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Clonal CD8+ TCR-Vβ expanded populations with effector memory phenotype in Churg Strauss Syndrome

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Abstract Churg Strauss Syndrome (CSS) is a systemic vasculitis in which oligoclonal T cell expansions might be involved in the pathogenesis. Combined analysis of TCR-Vβ expression profile by flow cytometry and of TCR gene rearrangement by heteroduplex PCR was used to detect and characterize T cell expansions in 8 CSS patients, 10 asthmatics and 42 healthy subjects. In all CSS patients one or two Vβ families were expanded among CD8+ cells, with an effector memory phenotype apt to populate tissues and inflammatory sites. Heteroduplex PCR showed the presence of one or more clonal TCR rearrangements, which reveals monoclonal or oligoclonal T cells subpopulations. After purification with a Vβ specific monoclonal antibody, each CD8+/Vβ+ expanded family showed a single TCR rearrangement, clearly suggestive of monoclonality. All CD8+ expansions were detectable throughout the disease course. TCR-Vβ expanded or deleted populations were not observed in asthmatic patients. Clonal CD8+/Vβ+ T cell expansions might be useful as a disease marker.
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Introduction

Churg Strauss Syndrome (CSS) is characterized by pulmonary and systemic small-vessel necrotizing vasculitis, vascular and extravascular granulomas, eosinophilia and tissue infiltration by eosinophils, occurring in patients with asthma and often allergic chronic rhinosinusitis with nasal polyps [1]. A central pathogenic role for T lymphocytes in vasculitides is suggested by different observations. First, T cells are commonly present in biopsies of active lesions, both vasculitic and granulomatous [2,3]. Second, T cell lines from CSS patients, generated *in vitro* by polyclonal stimulation, are polarized towards a Th2 phenotype (IL-4 and IL-13), which may explain the eosinophilia, the hallmark of the disease. The same cells have also been shown to release significant amounts of IFNγ [4], which may be related to the granuloma formation. Third, immunosuppressive therapy targeting lymphocytes is useful in CSS patients, strengthening the concept of CSS as a T cell driven disease [5]. Moreover, anti myeloperoxidase (MPO) autoantibodies (p-ANCA) may play a pathogenic role in a subgroup of patients (about 38%) [6,7] and T cells from p-ANCA positive patients have been shown to proliferate in response to MPO, suggesting their helper function in the production of autoantibodies [8]. Allergen desensitization [9], vaccination, and/or infections, resulting in lymphocyte activation, have been implicated in triggering some cases of CSS [10,11].

Lymphocyte activation by Superantigens (SAg) has been suggested to occur in Kawasaki disease, polyarteritis nodosa and microscopic polyangitiis [12,13]. SAg stimulation of T cells bearing specific Vβ T cell receptors (TCR) results in the polyclonal expansion of selected Vβ families in CD4+ and CD8+ cells, followed by T cell restricted deletion [14].

Antigen stimulation, on the other hand, may cause oligoclonal T cell expansions, that have been actually found in CSS patients by immunoscope technique, but it is not known whether these expansions are CD4+ or CD8+ [15]. Flow cytometry analysis, using antibodies to conventional TCR β-chain variable region families (TCR-Vβ), can be used for assessment of T cell expansions, even if sensitivity of this technique for detection of clonality is lower than analysis of TCR rearrangement by molecular biology [16]. One advantage of flow cytometry is that TCR-Vβ antibodies can be combined to other T cell markers, allowing a detailed analysis of such expansions in terms of CD4 and CD8 subsets and also of naive-memory differentiation [17–19]. It would be particularly important to know the immunophenotype of expanded clonal T cell population, since persistent clonal expansions of CD8+ memory T cells have been shown to populate inflammatory sites [20,21].

The aim of the study was to confirm the presence of clonal T cell expansions in peripheral blood samples from 8 CSS patients and to characterize them by flow cytometry and by molecular analysis of TCR rearrangement [22,23].

Materials and methods

Subjects

Eight patients with proved CSS diagnosed according to criteria of the American College of Rheumatology (ACR) [24], were included in the study. All patients had asthma, hypereosinophilia, rhinosinusitis and clinical manifestations consistent with systemic vasculitis, with ($n=4$) or without ($n=4$) histologic evidence [25]. P-ANCA antibodies were positive in 5/8 patients. Clinical data of the patients are summarized in Table 1. At the time of the study 6/8 patients were considered to be in remission, that is the absence of disease activity for at least 6 months according to Birmingham Vasculitis Activity Score (BVAS) item list [26] with the exception of asthma or neurologic and renal sequelae. They were all receiving low dose prednisone (<15 mg/die) for asthma. The remaining two patients (#7, #8) had active disease and were still receiving high dose corticosteroids. All eight patients were receiving regular inhaled therapy with corticosteroids combined to β2 agonists. No patient had laboratory evidence of CMV and EBV reactivation. In each patient blood samples were obtained in 2–6 occasions throughout 3–19 months of follow up.

Ten asthmatic patients (age range 25–70, M/F 5/5) served as pathological control group. They had severe ($n=7$) or moderate ($n=3$) asthma, with persistent rhinitis and/or chronic rhinosinusitis and mild eosinophilia (range 500–1300 cells/mm^3). All were receiving regular inhaled therapy with corticosteroids combined to β2 agonists and 7/10 patients were receiving low dose prednisone (<15 mg/die). Forty-two healthy subjects were used as normal controls (see below). All subjects gave their informed consent to participate to the study which was approved by local ethical committee.

Immunophenotypic analysis

Peripheral blood mononuclear cells (PBMC) were separated from whole blood by density gradient centrifugation (Lymphoprep,

Table 1 Demographical and clinical characteristics of patients with Churg Strauss Syndrome

Patient #	Sex	Age	p-ANCA	Eosinophils n/mm^3 (%)*	Involved organs	Treatment
1	M	59	–	14,060 (37)	PNS, gallbladder, ileum	CCS, CYC, AZA
2	F	55	–	22,230 (59)	PNS	CCS, AZA
3	M	57	+	6920 (30)	PNS, lung (DAH), kidney	CCS, CYC, MTX
4	M	67	+	9990 (37)	PNS, lung, kidney	CCS, CYC
5	M	55	+	15,630 (50)	PNS, skin, kidney	CCS, CYC
6	M	60	+	5500 (26)	PNS, skin	CCS, AZA, MTX
7	M	61	+	28,000 (70)	PNS, lung	CCS, CYC
8	F	85	–	17,000 (50)	PNS, heart, skin	CCS, MTX

*Values reported at diagnosis.
Abbreviations: peripheral nervous system PNS; diffuse alveolar hemorrhage DAH; Corticosteroids CCS; cyclophosfamide CYC; azathioprine AZA; methotrexate MTX.

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