CONCISE REPORT

IgG4 immune response in Churg–Strauss syndrome

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ABSTRACT

Objective T-helper type 2 responses are crucial in Churg–Strauss syndrome (CSS) and may enhance the production of IgG4 antibodies. The authors assessed the IgG4 immune response in CSS patients.

Methods The authors included 46 consecutive patients with CSS (24 with active and 22 with quiescent disease), 26 with granulomatosis with polyangiitis (GPA, Wegener's), 25 with atopic asthma and 20 healthy controls and determined serum IgG, IgM, IgA, IgE and IgG subclass levels. Tissue infiltration by IgG4 plasma cells was assessed in nine patients with CSS, 10 with GPA, 22 with chronic sinusitis (11 with and 11 without eosinophilia).

Results IgG4 levels were markedly higher in active CSS patients than in controls ($p < 0.001$ vs all control groups). Serum IgG4 correlated with the number of disease manifestations ($r = 0.52$, $p = 0.01$) and the Birmingham vasculitis activity score ($r = 0.64$, $p = 0.001$). Longitudinal analysis in 12 CSS cases showed that both the IgG4 level and IgG4/IgG ratio dropped during disease remission ($p = 3 \times 10^{-5}$ and $p = 6 \times 10^{-4}$, respectively). Tissue analysis did not show an increased IgG4 plasma cell infiltration in CSS biopsies compared with control groups.

Conclusions Serum IgG4 levels are markedly elevated in active CSS and correlate with the number of organ manifestations and disease activity.

Churg–Strauss Syndrome (CSS) is a rare vasculitis occurring in patients with eosinophilia, a history of asthma and frequently allergic rhinitis and sinusitis. It is histologically characterised by eosinophil-rich infiltrates, eosinophilic (extravascular) granulomas and small to medium-sized vessel vasculitis.^{1,2} The pathophysiology of CSS is largely unknown; genetic association with HLA-DRB4 has been reported.³ The allergic history of CSS patients strongly suggests a T-helper (Th) type 2-mediated disease, a view reinforced by the evidence that Th2 cytokines (eg, interleukin (IL)-4, IL-5, IL-13 and IL-25) and chemokines (eg, eotaxin-3, TARC/CCL17) are involved in CSS.^{4,5}

The humoral immune response in CSS is relatively unexplored. High serum IgE levels are found in approximately 90% of active CSS patients.⁶ On the other hand, little is known about IgG production and IgG subclass distribution. IgG subclass analysis is often relevant to the pathogenesis of immune-mediated diseases, as a skewing in Th responses may affect IgG subclass switching. For example, in allergic asthma, allergen-specific IgG4 can often be found, although total IgG4 serum levels may not be significantly high.⁷

A group of diseases characterised by fibrosis, tissue eosinophilia, IgG4-positive plasma cell infiltration and high serum IgG4 levels have recently been grouped under the umbrella term 'IgG4-related systemic disease'.⁸ This entity encompasses sclerosing pancreatocholangitis, retroperitoneal fibrosis, chronic sialadenitis and other manifestations, most of which show Th2 and regulatory T-cell responses.⁹ Therefore, we hypothesised that CSS may share a similar IgG4 overproduction pattern, and decided to investigate local and systemic IgG4 responses in CSS patients.

METHODS

Study subjects and serum samples

We studied 24 consecutive patients with active CSS, 22 with quiescent CSS, 26 with active granulomatosis with polyangiitis (GPA, Wegener's), 25 with atopic asthma and 20 healthy controls. The patients were recruited at the Department of Internal Medicine 3, University of Erlangen-Nuremberg, Germany, and the Department of Clinical Medicine and Nephrology, University Hospital of Parma, Italy. All patients fulfilled the 1990 American College of Rheumatology criteria and/or the Chapel Hill Consensus Conference definitions for CSS or GPA. The characteristics of the active CSS patients are shown in table 1 and those of GPA patients in supplementary table S1 (available online only). Six of the active CSS patients and all but two patients with quiescent CSS were receiving immunosuppressive therapies. The healthy control group consisted of 10 men and 10 women (mean age 48 ± 10.5 years).

Serum levels of IgG, IgM, IgA, IgE and IgG subclasses were measured using standard nephelometric methods. Clinical manifestations and laboratory markers were assessed as previously described;³ the criteria for organ involvement in CSS are reported in the supplementary text (available online only). The ethics committees of the University of Erlangen-Nuremberg and of the University Hospital of Parma approved the study, and written consent was obtained from the study participants.

Tissue samples, histological and immunohistochemical analyses

Nine biopsies obtained from the ear–nose–throat (ENT) system (maxillary sinus $n = 6$, ethmoid sinus $n = 2$, nasal septum $n = 1$) of nine active CSS patients were studied, together with ENT samples from 10 GPA patients and from 22 patients with chronic sinusitis (11 with eosinophilia and 11 without).

Detailed histological and immunohistochemical methods are reported in the supplementary text (available online only).

Statistical analysis

Data are presented as mean±SEM, except where stated otherwise. For group comparisons, we used one-way factorial analysis of variance with the Bonferroni–Dunn test or the Mann–Whitney U test. Categorical variables were compared with the Fisher's exact test. For correlations between IgG4 and the number of involved organs or disease activity scores, Pearson's correlation coefficient was used. p Values less than 0.05 were considered statistically significant.

RESULTS

The clinical features of the 24 active CSS patients are given in table 1.

Total immunoglobulin levels are shown in figure 1. IgG levels were significantly higher in active CSS patients than in healthy controls (11709±384 mg/l vs 9631±405 mg/l, $p=0.001$), inactive CSS ($p<0.001$) and controls with asthma ($p=0.032$). IgG levels were also higher in GPA patients (11326±794 mg/l) than in healthy subjects, but this difference was not statistically significant ($p=0.063$); also, IgG levels were significantly higher in subjects with asthma (10769±271 mg/l) than in healthy controls ($p=0.033$). With respect to IgM and IgA levels, no clinically meaningful differences were detected between the groups. As

Table 1 Clinical characteristics of the 24 active Churg–Strauss syndrome patients

M/F, n	15/9
Mean±SEM age at onset, years	47.3±2.8 (range 18–68)
ENT involvement, n (%)	23 (96)
Skin involvement, n (%)	8 (33)
Lung/lower airway tract involvement	
Migratory lung infiltrates, n (%)	13 (52)
Asthma, n (%)	24 (100)
Pleural effusions	3 (6.1)
Neurological manifestations	
Peripheral neuropathy, n (%)	14 (58)
CNS involvement, n (%)	1 (4)
Gastrointestinal involvement, n (%)	3 (13)
Heart involvement	6 (25)
Renal involvement, n (%)	5 (21)
Mean±SEM eosinophil count, cells/ μ l	5607±2047
Mean±SEM C-reactive protein level, mg/l	25.6±6.4
ANCA positivity, n (%)	10 (42)
No (%) of treated patients	6† (25)
Mean±SEM BVAS	16.9±1.66
Patients with FFS ≥ 1	10 (42)

†Four patients were receiving corticosteroids alone, one prednisone and azathioprine and one prednisone and cyclophosphamide.

ANCA, antineutrophil cytoplasmic antibodies; ANCA positivity was by immunofluorescence; BVAS, Birmingham vasculitis activity score; CNS, central nervous system; ENT, ear–nose–throat; FFS, five factor score.

Figure 1 Serum immunoglobulin and IgG subclass levels in Churg–Strauss syndrome (CSS) and control groups. Serum levels of total IgG, IgA, IgM and IgG subclasses (IgG1, IgG2, IgG3, IgG4) were measured in the following groups: active CSS patients ($n=24$), CSS patients in remission ($n=22$), active granulomatosis with polyangiitis (GPA, Wegener's, $n=26$) patients, patients with asthma ($n=25$) and healthy subjects ($n=20$). Data are given as box plots (median, 25th and 75th percentile, end of whiskers indicating 10th and 90th percentile). Statistically significant p values are hereafter reported. IgG: active CSS versus inactive CSS: $p<0.001$; active CSS versus healthy: $p=0.001$; active CSS versus patients with asthma: $p=0.032$; GPA versus inactive CSS: $p=0.024$; asthma patients versus inactive CSS: $p=0.002$; asthma patients versus healthy: $p=0.033$. IgA: active CSS versus inactive CSS: $p=0.031$; GPA versus inactive CSS: $p=0.036$. IgM: no significant differences between the groups. IgE: active CSS versus inactive CSS: $p=0.005$; active CSS versus GPA: $p=0.001$; active CSS versus healthy: $p<0.001$; asthma patients versus GPA, inactive CSS and healthy: all p values <0.001 . IgG4: active CSS versus inactive CSS, asthma patients, GPA and healthy: all p values <0.001 ; GPA versus healthy: $p=0.001$; asthma patients versus healthy: $p=0.033$; inactive CSS versus healthy: $p=0.013$. GPA, granulomatosis with polyangiitis.

Clinical and epidemiological research

Figure 2 IgG4 levels and disease activity in Churg–Strauss syndrome (CSS) patients. Upper panels: in CSS patients with active disease (n=24), IgG4 serum levels significantly correlate with disease activity, as assessed by the Birmingham vasculitis activity score (BVAS) and the number of organ manifestations. Lower panels: IgG and IgG subclass serum levels were longitudinally measured in 12 patients during their active and remission disease stages. A markedly significant reduction is observed during remission in both the absolute IgG4 levels and the IgG4/IgG ratio ($p=3\times 10^{-5}$ and $p=6\times 10^{-4}$, respectively).

expected, IgE were higher in active CSS (761.5 ± 246.6 IU/ml) than in inactive CSS, GPA patients and healthy controls; equally, they were higher in subjects with asthma (570.0 ± 165.7 IU/ml) than in healthy controls (p values in figure 1 legend).

IgG subclass analysis showed that serum IgG4 levels were higher in active CSS patients (2732.2 ± 363.3 mg/l) than in inactive CSS (787.6 ± 163.0 mg/l, $p<0.001$), GPA (1066.4 ± 246.0 mg/l, $p<0.001$), subjects with asthma (825.2 ± 170.8 mg/l, $p<0.001$) and healthy subjects (347.5 ± 70.3 mg/l, $p<0.001$). IgG4 were also higher in the GPA group and the group with asthma than in healthy controls ($p=0.001$ and $p=0.033$; figure 1). Using 1350 mg/l as a cut-off value for IgG4, 18 active CSS patients (75%) had elevated IgG4. We found no significant alterations in IgG1, IgG2 and IgG3 levels between the groups.

We also evaluated IgG4/IgG ratios to exclude the possibility that the increase in serum IgG4 levels in active CSS was due to the overall IgG increase (figure 1). Strikingly, the IgG4/IgG ratio was higher in active CSS than in inactive CSS (22.0% vs 9.4%, $p=0.001$), GPA (8.6%, $p<0.001$), subjects with asthma (7.5%, $p<0.001$) and healthy subjects (3.6%, $p<0.001$).

Next, we explored the correlation between serum IgG4 levels and the number of organ manifestations as well as the Birmingham vasculitis activity score (BVAS)¹⁰ in active CSS; as shown in figure 2, serum IgG4 strongly correlated with both the number of organ manifestations ($r=0.52$, $p=0.01$) and the BVAS ($r=0.64$, $p=0.001$). Likewise, the IgG4/IgG ratio in active CSS also correlated with the number of organ manifestations ($r=0.44$, $p=0.034$) and the BVAS ($r=0.59$, $p=0.002$). The five factor score (FFS) is a well-established prognostic score in CSS, based on the presence of heart, gastrointestinal and central nervous system involvement, proteinuria greater than 1 g/24 h and creatinine greater than 140 μ mol/l.¹¹ Interestingly, the IgG4/IgG ratio was higher in active CSS patients with a FFS of 1 or greater compared with a FFS of 0 ($p=0.046$); IgG4 serum levels were also higher in the former group, but the difference was not statistically significant ($p=0.053$). There was no difference in the

IgG4/IgG ratio and IgG4 serum levels between antineutrophil cytoplasmic antibody (ANCA)-positive and ANCA-negative CSS patients (data not shown). IgE levels did not correlate with IgG4 levels ($r=0.23$, $p=0.270$); also, they did not correlate with the number of organ manifestations ($r=-0.122$, $p=0.571$) or the BVAS ($r=-0.72$, $p=0.738$), nor did they differ in patients with a FFS of 1 or greater versus a FFS of 0 ($p=0.450$). We also measured IgG4 serum levels longitudinally and the IgG4/IgG ratio in 12 CSS patients at the time of active disease and during disease remission; notably, not only IgG4 levels but also the IgG4/IgG ratio significantly dropped during remission ($p=3\times 10^{-5}$ and $p=6\times 10^{-4}$, respectively; figure 2).

We next investigated differences in clinical characteristics between patients with high (>1350 mg/l) and normal IgG4 levels (see supplementary table S2, available online only). There were no statistically significant differences between the two groups.

Finally, we evaluated the degree of IgG4-bearing plasma cell infiltration in tissue biopsies obtained from the upper airway tract of nine active CSS patients. All biopsies showed moderate to severe lymphoplasmacellular inflammation (grade 2 in five cases and grade 3 in four). Most biopsies also revealed moderate to severe tissue eosinophilia (grade 1 in two cases, grade 2 in three and grade 3 in four). Therefore, they were considered to be representative of CSS-related chronic rhinosinusitis. The mean IgG4/IgG-positive plasma cell ratio was $34.1\pm 9.1\%$. Three of the nine examined biopsies revealed an IgG4/IgG-positive plasma cell infiltration exceeding 30%, a cut-off ratio commonly used to define 'IgG4-related systemic disorders'.¹² Representative images are shown in supplementary figure S1 (available online only).

Next, we compared the degree of IgG4-positive plasma cell infiltration of CSS biopsies with that of ENT biopsies taken from active GPA patients and patients with chronic rhinosinusitis with or without eosinophilia. No statistically significant difference was found in IgG4/IgG-positive plasma cell ratios between CSS and GPA cases ($p=0.278$) and between CSS and chronic sinusitis

without eosinophilia ($p=0.112$), whereas this ratio was lower in CSS than in chronic sinusitis with eosinophilia ($p=0.056$) (see supplementary figure S2, available online only).

DISCUSSION

Humoral immunity may be involved in the pathogenesis of CSS. The efficacy of B-cell depletion with rituximab in refractory CSS cases^{13 14} supports the view that B cells and, possibly, humoral responses, are instrumental in CSS. In this study, we found highly elevated serum IgG4 levels in CSS patients with active disease, thus extending preliminary findings from a recent small case series.¹⁵ We also showed that the increased IgG4 production is much more pronounced in CSS than in other diseases with vasculitic or allergic features such as GPA or atopic asthma. Previous reports showed that IgG4 are increased in GPA compared with healthy controls.¹⁶ This was also confirmed by our study; nevertheless, the difference between CSS and GPA was highly significant.

Serum IgG4 levels paralleled the disease course as they normalised during remission; notably, the IgG4/IgG ratio also dropped during remission, thus indicating that the IgG4 decrease was due to the overall decrease in IgG levels, and that this subclass quite specifically reflected disease activity. Serum IgG4 levels also correlated with the number of organ manifestations and the BVAS, a well-established disease activity score in vasculitis. In addition, they tended to be higher in patients with an adverse prognosis, as assessed using the FFS. ANCA, which are more frequent in CSS patients with 'vasculitic' complications,¹⁷ were detectable in both IgG4-normal and IgG4-high patients. Probably due to the relatively small sample size, we were unable to detect differences in clinical manifestations between IgG4-normal and IgG4-high CSS patients. In-situ analysis of IgG4 immune responses in tissue biopsies from active CSS patients showed marked IgG4⁺ plasma cell infiltration in only a few cases, suggesting that a systemic rather than a local perturbation in IgG4 responses occurs in CSS.

Whether IgG4 antibodies play any role apart from being a potential biomarker in CSS is unclear. Compared with the other IgG subclasses, IgG4 has a negligible ability to activate the classic complement pathway, and its binding to Fcγ receptors is much lower than that of IgG1. IgG4 antibodies are thought to be blocking antibodies neutralising antigens. Nevertheless, they do play a pathogenic role in some autoimmune conditions (eg, pemphigus vulgaris), or in the protective response against parasitic infections.¹⁸ The shift towards IgG4 production seems to be related to the inflammatory milieu conditioning B-cell maturation. Th2 responses certainly favour IgG4 production, as typical Th2 cytokines such as IL-4, IL-5 and IL-13 promote IgG4 switching. However, also the immunoregulatory IL-10 and the Th1-linked IL-12 contribute to such responses.¹⁸

A new entity called IgG4-related disease has recently been established.⁸ The disorders included in this entity display elevated serum IgG4 levels, tissue infiltration by IgG4-bearing plasma cells and eosinophils, obliterative phlebitis and marked fibrosis.¹⁹ Could CSS thus be considered a form of IgG4-related disease? Indeed, a few of our cases had a strong local IgG4 production in tissue biopsies, and serum IgG4 levels were elevated in active patients. Also, allergic symptoms, eosinophil infiltration and phlebitis are features of both diseases. However, as an increased tissue IgG4 production may occur in diverse chronic inflammatory diseases (eg, rheumatoid arthritis), its specificity

remains questionable.²⁰ Certainly, the possibility that IgG4-related diseases and CSS have common immunopathogenetic mechanisms has to be considered.

In conclusion, an increased IgG4 production is found in active CSS. Serum IgG4 levels correlate with the extent of organ involvement and with disease activity. Although a Th2-dominated inflammatory milieu may account for the enhanced IgG4 production, further studies are needed to elucidate the mechanisms underlying such immune response and to clarify the potential pathogenetic role of IgG4 in CSS.

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Competing interests None.

Ethics approval This study was conducted with the approval of the University of Erlangen and the University of Parma.

Patient consent Obtained.

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