Elevated Serum Levels of CCL17 Correlate with Increased Peripheral Blood Platelet Count in Patients with Active Tuberculosis in China

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The serum levels of Th2 markers, including CCL17 (thymus and activation-regulated chemokine [TARC]), CCL22 (macrophage-derived chemokine [MDC]), and soluble CD30, were measured in 101 HIV-negative tuberculosis patients, 103 healthy community controls, and 18 tuberculosis patients in recovery. The levels of CCL17/TARC (249.8 ± 19.91 versus 143.9 ± 10.54, P < 0.0001) and sCD30 (7.78 ± 0.44 versus 4.93 ± 0.23, P < 0.0001) were significantly higher in patients with active tuberculosis than in controls; however, the CCL22/MDC serum level had no statistical difference between the groups (579.9 ± 16.42 versus 556.5 ± 15.29, P = 0.298). The counts of platelet and eosinophil in the peripheral blood of patients with active tuberculosis are significantly increased as well (289.4 ± 8.14 versus 248.3 ± 5.34 [P < 0.0001] and 165.1 ± 14.33 versus 102.5 ± 10.72 [P = 0.0005], respectively), and the platelet counts were positively correlated with serum TARC levels (Pearson r = 0.456, P < 0.0001), which indicates a new source of Th2 bias showing in active TB patients.

Active tuberculosis (TB) has been associated with imbalance of Th1 and Th2 cytokine pattern. Activation of Th1 lymphocytes, gamma interferon (IFN-γ) production and macrophage activation are crucial in defense against mycobacteria, while Th2 activation and interleukin-4 (IL-4) production are associated with TB progressing and with poor clinical outcome after treatment (6).

After being secreted, IFN-γ and IL-4 are rapidly bound by their receptors and/or inactivated by proteases, which leads to difficulty in detecting these two typical Th1/Th2 cytokines in plasma or serum (26). Therefore, other markers have been investigated as alternative indicators of preferential Th1 or Th2 activity in vivo.

CCL17 (thymus and activation-regulated chemokine [TARC]) and CCL22 (macrophage-derived chemokine [MDC]) are two specific chemotactic factors for Th2 cells (12). CD30, a tumor necrosis factor (TNF) receptor II family member, is also correlated with Th2 activation (3). sCD30 is a soluble form of CD30, which is generated by proteolytic cleavage of the extracellular portion of this transmembrane molecule and can be measured in serum or plasma by enzyme-linked immunosorbent assay (ELISA). Previous reports indicate increasing expression of these factors in patients with Th2 bias status, such as asthma (27), arthritis (7), and lymphoma (2); however, very few reports examine the changes in these Th2 factors in TB patients (16).

For screening of new Th2 markers in active TB patients, we compared the serum levels of CCL17/TARC, CCL22/MDC, and sCD30 in HIV-negative TB patients with samples obtained from community healthy controls in China. The results indicate that CCL17/TARC and sCD30 might be more suitable as serum Th2 marker than CCL22/MDC in active TB patients. The correlation of these Th2 markers and the platelet and eosinophil counts in peripheral blood were also analyzed.

MATERIALS AND METHODS

Study subjects. Peripheral blood was obtained from 103 normal healthy subjects, 101 active pulmonary TB patients, and 18 TB patients in recovery. The age and sex information of patients and controls are presented in Table 1. The patients included were clinically and radiologically diagnosed for pulmonary TB, and diagnoses confirmed by sputum smear and culture for Mycobacterium tuberculosis. The patients were recruited from the clinics of Shanghai Pulmonary Hospital. All of the patients were HIV negative; none of them presented with other infectious diseases or immunosuppressive conditions. All of the active TB patients received anti-TB therapy for less than 1 week and were classified into three categories (5): mild TB was defined by the presence of scattered and nonconfluent pulmonary infiltrates of slight to moderate density in one or both lungs, with the total volume less than one lung, without cavities. Moderate TB was defined by scattered and nonconfluent pulmonary infiltrates present in one or both lungs and/or with dense and confluent lesions but not involving more than one-third of the volume of one lung, with or without cavities with a total diameter of <4 cm. Advanced TB was defined by lesions exceeding the criteria described above. Among them, 31 cases were multidrug resistant TB, from which strains of M. tuberculosis were demonstrated to be resistant to rifampin and isoniazid or more anti-TB drugs by drug susceptibility testing. Recovery patients were recruited after 3 months of standard treatment; sputum smear results for patients in this group showed positive-to-negative transition, with radiologically confirmed absorption of the pulmonary inflammatory infiltration.

The normal healthy subjects were determined to be clinically normal and were willing to participate in the study. All studies involving human subjects were approved by the institutional review board of Tongji University School of Medicine.

White blood cell (WBC), eosinophil, basophil, and platelet count analyses. Venous blood (obtained in EDTA tubes) was analyzed for the complete blood count by using a CELL-DYN 3700 (Abbott Laboratories) hematology analyser. The CELL-DYN 3700 system is a multiparameter, automated hemotology analyser designed for in vitro diagnostic use in clinical laboratories. Absolute eosinophil/basophil counts were calculated as the percentage of eosinophils/basophils × the total white blood cell count.
Measurement of serum chemokine concentrations. The sera were collected after centrifugation at 3,000 rpm at 4°C for 10 min and were stored at −80°C until use. Sandwich-type ELISA kits were used to measure levels of CCL17/TARC and CCL22/MDC (Antigenix America, Inc.); sCD30 was measured by ELISA using human sCD30-matched antibody pairs (Bender Medsystems).

Statistical analysis. Results are shown as means ± the standard error. Comparisons between groups were performed with an unpaired Student t test. The Pearson correlation coefficient was calculated between two parameters. A P of <0.05 was considered statistically significant.

RESULTS

Increasing eosinophil and platelet counts in the peripheral blood of patients with active TB. We compared the WBC, eosinophil, basophil, and platelet counts between the active-TB patients and healthy control groups. As shown in Fig. 1, the total WBC count tended to increase in patients with active TB but had no statistical difference compared to the normal group in our test (7.15 ± 0.19 versus 6.68 ± 0.16, P = 0.06); basophil counts showed no difference between the two groups (47.65 ± 2.16 versus 51.47 ± 2.40, P = 0.238). However, eosinophil (165.1 ± 14.33 versus 102.5 ± 10.72, P = 0.0005) and platelet (289.4 ± 8.14 versus 248.3 ± 5.34, P < 0.0001) counts increased markedly in patients with active TB, and patients in recovery tended to show a decline in the platelet count.

CCL17/TARC and sCD30 increased significantly in the sera of patients with active TB. As shown in Fig. 2, serum CCL17/TARC levels in patients with active TB increased greatly compared to those of healthy control (249.8 ± 19.91 versus 143.9 ± 10.54, P < 0.0001). The sCD30 levels in the sera of patients with TB were also remarkably higher than in healthy controls (7.78 ± 0.44 versus 4.93 ± 0.23, P < 0.0001), whereas the levels of CCL22/MDC showed no significant differences between these two groups (579.9 ± 16.42 versus 556.5 ± 15.29, P = 0.298).

Correlation of serum levels of CCL17/TARC and platelet counts. It has been reported that platelets contain high amounts of CCL17/TARC, which can be released during blood clotting (8). We therefore analyzed whether the increasing CCL17/TARC level is associated with increasing platelet counts. The data showed that the serum level of CCL17/TARC was moderately correlated with the peripheral platelet count (r = 0.456, P < 0.0001), whereas there were no correlations between CCL17 and sCD30 serum levels, between the sCD30 level and the platelet count, between the CCL17 level and the eosinophil count, or between the sCD30 level and the eosinophil count (Fig. 3).

DISCUSSION

CCL17/TARC and CCL22/MDC are a pair of chemokines and specific ligands of CCR4 that play important roles in polarized Th2 and CD4+CD25+ T regulatory cells (Tregs). Tregs are known to play a crucial role in downmodulating immune responses, contributing both to the maintenance of
self-tolerance and to the prevention of excessive responses against infection (12, 22). Increasing CCL17/TARC and CCL22/MDC levels would eventually amplify Th2 and Treg circuits and negatively modulate Th1 polarization, and so these chemokine pairs can be used as indicators of Th2 domination (10, 24).

Our data are the first to demonstrate increasing CCL17/TARC levels in the sera of patients with active TB, a finding consistent with the previous report of high expression of CCL17/TARC mRNA in the airways of mice injected with a mycobacterium-purified protein derivative (23). Increasing levels of CCL22/MDC and sCD30 have been observed in plasma samples of patients with active TB in South Africa (16); while our data for serum sCD30 are consistent with reported data for plasma samples from South Africa, the serum levels of CCL22/MDC in Chinese patients showed no statistical difference compared to the healthy control group. Differential blood samples (e.g., sera and plasma) used in these two studies might be one reason for the disagreement in results, since blood clotting process will influence the concentration of CCL22/MDC (8, 20). Our data indicate that the levels of CCL17/TARC and sCD30 can be more suitable serum Th2 markers than CCL22/MDC in TB patients.

In addition to Th2 cytokine (IL-4 and IL-13)-priming T cells, dendritic cells, and monocytes (11, 15, 21), platelets may be another major source of increasing serum CCL17/TARC, since they have been found to contain large amounts of CCL17/TARC, which can be released during cloting (8). In parallel with increasing CCL17/TARC levels in sera, higher platelet counts were found in patients with active TB compared to control groups, and there was significant positive correlation between platelet counts and CCL17/TARC levels in sera. Consistent with our finding with increasing numbers of platelets, increased platelet aggregation and a hypercoagulable state was reported in cases of TB (25). The release of CCL17/TARC from activated platelets may further enhance their aggregation and the release of CCL17/TARC in an autocrine manner (1).

Increased levels of CCL17/TARC also paralleled increasing eosinophil counts in peripheral blood of TB patients, a finding similar to those of a previous report about pleural effusion.

![FIG. 2](image1)

**FIG. 2.** Serum levels of CCL17, sCD30, and CCL22 from different subject groups. Short horizontal lines represent median values. Comparisons were made between healthy and active-TB groups. $P > 0.05$, without difference; $P < 0.001$, with a statistically significant difference.

![FIG. 3](image2)

**FIG. 3.** Correlation analysis. Correlations of serum CCL17 and sCD30 levels with platelet and eosinophil counts in peripheral blood were analyzed for the active-TB patients. Serum CCL17 levels correlated moderately and positively with peripheral platelet counts (Pearson $r = 0.456$; 95% confidence interval, 0.239 to 0.630; $P < 0.0001$).
samples from patients infected with *Paragonimus westermani* (19). Eosinophils can mediate antibody-dependent cellular cytotoxicity and interact closely with Th2-type CD4+ T cells in mediating cytokine production, chemokine receptor expression, and cellular influx into the respiratory tract. Recently, eosinophils have been found to have a role in chemokine generation and immune regulation of T cells (17). Eosinophils may contribute to increasing the CCL17/TARC level in blood, too, since they demonstrated increased mRNA expression and protein secretion of the CCL17/TARC and CCL22/MDC when *in vitro* cultured with TNF-α plus IL-4 (17), two cytokines that are detectable in patients with active TB (4, 9).

On the other hand, CCL17/TARC, the dominantly expressed type 2 chemokine, may be the major proinflammatory signal in eosinophilic inflammation seen in TB patients, as in the very early lesions of guinea pigs infected with *M. tuberculosis* (14). CCL17/TARC stimulation can induce the production of excess amounts of Th2 type cytokines, such as IL-5, and thus stimulate the proliferation and activation of eosinophils. In a mouse model of asthma, treatment with anti-TARC antibody attenuates the accumulation of eosinophils and lymphocytes into the lung (13).

In conclusion, patients with active TB in China showed a remarkable increase in CCL17/TARC and sCD30 in sera. Platelet might be one previously neglected source of CCL17/TARC in cases of active TB. Since increasing serum levels of CCL17/TARC are correlated with high levels of circulating Tregs recently found in patients with active TB (18), platelets may play important roles in TB pathogenesis by releasing CCL17/TARC. Circulating levels of CCL17/TARC and sCD30 and the platelet count may thus serve as useful markers for evaluating disease progression in patients with TB.

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