Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis

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Abstract

Background. The Th17 subset has been implicated in the pathogenesis of a number of autoimmune diseases. However, little is known about its role in anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV). We measured serum levels of IL-17A and associated upstream cytokines and the frequency of IL-17-producing autoantigen-specific T cells in patients with AAV.

Methods. ELISA on sera from acute (n = 28) and convalescent (n = 65) patients with AAV from Hammersmith Hospital was performed for IL-17A and the associated upstream cytokines IL-23, IL-6 and IL-1β, as well as the Th1 cytokine IFN-γ. ELISPOT was performed to measure autoantigen-specific recall T cell responses in convalescent patients and the frequency of IL-17- and IFN-γ-producing cells.

Results. Serum IL-17A and IL-23 levels were significantly elevated in acute AAV patients compared to healthy controls (P < 0.01 and P < 0.001, respectively), but importantly, remained elevated in a proportion of convalescent patients. By contrast, no significant differences in IFN-γ levels were detected between patient groups and controls. Patients with elevated levels of IL-23 compared to those with low IL-23 had more active disease as measured by Birmingham Vasculitis Activity Score (P < 0.05) and had higher ANCA titres (P < 0.05). Critically, immunosuppressive therapy did not always effectively suppress IL-23 or IL-17 production. Additionally, autoantigen-specific IL-17-producing, but not IFN-γ-producing, cells were significantly elevated in patients during disease convalescence compared to healthy controls.

Conclusions. These data implicate the Th17 axis and specifically IL-23 as mediators of more severe disease in AAV. Their persistence despite conventional treatment may contribute to high relapse rates.

Keywords: ANCA; cytokines; IL-17; IL-23; Th17; vasculitis

Introduction

Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) and Churg–Strauss syndrome (CSS) are idiopathic multi-system vasculitides affecting small-calibre blood vessels. They are characterized by the production of anti-neutrophil cytoplasm antibodies (ANCA), reactive to either proteinase-3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA), constituents of neutrophil granules and monocyte lysosomes. Over 70% of patients with ANCA-associated vasculitis (AAV) have renal involvement ranging from indolent disease to rapidly progressive glomerulonephritis and end-stage renal failure. Renal disease is the most important prognostic feature and renal failure constitutes the commonest cause of death after treatment-related infection. Despite advances in immunosuppression regimes, relapse rates in AAV remain high.
Although ANCA are thought to be pathogenic [1,2], T cells are also implicated in disease. Both PR3- and MPO-autoreactive T cells can be detected within peripheral blood mononuclear cells (PBMC) of patients [3] and a sub-population of T cells appears to be persistently activated, even during disease remission [4,5]. T lymphocytes and macrophages are found in renal lesions [6] and T cell-directed therapy can induce remission in refractory disease [7].

Recent studies in animal models and humans have identified a subset of T cells characterized by IL-17 production (Th17 cells), which are implicated as critical mediators of autoimmune disease. Th17 cells are a distinct lineage of CD4+ T cells [8] with a specific transcription factor ROR-γt [9], that are characterized by production of IL-17 [10], a cytokine with multiple inflammatory and haemopoietic effects [11], including release of pro-inflammatory cytokines (TNF-α, IL-6 and IL-8) [12], up-regulation of adhesion and MHC molecules and recruitment of monocytes and neutrophils—all changes typically found during the acute phase of AAV. Thus far, little is known about the role of IL-17 in AAV, although these pleiotropic pro-inflammatory functions make it an attractive candidate as a potential mediator of inflammation. Although IL-17 consists of a family of proteins, IL-17A–F, it is IL-17A that is found predominantly in a subset of T cells and has been implicated in autoimmunity. A number of studies have proposed a role for IL-17A (further referred to as IL-17) in the pathogenesis of certain experimental animal models and human immune-mediated diseases. IL-17-deficient mice are resistant to collagen-induced arthritis [13] and experimental autoimmune encephalomyelitis [14]. Most recently, IL-17- and IL-23-deficient mice have also demonstrated resistance in two mouse models of experimental glomerulonephritis [15,16].

In humans, IL-17 has been detected in the synovium of patients with rheumatoid arthritis [17] and the serum of those with systemic lupus erythematosus [18]. IL-17 has also been found to be over-expressed in inflammatory airways disease [19], transplant rejection [20], psoriasis [21], multiple sclerosis [22], inflammatory bowel disease [23] and systemic sclerosis [24].

In mice, differentiation of Th17 cells is driven by TGF-β together with IL-6 [25]. In humans, Th17 differentiation requires TGF-β [26] and is driven by IL-1 and enhanced by IL-6 and IL-23 [27,28], with IL-23 critically required for maintenance of the IL-17-secreting phenotype [29].

Therefore, to clarify whether the cytokine profile in patients with AAV was consistent with that of a Th17-mediated disease, we measured serum levels of IL-17A and IL-23, as well as the upstream cytokines IL-1β and IL-6, in acute and convalescent AAV patients and compared them to healthy and disease controls. Additionally, we measured serum levels of interferon (IFN)-γ reflecting the contribution of Th1 cells, which may inhibit the development of Th17 cells [8,30]. Secondly, in order to investigate whether there is an antigen-specific memory Th17 cell population in AAV, we stimulated PBMC from AAV patients and controls with MPO or PR3 (depending on patient ANCA specificity) and quantified the number of IL-17- and IFN-γ-producing cells by ELISPOT. To take account of the effect of immuno-suppression on T cell responses, we compared autoantigen-specific responses with those following stimulation with recall antigens and mitogen.

Materials and methods

Samples from 28 acute patients with de novo or relapsing disease were collected at presentation or within 10 days of presentation. Samples from 65 convalescent patients were collected during routine attendance at out-patient clinics. Serum or plasma samples were isolated immediately and stored at −80°C until required. Clinical diagnosis was in accordance with the Chapel Hill Consensus Criteria [31], and patients were classified as having WG, MPA or CSS. Renal-limited vasculitis was considered as part of MPA. Disease activity was assessed at the time of sample collection by calculating the Birmingham Vasculitis Activity Score (BVAS) 2003, as described previously [32,33]. Some historical acute patients had BVAS calculated retrospectively, while the rest had it collected prospectively. The BVAS 2003 score is reproducible and has been validated in clinical vasculitis trials to assess disease activity. Disease remission was based on clinical and biochemical parameters, as well as a BVAS 2003 of zero. Relapse was considered if there was a recurrence of symptoms/signs attributable to active vasculitis. It was classified as major if there was organ- or life-threatening disease and a significant change in therapy was instituted. Minor relapses did not involve organ- or life-threatening disease, and only dose adjustment of current immunosuppression. These criteria have been utilized in recent European vasculitis studies (see http://www.vasculitis.org/protocols).

The negative control group consisted of 28 healthy volunteers. Two positive control groups consisted of patients with sepsis or Goodpasture's (anti-GBM) disease. Sepsis patients were defined as having a systemic inflammatory response syndrome according to the consensus panel of 2001 [34] or a proven infective cause. All had CRP >100 mg/dl. The anti-GBM disease control group was a historical cohort with samples collected at the time of presentation.

Sample preparation

Blood was collected in ethylenediaminetetraacetic acid or plain tubes. Plasma or serum was isolated and stored at −80°C until analysis. The remainder was used for isolation of PBMC by Lymphoprep density centrifugation (Nycomed, Oslo, Norway).

Measurement of ANCA titres and specificity

ANCA positivity was determined by indirect immunofluorescence. ANCA antigenic specificity and titre were determined by Luminex-based technology, using the FIDIS vasculitis kit (Technoclone, Vienna, Austria), in the Hammersmith Hospital Immunology Laboratory.

ELISA

IL-1β and IL-6 were analysed using Duoset ELISA Development System (R&D Systems, Abingdon, UK), while IL-17A and IL-23 ELISA used mouse anti-human IL-17A and IL-23 p19 (R&D Systems, Abingdon, UK). MaxiSorp-IgM plates (Nunc, Roskilde, Denmark) were coated with monoclonal mouse anti-human IL-1β, IL-6, IL-17A or IL-23, blocked and then incubated with serum samples and standards in duplicate for a further 2 h. Biotinylated secondary antibodies were added for 2 h followed by streptavidin–horseradish peroxidase (Strep-HRP) and peroxide–chromogen substrate until colour development. INF-γ was assayed with human INF-γ ELISA Ready-SET-Go (eBioscience, Insight Biotechnology Ltd, UK) and performed following the manufacturer's instructions.

ELISPOT

ELISPOT plates (Millipore, Billerica, MA, USA) were coated with human anti-IFN-γ coating (Mabtech, Sweden) overnight. Plates were blocked and PBMCs (0.5 × 10⁶/well) were incubated with stimuli for 48 h. Cells were stimulated with medium alone; phytohaemagglutinin (PHA) (Sigma, MO, USA) at 1 μg/ml; diphtheria, tetanus and pertussis antigens (DTP) (marketed under Revaxis; Sanofi Pasteur MSD Limited, UK) 1:250 and 5 μg/ml autoantigen PR3.
Th17 axis in ANCA-associated vasculitis

**Table 1. Clinical and demographic characteristics of patients and controls**

<table>
<thead>
<tr>
<th>AAV patients</th>
<th>Patients (n)</th>
<th>Male</th>
<th>Female</th>
<th>Mean age (yrs)</th>
<th>WG</th>
<th>MPA</th>
<th>CSS</th>
<th>Limited disease</th>
<th>Systemic disease</th>
<th>ARF/HD</th>
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<tr>
<td>1. Acute PR3-ANCA/MPO-ANCA</td>
<td>28</td>
<td>16</td>
<td>12</td>
<td>55</td>
<td>17</td>
<td>11</td>
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<td>32</td>
<td>33</td>
<td>53</td>
<td>42</td>
<td>20</td>
<td>3</td>
<td>12</td>
<td>53</td>
<td>29</td>
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<tr>
<td>3. Prospective cohort</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>50</td>
<td>4</td>
<td>1</td>
<td>–</td>
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<td>5</td>
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<tr>
<td>Relapse</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>31</td>
<td>2</td>
<td>0</td>
<td>–</td>
<td>0</td>
<td>2</td>
<td>–</td>
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<tr>
<td>Time to relapse (months)</td>
<td>13, 15</td>
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<td>Controls</td>
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<td>Healthy controls</td>
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<td>Septic patients</td>
<td>9</td>
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<td>4</td>
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<tr>
<td>Anti-GBM + patients</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>43</td>
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**Statistical analysis**

All statistical analyses were performed using GraphPad prism 4.0 (GraphPad Software, San Diego, CA, USA). One-way analysis of variance was carried out using non-parametric Kruskal–Wallis test and Dunn’s multiple comparison post-test, and Mann–Whitney for ELISPOT analysis. Correlations were assessed using the non-parametric Spearman rank correlation analysis. Two-tailed analysis was carried out with significance defined as \( P < 0.05 \) with 95% confidence.

**Results**

The study received ethical approval from the Hammersmith, Queen Charlotte’s and Chelsea Hospitals research ethics committee. Twenty-eight acute and 65 convalescent patients with AAV were included. Some patients provided more than one sample during their disease follow-up. All patients were under follow-up in the multi-disciplinary vasculitis clinic at Hammersmith Hospital and fulfilled the Chapel Hill Conference criteria for disease [31]. All gave informed consent.

Mean age of patients was 54 years (range 19 to 84 years) and 61% were male. Patients were sub-divided according to ANCA specificity, clinical syndrome and extent of disease (limited or systemic) (Table 1). Overall, mean BVAS in the acute patients was 15.6 (range 7–27). Median BVAS in convalescent patients was 0 (range 0–5), with two individuals scoring >0 for chronic symptoms, thought not to be related to new or acute disease. Of the acute patients, two were entirely naïve to treatment. The others had received the first doses of immunotherapy: 16 had received a dose of cyclophosphamide and prednisolone (P), of whom 10 additionally received plasma exchange and three received additional rituximab. Ten of the acute patients had relapsed on therapy, three on P and mycophenolate mofetil (MMF); five P and azathioprine (AZA); one P and methotrexate (MTX) and one P alone. In the convalescent patients, their maintenance immunosuppressive regimes were P + AZA (n = 27); P + MMF (n = 7); P + MTX (n = 4); P + tacrolimus (n = 2); AZA only (n = 12); P only (n = 4); MTX only (n = 1) and no treatment (n = 8).

**Serum cytokine levels and correlations**

**IL-17 and IL-23.** Both acute and convalescent patients with AAV had significantly higher IL-17 levels than healthy controls (acute: mean 3581 pg/ml, median 64 pg/ml, range 0–72 940 pg/ml; convalescent: mean 329 pg/ml, median 0 pg/ml, range 0–2732 pg/ml; healthy controls: mean 21 pg/ml, median 0 pg/ml, range 0–252 pg/ml; \( P < 0.01 \) (Fig. 1A). Importantly, patients with AAV demonstrated a very wide variation in levels of IL-17. By defining the upper limit of normal (>133 pg/ml, derived from the mean + 2 SD of the healthy control population), we found that 36% of acute patients and 34% of convalescent patients had increased IL-17 levels.

Interestingly, IL-23, which plays a role in division and maintenance of IL-17-producing cells, demonstrated a similar pattern of levels in our different groups. Acute and convalescent patients with systemic disease had significantly higher IL-23 levels than healthy controls (acute: mean 941 pg/ml, median 282 pg/ml, range 0–5950 pg/ml; convalescent: mean 775 pg/ml, median 155 pg/ml, range 0–11 033 pg/ml; healthy controls: mean 66.4 pg/ml, median 0 pg/ml, range 0–645 pg/ml; \( P < 0.001 \); Fig. 1B). Again, only a proportion of patients had elevated levels compared to the upper limit of normal derived from healthy controls (>373 pg/ml), consisting of 43% of acute patients and 25% of convalescent patients (Fig. 1B).

Prospective serial measurements of IL-17 and IL-23 were carried out in five acute AAV patients with systemic disease and elevated cytokine levels, from presentation until remission. Levels of both cytokines fell following treatment, but only returned to normal levels in three out of...
five patients (Fig. 2A and B). Interestingly, the two in whom levels remained elevated have had major relapses (at 13 and 15 months), while the other three have maintained disease-free remission (at 18, 20 and 23 months of follow-up, \( P = 0.039 \) by chi-squared test). The low levels of IL-17 and IL-23 found in some patients was not simply due to immunosuppressive medication as levels were equivalent in those on and off treatment (Fig. 2C and D).

In patients with AAV, IL-17 levels correlated significantly with IL-23 \((r = 0.54, P < 0.0001)\), IL-1\( \beta \) \((r = 0.56, P < 0.0001)\) and IL-6 \((r = 0.59, P < 0.0001)\) (Fig. 3A–C). By contrast, no such correlation was found for IFN-\( \gamma \) \((P > 0.05)\) (Fig. 3D). Interestingly, in the positive control groups, there was no correlation between IL-17 production and any of the other cytokines \((P > 0.05)\).

**IL-1\( \beta \) and IL-6.** Generation of Th17 cells requires IL-6 and IL-1\( \beta \), so these cytokines were measured in the same serum samples. IL-1\( \beta \) levels in acute patients (mean 63 pg/ml, median 0 pg/ml, range 0–562 pg/ml) and convalescent patients (242 pg/ml, median 0 pg/ml, range 0–5124 pg/ml) did not vary statistically from healthy controls (12 pg/ml, median 0 pg/ml, range 0–113 pg/ml) (Fig. 1C) or disease controls. IL-6 levels in acute patients (mean 35 pg/ml, median 6.8 pg/ml, range 0–358 pg/ml) and convalescent patients (161 pg/ml, median 0 pg/ml, range 0–4278 pg/ml) tended to be higher than those of healthy controls (11.5 pg/ml, median 0 pg/ml, range 0–113 pg/ml) but only the acute samples were statistically significant \((P < 0.05)\) (Fig. 1D).

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**Fig. 1.** Serum cytokine levels in acute \((n = 28)\) and convalescent \((n = 50)\) AAV patients compared to healthy controls \((n = 28)\) and disease controls \((n = 19)\): (A) IL-17, (B) IL-23, (C) IL-1\( \beta \), (D) IL-6 and (E) IFN-\( \gamma \); IL-17 and IL-23 levels were significantly higher in both acute and convalescent patients with AAV than in disease and healthy controls (HC, healthy controls; septic, septic patients; Anti-GBM+, anti-GBM disease); bars represent medians; *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \).

**Fig. 2.** Changes in IL-17 and IL-23 levels following remission induction: box and whisker plots of (A) levels of IL-17 and (B) levels of IL-23 in five patients from their acute presentation to disease remission (dotted lines represent upper limit of normal range); (C) influence of immunosuppression on IL-17 and (D) IL-23 cytokine levels in samples from patients [no difference between samples from patients taking \((n = 80)\) or not taking \((n = 11)\) immunosuppressive medication is seen].
Compared to the other cytokines, IFN-γ levels were generally low in all the groups. Levels in the patient groups were not significantly different from healthy or disease controls.

Cytokine levels according to ANCA epitope specificity. In acute and convalescent patients, there was no difference in any of the cytokines (IL-17, IL-23, IL-1β and IL-6) between MPO-ANCA and PR3-ANCA patients.

Cytokine levels and clinical features. Patients with elevated levels of IL-23 (greater than the mean + 2SD of controls) had significantly more active disease (mean BVAS 9.5) than patients with levels of IL-23 within the normal range (mean BVAS 4.5) (P < 0.05; Fig. 4A) and higher levels of ANCA titre (P < 0.05; Fig. 4B). In this cross-sectional cohort, other cytokine levels did not appear to correlate with BVAS score, disease extent, number of previous relapses, current renal function, proteinuria or renal biopsy findings.

IL-17-secreting autoantigen-specific memory T cells
To understand the role of IL-17 in disease relapse, we investigated whether there is a predominant IL-17-producing autoantigen-specific cells compared to 0% of controls (P < 0.05) (Fig. 5A). When the number of IL-17-secreting cells was expressed as a percentage of the total PHA or DTP response, as a means of controlling for the degree of immunosuppression, a similar difference between patients and controls was found (percent autoantigen-specific T cells relative to DTP response in controls 0%; patients mean 22%, range 0–143%, P = 0.05) (Fig. 5B) or relative to PHA response (controls 0%; patients mean 11.2%, median 4%, range 0–63%, P < 0.01) (Fig. 5C). Overall, there was no difference in Th17 frequency between patients stimulated with PR3 or MPO autoantigens.

With respect to IFN-γ, there was no difference in numbers of IFN-producing cells in response to autoantigen between patients and controls (Fig. 5D), confirming our previous findings [35].

Critically, 53% of convalescent patients had IL-17-producing autoantigen-specific cells compared to 0% of controls (P < 0.05) (Fig. 5A). When the number of IL-17-secreting cells was expressed as a percentage of the total PHA or DTP response, as a means of controlling for the degree of immunosuppression, a similar difference between patients and controls was found (percent autoantigen-specific T cells relative to DTP response in controls 0%; patients mean 22%, range 0–143%, P = 0.05) (Fig. 5B) or relative to PHA response (controls 0%; patients mean 11.2%, median 4%, range 0–63%, P < 0.01) (Fig. 5C). Overall, there was no difference in Th17 frequency between patients stimulated with PR3 or MPO autoantigens.

Of the patients with a positive ELISPOT, 63% had detectable contemporaneous serum IL-17 levels (mean level 133 pg/ml) with only 25% expressing significantly elevated levels compared to the control population. By contrast, 40% of patients without T cell reactivity on ELISPOT had detectable levels of IL-17 (mean level of 35pg/ml), with 20% expressing significantly elevated levels. These data demonstrate that there is no direct correlation between serum IL-17 levels and isolation of antigen-specific autoreactive Th17 cells. This is also true for IL-23 levels and Th17 cells. This may be as a result of other antigens stimulating a T cell IL-17 response at the time of sample collection or other immune cells contributing to the circulating IL-17 levels (as has been suggested for neutrophils, macrophages and NK T cells) [19,36,37] in the ELISPOT-negative patients. The lack of circulating IL-17 levels in a proportion of ELISPOT-positive patients suggests that the appropriate antigenic stimuli were not pres-
Discussion

Our data show that a proportion of both acute and convalescent patients with AAV have significantly higher IL-17 and IL-23 levels than healthy controls. Reduction in IL-17 and IL-23 levels occurs in some, but not all patients following treatment. High IL-23 levels correlated with higher levels of clinical disease activity as defined by BVAS and with higher ANCA titres. In addition, over half of our convalescent patients have circulating memory T cells which respond to ANCA autoantigen by producing IL-17. These findings are in keeping with a role for the Th17 axis in the pathogenesis of AAV in at least some patients. The range of IL-17 and IL-23 found in AAV patients was extremely wide, reminiscent of other cytokine studies performed in AAV patients [38,39]. Interestingly, our data demonstrate that despite being in clinical and biochemical remission, many of these convalescent patients demonstrate significant immunological activity, underscoring their high relapse potential.

It is intriguing that IL-23 levels but not IL-17 levels appeared to correlate with disease severity. Recently, IL-23-deficient animals with experimental glomerulonephritis demonstrated a non-selective deficit in T cell cytokine production, with less IFN-γ, IL-4, TNF and MCP-1, not just a decrease in IL-17 [15]. Therefore, the mechanisms by which IL-23 modulates disease may be broader than simply through IL-17. High levels of IL-17 and IL-23 were found in acute patients, although in this cross-sectional study, these were not statistically different from levels in convalescent patients. However, our sequential data demonstrate that falls in levels of IL-17 and IL-23 over time may be associated with better outcome than in those patients in whom levels do not reach the normal range. A larger prospective cohort is being investigated to confirm the role of IL-17 and IL-23 in disease relapse.

The range of IL-17 levels in patients was large, between 0 and 72,940 pg/ml in the acute cohort, with 36% of them demonstrating levels above the normal range. Similarly, the range of IL-23 in acute patients was large, 0–5,950 pg/ml, and 43% had levels above the normal range. Thus, there are a significant proportion of patients with extremely high cytokine levels and a group in which IL-17 and IL-23 were not elevated at the measured time-point. There are a number of possible explanations for this. Since most patients with acute presentations received some initial immunosuppressive treatment in the first 10 days, IL-17 levels may have dropped rapidly due to the effect of therapy. However, our analysis of cytokine levels and immunotherapy (Fig. 2C and D) does not support this conclusion. Alternatively, the Th17 population may be unstable and, at least in experimental systems, Th17 cells may undergo phenotype switching depending on the cytokine milieu [40]. Lastly, whilst IL-17 may be important for pathogenesis in some patients, this may not apply to all. Importantly, IL-17 and IL-23 levels remain significantly elevated during convalescence in a proportion of patients, along with persistent autoantigen-specific IL-17-producing cells. Increased percentages of circulating CD4 effector memory T cells have previously been identified in convalescent WG patients [41], and regulatory T cells have been found to be functionally defective [42]. Our data suggest that these changes may result in a persistent CD4+ Th17 memory cell population.

IL-1 and IL-6 levels were not significantly elevated in acute patients. Since both are thought to be important in the differentiation of Th17 cells in humans, it is possible that the peak in IL-1 and IL-6 levels preceded that of IL-17 and was missed in our study. In contrast, IL-23, which is required for the maintenance and proliferation of Th17 cells, was elevated at the time of sampling. Amongst
AAV patients, we found that IL-17 production correlated closely with the levels of IL-1, IL-6 and IL-23 \((P < 0.0001)\), but not IFN-\(\gamma\), which again underscores the role of those cytokines in Th17 population development and survival. Our patient population is typically heterogeneous in terms of pattern of organ involvement, degree and duration of disease activity, as well as immunosuppression regime. We found no correlation of IL-17 with CRP, ANCA specificity or ANCA titre. However, IL-23 was associated with clinical disease severity, as assessed by BVAS and ANCA titre. We recognize the constraints of a retrospective study and believe that some clinical correlations may be better assessed prospectively.

Serum levels of IFN-\(\gamma\) were not elevated in patients and ELISPOT data demonstrated no increase in IFN-\(\gamma\)-producing cells in response to autoantigen in convalescent patients, as we have previously described [35]. Since the characterization of the Th17 lineage, it has been recognized that IFN-\(\gamma\) is inhibitory to the differentiation of Th17 cells [8,30]. Therefore, in keeping with these findings, if Th17 are important in the pathogenesis of vasculitis, it is consistent that IFN-\(\gamma\) levels should be low.

Our data are in keeping with and extend those of Abdulahad et al. [43] who limited their analysis to patients with WG and PR3-ANCA and found increased PR3-specific IL-17- and IL-4-producing T cells in convalescent WG patients. Unlike our data, they did not address the role of IL-23, but similarly found no significant difference in IFN-\(\gamma\) production, confirming a relative skewing toward Th17 (and Th2) cells in WG. Moreover, consistent with our data, following stimulation with PR3, they found an increase in Th17 cells in ANCA-positive compared to ANCA-negative patients and healthy controls.

Our data support the current evidence which suggests that T cells are likely responsible for IL-17 production in AAV and that a Th17 memory cell population may persist in disease convalescence. Clinical trials investigating the efficacy of IL-17 or IL-23 blockade in a number of autoimmune diseases are currently underway (http://www.clinicaltrials.gov.uk), as are agents inhibiting the upstream cytokines IL-1 and IL-6. Our data suggest that the IL-23 and IL-17 axis may represent a potential future target for therapeutic intervention in some patients with AAV.

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**Conflict of interest statement.** None declared.
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