The critical role of IL-6 in the pathogenesis of Takayasu arteritis

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ABSTRACT

Objective. *To investigate T cell subsets and immune cytokine profiles in untreated Takayasu arteritis (TAK) patients and the underlying immunopathological mechanism.*

Methods. *We enrolled 50 untreated TAK patients and 40 age-matched controls (20 healthy controls, 20 untreated SLE patients). Enzyme-linked immunosorbent assays (ELISAs) were used to define cytokine profiles in all patients, and flow cytometry was performed for 9 TAK patients and 12 healthy controls. Haematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) were performed in aortic tissues of 9 TAK and 9 atherosclerosis patients; clinical data were also collected.*

Results. *Circulating CD4$^+$ T cells were more frequent in TAK patients ($p<0.05$). Frequencies of Th1, Th2, and Th17 cells were higher, whereas Treg cells were reduced in TAK. Significantly higher levels of IL-6 and lower levels of IFN-γ, IL-4, and IL-17 were detected in TAK patients ($p<0.05$). By H&E staining, thickened vascular walls with proliferation of collagen fibre were observed in most patients. Inflammatory sites with infiltrating macrophages, lymphocytes, and neutrophils were located in adventitia. IHC revealed T cells (mainly CD4$^+$ T cells) in vascular lesions. Additionally, IL-6 was positive throughout the vascular wall in most specimens, whereas IFN-γ, IL-12, and IL-17 were detected in inflammatory sites of active patients. IL-6 levels were positively related to ESR, CRP, and Kerr scores ($p<0.05$).*

Conclusion. *Significantly increased levels of IL-6 were detected in peripheral blood and aortic tissues of untreated patients. IL-6 might be a sensitive biomarker to assess disease activity and could be critical in the immunopathogenesis of TAK.*

Introduction

Takayasu arteritis (TAK) is a type of chronic non-specific granulomatous vasculitis that mainly affects the aorta and its branches in reproductive stage females. A higher rate of incidence of TAK has been reported in some Asian regions, including China. TAK often occurs insidiously, and thickness, stenosis, occlusion, or aneurysm of the involved vessels can develop progressively, which can ultimately lead to ischaemia of related organs and life-threatening events. Thus, it is essential to characterise the pathogenesis of this condition. Currently, an increasing number of experts have suggested that TAK and giant cell arteritis (GCA) share a similar course of pathogenesis, as they are both types of large vessel vasculitis. In contrast to TAK, GCA exhibits a higher incidence in Caucasians and has received more attention from rheumatologists. In GCA, two dominant cytokine clusters – the IL-6–IL-17 and IL-12–IFN-γ axes – have been considered to be related with disease activity, and Th1 and Th17 cell subsets have been proposed to promote vascular inflammation, especially Th1 cells, which are chronically present at higher frequencies in vascular lesions independent of therapy (1-3). However, no systematic study has been previously published that delineates the distribution patterns of T cell subsets and these two immune cytokine axes in TAK.

Previous studies have shown that local vascular lesions of TAK occurred from adventitia, which are infiltrated with multiple types of inflammatory cells, predominantly γδ T cells, cytotoxic T cells, CD4$^+$ T cells, natural killer cells, and macrophages (4-5). Clifford and Hoffman outlined three possible pathways involved in TAK pathology: exogenous or modified endogenous antigens trigger the innate and/or adaptive

immune response, and then tumour necrosis factor (TNF)-α and interleukin (IL)-6, two important pro-inflammatory cytokines, are produced (6). Indeed, most studies of TAK have been aimed at identifying specific biomarkers for disease activity, because vascular inflammation can persist in patients with a normal C-reactive protein (CRP) level and/or erythrocyte sedimentation ratio (ESR).

Previously, we observed high levels of IL-6 and MMP-9 in patients with TAK, which might represent useful markers to predict imaging outcomes and monitor disease progression (7). Noris and colleagues also reported that serum IL-6 levels showed close correlations with disease activity and suggested that this cytokine might contribute to vasculitic lesions in TAK (8). Park and coworkers investigated the serum profiles of inflammatory cytokines in TAK, including TNF-α, interferon (IFN)-γ, IL-6, IL-12, and IL-18. They found that serum IL-18 and IL-6 levels were elevated and that IL-18 could be a useful marker for monitoring disease activity (9). Recently, PTX3 was suggested to be a biomarker that reflects localised inflammation in TAK patients (10,11). Furthermore, a recent study reported that IL-6, IL-8, and IL-18, which have been associated with neutrophilic, pro-inflammatory responses, could be potential biomarkers for assessing disease activity in TAK (12).

As mentioned above, the immunopathology of TAK remains incompletely characterised. This study aimed to assess T cell subset distributions and immune cytokine profiles in untreated patients in TAK and to explore the immune mechanism that drives pathology in TAK. Based on our findings, we offer insights that may assist future studies of the immunopathogenesis and treatment of TAK.

Materials and methods

Patients and clinical assessments

A total of 50 untreated patients with TAK and 40 age-matched controls (20 untreated systematic lupus erythematosus (SLE), 20 healthy controls) were enrolled as Group 1 to detect serum cytokine profiles and T cell frequencies. Another nine patients with TAK (two GC [glucocorticoids]-treated before and seven untreated) and atherosclerosis patients treated with surgery were admitted as Group 2 for immunohistochemistry (IHC) analysis. All TAK patients fulfilled the American College of Rheumatology 1990 criteria for the classification of TAK (13) and were admitted from Zhongshan Hospital, Shanghai from January 1st, 2009 to September 1st, 2014. Clinical and laboratory data, including haemoglobin (Hb), white blood cells (WBC), platelets (PLT), ESR, and CRP, were recorded. The disease activity of patients with TAK was assessed using both the Kerr score (14) and Indian Takayasu Activity Score (ITAS) (15). The study protocol was approved by Ethics Committees of Zhongshan Hospital and conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee. All patients provided written informed consent for inclusion in this study.

H&E staining and immunohistochemistry

Specimens of the aortic valve (n=5) or ascending aorta (n=4) were obtained from nine patients with TAK. As controls, six valve specimens (including four aortic valve and two bicuspid valve) and three specimens of the aortic root were obtained from atherosclerotic patients. Each specimen was fixed in 10% neutral buffered formalin for 24 h at room temperature and embedded in paraffin. Sections (3-um thick) were cut, deparaffinised in xylene, and rehydrated in ethanol (100% ethanol for 2 min, followed by 95% ethanol for 2 min). Antigen retrieval was carried out using citrate buffer solution (0.01 mol/L, pH 6.0). Endogenous peroxidase activity was blocked by soaking slides in a solution of 3% H_2O_2 for 30 min at room temperature. Each slide was blocked with 75 μl goat serum and incubated for 30 min at room temperature. Subsequently, 100 μl diluted antibody (IFN-γ, IL-4, IL-6, IL-10, IL-12, IL-17, CD4, CD8, or Foxp3 antibody, all from Abcam) were added to each slide for 1 h at 37°C in a humidified chamber, then these slides were transferred from the chamber to 4°C overnight. The next day, slides were warmed to room temperature and 75 μl secondary antibody was added to each slide. Then, slides were incubated for 1 h at 37°C. Slides were developed with DAB (1 ml solution B, 20 μl solution C) by adding 25 μl to each slide; the reaction was monitored under a microscope to assess colour changes. The reaction was stopped by incubating slides in TBS buffer. Slides were counterstained with haematoxylin, dehydrated, and mounted with neutral gums. For IHC, each slide was washed with TBS (pH 6.5) three times for 5 min after each step, except for the serum blocking step. A slide was blocked with serum instead of primary antibodies as a negative control for each stain. Tissue sections are also stained with H&E for each patient.

For IHC analysis, positive cells were assessed and scored in four randomly chosen fields (magnification 400×) at inflammatory sites for each slide. A cell was counted as positive if staining was present on all or part of the cell surface membrane or in the cytoplasm. Based on the frequency of cells, findings could be classified as strongly positive (>75%), moderately positive (30–75%), weakly positive (1–30%), or negative (0%).

Flow cytometric analysis

Distributions of T cell subsets were determined in TAK patients (n=9) and age-matched healthy controls (n=12) in Group 1. PBMCs were isolated by standard Ficoll-Hypaque (Histopaque, Sigma) density gradient centrifugation. After 6 h stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin in the presence of brefeldin A, cells were stained with anti-CD4 (APC-H7, clone RPA-T4), anti-IL-4 (PerCP-Cy5.5, clone 8D4-8), anti-IFN-γ (APC, clone 4S.B3), and anti-IL-17 (PE, clone SCPL1362) antibodies, or alternatively were stained with anti-CD4 (APC-H7), anti-CD25 (PerCP-Cy5.5), and CD127 (PE). Normal mouse IgG antibody instead of primary antibody was used as an isotype control. PMA, ionomycin, and brefeldin A, as well as all primary antibodies and their isotype controls were purchased

Table I. Patient characteristics and cytokine profiles in the peripheral blood of Group 1.

Characteristics	TAK group (n=50)	Healthy group (n=20)	SLE group (n=20)
Sex ratio (female:male)	4:1	11:9	9:1
Age (years)	39.92 ± 14.70 (35.60, 44.23)3	31.8 ± 9.82 (27.21, 36.39)	38.25 ± 14.57 (31.43, 45.07)
Hb (g/L)	116.81 ±17.26 (111.74,121.88)	110 ± 15 (103.42,116.58)	102 ± 40.89 (83.38, 121.65)
WBC ($\times 10^9$/L)	7.73 ± 2.63 (6.96, 8.50)	6.5 ± 2.5 (5.4, 7.6)	6.32 ± 2.17 (5.30, 7.34)
PLT ($\times 10^9$/L)	269.60 ± 91.55 (242.72, 296.48)	196 ± 87.05* (157.85, 234.15)	221.75 ± 86.56* (181.24, 262.26)
ESR (mm/h)	53.54 ± 33.75 (43.63, 63.45)	8 ± 12.35* (2.58, 13.42)	38.45 ± 30.73 (24.07,52.83)
CRP (mg/L)	24.49 ± 29.21 (15.91, 33.07)	3 ± 6.5* (0.15, 5.85)	8.38 ± 15.26* (1.25, 15.53)
Cytokines (pg/ml)			
IFN-γ	–	2.39 ± 1.1 (1.86, 2.92)	–
IL-4	3.77 ± 0.87# (2.97, 4.58)	15.51 ± 2.17 (14.49, 16.52)	–
IL-6	14.61 ± 17.46# (9.75, 19.47)	1.09 ± 1.32 (0.41, 1.77)	5.78 ± 3.03 (4.10,7.46)
IL-10	12.34 ± 13.01 (6.85, 17.83)	–	11.69 ± 11.75 (6.47, 17.46)
IL-12p70	2.99 ± 1.35 (1.85, 4.12)	–	2.99 ± 0.98 (2.53, 3.45)
IL-17	1.69 ± 1.5# (1.20, 2.17)	18.66 ± 2.27 (17.60, 19.73)	–

*Significant differences ($p<0.05$) between data of the TAK and healthy or SLE groups.
#Significant differences ($p<0.05$) in IL-4, IL-6, and IL-17 levels between patients with TAK and healthy controls. – : Less than minimal detection levels.

Table II. General features of TAK patients in Group 2 and cytokine profiles in aortic tissues.

Number	Sex	Age (years)	Specimen	IFN-γ	IL-4	IL-6	IL-10	IL-12	IL-17
Active patients									
1	M	46	V	+++	+++	+++	–	+	+++
2	M	45	A	+++	–	++	+	+++	+++
3	M	46	A	+	–	+++	–	++	+
4	M	46	V	+	++	++	–	–	+++
5	F	48	V	+	+	+++	–	+++	+
6	M	39	V	+++	++	++	–	++	+++
Inactive patients									
1	M	60	V	–	–	++	–	++	+
2 (GC-treated)	F	32	A	–	–	+	–	++	–
3 (GC-treated)	F	25	A	–	–	+++	–	++	+

M: Male; F, female; A: aorta; V: aortic valve; +: weakly positive; ++: moderately positive; +++: strongly positive; – : negative.

from Becton Dickinson (San Jose, CA, USA). Stained cells were analysed using a Conto II flow cytometer (BD Biosciences). Data analysis was performed with Diva software (BD Biosciences) and FlowJo v.7.6.4 (Treestar Inc., Ashland, OR, USA). A minimum of 1×10^6 cells were analysed from each sample. Data were expressed as the percentage of positive cells (%).

ELISA assays

Serum was collected from each patient and controls of Group 1 and stored at –80°C until later use. Commercial ELISA kits were used to measure the levels of serum IFN-γ, IL-4, IL-6, IL-10, IL-12, and IL-17 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Flow cytometry and ELISA data were expressed as means ± SD. Differences between groups were analysed by Student's *t*-test. Correlation analysis was performed between serum cytokine levels and disease activity, which included clinical parameters such as the ESR, CRP, Kerr score, and ITAS score (analysed by Pearson's correlation analysis). All statistical analyses were performed using SPSS v.18.0 (Chicago, IL, USA). A p-value <0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics

Demographic and clinical characteristics of Group 1 are shown in Table I, and those of Group 2 are presented in Table II. All patients with TAK underwent magnetic resonance imaging of systemic arteries (MRA) at the time of diagnosis and corresponding angiographic findings were classified according to the International Takayasu Arteritis Conference of 1994 (16). A total of 17 (34.0%) of the 50 patients had type I, and 11 patients (22.0%) had type II, which included 5 type IIa (10.0%) and 6 type IIb (12.0%) patients. There were 4 patients (8.0%) who had type III, 3 (6.0%) who had type IV, and 15 (30.0%) who had type V. According to the Kerr score for disease activity assessment, 45 patients were in the active phase of disease and 5 were inactive.

Activation of the IL-6–IL-17 and IL-12–IFN-γ axes in aortic tissues

Pathological findings showed visible differences in IHC staining for samples from different patients with TAK (Table II; Fig. 1). IL-6 staining was strongly positive in three vascular layers for all TAK specimens, independent

of vascular changes. Other cytokines, such as IFN-γ, IL-12, and IL-17, were mainly positive in sites with inflammatory cell infiltrates. Expression levels of IL-4 and IL-10 were low. Most specimens from active patients exhibited positivity for IFN-γ, IL-6, IL-12, and IL-17. Inactive patients showed negative staining for IFN-γ and weakly positive staining for IL-17. Various expression levels of cytokines could also be detected in different specimens from atherosclerotic patients.

High levels of IL-6 in the peripheral blood of TAK patients

Mean serum IL-6 levels were significantly higher in patients with TAK compared with those of healthy controls; however, IL-4 and IL-17 levels were obviously lower in the TAK group than in the healthy control group ($p<0.05$). In the TAK group, levels of IL-10 and IL-12 tended to be higher than those of the healthy control group, whereas IFN-γ levels tended to be lower. No significant difference was observed for any cytokine level between the TAK and SLE groups. Additionally, levels of IFN-γ, IL-4, and IL-17 in the SLE group, IL-10 and IL-12 in the healthy control group, and IFN-γ in the TAK group were lower than the minimum detectable concentration for the ELISA kits (Table I).

More CD4⁺ T cells than CD8⁺ T cells infiltrated into inflammatory sites

Specimens from six active patients showed obvious inflammation other than fibrosis, whereas specimens from three inactive patients predominately showed increased wall thickness and collagen fibre hyperplasia (Fig. 2A and 2B). The muscular layer was injured in one specimen because of severe inflammation (Fig. 2C). Typically, inflammation was observed in the adventitia (Fig. 2D), in which newly formed vessels were surrounded by macrophages and CD4⁺ T cells (Fig. 2E-F). Most macrophages aggregated together and some formed multinuclear giant cells (Fig. 2E-F). As observed by IHC staining, many CD4⁺ cells existed in local lesions, especially in adventitia (Fig. 1). By contrast, CD8⁺ cells were distributed sparsely (Fig. 1), and few Foxp3⁺ cells were detected. Variable frequencies of CD4⁺ T cells and CD8⁺ T cells were distributed in specimens from atherosclerotic patients.

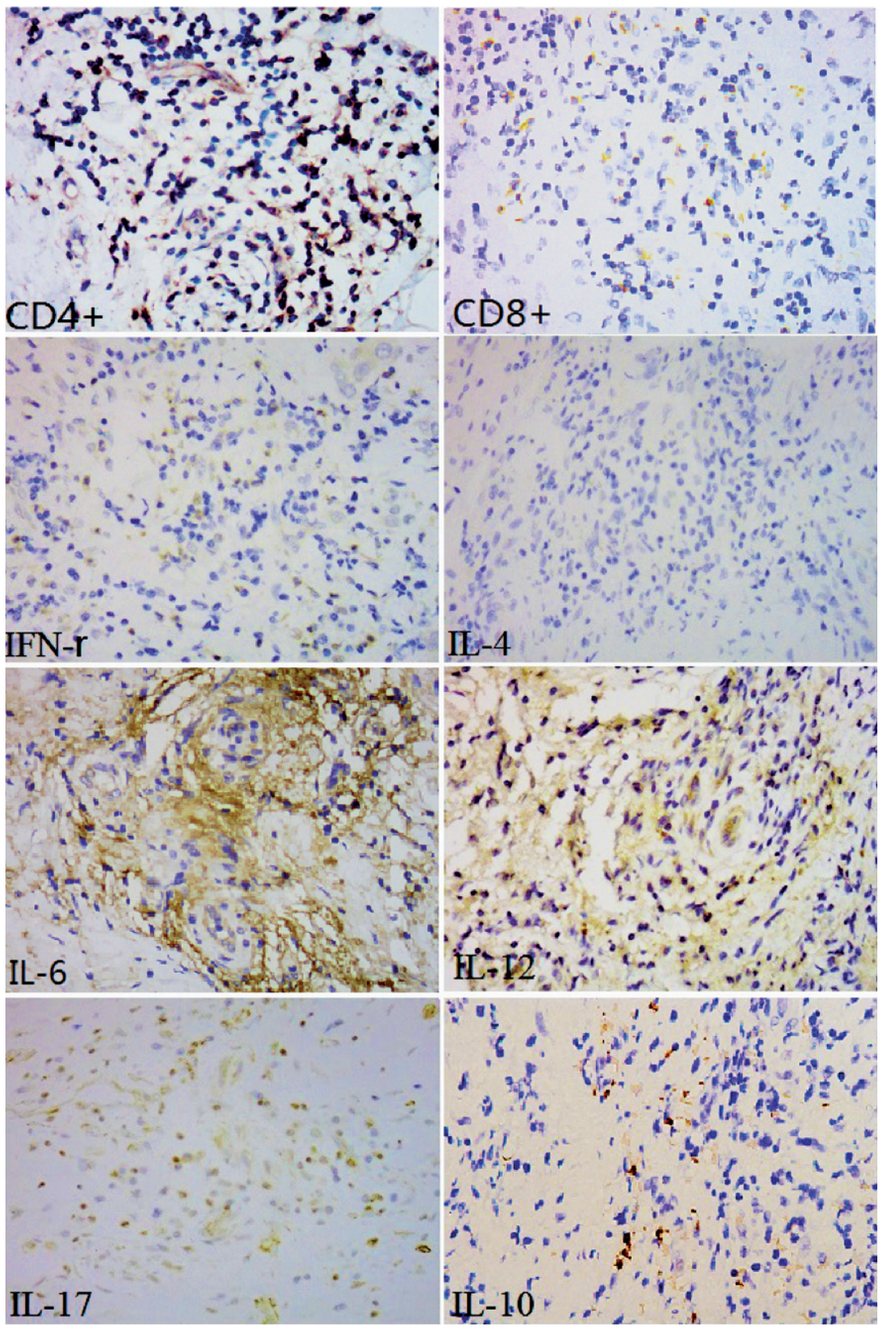

Fig. 1. Immunohistochemical staining of different markers in inflammatory sites of the second active patient with TA (Table II; magnification 400×). More CD4⁺ T cells than CD8⁺ T cells infiltrated local lesions. IL-6, IL-17, and IL-12 were strongly positive (+++), IFN-γ was moderately positive (++), while IL-10 was weakly positive (+). IL-4 was negative in local lesions (–).

Expanded CD4⁺ T cells in peripheral blood

The frequency of CD4⁺ T cells in the peripheral blood of TAK patients was higher than that in healthy controls ($p<0.05$). Frequencies of circulating IFN-γ-producing Th1 cells, IL-4-producing Th2 cells, and IL-17-producing T cells showed a trend to be expanded in TAK patients compared with healthy controls. By contrast, CD4⁺CD25⁺CD127⁺ Treg cell frequencies tended to be reduced in patients with TAK compared with healthy controls (Table III).

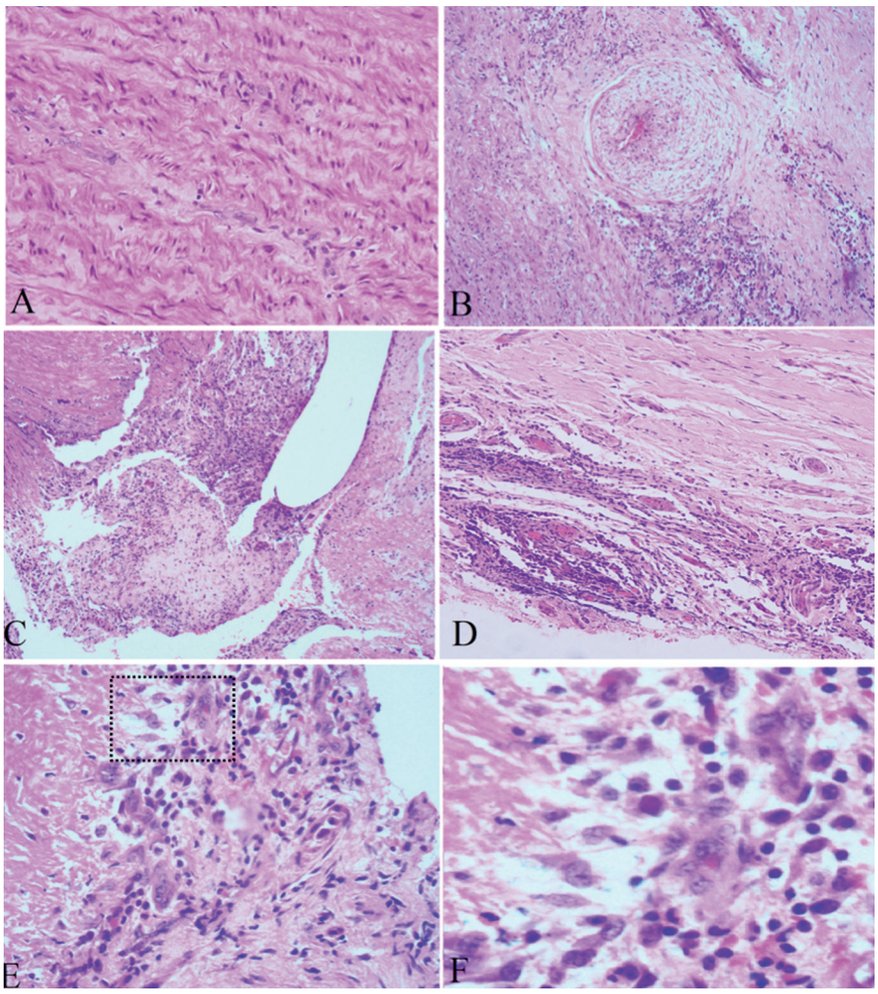

Fig. 2. Pathological manifestations of Takayasu arteritis (H&E staining).
Images with a magnification of 100× are shown in **B**, **C**, and **D**; images with a magnification of 400× are shown in **A** and **E**. In F, the image was cropped from panel **E** in the area marked by the black rectangle. **A**: There was extensive proliferation of collagenous fibre in the arterial media. **B**: Annular thickening of the vasa vasorum that was surrounded with inflammatory cells could be observed in the adventitia. **C**: Medial injury resulted from serious inflammation. **D**: Typical adventitial inflammation in the early stages of TA included newly formed vessels and pro-inflammatory cell infiltrates. **E** & **F**: Many macrophages, lymphocytes, and a few neutrophils/granulocytes existed in local lesions. Some macrophages aggregated together, which were inclined to form multinucleated giant cells.

Positive correlations between levels of IL-6 and the ESR, CRP, or Kerr score

Associations between serum IL-6 levels and clinical activity markers (ESR and CRP), activity scores (Kerr score and ITAS), and imaging types were analysed (Fig. 3). IL-6 levels were positively correlated with ESR, CRP, and the Kerr score ($p<0.05$). No associations between IL-6 levels or ITAS or imaging types were observed ($p>0.05$).

Discussion

TAK is an autoimmune disorder that is mediated by macrophages and T cells. However, no previous study has clarified the repertoire of disease-relevant T cells in TAK. Data from this study confirmed the proliferation of $CD4^+$ T cells in the peripheral blood and aortic tissues of untreated patients with TAK. The Th1 and Th17 subsets appeared not to be activated in the peripheral blood, as indicated by the low levels of effector cytokines (IFN-γ and IL-17). However, the excessive production of IL-6, IL-17, IL-12, and IFN-γ in local lesions indicated the expansion of Th1 and Th17 cells, which are two critical players in vasculitic pathogenesis. Differences in the cytokine production patterns in circulation and vasculitic lesions might contribute to the migration of T cells from the peripheral blood to local vascular tissues and subsequently to activation *in situ*. Furthermore, this phenomenon could also be related with different disease statuses in those patients and might indicate that cytokines in the peripheral blood are not always in accord with those in vasculitic lesions.

IL-17 is mainly produced by Th17 cells. Seen from our results, there was discrepant observation between serum IL-17 levels and tissue IL-17 signals, which can be explained from three aspects: Firstly, Takayasu arteritis is a large-vessel vasculitis with chronic inflammation, which dominates in local vascular tissue, while systemic inflammation is not obvious. Secondly, a comparable percentage of peripheral Th17 cells between TA patients and healthy controls implied an inactive state of peripheral Th17 subset in TA patients, which may directly caused low serum IL-17 levels. Thirdly, migration of T cells from peripheral blood to vascular tissue and

Table III. Percentages of $CD4^+$ T cell subsets in peripheral blood.

Percentage (%)	$CD4^+$ T*	Th1	Th2	Th17	Treg
Control group (n=12)	32.42 ± 6.42 (28.34, 36.50)	9.72 ± 5.14 (6.45, 12.99)	1.05 ± 0.67 (0.62. 1.48)	1.07 ± 0.74 (0.59, 1.54)	5.02 ± 1.64 (3.98, 6.07)
TAK group (n=9)	39.10 ± 6.84 (33.84, 44.36)	11.64 ± 5.81 (7.17, 16.11)	2.21 ± 1.92 (0.74, 3.69)	1.12 ± 0.97 (0.38,1.86)	4.57 ± 1.69 (3.27,5.87)

*Significant differences ($p<0.05$) in the percentage of $CD4^+$ T cells between the TAK and healthy groups.

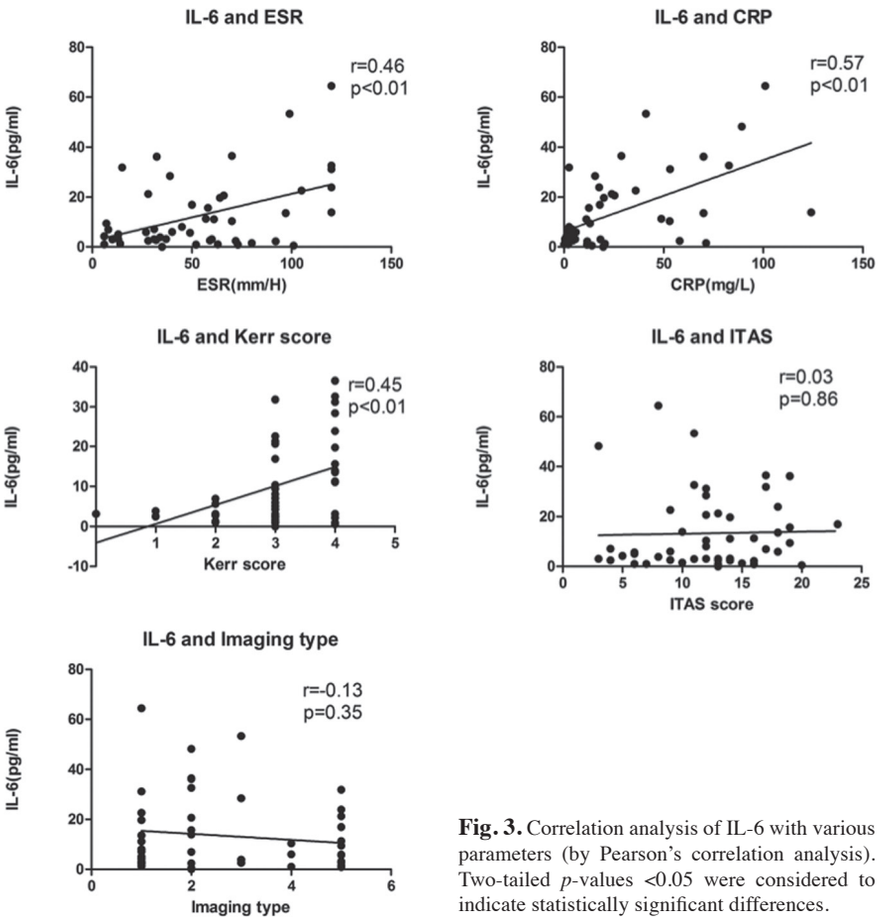

Fig. 3. Correlation analysis of IL-6 with various parameters (by Pearson's correlation analysis). Two-tailed *p*-values <0.05 were considered to indicate statistically significant differences.

differentiation of T cells to Th17 subsets induced by highly expressed IL-6 *in situ* contributed to strong IL-17 signals in local vascular. However, the specific underlying mechanism needs to be addressed in further studies.

The IL-6–IL-17 and IL-12–IFN-γ axes are two immune pathways that closely coincide with the presence of two T cell lineages, Th1 and Th17 cells. IL-6 is a pleiotropic cytokine that can promote T cell expansion and differentiation, especially for the Th17 cell subset. Currently, the consensus is that IL-6 is critical for Th17 cell differentiation. Excessive production of IL-17 in aortic tissues might result from enhanced expression of IL-6. Additionally, production of IL-12 by dendritic cells, macrophages, and various other immune cells is important for Th1 cell differentiation, which has been implicated in other granulomatous inflammatory diseases, such as sarcoidosis (17). From this perspective, the IL-6–IL-17 and IL-12–IFN-γ axes might play a critical role in vascular involvement, not only in GCA, but also in TAK. However, in contrast to GCA, IFN-γ expression was negative in the two GC-treated patients in this study, whereas staining for IL-6 and IL-12 remained positive, which might indicate that IL-6 and IL-12 are involved in vascular chronic fibrosis.

Based on the ELISA results, higher levels of IL-6, IL-10, and IL-12 were detected compared with healthy controls, particularly for levels of IL-6. The absence of significant differences in IL-6 levels between active and inactive patients with TAK might have resulted from a lower number of inactive TAK patients. Similar to our previous study, IL-6 levels were well correlated with disease activity parameters, such as ESR, CRP, and the Kerr score. IL-6 is an acute phase protein, which is produced by not only monocytes and lymphocytes, but also by fibroblasts. Highly activated infiltrating macrophages not only occupied the vessel wall, but they also circulated in the blood, resulting in high levels of serum IL-6. Diffuse positive areas of IL-6 were observed not only around inflammatory cells, but also in tissue cells of the aorta, which might indicate that fibroblasts or smooth muscle cells could also be sources of IL-6 in the pathogenesis of TAK. Stimulation of Toll-like receptors on advential fibroblasts that recognise distinct pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) can induce IL-6 expression. In previous studies, exogenous bacteria, such as tuberculosis and/or modified endogenous antigens, were reported to be closely linked to the occurrence of TAK (6, 18). Thus, it can be inferred that in early stages of TAK pathogenesis, monocytes and fibroblasts might be activated via TLR pathways. Furthermore, the production of high levels of IL-6 further promoted inflammation in the vascular wall. Therefore, IL-6 plays a crucial role in the initiation of TAK and could be a sensitive indicator of disease its early stages.

IL-10 is a prototypical anti-inflammatory cytokine that is primarily produced by macrophages and Treg cells. IL-10 has multiple effects on immunoregulation and inflammation, as it can inhibit the activation of macrophages and down-regulate the expression of Th1 cytokines. The slightly higher serum levels of IL-10 in TAK might regulate systemic inflammation in a feedback-dependent manner. In most aortic tissues, IL-10 expression was negative, which was consistent with the low frequencies of peripheral Treg cells. As a cytokine that is mainly produced by Th2 cells, IL-4 levels were lower in peripheral blood and were lowly expressed in local lesions. Thus, the Th2 subset was not prominent in the pathogenesis of TAK.

In the early stages of TAK, most patients showed no symptoms of obvious pathological changes, and those that occurred were confined to vascular lesions and accompanied with inflammation and chronic fibrosis. Thus, pathological studies might provide further insights into understanding the pathogenesis of this disease. Abundant levels of IL-6 were produced in three layers of vascular specimens, and in specimens with fibrosis, IL-6 (rather than other cytokines) was most prominent,

suggesting that IL-6 might contribute to chronic fibrosis of the vascular wall. Previous studies also suggested that IL-6 can drive fibrosis in unresolved chronic inflammatory disease (19), but the specific interactions between IL-6 and tissue cells in TAK need to be better characterised. Excess amounts IL-17 and IL-12 were detected around vessels that were newly formed in adventitia, whereas increased numbers of infiltrating $CD4^+$ and $CD8^+$ T cells were observed. Thus, various cytokines participate in the pathogenesis of TAK.

However, pathological changes could not be evaluated in patients without surgical treatment. A general assessment of disease can only be estimated based on the levels of serum cytokines. Levels of IL-6 were strongly correlated with TAK disease activity and could be detected in peripheral blood at an early stage. Therefore, it might be a sensitive biomarker for monitoring disease activity and assisting the diagnosis of TAK. What is more, recent studies have proved that anti-IL6 receptor monoconal antibody tocilizumab (TCZ) was effective in inflammatory aortitis refractory to corticosteroids or to other biologic immunosuppressive drugs (20). IL-6 can also be a promising therapeutic target in refractory patients with TA. Nevertheless, because the low TAK disease incidence makes it difficult to collect a large numbers of patients and perform detailed studies, the peripheral and pathological manifestations described above need to be verified in more cases of TAK.

Conclusions

Diverse $CD4^+$ T cell subsets and various cytokines participated in the initiation and development of TKA. Significantly increased levels of IL-6 were detected in peripheral blood and aortic tissues of untreated patients. IL-6 might be a sensitive biomarker for assessing disease activity and could play a vital role in the pathogenesis of TAK.

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