Silent arterial inflammation during the apparent remission state of Takayasu's arteritis. What do cytokines tell us?

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ABSTRACT

Objective. *To evaluate serum cytokines as biomarkers of smoldering disease activity in patients with Takayasu's arteritis (TAK) in remission.*

Methods. *Thirty-four TAK patients with stable disease during the last 6 months and 22 healthy controls (HC) were included in a cross-sectional study. Serum levels of pro-inflammatory, anti-inflammatory, Th1, Th2, Th9, Th17 and Th22 cytokines were measured by the multiplex technique.*

Results. *No significant differences regarding serum cytokine levels were found between TAK patients and HC. Serum TNF-α, IL-17F, IL-21 and IL-23 were higher in patients presenting angiographic type V than in those presenting other angiographic types. Serum IL-17E, IL-17F, IL-22 and IL-23 were higher in TAK patients with previous ischaemic events compared with those without previous ischaemia. No differences in serum cytokines were observed between TAK patients with and without aneurysmal disease in the aorta or among TAK patients without therapy, those under immunosuppressive agents and patients on biological therapy. Independent associations were found regarding angiographic type V and higher serum levels of IL-4, IL-6, IL-17A, IL-17E, IL-17F, IL-21, IL-22 and IL-23. Previous ischaemic events were independently associated with higher serum IL-4, IL-17E, IL-22 and IL-23. Daily prednisone dose had an inverse association with lower serum IL-4, IL-6, IL-17A, IL-17E, IL-22 and IL-23. The simultaneous use of immunosuppressive and biological agents led to lower serum IL-4, IL-17E and IL-23 levels.*

Conclusion. *A smoldering inflammatory response with predominantly cytokines involved in Th17 response seems to be ongoing in TAK patients in remission with extensive disease or with previous ischaemic events.*

Introduction

Takayasu's arteritis (TAK) is a chronic granulomatous large-vessel vasculitis that affects the aorta, its main branches and pulmonary arteries. The inflammatory process in the arterial wall leads to stenosis, occlusion, dilation and/or aneurysm formation (1). The inflammatory infiltration in arteries from TAK patients involves all layers and comprises mononuclear cells such as $CD4^+$ and $CD8^+$ lymphocytes, γδ T cells, dendritic cells, macrophages, multinucleated giant cells, natural killer cells, and neutrophils (2). Th1 and Th17 responses are predominantly observed when peripheral blood mononuclear cells (PBMC) from TAK patients are stimulated *in vitro* (3).

Several studies have evaluated serum or plasma levels of different cytokines as biomarkers of disease activity and disease status in TAK (4). Conflicting or disappointing results regarding disease activity and disease status were found for levels of most cytokines such as tumour necrosis factor (TNF)-α, interferon (IFN)γ, interleukin (IL)-1β, IL-2, IL-4, IL-10, IL-12, IL-17, and IL-23 (5-13). Only serum IL-6 and IL-18 levels were consistently higher in TAK patients than in healthy controls (HC) and higher in patients with active disease than those in remission (4, 10, 12, 14, 15). However, the longitudinal assessment of serum IL-6 in TAK patients showed that its levels remain high irrespective of disease activity, whereas serum levels of the soluble IL-6 receptor (sIL-6R) only increase during phases of active disease (15). In the only study that assessed serum IL-9 in TAK, its levels where higher in patients with active disease compared with HC (13).

Besides its role as a biomarker in TAK (14), IL-6 and IL-6 receptor have a relevant role in the pathogenesis of fibrosis in arterial wall of TAK patients,

since *in vitro* stimulation of human aortic adventitial fibroblasts by IL-6 and its receptor has a profibrotic effect by increasing the expression of collagen I, collagen III, fibronectin, α-smooth muscle actin and transforming growth factor (TGF)-β in these cells. Indeed, the expression of IL-6, IL-6 receptor, collagen I, collagen III, fibronectin, α-smooth muscle actin and TGF-β is enhanced in arteries from TAK patients compared with arteries from controls (16).

To date, studies assessing serum/plasma cytokines levels as biomarkers in TAK have compared patients and HC and/or TAK patients with active disease and those in remission. However, the definition of disease remission is a challenge in TAK and arterial lesions usually progress slowly in the absence of disease manifestations. Serum cytokine levels are promising biomarkers in TAK patients with stable disease, since high levels of certain cytokines may indicate silent ongoing arterial inflammation (4). Therefore, this study aims to evaluate if serum levels of pro-inflammatory, anti-inflammatory and Th1, Th2, Th9, Th17 and Th22 cytokines in TAK patients in remission would indicate a potential subclinical disease activity in TAK and to analyse associations with the extension of arterial involvement, aneurysmal disease, ischaemic events and therapy. TAK patients with overt signs of disease activity were not included because serum cytokines would not bring additional information to the already known status of active TAK.

Methods

Patients and healthy controls

A cross-sectional study was performed at the Vasculitis Outpatient Clinic of Universidade Federal de São Paulo, Escola Paulista de Medicina (Unifesp-EPM). Inclusion criteria were age above 18 years, the fulfilment of the 1990 American College of Rheumatology classification criteria for TAK and/or the Ishikawa diagnostic criteria modified by Sharma (17, 18), and the absence of signs and symptoms of disease activity or disease progression (*i.e.,* the development of new arterial lesions) during the past six months. Patients were excluded if they presented active disease according to Kerr's criteria (19), acute or chronic infectious illnesses, ongoing allergy, asthma and a history of cancer. In this study, remission was defined as the absence of new signs and symptoms of disease activity of TAK and the absence of new angiographic lesions. Isolated abnormal results of acute phase reactants in asymptomatic TAK patients were not enough to consider active disease. Forty-five consecutive TAK patients agreed to participate in the study. Eleven patients were excluded due to current active disease and finally 34 TAK patients met the inclusion criteria. Twenty-two HC with similar mean age (44.13±15.62 years *vs.* 41.64±13.68 years, p=0.532) and a similar frequency of females to TAK patients (90.9% *vs.* 88.2%, p=0.752) were also assessed in the study. The study was conducted in accord with the declaration of Helsinki, the institutional ethics committee approved study's protocol (Nr. 0396/14) and all participants gave written informed consent.

TAK-related variables

Disease extension was ascertained according to the angiographic classification by Hata *et al.* (20). Angiographic type V was regarded as the most extensive arterial involvement, whereas angiographic types I, IIa and IV were considered as localised arterial involvement in TAK. TAK patients were also evaluated for aneurysmal disease involving the aorta and previous ischaemic events (*i.e.,* stroke, transient ischaemic attacks, unstable angina, acute myocardial infarction, abdominal angina and limb ischaemia with gangrene) according to established guidelines (21-25). Information about current medical therapy for TAK was also collected from medical records.

Measurement of serum cytokines

Blood samples were collected from TAK patients and serum was stored at -80ºC with the Protease Inhibitor Cocktail Set I - Calbiochem (Merck, Millipore, USA). The multiplex technique was used to determine serum levels of TNF-α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17A, IL-17E, IL-17F, IL-21, IL-22 and IL-23 (Milliplex, Merck-Millipore, USA) using the Luminex® 200™ System (Luminex Corporation, USA), according to manufacturer's instructions.

Statistical analysis

Statistical analysis was carried out with the IBM SPSS Statistics for Windows v. 20.0 (Armonk, USA) and with GraphPad Prism v. 6.00 for Windows (La Jolla, USA). Continuous data were presented as mean and standard deviation or as median and interquartile range (IQR) and categorical data as number and percentage. Comparisons between groups were performed by the Student's *t*-test or by the Mann-Whitney's U-test for continuous variables and by the Chi square test or by the Fisher's exact test for categorical variables. For comparisons among 3 groups regarding continuous variables, one-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used. Multivariate linear regression models were built to analyse independent associations with serum cytokine levels in TAK. Age, female sex, ischaemic events, angiographic type V, daily prednisone dose and the simultaneous use of immunosuppressive and biological agents were independent variables in all models while serum levels of TNF-α, IL-4, IL-6, IL-17A, IL-17E, IL-17F, IL-21, IL-22 and IL-23 were analysed as dependent variables in each model. Results were expressed as unstandardised β coefficient and 95% confidence interval (95CI). Significance level accepted was 5% (p<0.05).

Results

Disease features and therapy in TAK patients

The median time since diagnosis of TAK was 102.0 months (21.0-192.0) and TAK patients presented median ESR and CRP levels at study as 23.5mm/hour (6.0-32.5) and 1.8mg/L (0.6-5.4), respectively. Angiographic type V was found in 21 (61.8%) TAK patients while the other angiographic types were type I in 7 (20.6%), type IIa in 3 (8.8%) and type IV in 3 (8.8%)

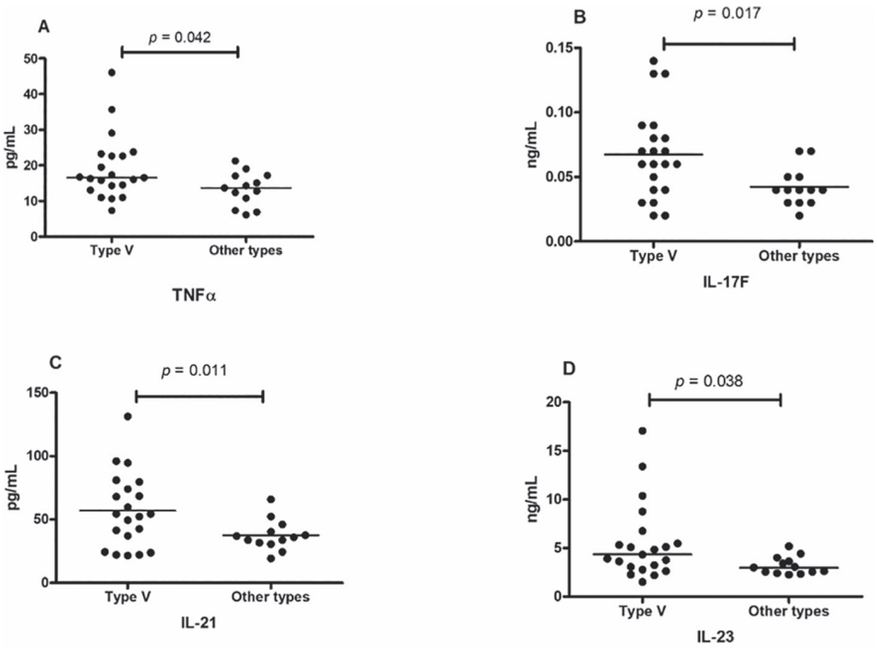

Fig. 1. Cytokine levels and the extension of arterial involvement in Takayasu's arteritis.
Serum levels of TNF-α, IL-17F, IL-21 and IL-23 were significantly higher in TAK patients presenting angiographic type V compared with TAK patients presenting other angiographic types. Transverse bars represent the median serum level of TNF-α and IL-23 and the mean serum level of IL-17F and IL-21.

Table I. Serum cytokine levels in Takayasu's arteritis according to the extension of arterial involvement.

Cytokines	Angiographic type V (n=21)	Other angiographic types (n=13)	*p*
IL-6, pg/ml	19.05 (13.58-35.71)	14.84 (10.89-17.58)	0.058
IL-1β, pg/ml	6.08 ± 1.99	5.21 ± 1.08	0.158
TNFα, pg/ml	16.60 (13.69-22.97)	13.69 (9.07-17.14)	0.042*
IL-2, pg/ml	32.78 (23.77-44.29)	29.18 (21.52-36.09)	0.272
IL-12p70, pg/ml	14.40 (10.14-24.97)	10.84 (9.21-14.28)	0.127
IFNγ, pg/ml	27.66 (19.18-36.22)	26.55 (22.17-32.10)	0.456
IL-4, ng/ml	0.09 (0.05-0.15)	0.07 (0.04-0.09)	0.239
IL-5, pg/ml	10.76 (7.41-15.60)	9.18 (7.83-11.28)	0.257
IL-9, pg/ml	18.99 (15.17-22.44)	17.48 (13.75-21.06)	0.467
IL-13, pg/ml	337.09 ± 152.49	270.44 ± 95.96	0.169
IL-17A, pg/ml	22.93 (14.81-29.59)	17.49 (13.37-23.46)	0.161
IL17E, ng/ml	0.22 (0.16-0.26)	0.17 (0.14-0.20)	0.067
IL-17F, ng/ml	0.067 ± 0.034	0.042 ± 0.014	0.006*
IL-21, pg/ml	57.12 ± 29.05	37.63 ± 11.99	0.011*
IL-22, ng/ml	0.29 (0.24-0.37)	0.24 (0.21-0.28)	0.065
IL-23, ng/ml	4.35 (2.92-6.13)	3.00 (2.49-3.82)	0.038*
IL-10, pg/ml	5.48 (4.17-8.82)	4.09 (3.62-6.45)	0.141

*Significant *p*-values; IFN: interferon; IL: interleukin; TNF: tumour necrosis factor.
Results are presented as mean and standard deviation or as median and interquartile range.

TAK patients. Nine TAK patients (26.5%) presented aortic aneurysms and 13 (38.2%) TAK patients had previous arterial ischaemic events including stroke in 53.8a%, acute myocardial infarction in 38.5%, mesenteric angina in 23.1% and limb ischaemia with gangrene in 7.7%.

Twenty-eight (82.3%) TAK patients were under therapy with an immunosuppressive agent with or without a biological agent at study. Prednisone was used by 19 (55.9%) TAK patients at a median daily dose of 12.5mg (5.0-30.0). The immunosuppressive agents were used by 23 (67.6%) TAK patients as follows: methotrexate (30.4%), leflunomide (30.4%), azathioprine (21.7%), mycophenolate sodium (13.0%) and cyclophosphamide (4.3%). Biological agents were prescribed for 10 (29.4%) TAK patients and they were associated with immunosuppressive agents in half of them. Adalimumab (50.0%), tocilizumab (30.0%) and infliximab (20.0%) were the biological agents used by TAK patients.

Serum cytokine levels

TAK patients in remission and with stable disease in the last 6 months presented similar serum cytokine levels compared with HC (supplementary Table S1). Amongst TAK patients, those presenting extensive vascular involvement (*i.e.,* angiographic type V) had significantly higher serum TNF-α, IL-17F, IL-21 and IL-23 than TAK patients with other angiographic types (Fig. 1). In addition, a trend for higher serum IL-6, IL-17E and IL-22 levels was also observed in TA patients with angiographic type V compared with other angiographic types (Table I). TAK patients with previous arterial ischaemic events presented significantly higher serum levels of IL-17E, IL-17F, IL-22 and IL-23 compared with TAK patients without previous ischaemia (Table II and Fig. 2). No significant differences were found between TAK patients with and without aneurysmal aortic disease regarding serum cytokine levels (Table III).

In order to analyse the influence of medical therapy on serum cytokine levels, TAK patients without any therapy (*i.e.,* without glucocorticoids, immunosuppressive or biological agents) were compared with TAK patients on immunosuppressive agents and with patients on biological therapy. No significant differences in serum cytokine levels were observed regarding therapy for TAK (Table IV).

Factors associated with serum cytokine levels in TAK

Detailed results of multivariate linear regression analysis are depicted in supplementary Table S2. Angiographic type V was independently associated with serum IL-4 (β=0.064; 95CI: 0.025 to 0.103; *p*=0.004), IL-6 (β=14.126; 95CI: 4.901 to 23.352; *p*=0.006), IL-17A (β=11.099, 95CI: 2.972 to 19.227; *p*=0.012), IL-17E (β=0.064,

Table II. Serum cytokine levels in Takayasu's arteritis and arterial ischaemic events.

Cytokines	Previous ischaemic events (n=13)	No ischaemic events (n=21)	*p*
IL-6, pg/ml	17.79 (13.86-31.42)	15.12 (10.89-20.03)	0.405
IL-1β, pg/ml	5.94 (4.91-6.76)	4.82 (4.44-6.49)	0.256
TNFα, pg/ml	16.60 (14.80-22.97)	14.30 (10.88-18.22)	0.127
IL-2, pg/ml	37.19 (24.82-44.08)	26.90 (23.54-35.87)	0.184
IL-12p70, pg/ml	15.01 (11.19-22.16)	10.84 (9.21-15.99)	0.178
IFNγ, pg/ml	32.38 (19.18-37.87)	26.55 (22.03-31.82)	0.221
IL-4, ng/ml	0.110 (0.050-0.150)	0.070 (0.045-0.095)	0.212
IL-5, pg/ml	11.02 (8.16-14.36)	9.18 (7.62-11.66)	0.357
IL-9, pg/ml	18.99 (14.76-20.18)	16.87 (14.39-21.79)	0.972
IL-13, pg/ml	335.87 (245.83-412.15)	231.63 (193.04-404.25)	0.425
IL-17A, pg/ml	22.93 (15.65-29.21)	17.49 (14.10-24,92)	0.264
IL17E, ng/ml	0.230 (0.195-0.265)	0.170 (0.140-0.195)	0.010*
IL-17F, ng/ml	0.070 (0.050-0.085)	0.040 (0.030-0.065)	0.027*
IL-21, pg/ml	54.56 (39.21-74.15)	37.17 (27.48-52.40)	0.082
IL-22, ng/ml	0.30 (0.27-0.35)	0.24 (0.21-0.30)	0.029*
IL-23, ng/ml	4.85 (3.44-6.13)	3.07 (2.49-3.96)	0.021*
IL-10, pg/ml	5.48 (4.35-7.49)	4.26 (3.62-7.99)	0.366

*Significant *p*-values; IFN: interferon; IL: interleukin; TNF: tumour necrosis factor.
Results are presented as median and interquartile range.

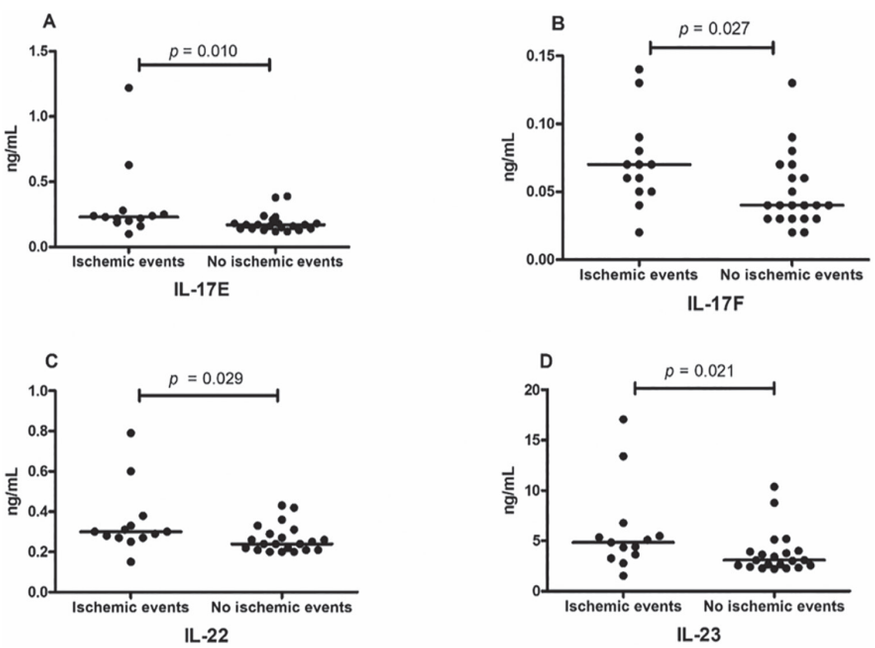

Fig. 2. Cytokines levels and previous ischaemic events in Takayasu's arteritis.
Serum levels of IL-17E, IL-17F, IL-22 and IL-23 were significantly higher in TAK patients with previous ischaemic events compared with TAK patients without previous ischaemic events. Transverse bars represent the median serum level of IL-17E, IL-17F, IL-22 and IL-23.

95CI: 0.027 to 0.102; *p*=0.003), IL-17F (β=0.028, 95CI: 0.006 to 0.050; *p*=0.016), IL-21 (β=26.166, 95CI: 8.669 to 43.663; *p*=0.007), IL-22 (β=0.062, 95CI: 0.016 to 0.108, *p*=0.012) and IL-23 levels (β=1.860, 95CI: 0.839 to 2.882; *p*=0.002). Ischaemic events were associated with serum IL-4 (β=0.030, 95CI: 0.025 to 0.103; *p*=0.048), IL-17E (β=0.044, 95CI: 0.016 to 0.072, *p*=0.005), IL-22 (β=0.039, 95CI: 0.005 to 0.072; *p*=0.029) and IL-23 levels (β=1.163, 95CI: 0.404 to 1.923; *p*=0.006). Daily prednisone dose had an independent influence in lowering serum IL-4 (β=-0.002, 95CI: -0.002 to -0.001, *p*=0.002), IL-6 (β=-0.286, 95CI: -0.491 to -0.081; *p*=0.010), IL-17A (β=-0.220, 95CI: -0.400 to -0.039; *p*=0.021), IL-17E (β=-0.001, 95CI: -0.002 to -0.001; *p*=0.003), IL-22 (β=-0.001, 95CI: -0.002 to 0.000; *p*=0.011) and IL-23 levels (β=-0.036, 95CI: -0.058 to -0.013; *p*=0.005). The simultaneous use of immunosuppressive and biological agents had an independent association with lower serum IL-4 (β=-0.044, 95CI: -0.082 to -0.007; *p*=0.024), IL-17E (β=-0.046, 95CI: -0.082 to -0.010; *p*=0.017) and IL-23 levels (β=-0.986, 95CI: -1.961 to -0.011; *p*=0.048). No significant associations were found with serum TNF-α levels.

Discussion

In this study, higher levels of cytokines involved in Th17 response were independently associated with a more extensive arterial involvement and with previous ischaemic events in TAK, despite therapy and the absence of manifestations of active disease. Moreover, daily prednisone dose was independently associated with lower serum levels of several cytokines, especially those involved in Th17 response but not with Th1 cytokines.

The evaluation of TAK patients with stable disease may disclose important information about biomarkers that would indicate smoldering arterial inflammation during the remission state, since disease progression (*i.e.*, the development of new angiographic lesions) may ensue even in the absence of overt signs and symptoms of disease activity in TAK. In addition, the differentiation between acute vascular inflammatory lesions and permanent vascular damage due to a fibrotic stenotic lesion is often difficult (26, 27). Therefore, surrogate markers of ongoing vascular inflammation would be promising tools to predict disease relapses and the progression vascular lesions in TAK.

Although serum IL-17A is not a biomarker for disease activity or disease status in TAK (11-13), there is evidence that the Th17 response plays a role in its pathophysiology (3, 28, 29). In a mouse model, the deficiency of IFN regulatory factor 4 (IRF4)-binding protein, a protein that controls IL-17 and IL-21 production, leads to high IL-17 and IL-21 levels and to the development of large vessel vasculitis (28). PBMC from TAK patients produce

Table III. Serum cytokine levels and aortic involvement with aneurysms in Takayasu's arteritis.

Cytokines	Aortic involvement with aneurysms (n=9)	Absence of aortic aneurysms (n=25)	*p*
IL-6, pg/ml	17.37 (13.00-43.01)	16.53 (11.03-25.86)	0.319
IL-1β, pg/ml	5.57 (4.63-8.53)	5.01 (4.44-6.40)	0.469
TNFα, pg/ml	17.22 (11.86-32.40)	15.07 (11.68-19.31)	0.339
IL-2, pg/ml	32.78 (22.23-53.80)	29.18 (23.77-38.93)	0.711
IL-12p70, pg/ml	14.64 (10.38-31.75)	11.55 (9.21-17.10)	0.178
IFNγ, pg/ml	27.66 (22.46-44.34)	27.11 (19.18-32.79)	0.584
IL-4, ng/ml	0.09 (0.06-0.20)	0.07 (0.04-0.11)	0.280
IL-5, pg/ml	11.79 (9.18-19.79)	9.45 (7.35-12.04)	0.066
IL-9, pg/ml	18.39 (14.85-28.39)	17.78 (14.39-21.06)	0.545
IL-13, pg/ml	328.76 ± 154.56	305.43 ± 131.73	0.666
IL-17A, pg/ml	18.87 (14.94-39.07)	18.05 (14.10-26.56)	0.494
IL17E, ng/ml	0.18 (0.16-0.31)	0.19 (0.14-0.23)	0.725
IL-17F, ng/ml	0.04 (0.04-0.07)	0.05 (0.03-0.07)	0.859
IL-21, pg/ml	58.53 ± 34.72	46.47 ± 21.45	0.231
IL-22, ng/ml	0.26 (0.24-0.36)	0.27 (0.21-0.32)	0.938
IL-23, ng/ml	3.64 (2.81-7.61)	3.64 (2.60-5.16)	0.785
IL-10, pg/ml	6.37 (3.92-11.86)	4.70 (3.92-7.08)	0.218

IFN: interferon; IL: interleukin; TNF: tumour necrosis factor.
Results are presented as mean and standard deviation or as median and interquartile range.

Table IV. Serum cytokine levels and therapy for Takayasu's arteritis.

Cytokines	No therapy (n=6)	Immunosuppressive agents (n=18)	Biological Therapy (n=10)	*p*
IL-6, pg/ml	15.95 (8.83-37.98)	16.53 (12.44-18.84)	22.22 (12.80-37.98)	0.725
IL-1β, pg/ml	6.22 (3.86-8.97)	5.47 (4.58-6.44)	4.82 (4.20-6.53)	0.536
TNFα, pg/ml	19.68 ± 13.96	17.52 ± 7.08	14.42 ± 5.01	0.431
IL-2, pg/ml	29.44 (18.28-63.54)	31.88 (24.24-37.94)	28.04 (24.00-43.97)	0.988
IL-12p70, pg/ml	13.51 (8.05-30.55)	13.56 (10.08-15.47)	12.25 (9.56-24.76)	0.924
IFNγ, pg/ml	36.24 ± 30.93	27.96 ± 10.18	28.29 ± 7.73	0.494
IL-4, ng/ml	0.08 (0.04-0.19)	0.07 (0.04-0.11)	0.08 (0.05-0.12)	0.822
IL-5, pg/ml	11.66 (6.99-22.27)	9.58 (7.83-11.28)	11.00 (7.34-13.49)	0.768
IL-9, pg/ml	18.65 (13.08-25.78)	18.69 (14.71-21.06)	17.32 (13.24-20.69)	0.836
IL-13, pg/ml	317.15 ± 198.40	304.36 ± 117.23	321.33 ± 140.91	0.949
IL-17A, pg/ml	20.82 (12.12-48.77)	18.59 (15.94-26.26)	20.49 (14.24-29.01)	0.922
IL17E, ng/ml	0.18 (0.11-0.44)	0.18 (0.15-0.23)	0.19 (0.15-0.24)	0.914
IL-17F, ng/ml	0.05 (0.02-0.13)	0.05 (0.04-0.07)	0.05 (0.03-0.07)	0.976
IL-21, pg/ml	60.00 ± 45.81	46.21 ± 19.66	49.68 ± 20.37	0.536
IL-22, ng/ml	0.26 (0.18-0.47)	0.27 (0.23-0.30)	0.26 (0.23-0.32)	0.947
IL-23, ng/ml	3.73 (2.03-9.93)	3.71 (2.91-4.91)	3.44 (2.62-5.21)	0.912
IL-10, pg/ml	4.79 (3.25-11.36)	5.30 (4.04-7.90)	4.74 (4.04-7.28)	0.863

IFN: interferon; IL: interleukin; TNF: tumour necrosis factor. Results are presented as mean and standard deviation or as median and interquartile range.

in vitro higher levels of cytokines involved in the Th1 and Th17 responses compared with stimulated PBMC from HC and from patients with other forms of vasculitis. Furthermore, sera from TAK patients with active disease lead to increased IL-17A production by $CD4^+$ T cells from HC and IL-17A is expressed within the aortic inflammatory infiltration from TAK patients (3). TAK patients with disease onset after 40 years of age present lower serum IL-17 levels and fewer relapses compared with TAK patients who developed disease manifestations before 40 years of age. These findings indicate that IL-17 levels are influenced by age and may be predictive of relapses in TAK (29). Therefore, we decided to evaluate not only IL-17A levels, but serum levels of all cytokines involved in Th17 response, including IL-17A, IL-17E, IL-17F and IL-21 levels, as well as cytokines that induce and/or maintain the Th17 response such as IL-6, IL-1β and IL-23 (30).

Actually, this is the first time that serum IL-17E and IL-17F levels were assessed in TAK patients. Surprisingly, independent associations with disease parameters were found with IL-17A, IL-17E and IL-17F in this study. IL-17A and IL-17F have a high sequence homology and both cytokines share pro-inflammatory effects (31). Recently, an association between the G allele at the single nucleotide polymorphism (SNP) rs763780 of the IL-17F gene and TAK has been described, highlighting the potential role of IL-17F in the pathogenesis of TAK (32). Serum IL-23, a cytokine involved in the maintenance of the Th17 response (30), was also higher in TAK patients presenting angiographic type V and this finding reinforces the role of the Th17 response in TAK.

The association between serum IL-17E levels and angiographic type V indicates another potential Th response associated with TAK in remission, since IL-17E (also known as IL-25) has a distinct role apart from the Th17 response which is the regulation of Th2 responses (31). Indeed, the Th2 response may play a role in the pathophysiology of TAK. Autoantibodies against endothelial cells and antiferritin antibodies have been described in TAK (33). Moreover, TAK patients with active disease present a higher frequency of antibody-secreting newly formed plasmablasts ($CD19^+CD20^-CD27^{high}$) in peripheral blood compared with HC (34). Although serum IL-4 levels do not seem to be a useful biomarker for TAK (12, 13), we found that serum IL-4 levels were independently associated with angiographic type V and with previous ischaemic events in TAK. It is possible that IL-4 may exert effects in TAK besides the activation of B cells and autoantibody production, such as the induction the M2 macrophage phenotype which has anti-inflammatory effects and is involved in tissue repair and remodelling (35, 36).

Arterial ischaemic events in TAK are multifactorial and anatomic abnormalities in arteries, atherosclerotic disease and a hypercoagulability state may all

play a role (37, 38). The independent associations between serum IL-17E and IL-23 levels with previous ischaemic events may indicate the influence of arterial inflammation on ischaemic events in TAK. On the other hand, considering the burden of atherosclerotic disease in TAK (39, 40), the Th17 response may influence either disease progression with new inflammatory lesions or the development atherosclerotic lesions in TAK patients. Indeed, the Th17 response is pro-atherogenic and contributes to vascular and systemic inflammation in atherosclerotic disease as well as to plaque instability (41-43).

Serum IL-21 levels were also associated with a more extensive arterial involvement in TAK in this study. IL-21 is a cytokine with pleiotropic effects on a broad range of cells and it is produced predominantly by follicular helper T cells and by Th17 cells (44). Thus, higher IL-21 levels in TAK patients with angiographic type V indicates that both cell types (*i.e.,* follicular helper T cells and Th17 cells) may be involved in disease progression of TAK. In fact, tertiary lymphoid organs have been found in the aorta of TAK patients with active disease (45) but the role of follicular helper T cells in the pathogenesis of TAK has not been assessed yet. The independent associations observed regarding serum IL-22 levels with angiographic type V and previous ischaemic events in TAK are intriguing. IL-22 has predominantly effects on tissue protection, survival, differentiation and remodelling, and to a lesser extent IL-22 has also pro-inflammatory effects (46). These associations might indicate either a potential reparative role or a pro-inflammatory stimulation exerted by IL-22 in these subgroups of TAK patients.

The influence of therapy on Th responses is another issue that is worth mentioning. In giant cell arteritis, glucocorticoid therapy was shown to suppress the Th17 response and spare the Th1 response (47). However, in TAK a previous study had shown that Th1 cytokines were affected by daily prednisone use rather than the Th17 response (3). In this study, daily prednisone dose had an independent effect on lowering serum levels of several cytokines such as IL-4, IL-6, IL17A, IL-17E, IL-22 and IL-23, but no effect was observed on serum levels of Th1 cytokines (*i.e.,* IL-12p70 and IFNγ). Although, prednisone use had a wide effect on cytokines from different responses in TAK, its predominant effects were observed on Th17 cytokines. Therefore, our findings are more consistent with the effects of prednisone on Th17 cytokines as previously described for giant cell arteritis (47).

Limitations of this study include its cross-sectional design and the inclusion of patients with the disease controlled by immunosuppressive and/or biological therapy. We believe that the inclusion of TAK patients free from any therapy would have strengthened the associations between the Th17 response and extensive arterial involvement in TAK. One caveat that needs to be taken into account is the fact that cytokines usually associated with specific Th responses are also produced by innate immune cells. For example, IL-17A and IL-22, the signature cytokines of their respective Th17 and Th22 responses, are also produced by NK cells, δγ T cells and by innate lymphoid cells (44). Thus, associations between cytokine production and the respective Th response may not be absolute.

Conclusions

In conclusion, a smoldering inflammatory process with predominantly cytokines involved in Th17 response seems to be ongoing in TAK patients with angiographic type V or previous ischaemic events despite clinical remission, this association is independent of therapy prescribed to prevent disease relapses. These findings highlight the role of Th17 response in the pathophysiology of TAK and may be the clue for novel therapeutic targets in TAK. A prospective study to confirm these findings is warranted.

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