Circulating cytokines and soluble CD23, CD26 and CD30 in ANCA-associated vasculitides

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Abstract

Objective

To assess circulating immunoregulatory cytokines and soluble surface markers of T and B cell activation in the plasma of patients with Wegener's granulomatosis (WG), Churg-Strauss syndrome (CSS) and microscopic polyangiitis (MPA) during active and inactive diseas, in order to establish their value in discriminating between disease entities and as markers of disease activity.

Methods

Plasma levels of IL-4, IL-5, IL-10, IL-12, IL-13, IFN-γ and soluble CD23, CD26 and CD30 were determined by enzyme-linked immunosorbent assay in patients with WG (n = 21), CSS (n = 19) and MPA (n = 14) during active disease and remission.

Results

Concerning cytokines, no differences were observed for IFN-γ, IL-4, IL-5 and IL-13. Plasma levels of IL-12 were decreased in all subgroups of patients. On the contrary, IL-10 levels were significantly elevated only in patients with CSS. Levels of sCD30 were significantly increased in patients with active generalized WG and CSS, but not in those with MPA and localized WG, correlating with the disease extent and activity. sCD26 levels were markedly decreased in patients with generalized WG, CSS and MPA and increased towards remission. sCD23 levels were slightly, but not significantly increased in CSS and generalized WG.

Conclusion

Regarding the investigated immunoregulatory cytokines (Th1/Th2 type), only the measurement of plasma levels of IL-10 discriminated CSS from WG and MPA. The reported data could indicate a similar status of T cell activation in generalized WG and CSS, and possibly a shift in peripheral immunity towards a more humoral dominated immune response. The differences observed between patients with the localized and generalized forms of WG seem to reflect the clinically known biphasic course of this disease.

Key words

Vasculitis, ANCA cytokine, soluble CD26, soluble CD30.

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Introduction

There is increasing evidence for the importance of T cells in the pathogenesis of systemic vasculitides, such as Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg-Strauss syndrome (CSS), which are characterized by the presence of antineutrophil cytoplasmic antibodies (ANCA) and therefore are referred to as ANCA-associated vasculitides (AAV) (1). For example, increased levels of T cells in bronchoalveolar lavage, activated CD4+ and CD8+ peripheral blood cells, high plasma levels of soluble markers of T cell activation, such as soluble IL-2 receptor, reflect the involvement of cell-mediated immune responses in WG (2, 3). However, studies of other AAV, i.e. CSS and MPA, are scarce (4). Therefore measurement of circulating cytokines and soluble surface markers seems to be helpful in distinguishing the predominance of cell-mediated (Th1) versus humoral responses (Th2) in AAV and to define similarities and differences between the different forms of AAV.

In the regulation of T and B cells a complex network of immunoregulatory cytokines is involved, polarizing the immune responses towards cell-mediated (Th1-type cytokines: interferon-γ (IFN-γ) and IL-12) or humoral immune responses (Th2-type cytokines: IL-4, IL-5, IL-10, and IL-13).

In vivo detection of the expression of CD30, a member of the tumor necrosis factor receptor family, is thought to be an excellent surrogate marker for the identification IL-4 responsiveness (5). Elevated levels of the soluble form of CD30 (sCD30) have been reported in various diseases with a proposed Th2-type cytokine pattern, like ulcerative colitis, systemic lupus erythematosus (SLE), generalized WG, and in rheumatoid arthritis (RA) (6-9).

The surface molecule CD26, exhibiting dipeptidyl peptidase IV enzyme activity (DPPIV), is known as the T cell activation antigen with co-stimulatory activity. Recently, it was concluded that CD26 up-regulation may be suggestive of a new cellular pathway of Th1-like immune reactions (10-12). Although soluble CD26 (sCD26) is not a breakdown product of the membrane form, it exposes significant structural similarity and functional properties and the release of sCD26 correlates with cell surface expression (13).

The expression of the low-affinity receptor for IgE (CD23) on lymphocytes can be modulated by cytokines, e.g. it is up-regulated by IL-4 and down-regulated by IFN-γ (14). Increased plasma levels of soluble CD23 (sCD23) have been reported in allergic disorders, acute viral diseases, lepromatous leprosy, B-CLL, and inflammatory diseases like RA, SLE and sarcoidosis (15-20).

It was the aim of this study to measure levels of regulatory cytokines (Th1-type: IFN-γ, IL-12; Th2-type: IL-4, IL-5, IL-10, IL-13) and soluble surface molecules of T and B cell activation (sCD23, sCD-26, sCD30) in the plasma of patients with AAV to assess their value in discriminating between different forms of AAV, their use as markers for following disease activity and, if possible, to establish links with pathogenetic implications.

Materials and methods

Patients and controls

Plasma samples from 58 patients with different forms of ANCA-associated vasculitides during active disease or in remission were investigated (CSS: 9 males, 10 females, 1 cANCA/ PR3-ANCA+ve, 1 pANCA/MPO-ANCA+ve; MPA: 6 males, 8 females, 9 pANCA+ve/ 7 MPO-ANCA+ve; generalized WG 7 males, 8 females, 14 cANCA+ve /13 PR3-ANCA+ve, 1 pANCA+ve; localized WG: 2 males, 4 females, 1 cANCA+ve, 1 pANCA+ve). All patients fulfilled the criteria for classification as described by the American College of Rheumatology (ACR) in 1990 (21) and the 1992 Chapel Hill Consensus Conference definitions (22). At the time of investigation patients were treated as follows: CSS with steroids 91%, cyclophosphamide 27%, methotrexate 67%, or interferon 27%; MPA with steroids 81%, cyclophosphamide 38%, or methotrexate/cyclosporine/azathioprine 43%; generalized WG with steroids 53%, cyclophosphamide 23%, or methotrexate/azathioprine/leflunomide 40%); and localized WG with steroids 25%, methotrexate 17%, trimethoprim/sulfamethoxazole 67%). Six patients were studied during the first mani-

Table I. Clinical and serological characteristics of patients with CSS, MPA and WG in active disease vs. remission.

		Active (median, range; *mean ± SEM)	Remission (median, range; *mean ± SEM)
CSS	Age (years)	46 (26-65)	44 (28-62)
	Disease duration (months)	14 (0-224)	28 (2-211)
	DEI	5 (2-13)	2 (0-4)
	ESR (mm/hr)*	20 ± 5	14 ± 3
	CRP (mg/dl)*	1.5 ± 0.7	< 0.5
	Leukocytes (10^3/ml)*	10.1 ± 1.3	6.9 ± 0,5
MPA	Age (years)	55 (39-66)	59 (40-77)
	Disease duration (months)	46 (1-110)	41 (3-107)
	DEI	4 (2-6)	2 (0-5)
	ESR (mm/h)*	47 ± 10	24 ± 5
	CRP (mg/dl)*	3.0 ± 1.9	0.6 ± 0.2
	Leukocytes (10^3/ml)*	8.6 ± 0.9	8.0 ± 0.4
Localized WG	Age (years)	51 (29-75)	60 (28-75)
	Disease duration (months)	22 (1-100)	55 (5-93)
	DEI	2 (2)	2 (0-2)
	ESR (mm/h)*	42 ± 14	23 ± 5
	CRP (mg/dl)*	1.0 ± 0.4	0.4 ± 0.2
	Leukocytes (10^3/ml)*	7.3 ± 0.7	6.4 ± 0.4
Generalized WG	Age (years)	64 (28-74)	65 (29-74)
	Disease duration (months)	18 (0-233)	38 (5-236)
	DEI	(2-13)	0 (0-4)
	ESR (mm/h)*	51 ± 7	34 ± 7
	CRP (mg/dl)*	3.4 ± 1.0	2.0 ± 1.0
	Leukocytes (10^3/ml)*	7.1 ± 0.7	6.1 ± 0.7
	ANCA (median)	1 : 256	1 : 32

festation of active disease without any prior immunosuppressive treatment (2 with CSS and 4 with generalized WG), further 7 patients after initiating immunosuppressive therapy within 4 months after establishing the diagnosis (2 with CSS, 1 with MPA, 2 with localized WG, and 2 with generalized WG). Fifteen healthy volunteers (7 males, 8 females, mean age 36 years, range 20 - 50 years) were enrolled as controls (Table I).

Assessment of disease extent and activity

All patients were examined by a team of specialized clinicians who assessed disease activity at each visit. Remission was defined as the improvement or arrest of disease progression (partial remission) or the absence of clinical, serologic and radiologic evidence of disease activity (complete remission). Disease extent was determined using a disease extent index (DEI), as previously described (23). Briefly, organ involvement was recorded as follows: upper respiratory tract (E), lung (L), kidney (K), rheumatologic symptoms (A), inflammatory eye involvement (Ey), peripheral nervous system (P), central nervous system (C), heart (H), gastrointestinal tract (Gi), skin (S) and constitutional symptoms (B). Each organ involved counted for 2 points and constitutional symptoms for 1 point; the maximum possible score was 21. The distribution of organ involvement in patients with active disease or in remission are given in Figure 1.

Plasma samples

Plasma samples were collected into EDTA Vacutainer tubes. All of the samples were aliquoted and stored at -20°C until used.

Cytokine assays

Plasma IL-12 and IFN-γ levels were measured with specific ELISA kits (DuoSet, Genzyme Corporation, Cambridge, USA). Levels of IL-4 were determined using a highly sensitive ELISA kit (Quantikine HS Human IL-4, R&D Systems, Wiesbaden, Germany). IL-5, IL-10 and IL-13 levels were measured by sandwich ELISA using 2 monoclonal antibodies (PharMingen, San Diego, USA) and alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch, dianova, Hamburg, Germany). The sensitivities of the various assays were as follows: IFN-γ 16 pg/ml; IL-4 0.25 pg/ml; IL-5, IL-10 and IL-13 3 pg/ml; IL-12, 13 pg/ml.

Soluble surface molecules

Levels of sCD23, sCD26 and soluble interleukin-2 receptor (sIL-2R) were measured by commercially available enzyme immunoassays (The Binding Site, Birmingham, UK; Bender MedSystems, Vienna, Austria; Milennia, Hermann Biermann GmbH, Germany). The detection limits were 0.78 µg/l for sCD23, 11 ng/ml for sCD26 and 16 U/ml for sIL-2R. sCD30 levels were determined by radioimmunoassay as previously described using the monoclonal antibody Ki-2 for detection (8).

Laboratory parameters

Determination of ANCA was performed by indirect immunofluorescence according to the recommendations of the European ANCA study group (24). All samples were also studied by antigen-specific ELISA for the presence of autoantibodies against proteinase 3 and myeloperoxidase, and other target antigens. The erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood count (WBC) were determined by routine laboratory procedures.

Statistical analysis

Analysis of significance was performed using Wilcoxon's rank test for paired data and the Mann-Whitney U test for non-paired data. Relationships between clinical variables and levels of soluble markers were evaluated by determining the Pearson correlation coefficient, and in cases of comparison with DEI by determining the Spearman's rank correlation coefficient. A p value < 0.05 was considered to be significant.

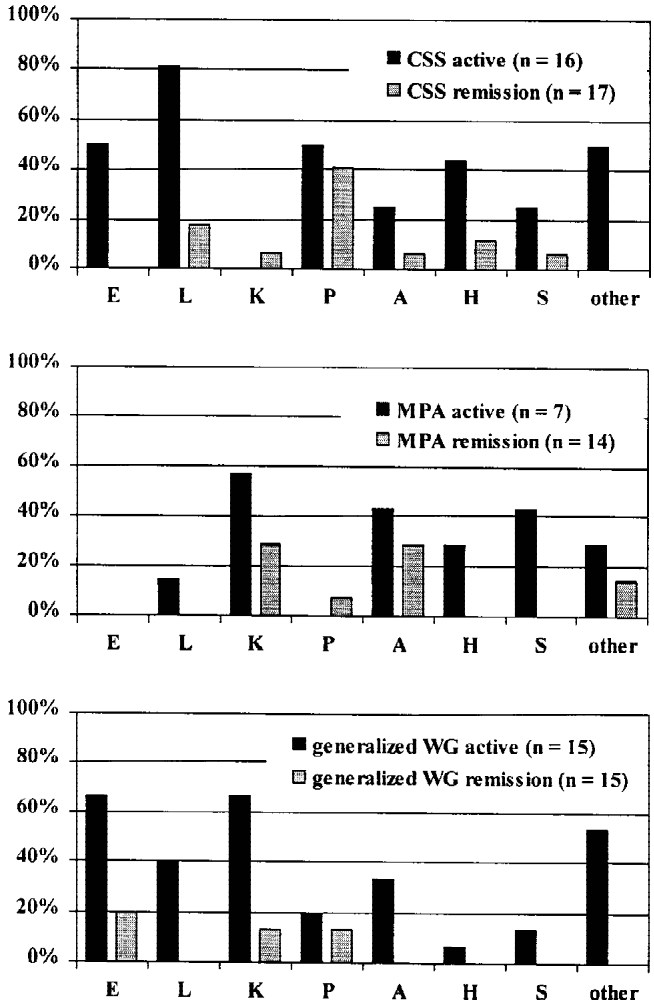


Fig. 1. Organ involvement of patients with Churg-Strauss syndrome (CSS), microscopic polyangiitis (MPA) and generalized Wegener's granulomatosis (WG) at time of investigation recorded as follows: **E** - upper respiratory tract, **L** - lung, **K** - kidney, **P** - peripheral nervous system, **A** - rheumatologic symptoms, **H** - heart, **S** - skin, **other** - comprising inflammatory eye involvement, involvement of central nervous system, gastrointestinal tract, and/or constitutional symptoms.

Results

Plasma levels of Th1-type cytokines

Only low levels of plasma IFN-γ were measured, no differences between the patient subgroups and healthy controls could be observed. Plasma levels of IL-12 were reduced in all patients in both active phases of disease and during remission (median 366 - 381 pg/ml) as compared to healthy controls (median 907 pg/ml, $p < 0.05$), although IL-12 values tended to be higher in active CSS (median 604 pg/ml).

Plasma levels of Th2-type cytokines

IL-4 was not detected in plasma samples from either patients and healthy controls. Elevated IL-5 levels were detected rarely in all subgroups with a maximum of 30% of patients with active CSS. Plasma levels of IL-13 were not significantly different regarding all groups of patients as compared to healthy controls. IL-10 plasma levels were significantly elevated in patients with CSS during active disease (median 35; range < 3 - 639 pg/ml, $p < 0.05$) and remission (median 16; range < 3 - 350 pg/ml, $p < 0.05$) as compared to healthy controls (median 3; range < 3 - 19 pg/ml). IL-10 levels were not significantly increased in patients with WG or MPA.

Levels of soluble surface molecules

Plasma levels of sCD23 were only slightly higher in patients with active generalized WG (median 3.5 µg/l) and active CSS (median 3.4 µg/l) than in controls (median 2.7 µg/l) or in remission (median 1.8), but without reaching statistical significance. No differences were observed in patients with MPA or localized WG (Table II).

Levels of plasma sCD26 (Table II) were inversely correlated with disease activity in generalized WG and CSS, i.e. sCD-26 levels were significantly decreased in active generalized WG (median 258 ng/ml, $p < 0.05$) and active CSS (median 188 ng/ml, $p < 0.05$) as compared to healthy controls (median 411 ng/ml) and increased during remission (generalized WG: median 295; CSS: median 257 ng/ml). Patients with MPA showed a similar reduction of sCD26 in both active disease (median 283 ng/ml, $p < 0.05$) and remission (median 228 ng/ml, $p < 0.05$). In contrast patients with localized WG had higher levels of sCD26 (median 316 ng/ml) than patients with active generalized WG and CSS, although these values were lower than in healthy controls (Fig. 2).

Plasma levels of sCD30 were significantly increased in patients with active generalized WG (median 8 U/ml, $p < 0.05$) as compared to controls (median 5 U/ml) and decreased during remission (median 7 U/ml, $p < 0.05$). sCD30 levels were also increased in active CSS (median 8.5 U/ml) in comparison to controls ($p < 0.05$) and remission (median 7 U/ml). In active MPA (median 6 U/ml) and in remission (median 5.5 U/ml) as well as in localized WG (median 4 U/ml) sCD30 plasma levels did not differ significantly from healthy controls.

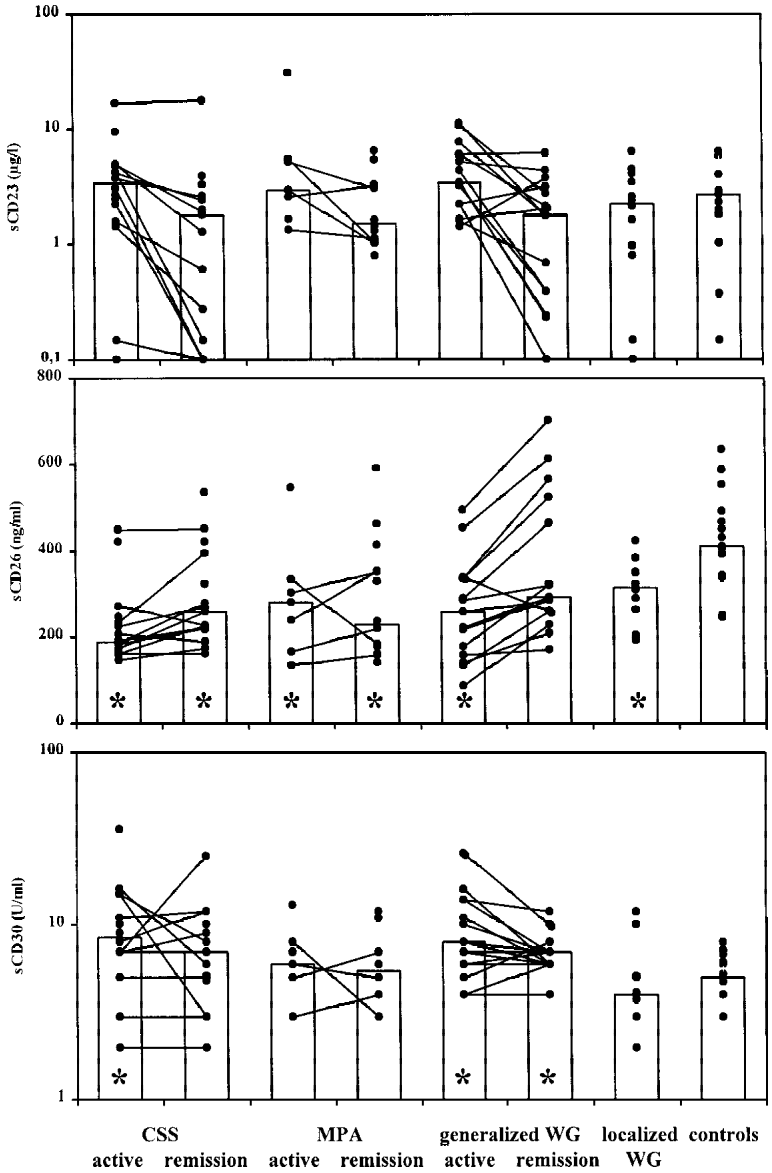
Correlations with parameters of disease extent and activity.

Taken together, in all patients with generalized WG, CSS and MPA the levels of plasma sCD30 correlated moderately with plasma sCD23 levels ($r = 0.33$, $p < 0.01$), DEI ($r = 0.36$, $p < 0.01$) and sIL-2R plasma levels ($r = 0.64$, $p < 0.001$). Considering patients with generalized WG alone sCD30 plasma levels correlated with sCD23 ($r = 0.43$, $p < 0.02$), ESR ($r = 0.61$, $p < 0.001$), CRP ($r = 0.50$, $p < 0.01$) and sIL-2R ($r = 0.56$, $p < 0.01$). Only among patients with CSS did sCD23 correlate significantly with IL-10 levels ($r = 0.63$, $p < 0.01$) and marginally with sCD30 ($r = 0.34$, $p = 0.05$). Significant positive correlations were observed between plasma values of sCD30

Table II. Plasma levels of soluble surface molecules and the cytokine IL-10.

	n	sCD30 (U/ml)	sCD26 (ng/ml)	sCD23 (µg/l)	IL-10 (pg/ml)
Controls	15	5 (3-8)	411 (246-634)	2.7 (< 0.8-6.4)	3 (< 3-19)
CSS					
- active	16	8.5 (2 - 36)[a]	188 (146 - 447)[a,b]	3.4 (< 0.8 - 16.7)	35 (< 3 - 639)[a]
- remission	17	7 (2 - 25)	257 (161 - 536)[a]	1.8 (< 0.8 - 17.9)	16 (< 3 - 350)[a]
MPA					
- active	7	6 (3-13)	283 (136-544)[a]	2.9 (1.3 - 30.5)	18 (< 3 - 51)
- remission	14	5.5 (3-12)	228 (142-591)[a]	1.5 (0.8 - 6.4)	7 (<3 - 145)
Generalized WG					
- active	15	8 (4 - 26)[a,b]	258 (86-494)[a,b]	3.5 (1.4 - 11.3)	6 (< 3 - 229)
- remission	15	7 (4-12)[a]	295 (171-700)	1.8 (< 0.8 - 6.3)	9 (< 3 - 63)
Localized WG	6	4 (2-12)	316 (192-423)[a]	2.3 (< 0.8 - 6.4)	12 (< 3 - 283)

Values are the median and range for sCD30, sCD26, sCD23 and IL-10.
[a] indicates a p value < 0.05 for comparison to healthy controls using Mann-Whitney U test for non-paired data; [b] indicates a p value < 0.05 for comparison of active disease and remission using Wilcoxon's rank test for paired data (CSS: 12 pairs; MPA: 5 pairs; generalized WG: 15 pairs). n: number of patients.



and sIL-2R levels (r = 0.73, p < 0.001). No correlation was seen with regard to the use of different immunosuppressive drugs.

Discussion

It was the aim of this study to analyse circulating immunoregulatory cytokines and soluble surface markers of T and B cell activation in patients with AAV to assess their value in discriminating between different forms of AAV and as marker for disease extent or activity.

Measurement of cytokines in plasma revealed no significant differences for IL-4 and IFN-γ (both undetectable or barely detectable) and levels of IL-13 and IL-5 between patients and controls. However, the plasma levels of cytokines may be affected by soluble cytokine receptors or the presence of corresponding autoantibodies and therefore are difficult to interpret. On the other hand, plasma levels of IL-12, known as Th1-type cytokine, were downregulated in all patients as compared to healthy controls and did

Fig. 2. Plasma levels of soluble surface molecules in patients with Churg-Strauss syndrome (CSS), microscopic polyangiitis (MPA) and Wegener's granulomatosis (WG) as compared to healthy controls. Bars represent median of patients with active disease or remission in each group. Values obtained from active disease and remission of the same patient are connected with thin lines.
*indicates a p value < 0.05 in comparison to healthy controls using the Mann-Whitney U test for non-paired data.

not discriminate between the different patient groups, although patients with active CSS tended to have higher IL-12 levels. On the contrary, plasma levels of IL-10, a Th2-type cytokine with both immunostimulatory and anti-inflammatory effects, were elevated only in patients with CSS, indicating a special role for this cytokine in the pathogenesis of CSS. The pathogenic role of IL-10 has been suggested in animal models of SLE, and elevated plasma levels correlating with disease activity have also been reported in inflammatory diseases like RA, systemic sclerosis and SLE (25-27).

Patients with CSS showed a similar elevation of plasma sCD30 during active disease and a tendency of normalization during remission as that in patients with generalized WG, whereas these levels did not differ significantly from controls in patients with MPA. The results demonstrating increased plasma levels of sCD30 in generalized WG, but not in localized WG confirm our previous findings (8). Increased levels of sCD30 have been reported in several diseases associated with presumed Th2-like immune responses, such as SLE, systemic sclerosis and ulcerative colitis (6, 9), suggesting a similar immune response in generalized WG and CSS, where sCD30 correlated with disease activity and markers of T cell activation. Although some authors have proposed that the CD30 molecule could be expressed by T cells producing both IL-4 and IFN-γ (28), recent studies have clearly shown that CD30 expression by activated T cells reflects IL-4 responsiveness at the single T cell level (5). Therefore, our data could indicate an *in vivo* involvement of Th2 or Th0 rather than Th1 cells in active generalized WG and CSS. However, recently a predominance of the Th1-type pattern in granulomatous tissue of patients with WG has been shown (29).

This study also demonstrates decreased levels of sCD26 in active generalized WG and CSS, which increase during remission, and in MPA, while sCD26 levels in localized WG are slightly higher than those from the other disease groups investigated. With the exception of a marginal negative correlation of sCD26 with WBC, no correlation with serological markers of disease activity or ANCA titers was observed. Similar data have been reported from studies of major depressed patients, where a significantly lower activity of DPPIV in peripheral blood was observed, but without any relationship to immunoinflammatory markers, such as sIL-2R (30). Recently it has been suggested from studies in tuberculoid leprosy, known as Th-1-like disease, that high expression of CD26 might be suggestive of a Th1-like immune reaction (11, 12, 31). However, the relevance and pathophysiological role of sCD26 in AAV remain to be established.

A slight, but not significant, increase of sCD23 levels was observed in patients with active generalized WG and CSS, but not in localized WG and MPA. In both patients with generalized WG and CSS, sCD23 correlated marginally with sCD30. It has been proposed that sCD23 is helpful in determining the overall balance of cellular and humoral immunity due to its modulation by cytokines (14, 17). However, the data concerning sCD23 do not allow a conclusive discrimination. Moreover, as sCD23 might be downregulated during therapy with corticosteroids (18), an influence of the administered therapy can not be excluded, also no differences were observed between patients receiving corticosteroids or not.

In conclusion, regarding the immunoregulatory cytokines studied here (Th1/Th2-type), only the measurement of plasma levels of IL-10 discriminated CSS from WG and MPA. The determination of soluble surface markers of T and B cell activation demonstrated similar results in patients with generalized WG and CSS, i.e. elevated levels of sCD30, correlating with markers of disease activity, as earlier described, a slight but not significant increase of sCD23 and lower levels of sCD26 during active disease. These data are partially similar to other reports of diseases with proposed Th2-like immune reactions, such as SLE, and therefore could indicate a similar status of T cell activation in generalized WG and CSS, and possibly a shift in peripheral immunity towards a more humoral dominated immune response. The differences observed between patients with the localized and generalized form of WG seem to reflect the clinically known biphasic course of this disease. Although patients with MPA exhibited a similar decrease in sCD26, the results revealed no difference regarding sCD30 or sCD23. Therefore, an arrangement according to the Th1/Th2 scheme is not possible. However, the relationship between increased sCD30 and decreased sCD26 levels and their specific role in the pathophysiology of AAV remain to be elucidated.

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