Clinical Immunology (2013) 149, 65–72

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Vδ2 T cell deficiency in granulomatosis with polyangiitis (Wegener's granulomatosis)

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Received 21 November 2012; accepted with revision 9 June 2013
Available online 18 June 2013

KEYWORDS
Granulomatosis with polyangiitis;
γδ T cells;
Phosphoantigen;
Vδ2 T cells

Abstract Previous studies have characterized phenotypic and functional alterations within T-cell receptor αβ-expressing T cells in patients with granulomatosis with polyangiitis (GPA). We analyzed the frequency, subset composition and *in vitro* activation of blood γδ T cells in GPA patients. We observed a significant reduction of γδ T cell numbers, due to the selective depletion of the Vδ2 subset which remained consistent over time upon repeated analysis. The loss of Vδ2 T cells was not due to migration into the inflamed lesions as very few γδ T cells were detected in inflammatory infiltrates. The memory subset distribution did not differ among Vδ2 T cells from healthy donors and GPA patients. Importantly, the remaining Vδ2 T cells were capable of responding to phosphoantigen stimulation *in vitro*. The marked depletion of blood Vδ2 T cells in GPA suggests cellular exhaustion, possibly due to chronic exposure to and continuous overstimulation by microbial phosphoantigens.

1. Introduction

Granulomatosis with polyangiitis (GPA, previously Wegener's granulomatosis) is a severe chronic inflammatory disease of unknown etiology characterized by necrotizing granulomatous inflammation predominantly involving the upper and/or lower respiratory tract, and a systemic necrotizing vasculitis of small to medium-sized vessels [1]. The serine protease proteinase 3 (PR3) contained in azurophilic granules within neutrophils has been identified as the major GPA-associated autoantigen, and anti-neutrophil cytoplasmic autoantibodies against PR3 (PR3-ANCA) are strongly associated with GPA [2]. Clinical observations and data from *in vitro* and *in vivo* studies suggest a pathogenic role for ANCA in ANCA-associated vasculitides (AAV) [3]. Although the pathogenesis of GPA has not been clearly elucidated, there is clinical evidence for a role of

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1521-6616/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.clim.2013.06.003

sustained exposure to microbes such as *Staphylococcus aureus* (*S. aureus*). Thus, chronic nasal *S. aureus* carriage is associated with GPA relapses [3,4]. Barrier dysfunction of the mucosa may facilitate granulomatous inflammation predominantly affecting the upper and/or lower respiratory tract in GPA [5,6]. Recently, the pathogenicity of PR3-ANCA resulting in acute vascular pulmonary damage and glomerulonephritis has been demonstrated in animal models. However, necrotizing granulomatous inflammation was not observed in these models suggesting that the pathogenesis of granulomatous lesions predominantly found in the respiratory tract is separate from acute systemic vasculitis, and in particular seems to be T cell-driven in GPA [7–10]. In fact, multiple alterations within the T cell compartment have been reported in GPA, including the expansion of CD4 effector memory (T_{EM}) T cells during remission and their disappearance during active disease [11], the expansion of CD28-negative CD4 T cells [12], the appearance of IL-17 producing PR3-reactive T cells [13], or the functional deficiency of regulatory T cells (Treg) [14,15]. The aforementioned T cells all belong to the major population of T cells expressing the αβ T-cell receptor (TCR). A minor proportion (3–6%) of blood T cells, however, expresses the alternative γδ TCR. Within the γδ T cell compartment in the blood of healthy adult donors, most (50–95%) γδ T cells express a particular TCR composed of Vδ2 paired with Vγ9, whereas Vδ1 (paired with various Vγ elements) usually account for <20% [16]. Of particular interest is the fact that γδ T cells differ from αβ T cells with regard to the type of recognized antigens and the general lack of MHC restriction [17]. The ligands for Vδ2 T cells have been identified as non-peptidic and phosphorylated intermediates of the microbial and eukaryotic isoprenoid biosynthesis pathway [18,19]. The recognition of such phosphoantigens is restricted to human Vδ2Vγ9 T cells (there is no homologous TCR in mice) and does not require presentation by classical or non-classical MHC molecules [17]. Recently, the butyrophilin family member CD277 was shown to play a pivotal role in the phosphoantigen-dependent activation of human γδ T cells [20]. The prototype microbial phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) is produced by many bacteria including *S. aureus*, and is active at pico- to nanomolar concentrations. In contrast, the eukaryotic phosphoantigen isopentenyl pyrophosphate (IPP) requires 3-log higher (micromolar) concentrations which are not achieved in normal non-transformed cells but can be produced by transformed tumor cells [19] thus accounting for the central role of human Vδ2Vγ9 T cells in both anti-infective and anti-tumor immunity. Furthermore, Natural Killer Group 2 Member D (NKG2D), a receptor for stress-inducible MHC class I-related molecule including MICA/B, is expressed on most if not all γδ T cells and provides an activating signal [17]. In addition to cytokine production and cytolytic effector function, γδ T cells can also display regulatory activity (*i.e.*, down-regulate αβ T cell responses) and can exert antigen-presenting capacity [21,22].

Alterations in γδ T cell numbers, subset distribution, and/or function have been described in various autoimmune diseases [23]. As an example, an increase in Vδ1 γδ T cells was reported in patients suffering from Takayasu's arteritis [24], and alterations within the Vδ2 T cell subset distribution were observed in patients with Behçet's disease [25,26]. So far, however, no studies have been reported addressing the potential role of γδ T cells in GPA. The objective of the current study was to address the present dearth of information regarding the potential role of γδ T cells in GPA. Here we report a significant reduction of total γδ T cells in the blood of GPA patients, resulting from a selective deficiency of Vδ2 T cells that was found to be sustained over time when reassessed over a period of one to three years. Such a reduction of Vδ2 T cells would be in line with a possible chronic overexposure to phosphoantigens, potentially also derived from or induced by *S. aureus* [27].

2. Materials and methods

2.1. Blood samples

Heparinized blood was obtained from 43 healthy donors (HD), 44 GPA patients according to the ACR classification criteria and the CHCC definition [28,29] (22 males, 22 females, age range 27 to 77 years; see Table 1 for details), 10 patients with Churg–Strauss syndrome (CSS; 6 females, 4 males, age range 20 to 73 years), and 10 patients suffering from chronic sinusitis (CS; 4 females, 6 males; age range between 35 and 83 years). Blood samples were provided by the Klinikum Bad Bramstedt (GPA and CSS patients) or the Dept. of Otorhinolaryngology, UKSH Campus Kiel (CS patients). The blood of healthy donors was obtained from donors of the Institute of Immunology or was provided by the Dept. of Transfusion Medicine, UKSH Campus Kiel. The study was approved by the appropriate institutional clinical ethics board and informed consent was obtained from all donors.

2.2. Cell isolation

Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using the Ficoll-Hypaque density gradient centrifugation (Biochrom AG, Berlin, Germany). The purification of CD4 T cells was performed utilizing CD4 MicroBeads (Miltenyi, Bergisch Gladbach, Germany) following the manufacturer's protocol.

2.3. Flow cytometry

The following monoclonal antibodies (mAb) were used: PE-anti-CD3 (clone SK7), APC- or FITC-anti-pan-γδ TCR (clone 11F2), PE-anti-CD27 (clone M-T271; all from BD Biosciences, Heidelberg, Germany), FITC-anti-CD45-RA (clone HI 100) and PerCP-anti-Vδ2 (clone B6; both from Biolegend, Fell, Germany) and FITC-anti-Vδ1 (clone TS8.2; Thermo Scientific, Schwerte, Germany). All samples were measured on a FACSCalibur

Table 1 Clinical data of GPA patients.

Age range	27 to 77 years
Sex	22 males, 22 females
Organ involvement	Nasopharyngeal zone (43), lung (24), kidney (19), eye (17), nervous system (18), skin (12)
Clinical status	Generalized disease (18), localized disease (3), relapse/active disease (23)
Treatment	Glucocorticoids (37), methotrexate (21), azathioprine (11), others (12) [a]

[a] Including cyclophosphamide, leflunomide, certolizumab, and rituximab.