CCL17/Thymus and Activation–Related Chemokine in Churg-Strauss Syndrome

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Objective. Churg-Strauss syndrome (CSS) is a Th2-mediated systemic vasculitis characterized by eosinophilic infiltration, blood eosinophilia, and high IgE levels. CCL17/thymus and activation–regulated chemokine (TARC) is a chemokine responsible for the recruitment of Th2 cells. This study was undertaken to explore a possible role of CCL17/TARC in CSS.

Methods. CCL17/TARC levels in serum from patients with active or inactive CSS, hypereosinophilic syndrome, systemic small-vessel vasculitis other than CSS, other types of eosinophilia, and healthy controls were determined by enzyme-linked immunosorbent assay. Biopsy samples of affected tissue from CSS patients were examined by immunohistochemical staining for Th2 infiltration and CCL17/TARC expression.

Results. Serum CCL17/TARC levels were significantly elevated in CSS patients with active disease (mean ± SEM 1,122.0 ± 422.7 pg/ml) compared with controls (220.6 ± 27.9 pg/ml) and patients with inactive disease (388.9 ± 72.6 pg/ml) (P < 0.001 and P < 0.05, respectively). These levels correlated with the clinical disease course of CSS and with absolute eosinophil counts as well as IgE levels. Infiltrating Th2 cells in active CSS lesions were evidenced by CD294 staining. Elevated CCL17/TARC levels were also noted in patients with hypereosinophilic syndrome (794.5 ± 294.8 pg/ml) and other disorders associated with eosinophilia (1,096.0 ± 345.3 pg/ml) (both P < 0.005 versus controls).

Conclusion. CCL17/TARC may contribute to CSS pathogenesis by recruitment of Th2 cells into affected tissue. Serum CCL17/TARC levels reflect disease activity, and further studies to validate its use as an activity marker in CSS are warranted.

Churg-Strauss syndrome (CSS) is a necrotizing systemic small-vessel vasculitis associated with substantial morbidity and mortality (1). Although CSS belongs to the group of antineutrophil cytoplasmic antibody (ANCA)–associated vasculitides, some disease features are specific for CSS and distinguish it from Wegener’s granulomatosis or microscopic polyangiitis, i.e., 1) almost all patients with CSS present with a history of allergic disease (“allergic granulomatous angiitis”), and the onset of CSS is sometimes associated with a change in asthma therapy (2); 2) dense eosinophilic infiltration and extravascular granulomas are found on tissue biopsy of CSS-affected organs (1), and high eosinophil counts are commonly found in peripheral blood; and 3) as opposed to other ANCA-associated vasculitides, cardiac and peripheral nerve involvement is frequent in CSS, while renal disease is rare (2).

The pathogenesis of eosinophilia and tissue damage in CSS remains unclear; however, it is suspected that an unknown allergen or environmental trigger provokes CSS in presensitized asthma patients. Genetic suscepti-
bility might play an important role, as recently shown by two independent groups of investigators. HLA–DRB4, in particular, is linked to CSS development and may provide a link between adaptive immunity and the pathogenesis of CSS (3,4). On a cellular level, a strong shift toward a Th2-like response with massive T cell activation is evident. In vitro, T cell lines from CSS patients produce significant amounts of interleukin-4 (IL-4) and IL-13 (5). This is underscored by the fact that patients with active CSS usually have high serum levels of IgE and IgE-containing immune complexes (6). Also, Th2 cytokines contribute to the recruitment, activation, and survival of eosinophils (7) and, in accordance with this, granulomatous vasculitic lesions filled with eosinophils, macrophages, and lymphocytes are observed on biopsy (8). Local activation with degranulation of eosinophils and subsequent release of granule proteins such as eosinophil-derived neurotoxin and major basic protein likely contributes to vasculitic tissue damage. However, the molecules that specifically contribute to eosinophilia, eosinophilic infiltration, and subsequent degranulation of eosinophils in CSS remain enigmatic.

We recently demonstrated that the chemokine eotaxin 3 is not only strongly expressed in the affected tissue of CSS patients but is also specifically related to disease activity and eosinophilia (9). CCL17/thymus and activation–related chemokine (TARC) is a CC chemokine secreted from peripheral blood mononuclear cells (PBMCs) and various subsets of monocyte-derived dendritic cells (DCs), including thymic DCs and endothelial cells (10). It is the ligand for CCR4 and CCR8 chemokine receptors on human PBMCs and T cells, respectively (11). Given the expression profile of its receptors and the ability of CCL17/TARC to selectively induce migration of CD4+CD45RO+ T cells producing Th2 cytokines (12), it may be crucial for the specific recruitment and trafficking of Th2 cells that are activated in CSS. Recently, marked elevation of CCL17/TARC was demonstrated in sera of patients with the lymphocytic subtype of idiopathic hypereosinophilic syndrome (HES), which is defined by the presence of circulating IL-5–producing clonal Th2 cells (13).

Considering the Th2-dominated eosinophilic infiltration in tissue affected by CSS, we hypothesized that CCL17/TARC plays a role in recruitment of Th2 cells to sites of inflammation. We thus compared serum levels of CCL17/TARC in patients with active CSS, patients with inactive CSS, and healthy controls, as well as in patients with other conditions involving persistent eosinophilia. Additionally, we determined local expression of CCL17/TARC in tissue affected by active CSS.

PATIENTS AND METHODS

Patients. Twenty-five CSS patients seen at the departments of rheumatology and internal medicine at 3 different centers (University of Erlangen–Nuremberg, Medical University of Vienna, and University of Schleswig-Holstein) were included in this study. All patients fulfilled the 1990 American College of Rheumatology (ACR) criteria for CSS (14) and/or the Chapel Hill Consensus Conference definition of CSS (15). Medical records of patients with clinically diagnosed CSS were retrieved, and clinical, laboratory, and histopathologic data were reviewed. CSS patients had routine laboratory investigations including complete blood cell count, liver function tests, testing for ANCA by indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA), and determination of serum creatinine, C-reactive protein (CRP), and serum IgE levels.

Patients with active HES (n = 18), newly diagnosed systemic small-vessel vasculitis (SVV) other than CSS (Wegener’s granulomatosis [WG] and microscopic polyangiitis [MPA] [n = 12]), other types of eosinophilia (n = 14), and healthy age- and sex-matched controls without a known history of allergic disease (n = 21) served as controls. WG patients were classified according to the ACR criteria (16), MPA was diagnosed according to the recommendations of Guillevin et al (17), and HES was diagnosed based on the results of clinical and laboratory studies after exclusion of other causes of persistent eosinophilia. Other types of hypereosinophilia included parasite infestation (n = 2), eosinophilia in patients with human immunodeficiency virus infection (n = 4), paraneoplastic eosinophilia (n = 1), eosinophilic leukemia (n = 1), mastocytosis (n = 1), atopic dermatitis (n = 1), and eosinophilia of unknown origin (n = 4).

The local ethics committees approved the study, and informed consent was obtained from all patients and healthy controls.

Detection of CCL17/TARC in serum. Serum samples were collected at routine visits and immediately stored at −80°C. CCL17/TARC was detected by ELISA (R&D Systems). Briefly, polystyrene microplates coated with a monoclonal antibody against CCL17/TARC were incubated with serum samples for 2 hours at room temperature. For detection, horseradish peroxidase (HRP)–conjugated polyclonal antibody specific to CCL17/TARC was added for 1 hour, and color reaction was developed using tetramethylbenzidine and hydrogen peroxide. Optical density at 450 nm was measured with a plate reader, and absolute values were calculated from the CCL17/TARC 4-parameter logistic standard curve.

Tissue samples. Tissue samples were obtained from CSS patients at the time of diagnosis. To facilitate comparison with other diseases, we investigated nasal biopsy specimens (n = 5). As controls, we used biopsy samples from patients with chronic rhinosinusitis associated with eosinophilia (n = 5) or without eosinophilia (n = 5). Tissue samples were fixed for 12 hours in 4% paraformaldehyde. Specimens were processed for paraffin embedding, and 1–2-μm serial sections were cut for routine histologic analysis and immunohistochemical staining. One section from each sample was stained with hematoxylin and eosin for histologic assessment.

Immunohistochemistry. Immunohistochemistry analysis was performed on formalin-fixed, paraffin-embedded sec-
Table 1. Characteristics of the study subjects*

<table>
<thead>
<tr>
<th></th>
<th>Active CSS (n = 12)</th>
<th>Inactive CSS (n = 13)</th>
<th>HES (n = 18)</th>
<th>WG/MPA (n = 12)</th>
<th>Other HE (n = 14)</th>
<th>Healthy controls (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, no. (%)</td>
<td>3/9 (25/75)</td>
<td>4/9 (31/69)</td>
<td>4/14 (22/78)</td>
<td>4/8 (33/67)</td>
<td>8/6 (57/43)</td>
<td>8/13 (38/62)</td>
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<td>Age, years</td>
<td>39.1 ± 16.1</td>
<td>42.3 ± 14.0</td>
<td>48.3 ± 14.9</td>
<td>48.4 ± 20.5</td>
<td>46.4 ± 20.4</td>
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<tr>
<td>CRP, mg/liter</td>
<td>46.0 ± 39.2</td>
<td>10.1 ± 23.4</td>
<td>8.1 ± 7.8</td>
<td>82.4 ± 96.0</td>
<td>18.1 ± 27.8</td>
<td>NA</td>
</tr>
<tr>
<td>ANCA positive, no. (%)</td>
<td>3 (23)</td>
<td>3 (23)</td>
<td>1 (6)</td>
<td>10 (83)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Absolute eosinophil count, cells/mm³</td>
<td>7,164 ± 12,431</td>
<td>538 ± 438</td>
<td>4,721 ± 6,871</td>
<td>260 ± 276</td>
<td>1,869 ± 2,127</td>
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</tr>
<tr>
<td>IgE, units/ml</td>
<td>748.7 ± 1,134.0</td>
<td>72.9 ± 107.7</td>
<td>709.0 ± 1,071.0</td>
<td>NA</td>
<td>1,104 ± 1,718</td>
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</tr>
</tbody>
</table>

* Except where indicated otherwise, values are the mean ± SD. CSS = Churg-Strauss syndrome; HES = hypereosinophilic syndrome; WG/MPA = Wegener’s granulomatosis/microscopic polyangiitis; other HE = other types of hypereosinophilia (parasite infestation [n = 4], paraneoplastic eosinophilia [n = 1], eosinophilic leukemia [n = 1], mastocytosis [n = 1], atopic dermatitis [n = 1], and eosinophilia of unknown origin [n = 4]); ANCA = antineutrophil cytoplasmic antibody; NA = not assessed; CRP = C-reactive protein.

Statistical analysis. For comparisons of CCL17/TARC levels between groups and before and after therapy, the Mann-Whitney U test was used; to compare clinical and serologic parameters, Pearson’s correlation coefficient was used. P values less than 0.05 were considered significant.

RESULTS

Patient characteristics. Demographic and clinical characteristics of the CSS patients and the controls are shown in Table 1. To explore a possible relationship of CCL17/TARC to eosinophilia and disease activity, the 25 CSS patients were divided into an active disease group (n = 12) and an inactive disease group (n = 13). Active CSS was defined as an increased eosinophil count (≥10% eosinophils or >1,000 eosinophils/µl) and active vasculitis in at least 1 organ as confirmed histologically.

Figure 1. Serum levels of CCL17/thymus and activation–related chemokine (TARC) in patients with active (untreated) or inactive (treated) Churg-Strauss syndrome (CSS), patients with active hypereosinophilic syndrome (HES), patients with active systemic small-vessel vasculitis other than CSS (Wegener’s granulomatosis [WG] or microscopic polyangiitis [MPA]), patients with other types of eosinophilia, and healthy controls. Values are the mean and SEM. * P < 0.005 versus controls; * with horizontal bar = P < 0.05.
or evidenced by surrogate (radiologic, clinical, or laboratory) parameters.

Disease manifestations were typical for CSS and were equally distributed in the 2 groups. All patients had eosinophilia, 88% had asthma, 89% had sinusitis, 52% had vasculitic polyneuropathy, 32% had pulmonary involvement, 33% had cardiac disease, and 28% had skin involvement. With respect to therapy, all patients in the inactive disease group were receiving low-dose corticosteroids, and 9 were receiving immunosuppressive drugs (azathioprine [4 patients], cyclophosphamide [3 patients], methotrexate [1 patient], and rituximab [1 patient]). In the active disease group, 1 patient was receiving corticosteroids, and 1 was receiving an immunosuppressive drug (cyclophosphamide) at the time serum samples were obtained. Compared with the inactive CSS group, the active CSS group was characterized by higher levels of acute-phase reactants (mean ± SD CRP level 46.0 ± 39.2 mg/liter versus 10.1 ± 23.8 mg/liter; \( P < 0.0001 \)), eosinophil counts (7,164 ± 12,431 cells/mm³ versus 538 ± 438 cells/mm³; \( P < 0.0001 \)), and serum IgE levels (748.7 ± 1,134.0 units/ml versus 72.9 ± 107.7 units/ml; \( P < 0.05 \)).

To evaluate the specificity of serum CCL17/TARC levels, we included sex- and age-matched groups of patients with other diseases, i.e., systemic SVV other than CSS (\( n = 12 \)), HES (\( n = 18 \)), and other hypereosinophilias (\( n = 14 \)), as well as healthy controls (\( n = 21 \)). The CSS group did not differ significantly from control groups in age and sex distribution, except for the smaller proportion of female subjects in the group of patients with other eosinophilias.

**Serum levels of CCL17/TARC.** Levels of CCL17/TARC in sera of CSS patients and controls were measured by ELISA (Figure 1). CCL17/TARC levels were significantly elevated in CSS patients with active disease.
compared with controls (mean ± SEM 1,122.0 ± 422.7 pg/ml versus 220.6 ± 27.9 pg/ml; P < 0.005). Although CCL17/TARC levels were also elevated in treated CSS patients with inactive disease (388.9 ± 72.6 pg/ml), this elevation was rather mild, and the level was not significantly different from that in healthy controls. However, CCL17/TARC levels were significantly higher in CSS patients with active disease than in those with inactive disease (P < 0.05). This observation was confirmed upon long-term monitoring of the disease course and CCL17/TARC levels in the 6 individual patients who had the highest levels of CCL17/TARC at the time of diagnosis, before treatment was instituted (Figure 2).

After initiation of high-dose corticosteroid therapy, CCL17/TARC levels declined significantly in all patients (mean ± SEM level before therapy 779.2 ± 267.9 pg/ml, lowest level after therapy 183.0 ± 42.6 pg/ml; P < 0.05). This decline occurred within a mean ± SEM of 7.8 ± 3.7 months after initiation of therapy and paralleled the clinical improvement (Figure 2). Interestingly, in each of the 2 patients who experienced a clinical relapse, an increase in CCL17/TARC levels preceded the relapse (by 5 months and 2 months, respectively) (Figures 2e and f).

CCL17/TARC levels were also elevated in the sera of patients with idiopathic HES (794.5 ± 294.8 pg/ml), SVV other than CSS (440.6 ± 76.6 pg/ml), and other types of eosinophilia (1,096.0 ± 345.3 pg/ml) (all P < 0.005 versus healthy controls).

**Clinical correlations of CCL17/TARC levels.** To determine possible correlations between CCL17/TARC levels and established laboratory markers of disease activity in hypereosinophilic syndromes, CCL17/TARC levels were compared with serum IgE levels, absolute eosinophil counts, and CRP levels in patients with CSS, patients with HES, and patients with other types of eosinophilia. We found a significant correlation of CCL17/TARC levels with eosinophil counts (r = 0.51, P < 0.0001) and IgE levels (r = 0.42, P < 0.005), whereas no correlation with the acute-phase reactant CRP was observed (r = 0.12, P = 0.26) (Figure 3).

We also investigated whether CCL17/TARC levels are associated with certain clinical manifestations in active CSS. We did not identify any significant differences in CCL17/TARC levels in relation to ANCA status, skin purpura, multiplex neuropathy, or biopsy-confirmed vasculitis (data not shown).

**Th2 cells in active CSS lesions.** Because CCL17/TARC is a chemoattractant for Th2 cells, we investigated whether Th2 cells could be localized to affected tissue in CSS patients and disease controls. We per-
formed immunohistochemical staining for CD294/CRTH2, which is localized exclusively on the Th2 subset and not found on other T cells. Interestingly, we observed strong expression of CD294-positive lymphocytes in nasal biopsy specimens from CSS patients (mean ± SEM 260 ± 37 cells/mm²). However, this was not specific to CSS; CD294-positive T cells were also identified in biopsy specimens from patients with chronic rhinosinusitis with eosinophilia (232 ± 28 cells/mm²). Interestingly, many fewer Th2 cells were found in biopsy samples from patients who had chronic rhinosinusitis without eosinophilia (94 ± 18 cells/mm²; P < 0.05 versus the patients with CSS and the patients with sinusitis with eosinophilia). Thus, Th2 cells can be found in conditions with eosinophilic infiltration, including active CSS lesions. Representative images are shown in Figure 4.

**CCL17/TARC expression in active CSS lesions.** To investigate the origin of elevated serum CCL17/TARC levels, we stained biopsy specimens from 5 patients with active CSS with a TARC-specific antibody. Biopsy samples were obtained, for diagnostic purposes, from nasal conchae or sinuses at the time of the first disease manifestation. CCL17/TARC was expressed in all samples of affected tissue that were examined (Figure 5). CCL17/TARC-positive cells were usually associated with a dense inflammatory infiltrate and were usually located around blood vessels. Occasionally, respiratory epithelium also stained weakly positive, while other tissue compartments remained negative. We also examined nasal biopsy samples from patients with chronic rhinosinusitis with or without eosinophilia (n = 5 in each group). Interestingly, we detected CCL17/TARC-positive cells in specimens from rhinosinusitis patients with significant tissue eosinophilia but not in those from patients without eosinophilia. Thus, infiltrating CCL17/TARC-expressing cells are likely associated with tissue eosinophilia. Furthermore, CCL17/TARC is expressed in tissue at sites of active disease in CSS and may also play a role in eosinophil infiltration.

**DISCUSSION**

Dense eosinophilic tissue infiltration, eosinophilic granulomas, and eosinophilia are pathologic hallmarks of CSS. The predominant Th2 response with resulting high levels of Th2 cytokines and IgE and the known ability of Th2 lymphocytes to support the activation and survival of eosinophils suggest that both eosinophils and Th2 cells have a substantial role in CSS pathogenesis.

CCL17/TARC is a chemokine that is secreted from monocyte-derived DCs and endothelial cells and is responsible for selective recruitment and migration of activated Th2 lymphocytes to affected tissue. The ex-
pression of both of its receptors (CCR4 and CCR8) is transiently up-regulated on activated T cells and preferentially on cells of the Th2 subset (18), and conversely, production of CCL17/TARC by DCs is stimulated by Th2 cytokines (19). Hence, analogous to previously reported observations in a murine model of atopic dermatitis (20), CCL17/TARC production by DCs in affected tissue may be up-regulated by Th2 cytokines and thus provide additional chemoattraction for Th2 cells (19). Expression of CCL17/TARC has been demonstrated in Langerhans’ cells and keratinocytes in the skin of patients with atopic dermatitis (21), which, in its acute phase, is considered to be a Th2-dominated disease. Also, CCL17/TARC is expressed in CD11c+ DCs in murine lung tissue (22) and plays a crucial role in Th2-mediated experimental allergen-induced asthma in mice, and development of murine asthma could be inhibited by a monoclonal antibody against CCL17/TARC (23). This is also supported by the finding of production of high levels of CCL17/TARC by bronchial epithelial cells of asthma patients (24).

To our knowledge, this is the first demonstration of CCL17/TARC expression in tissue from patients with CSS. Consistent with previously reported findings (21), we observed expression of CCL17/TARC by infiltrating cells and occasionally the respiratory epithelium. Hence, it is likely that CCL17/TARC contributes to trafficking of Th2 cells and eosinophils to affected tissue in CSS. Th2 infiltration may promote activation and degranulation of eosinophils, resulting in tissue damage. In the present study, we also clearly demonstrated infiltration of Th2 cells in active CSS lesions, as evidenced by the presence of CD294+ lymphocytes. The observation that CCL17/TARC could be detected only in biopsy samples from tissue infiltrated by eosinophils indirectly supports the notion that it has a role in establishing eosinophilic infiltration.

We also found significantly elevated serum levels of CCL17/TARC in patients with untreated CSS compared with healthy controls. Similar findings in patients with atopic dermatitis (21) and lymphocytic-type HES (13) have been reported. In the present investigation, HES patients were studied as a positive control. Although we were able to reproduce the elevated CCL17/TARC levels in HES patients observed by de Lavareille and colleagues (13), levels were not as high as those observed in their study, due to lack of selection for the lymphocytic type of HES.

More importantly, we were able to demonstrate that, upon initiation of therapy, CCL17/TARC concentrations declined to a level that was not significantly different from that in healthy controls. This is consistent with findings in patients with atopic dermatitis (21). Although individual levels of CCL17/TARC varied considerably, in particular among patients with active disease, they paralleled the clinical disease course in all 6 patients from whom sequential serum samples were obtained. In accordance with this, CCL17/TARC concentrations correlated well with IgE levels and eosinophil counts. Interestingly, in both patients who experienced a clinical relapse of CSS, an increase of CCL17/TARC levels preceded clinical manifestations. It could well be that this pattern of secretion reflects the pathophysiologic role of CCL17/TARC; i.e., the recruitment of Th2 cells and eosinophils to affected tissue before the actual eosinophil-mediated tissue damage that leads to the clinical symptoms occurs.

Accumulating evidence on the role of CCL17/TARC in allergic diseases (asthma, atopic dermatitis) and other diseases characterized by eosinophil-mediated tissue damage (HES, CSS, parasitic disease) confirms that CCL17/TARC is a hallmark of local DC stimulation in the affected peripheral organs in all of these conditions (25). To explore the specificity of our findings in CSS, we also measured levels of CCL17/TARC in patients with systemic small-vessel vasculitides other than CSS and patients with other types of eosinophilia. We found increased levels of CCL17/TARC during active disease in these conditions as well. Thus, as opposed to eotaxin 3 (9), CCL17/TARC involvement is not restricted to CSS but rather represents a general, but potentially crucial, mechanism in the pathogenesis of eosinophil-mediated tissue damage.

In conclusion, CCL17/TARC is expressed in affected tissue of patients with CSS. Its levels in the serum of patients with active disease correlate with disease activity, and increased concentrations precede clinical relapse of CSS in treated patients. Although CCL17/TARC expression is not entirely specific to CSS, it provides a clue for understanding the pathogenesis of CSS and also may serve as a biomarker for eosinophilic tissue damage.

ACKNOWLEDGMENT

We thank Isabel Schmidt for technical assistance with ELISA.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Zwerina had full access to all of
REFERENCES


