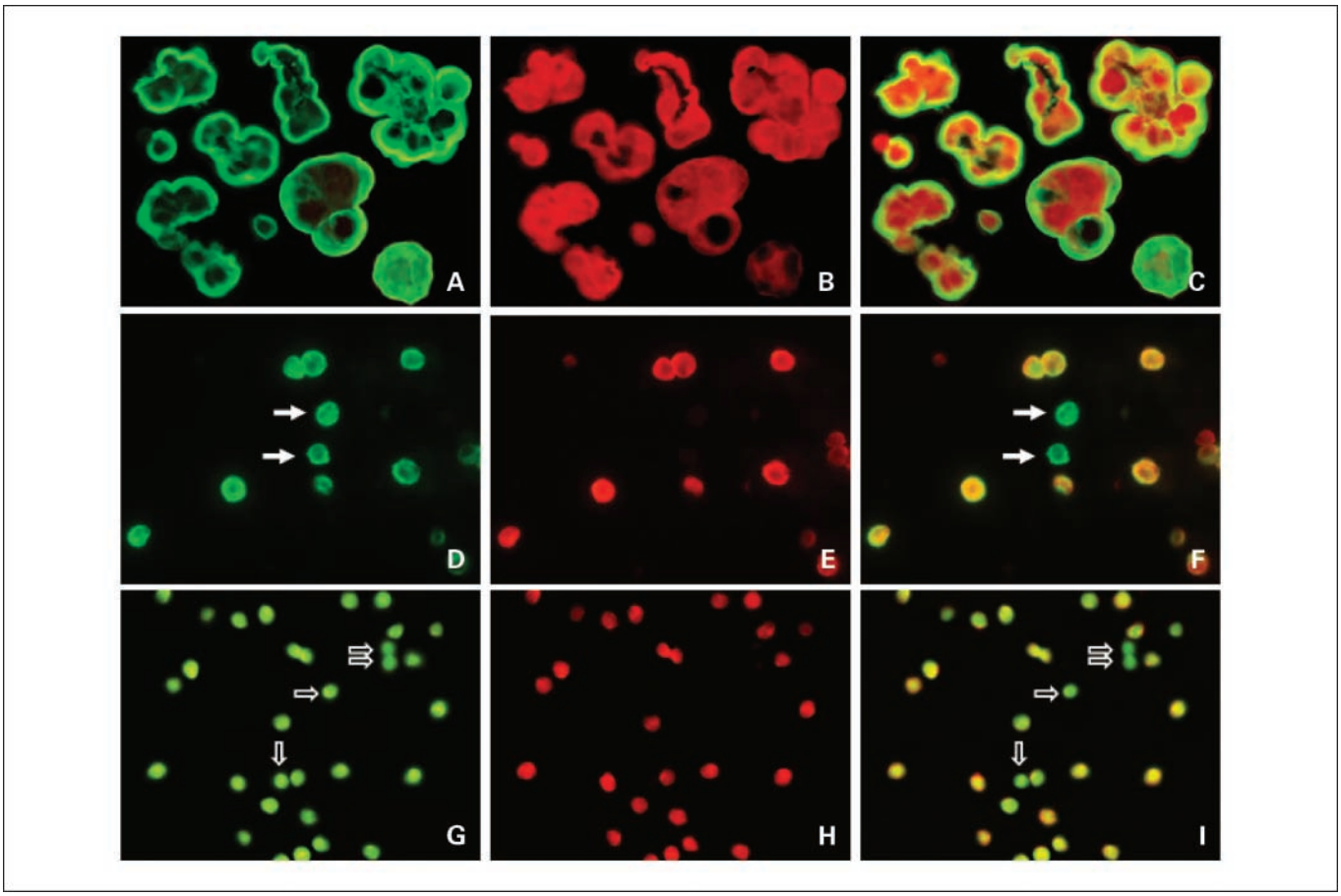

Fig. 4. Expression of CCL22 in malignant cells (*top*), macrophages (*middle*), and T lymphocytes (*bottom*). Malignant cells (*A*), macrophages (*D*), and T cells (*G*) were incubated with mouse antiepithelial membrane antigen, anti-CD163, and anti-CD3 mAbs, respectively, and then were stained with fluorescein-labeled goat anti-mouse IgG (*green*). CCL22 expression was detected by rabbit polyclonal Ab targeted against CCL22 and then rhodamine-labeled goat antirabbit IgG (*red*; *B, E, H*). Immunoflorescence double-staining indicate that all malignant cells (*C*), most of macrophages (*F*), and most of T cells (*I*) express CCL22. Closed arrows, CD163-positive macrophages that do not express CCL22; open arrows, CD3-positive T cells that do not express CCL22. Original magnification: ×1,000.

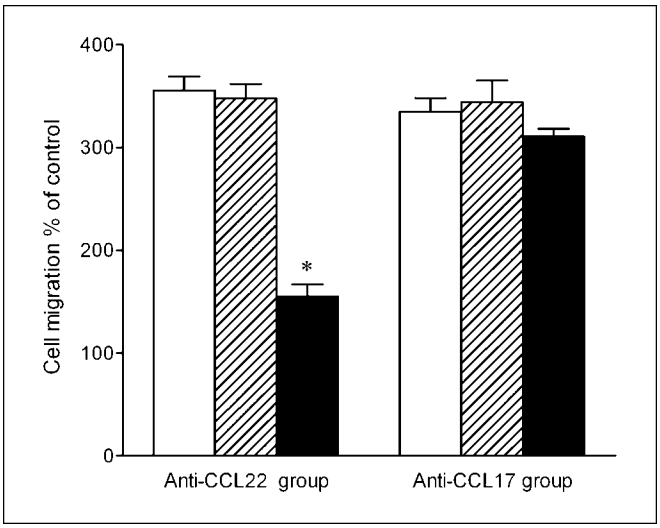

Fig. 5. Malignant pleural effusion is chemotactic for CD4-positive CD25-positive T cells. Pleural effusions from patients with lung cancer ($n = 5$) were used to stimulate chemotaxis of peripheral blood CD4-positive CD25-positive T cells isolated from healthy adults. Data are expressed as percent of control. Open bars, chemotaxis in the absence of anti-CCL22 or anti-CCL17 mAb; hatched bars, irrelevant isotype controls; closed bars, chemotaxis in the presence of anti-CCL22 or anti-CCL17 mAb. * $P < 0.05$, compared with the corresponding group without anti-CCL22 mAb were determined by Kruskal-Wallis one-way ANOVA on ranks.

Fig. 3A); in contrast, the concentration of CCL17 in malignant pleural effusion (163.0 ± 90.0 ng/L) was much lower than its corresponding compartments in serum (560.6 ± 70.4 ng/L; $P < 0.001$; Fig. 3B).

Identification of pleural cells able to express CCL22. To identify the phenotype of pleural cells expressing for CCL22 protein, we did double immunofluorescence staining on cells recovered from malignant pleural effusion. In the present study, the significant expression of CCL22 was found in all cancer cells identified by anti-EMA mAb, most of mononuclear phagocytes identified by anti-CD163 mAb, and most of T lymphocytes identified by anti-CD163 mAb (Fig. 4). These data suggested that malignant cells, macrophages, and T cells might be the cell sources of pleural CCL22.

Malignant pleural effusion was chemotactic for regulatory T cells. Our results showed that malignant pleural effusion exerted a potent chemoattractant activity for regulatory T cells (Fig. 5). To determine whether CCL22 or CCL17 was responsible for the recruitment of regulatory T cells, the ability of an anti-CCL22 or CCL17 mAb to neutralize the chemoattraction of regulatory T cells was tested. We noted that the anti-CCL22, but not the CCL17 mAb, significantly suppressed regulatory T cell chemotaxis. These data provided indirect evidence that CCL22 might be capable of inducing regulatory T cell recruitment into the pleural space of patients with malignant pleural effusion.

Recruitment of regulatory T cells into malignant pleural effusion caused by CCL22. To investigate the direct chemoattractant capacity of CCL22 to recruit regulatory T cells, we injected human recombinant CCL22 into the pleural space of the patients with malignant pleural effusion, and then observed the changes in regulatory T cell numbers using flow cytometry. Compared with baseline value (18.6 ± 4.4%), no significant increase in the number of regulatory T cells could be seen at 6 hours (18.7 ± 4.6%; $P > 0.05$) after intrapleural CCL22 injection. The number of regulatory T cells increased with time, reaching statistical significance at 12 hours (22.7 ± 4.2%; $P < 0.01$), and reaching a maximum at 24 hours (29.9 ± 3.3%; $P < 0.01$). This significant regulatory T cell infiltration lasted at least 48 hours (28.7 ± 3.5%; $P < 0.01$; Fig. 6). After vehicle only was injected into the pleural cavity, we did not observe increases of regulatory T cell counts in malignant pleural effusion obtained at 4 time points when compared with baseline measurement before injection (all $P > 0.05$).

Discussion

Malignant pleural effusion is frequently observed in lung cancer, and a diagnosis of malignant pleural effusion in lung cancer carries a poor prognosis (14, 15). In malignant pleural effusion, CD4-positive T cells are dominant, and the proportion of CD8-positive T cells is significantly lower than that of CD4-positive T cells (16). In contrast, the proportion of CD4-positive T cells in the pleural cavity of patients with lung cancer without malignant pleural effusion is significantly lower than that of CD8-positive T cells (17). Invasion of cancer cells into the pleural cavity may be affected by both the nature of the cancer cells and the host factors of patients with lung cancer. Our previous study has shown that regulatory T cell numbers in malignant pleural effusion were much higher than those in pleural lavage fluid from lung cancer patients without malignant effusion, as well as those in peripheral blood (10). Our data further revealed that pleural regulatory T cells could potently suppress the proliferation of responding T cells, and cytotoxic lymphocyte-associated antigen-4 was involved in the suppressive activity of pleural regulatory T cells (10). In the present study, we also observed the similar results that regulatory T cells were overrepresented in malignant pleural effusion. This raised the question of whether these regulatory T cells could be recruited from the circulation. We therefore determined what chemokines and chemokine receptors were involved in the chemotaxis of these pleural regulatory T cells.

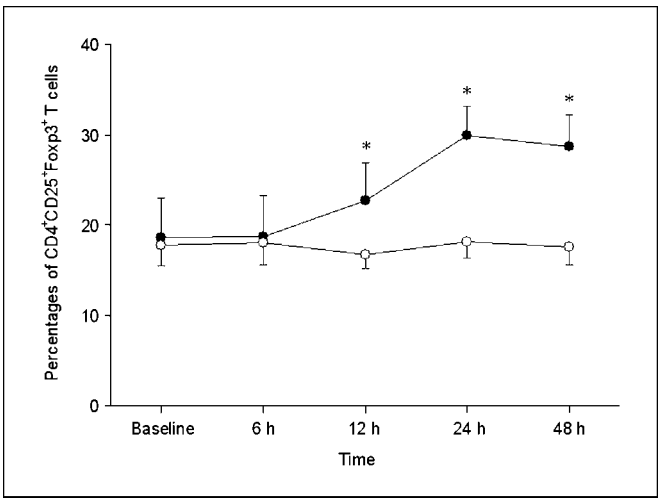

Fig. 6. Changes of CD4-positive CD25-positive T cell numbers in malignant pleural effusion from patients with lung cancer, who were intrapleurally injected with vehicle and recombinant human CCL22. $n = 5$ for each group. Points, mean values; error bars, SE at each time point. * $P < 0.05$ compared within-group change from baseline measurements determined by one-way repeated-measures ANOVA.

It has been reported that the long-term effects of adoptively transferred regulatory T cells induced *ex vivo* are due to their ability to generate new cytokine-producing regulatory T cells *in vivo* (18). It is possible that tumor-related factors induce transient Foxp3-positive T cells from CD4-positive CD25-positive Foxp3-negative effector T cells; however, their suppressive function is thought to be temporary, not intrinsic and unstable (19, 20). On the other hand, tumor-induced expression of addressins on the surface of endothelial cells allows a selective transmigration of regulatory T cells from peripheral blood to tumor tissues (21). It has been shown that human regulatory T cells preferentially move to and accumulate in tumors and ascites, but rarely enter draining lymph nodes in later cancer stages (22). We speculated that an increased percentage of regulatory T cells in malignant pleural effusion might be due to active recruitment or local differentiation. In the previous study, we provided direct evidence that interleukin-16 is capable of inducing CD4-positive T cell infiltration into the pleural space (23). Therefore, as a subpopulation of CD4-positive T cells, regulatory T cells might also be recruited into malignant pleural effusion by local production of interleukin-16, because interleukin-16 level is significantly higher in malignant pleural effusion than in serum (23).

Although chemokine receptors are important for T cell migration, it has been unclear how they are regulated in regulatory T cells. It has been reported that regulatory T cells migrate toward the malignant tumor microenvironment in a process mediated by chemokines CCL22 and/or CCL17 (11, 22, 24). In the present study, we were prompted to evaluate whether pleural CCL22 or CCL17 might be responsible for the influx of regulatory T cells into the pleural space. Our results showed that CCL22 level in malignant pleural effusion was significantly higher than that in corresponding serum, and that malignant cells, macrophages, and T cells might be the cell sources of pleural CCL22. Moreover, consistent with the previous results (25, 26), our data also showed that regulatory T cells in peripheral blood strongly express CCR4, a chemokine receptor for CCL17 or CCL22, on their surface as compared with effector T cells. The above data suggested that CCL22 in malignant pleural effusion might be related to the accumulation of regulatory T cells in malignant pleural effusion. Indeed, an *in vitro* migration assay in the present study further confirmed that malignant pleural effusion could induce the migration of regulatory T cells, and that an anti-CCL22 mAb inhibited the ability of the malignant pleural effusion to stimulate regulatory T cell chemotaxis. The important findings in this study also included that intrapleural administration of 10 μg human recombinant CCL22, not vehicle, of patients with malignant pleural effusion produced a marked progressive influx of regulatory T cells into pleural space. Therefore, in the present study we have provided the direct evidence for the first time that CCL22 was able to chemoattract regulatory T cell recruitment into pleural space.

Although it cannot be excluded that overrepresentation of regulatory T cells in malignant pleural effusion may be due to increased local antigen stimulation, our findings have shown that CCL22 in the pleural space is an important phlogistic agent, and that CCL22 is capable of inducing migration of regulatory T cells to the pleural space. Because regulatory T cells have an inhibitory effect on the surrounding effector T cells, the elimination of the local regulatory T cells might be an effective therapeutic approach against malignant pleural effusion. Now, an anti-CCR4 mAb could be an ideal treatment modality for patients with CCR4-positive neoplasms (27, 28) and could also be used as a novel strategy for treatment of a variety of other diseases, such as malignant pleural effusion, to overcome the suppressive effect of CCR4-positive regulatory T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the staff of Guangxi Key Laboratory of Subtropical Bioresource Conservation and Utilization at Guangxi University for their excellent technical assistance, and the patients who participated in the study.

References

1. Light RW. Clinical practice. Pleural effusion. N Engl J Med 2002;346:1971–7.
2. Dalbeth N, Lee YC. Lymphocytes in pleural disease. Curr Opin Pulm Med 2005;11:334–9.
3. Yang HB, Shi HZ. T lymphocytes in pleural effusion. Chin Med J (Engl) 2008;121:579–80.
4. Shevach EM. $CD4^+CD25^+$ suppressor T cells: more questions than answers. Nat Rev Immunol 2002;2:389–400.
5. von Boehmer H. Mechanisms of suppression by suppressor T cells. Nat Immunol 2005;6:338–44.
6. Beyer M, Schultze JL. Regulatory T cells in cancer. Blood 2006;108:804–11.
7. Woo EY, Chu CS, Goletz TJ, et al. Regulatory $CD4^+CD25^+$ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res 2001;61:4766–72.
8. Woo EY, Yeh H, Chu CS, et al. Cutting edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. J Immunol 2002;168:4272–6.
9. Meloni F, Morosini M, Solari N, et al. Foxp3 expressing $CD4^+CD25^+$ and $CD8^+CD28^-$ T regulatory cells in the peripheral blood of patients with lung cancer and pleural mesothelioma. Hum Immunol 2006;67:1–12.
10. Chen YQ, Shi HZ, Qin XJ, et al. $CD4^+CD25^+$ regulatory T lymphocytes in malignant pleural effusion. Am J Respir Crit Care Med 2005;172:1434–9.
11. Mizukami Y, Kono K, Kawaguchi Y, et al. CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of $Foxp3^+$ regulatory T cells in gastric cancer. Int J Cancer 2008;122:2286–93.
12. Atanackovic D, Block A, de Weerth A, Faltz C, Hossfeld DK, Hegewisch-Becker S. Characterization of effusion-infiltrating T cells: benign versus malignant effusions. Clin Cancer Res 2004;10:2600–8.
13. Beyer M, Kochanek M, Darabi K, et al. Reduced frequencies and suppressive function of $CD4^+CD25^{hi}$ regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood 2005;106:2018–25.
14. American Thoracic Society. Management of malignant pleural effusions. Am J Respir Crit Care Med 2000;162:1987–2001.
15. Haas AR, Daniel H. Sterman DH, Musani AI. Malignant pleural effusions. Management options with consideration of coding, billing, and a decision approach. Chest 2007;132:1036–41.
16. Lucivero G, Pierucci G, Bonomo L. Lymphocyte subsets in peripheral blood and pleural fluid. Eur Respir J 1988;1:337–40.
17. Takahashi K, Sone S, Kimura S, Ogura T, Monden Y. Phenotypes and lymphokine-activated killer activity of pleural cavity lymphocytes of lung cancer patients without malignant effusion. Chest 1993;103:1732–8.
18. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced $CD4^+CD25^+$ cells educate $CD4^+CD25^-$ cells to develop suppressive activity: the role of IL-2, TGF-β, and IL-10. J Immunol 2004;172:5213–21.
19. Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. Clin Immunol 2007;123:18–29.
20. Allan SE, Crome SQ, Crellin NK, et al. Activation-induced Foxp3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol 2007;19:345–54.
21. Nummer D, Suri-Payer E, Schmitz-Winnenthal H, et al. Role of tumor endothelium in $CD4^+$ $CD25^+$

regulatory T cell infiltration of human pancreatic carcinoma. J Natl Cancer Inst 2007;99:1188–99.
22. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942–9.
23. Qin XJ, Shi HZ, Huang ZX, Kang LF, Mo WN, Wu C. Interleukin-16 in tuberculous and malignant pleural effusions. Eur Respir J 2005;25:605–11.
24. Ishida T, Ishii T, Inagaki AY, et al. Specific recruitment of CC chemokine receptor 4-positive regulatory T cells in Hodgkin lymphoma fosters immune privilege. Cancer Res 2006;66:5716–22.
25. Iellem A, Mariani M, Lang R, et al. Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by $CD4^+CD25^+$ regulatory T cells. J Exp Med 2001;194:847–53.
26. Hirahara K, Liu L, Clark RA, Yamanaka K, Fuhlbrigge C, Kupper TS. The majority of human peripheral blood $CD4^+CD25^{high}Foxp3^+$ regulatory T cells bear functional skin-homing receptors. J Immunol 2006;177:4488–94.
27. Ishida T, Utsunomiya A, Iida S, et al. Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. Clin Cancer Res 2003;9:3625–34.
28. Ishida T, Inagaki H, Utsunomiya A, et al. CXC chemokine receptor 3 and CC chemokine receptor 4 expression in T-cell and NK-cell lymphomas with special reference to clinicopathological significance for peripheral T-cell lymphoma, unspecified. Clin Cancer Res 2004;10:5494–500.

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Clin Cancer Res 2009;15:2231-2237.