Review

Update on the pathogenesis of Churg-Strauss syndrome

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ABSTRACT

Churg-Strauss syndrome (CSS) is a rare form of systemic vasculitis occurring in patients with asthma. The cause of CSS is unknown, and yet little data are available regarding its pathogenesis. The presence of a marked tissue- and blood-eosinophilia, as well as secretory products of eosinophils in blood and tissues, implicates a pathogenetic role of eosinophil granulocytes. Prolonged survival of eosinophils due to inhibition of CD95-mediated apoptosis by soluble CD95 seems to contribute to eosinophilia in CSS. Although the mechanisms involved in eosinophil-activation in CSS have not been elucidated, recent data suggest a possible role of T lymphocytes secreting eosinophil-activating cytokines. This review describes the current insights into the pathogenesis of CSS in the light of its putative nature as a type 2 granulomatous disease. Recent clinical, experimental and epidemiologic data regarding the possible role of inflammatory cells and their secretory products, anti neutrophil cytoplasm antibodies (ANCA), epidemiologic factors and anti-asthma treatments are summarized.

Introduction

In 1951, the pathologists Jacob Churg and Lotte Strauss identified 13 patients with disseminated necrotizing vasculitis previously diagnosed as "Periarteriitis nodosa" occurring in patients with severe asthma and hypereosinophilia (1). In autopsies and tissue biopsies Churg and Strauss found tissue eosinophilia, necrotising vasculitis and extravascular granuloma to be the three distinctive features distinguishing their patients from other patients with "periarteriitis nodosa" without asthma (1). Churg and Strauss suggested that these cases represent a different entity which they termed "allergic granulomatosis and angiitis". Later, this syndrome was denominated "Churg-Strauss Syndrome" (CSS) to describe the development of vasculitis in patients with eosinophilia and asthma (2, 3). CSS is a very rare disease with an annual incidence of no more than 1 to 3.4 per million per year in Western Europe (4-8).

Clinical and laboratory diagnosis

Vasculitis of small and sometimes medium-sized vessels, blood- and/or tissue eosinophilia and asthma are the key features of CSS. In most patients, CSS develops in three stages. The first step is the development of asthma which is often accompanied by sinusitis and sometimes blood eosinophilia (3,9,10). In the second phase, tissue eosinophilia develops which can present in form of pulmonary infiltrates or eosinophilic infiltration of the gastrointestinal mucosa. At this stage, the clinical picture is indistinguishable from other hypereosinophilic disorders like chronic or acute eosinophilic pneumonia, eosinophilic gastroenteritis or the idiopathic hypereosinophilic syndrome. The third phase of disease is characterized by the onset of signs or symptoms attributable to vasculitis and usually develops several years after the onset of asthma (11). Only at this stage a diagnosis of CSS is possible. Histological confirmation of vasculitis is desirable whenever possible in order to distinguish CSS from other hypereosinophilic disorders (9). Biopsies should be taken a sites of suspected vasculitis which are easy accessible (like the ENT-tract, skin or sural nerve in case of mononeuritis multiplex). Beside asthma and eosinophilia, the most common clinical signs of CSS are fleeting pulmonary infiltrates, peripheral neuropathy, purpura, gastrointestinal and cardiac involvement (Table II) (2, 3,10, 12-15).

For the interpretation of studies regarding the pathophysiology of CSS, the definitions used to identify patients

with CSS in the particular studies are crucial. In the past, several attempts were made to develop definitions for CSS in order to facilitate diagnosis and to distinguish this entity from other forms of vasculitis (Table I) (1,3,16, 17). The initial definition from Churg and Strauss resulted from an examination of autopsy material obtained from untreated patients in the pre-glucocorticoid-aera, thus reflecting the most severe form of the disease (1).

However, in patients diagnosed according to the newer definitions, the three histological hallmarks of CSS described by Churg and Strauss (necrotizing vasculitis, extravascular accumulation of eosinophils and extravascular granuloma) are only rarely found together in one biopsy sample (3). Furthermore, in only 50% of cases with a clinical diagnosis of CSS it is possible to verify vasculitis bioptically (3,14,15). In these cases, surrogate parameters of vasculitis (e.g. microhaematuria with blood casts and glomerular proteinuria) or typical clinical findings suggestive of vasculitis (e.g. mononeuritis multiplex) can substitute for a histological confirmation, as proposed by Lanham *et al*. (3). However, as these criteria developed by Lanham require the simultaneous presence of asthma, eosinophilia and vasculitis in two or more extrapulmonary organs (3), a number of patients, especially in early phases of the disease, may be missed as: i) in a few cases asthma occurs not before but after the onset of vasculitis, ii) blood eosinophilia may be blunted by pretreatment with glukocorticoids in some cases, and iii) clinically overt vasculitis may be detectable in one organ system only.

Therefore, today the classification criteria developed by the American College of Rheumatology (ACR) (16) are generally regarded as the best approach to identify patients with CSS, as they include a spectrum of common clinical features of the disease, but also cover individual patients in which typical features like asthma are absent or not clinically overt at the time of presentation. However, these criteria can only be applied to patients with an already established diagnosis of vasculitis, as they were developed for the purpose of classification of vasculitides (16). In fact, these criteria perform poorly in terms of sensitivity and specificity when used as primary diagnostic criteria (18). The same limitations apply to the definition proposed by the Chapel Hill consensus conference (18), which require the presence of typical histological findings which are absent in a large number of patients.

Table I. Criteria for diagnosis or classification of Churg-Strauss syndrome.

	Churg & Strauss (1951)*	Lanham *et al.* (1984)*	ACR (1990) +¶	Chapel Hill (1994)*
Blood eosinophilia	+	+	+	+
Asthma	+	+	+	+
Vasculitis	+	+$	+¶	+
Granuloma (biopsy)	+		+	
Tissue Eosinophilia	+		+	+§
Fibrinoid necrosis	+			
Neuropathy			+	
Pulmonary infiltrates			+	
Parnasal sinus abnormality			+	

* all features must be present; + at least 4 features must be present; ¶ classification criteria for patients with an already established diagnosis of vasculitis; $ extrapulmonary, at least two organs; § respiratory tract.

Table II. Clinical manifestations of Churg-Strauss syndrome [data from [4, 7, 8)].

Organ system	Clinical findings	Frequency (%)
Respiratory	Asthma	96 - 100
	Pulmonary infiltrates	62 - 77
	Sinusitis	60
	Pleural effusions	29
	Alveolar hemorrhage	3
Peripheral nervous system	Mononeuritis multiplex, polyneuropathy	75
Central nervous system	Cerebral ischemia, epilepsia	8
	Cranial nerve dysfunction	4
Skin	Palpable purpura	31
	Skin nodules	19
	Urticaria	8
Musculoskeletal	Arthralgia	40
	Myalgia	54
	Non-erosive arthritis	15
Cardiovascular	Pericardial effusions	23
	Myocarditis, coronary vasculitis	22 - 26
Gastrointestinal	Abdominal pain	17 - 44
	Bloody diarrhoea	
Renal	Glomerulonephritis	20 - 47

Antineutrophil cytoplasm antibodies and their association with CSS

ANCA (antineutrophil cytoplasm antibodies) are reported to be present in about 10-80% of patients with CSS (11, 19, 20). Due to the relative rarity of CSS ("orphan disease") many different reports are based on a small numbers of patients and various studies used different diagnostic criteria for CSS. This together with different methods applied for ANCA detection in the respective investigations may contribute to these variable results concerning the prevalence of ANCA in CSS. Eustace and colleagues showed that in 82 CSS patients (summarized from 4 studies) tested for ANCA 52% (!) were nega-

tive; 23 patients were P-ANCA positive and 12 patients C-ANCA positive in the indirect immunofluorescence test (20). In the P-ANCA positive patients the antibody was in most cases directed against myeloperoxidase (MPO-ANCA) (20).

Recently, our group performed a prospective study on sera of 75 patients with CSS (19). Screening of ANCA was done using different methods: indirect immunofluorescence technique, direct and capture ELISAs for detection PR3 and MPO-ANCA, direct ELISAs for common antigens such as elastase, lactoferrin, cathepsin G, bactericidal/permeabilty increasing protein, and immunoblotting. In total 10 patients (13.3%) with CSS were ANCA-positive at one point in their disease, regardless of the antigen and the method applied for screening (20). Compared with the literature, we found a lower association of ANCA with CSS. Our results do not support the notion that ANCA is of immunodiagnostic value in CSS (20). Summarizing these data, we conclude that the clinical value of ANCA and its possible pathogenic role in CSS is questionable. This in contrast to WG or MPA, were ANCAis reported to be present in high percentage and seems to play an important role in induction of vasculitis (21).

Epidemiologic factors and the role of anti-asthma treatments

Antagonists against the cysteinyl-leukotriene-receptor such as zafirlukast, pranlukast or montelukast are widely used drugs in patients with asthma which often reduce the severity of asthmatic symptoms and allow a taper of systemic glukocorticoids and other asthma treatments. Since 1998, a growing number of reports described the development of CSS in patients with asthma who were being treated with any of the three leukotriene receptor antagonists (11, 22, 23). Therefore, some authors have suggested that in some asthma patients CSS might be caused by an "idiosyncratic or hypersensitivity reaction" to this class of drugs. Today, vasculitis and eosinophilia are listed as possible adverse events in the product labels of zafirlukast and montelukast (8). However, the fact that development of CSS has also been reported in association with other drugs of different classes commonly used in patients with asthma such as fluticasone dipropionate, zileuton, budenoside, macrolide antibiotics or carbamazepine (11, 23) makes a class-effect unlikely.

Thus, during a workshop of the US National Institute of Health held in 2001, members of the US Food and Drug Administration investigated a total of 165 cases of possible CSS (two or more ACR classification criteria positive), which were reported in association with anti-asthma treatments (23). Among these 165 cases, 83 were reported in association with zafirlukast (52 of these patients received also fluticasone), 63 with montelukast (41 of these patients received also fluticasone), 12 with fluticasone (2 with concomitant zafirlukast and/or montelukast), five with the 5-lipoxygenase inhibitor zileuton (2 with concomitant fluticasone) and two with salmoterol alone (23). Of all 126 patients, 88% developed CSS while the dose of systemic glukocorticoids was reduced, while the other 12% developed CSS with no or very distant use of glucocorticoids. It was concluded that CSS developed in association with a variety of structurally distinct agents and no single drug or one class of drug was identified as a causative factor (23).

Given the low incidence of CSS of 1 to 6.8 cases per million per year (64 per million per year in patients with asthma) (4, 8) and the high frequency of usage of the above mentioned drugs in patients with asthma, the FDA panel considered these antiasthmatic medications as "generally safe" (23). In view of the high percentage of CSS cases developing during taper of glukocorticoids, it has been hypothesised that an incipient CSS was unmasked in response to steroid use (11). Cases in which clinical sings of CSS were suppressed by glukocorticoids and became overt after reduction of the glukocorticoid dose have also been coined "formes frustes" of CSS (24). It is not unlikely that e.g. blockade of the leukotriene receptor reduces local airway inflammation, and thus signs and symptoms of asthma, but has no steroid-sparing properties towards the underlying inflammatory processes of CSS (e.g. eosinophilia, lymphocyte activation).

In fact, little is known about the immune regulatory properties of these drugs not related to blockade of the leukotriene receptor. For example, MK-571, a prototypical cysteinly leukotriene antagonist, inhibits specific organic anion transporters, like the multi-drug resistance-associated protein (25). These anion channels are differentially expressed on T cells with a Th1 and Th2 cytokine pattern (26), which might somehow interfere with the modulations of the immune systems operative in CSS, which are characterized by a Th1 and Th2 cytokine profile with Th2 predominance (27), as listed above in more detail. For instance, these ion channels were shown to alter inflammatory reactions and interfere with the migration of dendritic cells (28). Thus, in view of these uncertainties regarding the effect of certain anti-asthma drugs on some immune regulatory immune-responses, an effect on the development of CSS in a subset of patients with asthma cannot yet be fully excluded (23).

Pathogenesis of CSS

Pathologic features

Blood- and/or tissue eosinophilia are two of the defining feature of CSS which distinguish CSS from other types of small vessels vasculitis. Although a certain degree of blood eosinophilia can occur in individual patients with Wegener's granulomatosis (29), demonstration of tissue eosinophilia in a patient with small vessel vasculitis makes a diagnosis of CSS very likely, especially in patients with a history of asthma (10). In the absence of vasculitis, tissue infiltration with eosinophils in certain organs like lung or gut can hardly be distinguished from the histological picture seen in chronic eosinophilic pneumonia, eosinophilic gastroenteritis or the idiopathic hypereosinophilic syndrome.

Granulomas with central tissue necrosis and the presence of surrounding giant cells, as described by Churg and

Strauss (1), are seen only in a minority of tissue samples from patients with CSS (3,11,14). In a series from the early 1980s, Lanham found granulomas only in 40% of the cases studied (3). In our experience, granuloma formation is even rarer, possibly related to the fact that today most patients receive glukocorticoid treatment for their asthma before they develop CSS. The third histological feature of CSS, vasculitis, is characterized by an infiltration of eosinophils in the vessel walls of small and medium-sized vessels. Immunohistochemical analysis of tissue samples usually demonstrates the absence of larger amounts of immune complexes. Thus, CSS, along with WG and MPA, has been classified in the group of the so-called "Pauci-immune" vasculitides (30-32) which are distinguished from other types of small vessel vasculitis that are characterized by the presence of immunecomplexes (e.g. Schoenlein Henoch purpura, cryoglobulinemic vasculitis).

CSS: Lack of a causative antigen

The causative factors leading to the development of CSS are unknown. Most likely, in each stage of the disease, asthma, eosinophilia and vasculitis, a cascade of events is required to induce a progression to the next higher stage. Like in asthma or other forms of atopic disease, the first event in the pathogenesis of CSS is an allergic inflammatory response after exposure of the respiratory tract to an inhaled antigen (33). During progression to the stage of vasculitis, high serum levels of IgE were found which normalize during disease remission, implicating a role of allergic inflammation also for the vasculitic phase of the disease (34). The fact that development of CSS has been observed after vaccination, hyposensitation or administration of drugs (3,10,14) suggests that antigenic stimuli in subjects with chronic atopic disease can trigger the onset of vasculitis and that these events involve an increased production and activation of eosinophils.

Causative factors of eosinophilia

The histological finding of severe infiltration of tissues and blood vessel walls by eosinophils and the fact, that disease activity is closely linked to eosinophil blood counts, suggests that eosinophils play a central role in the pathogenesis of CSS.

Mild eosinophilia can be found in about 1% of patients with asthma, especially in subjects with a history of atopy (8). However, results of a recent large epidemiological study showed that severe eosinophilia (eosinophil blood counts >10%) is rare in patients with asthma and was found only in 12 of 36,230 asthma patients in that study

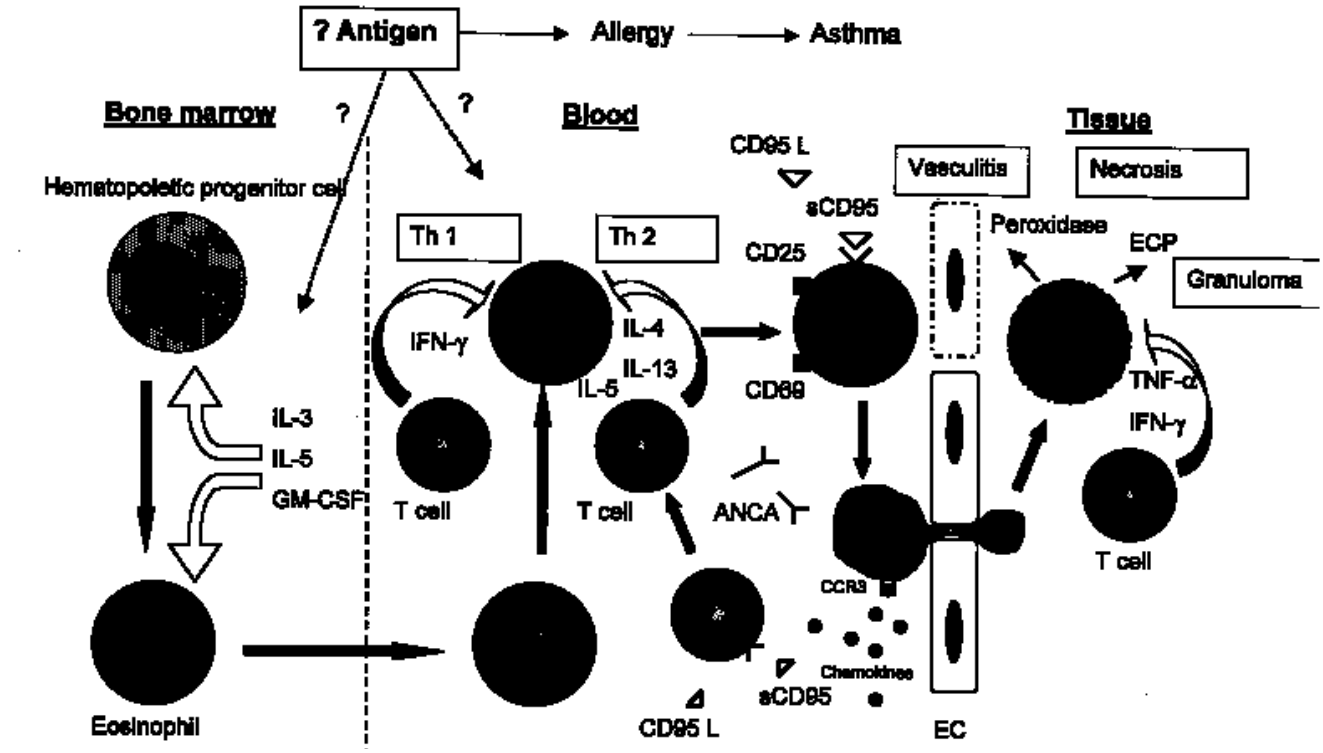

Fig. 1. Schematic concept of mechanisms involved in the pathogenesis of Churg Strauss Syndrome.
In the bone marrow, as yet unknown antigenic stimuli induce an increased development from pluripotential stem cells over eosinophil/basophil progenitor colony forming units to mature eosinophils. This process is regulated by three cytokines, IL-3, IL-5 and GM-CSF. In the circulation the mature eosinophils are stimulated by activated T cells which secrete increased amounts of Th1- (IFN-γ) and Th2-cytokines (IL-4, IL-13). Soluble CD95 (Fas) competes with the natural ligand (CD95L) for the binding to CD95 expressed on both T cells and eosinophils. This prevents aopotosis, prolongs the survival of eosinophils and T cells and results in the formation of clonal T cell expansions. Activated eosinophils which express CD69 and CD25 invade the endothelium and tissue. Degranulation of eosinophils results in a release of peroxidase and ECP which finally cause tissue damage and necrosis in tissue and blood vessels. This process is stimulated by the release of proinflammatory cytokines by activated T cells which are part of the inflammatory infiltrate. Possibly, antineutrophil cytoplasm antibodies (ANCA) participate in neutrophil activation in a subset of patients.

(8). These epidemiological data show a low prevalence of severe eosinophilia in asthma patients and given the high prevalence of eosinophilia in patients with CSS these observations suggest, that the development of CSS in asthma patients is closely related to the development of eosinophilia. Thus, factors leading to eosinophilia are likely to contribute to the pathogenesis of CSS.

Three haematopoietic cytokines, interleukin-3 (IL-3), interleukin-5 (IL-5) and granulocyte-macrophage colony-stimulating factor (GM-CSF), regulate the maturation, production and release of eosinophils in the bone marrow (35, 36). Elevated serum levels of IL-5 were found in patients with CSS, while simultaneous elevations of IL-3 and GM-CSF serum levels were reported in three of the five patients tested in a small series of patients (37). In our own series of 33 patients with CSS, IL-5 serum levels were increased in only 30% of patients with active disease (38). Interestingly, data from our own laboratory also show that polyclonal T cell lines from patients with CSS do not produce large amounts of IL-5 upon stimulation (27), suggesting that the increased IL-5 levels in some CSS patients are rather derived from local production than from a systemic release by T cells in the circulation.

Recent data suggest, that a prolonged survival of eosinophils due to prevention of apoptotic cell death is major contributory factor for chronic eosinophilia in CSS (39). Since IL-5 is an effective inhibitor of eosinophil apoptosis which is not detectable in large amounts in healthy subjects, the increased release of IL-5 in a subset of CSS patients (38) may be partially responsible for the inhibition of apoptosis. Furthermore, it was also shown that sera from patients with CSS contain high levels of soluble CD95 (39). The CD95/CD95 ligand system is one of the major pathways regulating apoptotic cell death. The soluble form of CD95 competes with CD95 for the binding to the natural ligand on eosinophils, CD95L (39). Thus, in CSS the presence of soluble CD95 may prevent the induction of eosinophil apoptosis via this pathway, even in the absence of other survival factors like IL-5 or IL-3. It was shown, that in CSS soluble CD-95 is released by mononuclear blood cells, especially T cells, pointing again to a regulatory role of lymphocytes in the pathogenesis of CSS (39). In view of the published data, one may speculate that the overexpression of soluble CD95 might then equally favor survival and proliferation of eosinophils and self-reactive T cells.

Activation and recruitment of eosinophils at the site of inflammation

In CSS, blood eosinophil counts do not necessarily reflect the degree of tissue eosinophilia. It was found that eosinophilia in the bronchoalveolar fluid can persist in patients with CSS despite treatment with glukocorticoids, while blood eosinophil counts are suppressed effectively (40). Thus, additional local factors must be responsible for the recruitment and local accumulation of eosinophils in CSS. Till to date, there are only few data on the functional characteristics and the activation of eosinophils in CSS. Recruitment of inflammatory cells may be modulated by four distinct single nucleotide polymorphisms of the CD18 gene which have been associated with anti-MPO-ANCA-positive vasculitis (41, 42).

Eosinophils obtained from the peripheral blood of patients with CSS express CD25, CD69 and CD4 on their surface (37), which suggests that these cells are activated as CD69 is expressed by eosinophils only upon activation by cytokine like IL-3, IL-5 or GM-CSF (43). In addition, eosinophils of a subset of patients is characterized also by surface expression of CD54 and HLA-DR (37). While CD4, CD54 and HLA-DR are involved in antigen-presentation and degranulation of eosinophils, the biological functions of CD25 and CD69 on eosinophils are not fully elucidated.

In addition to these surface markers, elevated serum levels of eosinophilic cationic protein (ECP) in active CSS indicate activation of eosinophils (44). ECP is a constituent of the specific granule content of eosinophils and is released upon cellular activation along with other granule constituents. High levels of ECP were found in tissue (45) and bronchoalveolar lavage fluid (40) of CSS patients, demonstrating activation of eosinophils not only systemically, but especially at the site of inflammation. Activated eosinophils secrete granule components like eosinophil major basic protein (MBP) and eosinophil derived neurotoxin (EDN), which are characterized by the capability to induce damage of tissues and endothelial cells. Immunofluorescene studies of skin biopsy specimens from CSS patients showed deposition of EDN and, to a lesser extent, of MBP (46). Extracellular deposition of MBP, one of the major constituents of eosinophil granules, was found in infracted areas of small bowel tissue from a patients with CSS (47). In contrast, no large amounts of MBP were found in nerve biopsies of CSS patients (48).

Elevated levels of peroxidase were found in BAL fluid from some CSS patients (40). Activated eosinophils are likely to be the major source of peroxidase in CSS, as beside eosinophils only neutrophils, which usually are present only in low numbers in BALfluid from CSS patients, are capable to secrete peroxidase. Nevertheless, in few patients with highly active CSS an activation of neutrophils occurs also, as shown by increased amounts of myeloperoxidase (MPO) in the BAL fluid of these patients (40). As raised MPO levels were found in patients with both elevated and normal BAL neutrophil counts (40), this MPO is likely to be derived from neutrophil degranulation in the tissue than from cells present on epithelial surfaces. In CSS, endothelial cell damage caused by peroxidase and other granule components is reflected by an increased level of thrombomodulin (44), which is released by endothelial cells after exposure to H_2O_2 or granulocyte proteases like cathepsin G,leukocyte elastase. While the above data give some initial insights about the local activation of eosinophils, little is known about factors mediating the initial steps of vasculitis like endothelial cell adhesion, chemoattraction and migration of eosinophils in CSS.

Some data indicate that activated T cells induce an activation of eosinophils not only systemically but also

locally at the site of inflammation. An infiltration of CD4+ and CD8+ T cells has been observed in histochemical studies of neuronal biopsy specimens obtained from patients with CSS (12). Only few data are available about the *in situ* cytokine secretion in CSS. In skin biopsy samples from two patients with CSS, Fujioka *et al*. confirmed mRNA expression of both Th1 (INF-γ, IL-12) and Th2 cytokines (IL-6, IL-10) (49), like shown in blood T cell clones from CSS patients on the protein level (27). It has been hypothesised that the increased INF-γ production supports the formation of the eosinophilic granuloma, one of the histological hallmarks of CSS (49). Elevated serum levels of soluble CD26, a T cell activation antigen with co-stimulatory activity have been demonstrated in patients with CSS(38), suggesting a role of-costimulatory molecules in leukocyte-stimulation in CSS. Chemokine receptor usage by eosinophils has generated considerable interest, because eosinophils are selectively recruited to certain inflammatory sites, and receptor antagonists may be useful for blocking eosinophil entry and degranulation. Many chemokines have been reported to act on human eosinophils: RANTES, MCP-2, MCP-3, eotaxin, etc. Furthermore, this array of ligands sugested a complex pattern of receptor expression on eosinophils. CCR3, the eotaxin receptor, has been identified as a major chemokine receptor on eosinophils. Antibody blockade of CCR3 inhibited eosinophilic response to helminth infection in a murine parasite model (50). Future studies attempt to determine the role of chemokines and their receptors in eosinphil chemotaxis in CSS, that could be attractive targets for therapy.

Cytokines and lymphocyte activation

The three clinical stages of CSS, asthma, tissue eosinophilia and vascular inflammation develop over time and yet unknown factors need to be present to cause a progression of the disease. As seen in other disorders associated with hypereosinophilia, like chronic hypereosinophilic pneumonia, hypereosinophilic gastroenteritis and idiopathic hypereosinophilic syndrome, blood- and tissue eosinophilia alone does not cause vasculitis (35). Thus, in CSS additional mechanisms need to be operative which trigger the diapedesis of leukocytes into the vessel walls, induce an activation of inflammatory cells and thereby contribute to the development of vasculitis.

Like in other types of vasculitis, cytokines secreted by activated T lymphocytes orchestrate the immune response in CSS. In patients with CSS and active disease Schmitt *et al*. found increased levels of soluble interleukin-2 receptor (sIL-2R), suggesting an activation of proinflammatory cells (44). Eosinophils activated by IL-3, IL-5 or GM-CSF show an increased cell surface expression of the IL-2R p55 subunit (51), which could be a possible source of increased sIL-2R levels in CSS. However, the majority of sIL-2R is generated by activated T cells and, to a lesser degree by activated B cells, suggesting a pathogenetic role of lymphocytes in CSS (44).

Clonal expansions of T cell have been shown in patients with CSS (27,39). These T cell clones show a preferential usage of a single gene from the Vβ21 family. In addition, these T cell clones possibly have similar T cell receptor (TCR) specificities as sequence analysis of clonal T cell expansions in CSS revealed two recurrent motifs of their TCR-β−VDJ junction (39). Thus, it has been suggested, that expanding T cell clones in CSS share the recognition of only a limited number of antigens (39), a hypothesis which has not yet been confirmed in clinical or epidemiological studies. Müschen *et al*. demonstrated in eight patients with CSS that such clonal T cell expansions were associated with an increased surface expression of CD95 and high serum levels of soluble CD95 (39). As has been shown for eosinophils, high levels of soluble CD-95 might protect CD95-positive T cells clones from apoptosis and thus favour the survival and clonal expansion of these T cells.

T cells contribute to the pathogenesis of autoimmune disorders by the secretion of proinflammatory cytokines. In a subset of CSS patients with active disease an increased activity of proinflammatory cytokines like tumor-necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) has been reported (37,52). Increased serum levels of TNF-α were found by Grau *et al.* in 18 of 33 patients (52), a finding which was confirmed by Tsukadaira *et al*. in 5 of 5 CSS patients (37). In these studies, elevated serum levels of IL-1β were found in 12 of 33 cases (52) and 3 of 5 cases (37), respectively. The importance of TNF-α in the pathogenesis of CSS is further emphazised by the good clinical response to inhibitors of TNF-α given to patients with CSS being refractory to standard treatment (53).

However, recent data suggest that not a TH1, but a TH2 cytokine response predominates in CSS. *In vivo* activated T cells from patients with CSS consist mostly of CD4+ T cells (27). In contrast, the majority of T cell lines from patients with WG are CD8+ (27). Compared to T-cells from healthy probands, T cell lines from CSS patients produce large amounts of interferon-γ (IFN-γ), but even more interleukin-4 (IL-4) and interleukin-13 (IL-13) (27). These data indicate that T cell lines in CSS are characterized by both a Th1 (IFN-γ) and a Th2 (IL-4, IL-5, IL-13) response. However, quantitatively the Th2 cytokine pattern predominates in CSS (27). Thus, there is a substantial difference in the cytokine profile in CSS compared to WG where a Th1 cytokine pattern predominates. Elevated serum levels of sCD30, a member of the tumor necrosis factor receptor family, have been found in CSS patients with active disease (38). Given the fact, that CD30 expression by activated T cells reflects IL-4 responsiveness at the single T cell level (54), the increased sCD30 levels support the concept of a primarily Th2 driven process in CSS. The following section will focus on the possible consequences of this TH2 response for the formation of eosinophil-rich granuloma, one of the histological and clinical hallmarks of CSS.

CSS: A type 2 granulomatous inflammation?

At this stage it is perhaps useful to introduce the concept of type 1 and type 2 granulomas, as it has been developed

in mice with model antigens such as PPD of *Mycobacterium bovis* (TH1-induced type 1 granulomas) or soluble *Schistosoma mansoni* egg antigens (TH2-induced type 2 granulomas) (55). This theoretical framework may prove helpful in stratifying the few data available on the pathogenesis of CSS, because within this framework, predictions and experimental models may be developed that should allow progress from the analysis of immunological phenomena to cause-and-effect relationships.

Granulomas are generally defined as focal accumulations of predominantly mononuclear cells in response to a chronic antigenic stimulus. Histopathologically, most granulomas are characterized by aggregates of epithelioid macrophages. However, depending on the eliciting agent, neutrophil and eosinophil granulocytes are also present to varying degrees. In contrast to foreign body granulomas, which occur T cell-independently and are characterized by little cellular turnover, T cell-mediated (DTH-type) granulomas are highly dynamic, and continuous recruitment of inflammatory cells is maintained by T cell-derived cytokines and T cell-induced chemokines.

It is the nature of the cytokine environment present during initial antigen encounter which ultimately determines the differentiation of the CD4+ effector cell subtype: the presence of IL-12 and IFNγ will promote the development of TH1 cells, capable of secreting IL-2, IFNγ and lymphotoxin (LT-)α, whereas the predominance of IL-4 and IL-10 will favor the differentiation of TH2 cells which preferentially produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 (56). Since the quality and quantity of the cytokine profile depends, to a large extent, on signalling via toll-like receptors, it is the nature of the antigen engaging toll-like receptors that determines the bias of the TH response (57). TH1 cells secrete copious quantities of TNF and IFNγ capable of attracting additional monocytes and T cells into the lesions, mostly by increasing the binding of these cells to inflamed endothelium (58). The essential role of TNF and IFNγ for granuloma initiation and maintenance has been amply demonstrated in many models of infection in which neutralization of TNF and IFNγ effectively abrogates granuloma formation, or converts it to a TH2 pattern with increased eosinophil infiltration and fibrotic degeneration (59-62).

TH2 cells orchestrate the development of a different kind of granuloma. IL-4, which is secreted in large amounts by T cells in CSS, leads to a significant increase in cellularity, size, procollagen type III expression, and accumulation of eosinophils (even in pre-existing TH1 granulomas). In addition, endothelial cells may become activated by IL-4 and secrete CCL7 which also induces the recruitment of eosinophils (63). Possibly, this interaction of IL-4 with endothelial cells contribute to the development of vascular inflammation in CSS. IL-4, in the presence of TNF, can induce CCL2 and CCR2 expression in fibroblasts. CCL2 is involved in fibrosis through the regulation of profibrotic cytokines, e.g. TGFγ, and the generation and deposition of a collagen matrix (64). Generally speaking, CCL2, CCL7, and CCL8 are more involved in type 2 than type 1 inflammation (65, 66). Likewise, CCL11 (eotaxin) and CCR3 (eotaxin receptor) levels are higher in TH2 than TH1 granulomas. CCR8 (the receptor for CCL1, CCL4, and CCL17 also found predominantly in type 2 granulomas) is exclusively expressed on TH2 cells and contributes to a functional TH2 granuloma, since, in its absence, there is impaired IL-5 production and eosinophil recruitment (67). Whereas NOS-2 dominates TH1 immune reactions, arginase is predominantly expressed in TH2 responses and is responsible for fibrosis in type 2 granulomas. Arginase production is mediated by IL-13 and regulated by substrate depletion through TH1-induced NOS-2 activity.

Evidently, there is extensive opportunity for cross-regulation between these two pathways of granuloma formation. For instance, TH2 cells may – via the secretion of IL-10, IL-4 and IL-13 - effectively diminish a TH1 response by downregulating IL-12, IFNγ, TNF and chemokine production in TH1 cells (68). In contrast, the type 1 cytokine IL-12 dramatically reduces type 2 lesion size, primarily curtailing eosinophil recruitment (69). This possibly explains the therapeutic efficacy of IFN-γ in CSS which shifts the cytokine profile towards a Th1 pattern (70). In the absence of IL-4, type 1 inflammation is exacerbated, but an established TH2 granuloma will not convert to a TH1 granuloma, since persistent expression of IL-13, IL-5 and CCL7 can compensate for the defect (69). CCL5 promotes the TH1 response and mediates cross-regulatory inhibition of type 2 granulomas (71). However, chemokines not only influence granuloma formation through a direct effect on leukocyte chemotaxis, but also through altering the TH1/TH2 cytokine balance. For instance, CCL2 promotes IL-4 production by T cells and inhibits IFNγ production by TH1 cells (64) and CCL5 downregulates IL-4, IL-5, IL-10 and IL-13 production by TH2 cells (71). If these chemokines involved in the formation of type 2 granuloma play a role in the pathogenesis of CSS has not yet been studied.

Conclusion

In summary, both the predominance of a TH2 cytokine response and the formation of eosinophil-rich granuloma are suggestive of a type 2 granulomatous inflammation in CSS. Further studies are needed to characterize the cells, cytokines and chemokines which are operative in inflamed lesions in CSS. A better understanding of the factors involved in the recruitment and activation of eosinophils and lymphocytes in situ may possibly lead to the identification of new therapeutic targets for this rare form of systemic vasculitis.

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