Concise Report

Serum cytokine profiles and their correlations with disease activity in Takayasu's arteritis

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Objective. To investigate serum profiles of inflammatory cytokines in patients with Takayasu's arteritis (TA) and to determine their correlations with disease activity of TA.

Methods. Forty-nine patients with TA and 12 age- and sex-matched controls were studied. Blood samples were obtained and were divided into active and stable disease groups. Paired blood samples were available in 19 patients at the active stage before treatment and at the remitted stage after treatment. Serum tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, IL-12 and IL-18 levels were determined by enzyme-linked immunosorbent assay.

Results. Serum TNF-α, IL-6 and IL-18 levels of patients with TA were significantly higher than those of controls ($P < 0.05$), but IFN-γ and IL-12 levels were not. Serum IL-6 and IL-18 levels were significantly higher in the active disease group than in the stable disease group ($P < 0.05$), but the levels of TNF-α were not different between the groups. In the 19 patients with paired samples, serum IL-18 levels at the remitted stage after treatment were significantly decreased compared with the active stage before treatment ($P < 0.001$). The changes in IL-18 levels between active and remitted stages correlated well with changes in erythrocyte sedimentation rate ($P < 0.001$).

Conclusion. Serum IL-18 and IL-6 levels were elevated in patients with TA, especially in those with active disease. Serum IL-18 levels correlated well with disease activity of TA. These results suggest that IL-6 and IL-18 might contribute to the pathogenesis of TA and that IL-18 could be a useful marker for monitoring disease activity of TA.

KEY WORDS: Takayasu's arteritis, Cytokines, Interleukins.

Takayasu's arteritis (TA) is a chronic granulomatous vasculitis that involves large vessels such as the aorta and its branches [1]. Vascular inflammation of TA originates in the vasa vasorum and is followed by infiltration of various inflammatory cells, leading to the formation of granulomas. At this stage, the production of inflammatory mediators is markedly increased [2, 3]. Previous studies have reported increased expression of interleukin (IL)-1 and IL-6 in aortic tissues from patients with TA, which were locally produced at the site of inflammation in TA [4], and serum levels of IL-6 correlated well with the disease activity of TA [5]. A recent report showed up-regulated mRNA expression of TNF-α from peripheral blood mononuclear cells (PBMCs) from patients with TA [6].

TA has a feature of granuloma formation on vessel walls, and several proinflammatory cytokines, including tumour necrosis factor (TNF)-α, interferon (IFN)-γ, IL-6, IL-12 and IL-18, are known to be associated with granuloma formation in inflammatory diseases, such as pulmonary tuberculosis, giant cell arteritis and Wegener's granulomatosis [7–12]. However, studies on the comprehensive profiles of these cytokines and on the changes in their levels along with disease activity of TA are lacking. Moreover, the use of biological agents that block the inflammatory cytokines has not been established in TA.

To investigate serum profiles of inflammatory cytokines that are involved in granuloma formation and their correlations with disease activity in TA, and to provide evidence for the use of biological agents in TA, we evaluated serum TNF-α, IFN-γ, IL-6, IL-12 and IL-18 levels in patients with TA.

Patients and methods

Patients and clinical assessments

Forty-nine patients with TA fulfilling the American College of Rheumatology 1990 criteria for the classification of TA [13] and 12 age- and sex-matched healthy controls were studied. All patients with TA were divided into two subgroups according to their disease activity at study enrolment: an active disease group ($n = 34$) and a stable disease group ($n = 15$). The disease activity was assessed using the National Institutes of Health criteria for active disease, as suggested by Kerr *et al*. [1]. These criteria include systemic features, such as fever and musculoskeletal symptoms; an elevated erythrocyte sedimentation rate (ESR; reference value <15 mm/h in men and <20 mm/h in women); features of vascular ischaemia or inflammation, such as claudication, diminished or absent pulse, bruit, vascular pain, and blood pressure difference

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in either upper or lower extremities; and typical angiographic findings. New onset or worsening of two or more features was defined as active disease, and reduction of symptoms and signs or complete resolution of clinical features were defined as stable disease. Among the 34 patients who initially had an active disease, 19 were followed for the remainder of the study, with a mean duration of 10.9 ± 6.9 months. All of the 19 patients were treated with glucocorticoid, while a subset of 11 were also given immunosuppresive agents, such as methotrexate ($n=9$) or azathioprine ($n=2$). After the follow-up period, remissions were achieved in the 19 patients and their paired blood samples were obtained. Achievement of remission was defined as having a stabilized disease for more than 6 months in patients who previously had active disease.

Angiographic assessment

All patients with TA underwent aortic angiography at the time of diagnosis and the angiographic findings were classified according to the International Takayasu's Arteritis Conference of 1994 [14]. This classification was as follows: type I, involvement of the main branches from the aortic arch; type IIa, involvement of the ascending aorta, aortic arch and its branches; type IIb, involvement of the ascending aorta, aortic arch and its branches, thoracic descending aorta; type III, involvement of the thoracic descending aorta, abdominal aorta, and/or renal arteries; type IV, involvement of the abdominal aorta and/or renal arteries; and type V, the combined features of type IIb and IV. Sixteen (32.7%) of the 49 patients had type I and three (6.1%) had type II, which included one type IIa (2.0%) and two type IIb (4.1%). Five patients (10.2%) had type III, 10 (20.4%) had type IV and 15 (30.6%) had type V.

Measurement of cytokines and acute-phase reactants

Blood samples were obtained from all patients and controls. Serum specimens were separated by centrifugation at 3000 r.p.m. for 7 min and stored at -20°C until assayed. Commercial enzyme linked immunosorbent assay (ELISA) kits were used for the measurement of serum TNF-α, IFN-γ, IL-6, IL-12 (R&D Systems, Minneapolis, MN, USA) and IL-18 levels (Medical and Biological Laboratories, Nagoya, Japan) according to the manufacturer's instructions. The white blood cell (WBC) count, ESR (modified Westergren method) and C-reactive protein (CRP) levels were measured at the same time point as the collection of samples for measurement of cytokine levels.

Statistical analysis

The t-test, χ^2 test and analysis of variance with multiple comparisons were used to evaluate the differences in demographic, laboratory and angiographic features between the study groups. Mann–Whitney U-tests were used to evaluate the differences in serum cytokine levels between groups and between stages, and the correlation coefficient was obtained by Pearson's correlation test. P values less than 0.05 were considered statistically significant.

Results

Subject characteristics

Demographic, laboratory and angiographic characteristics of subjects are summarized in Table 1. Mean ESR of the patients with active disease was significantly higher than those of the patients with stable disease and the controls ($P < 0.01$). However, the mean ESR level of those with stable disease was not significantly different from that of controls. Sex distribution, mean age, CRP levels, WBC counts, mean disease duration from diagnosis of TA to study enrolment, and angiographic types were not different between those with active disease and those with stable disease. In 19 patients who were followed for the remainder of the study, ESRs measured after the follow-up period were significantly decreased compared with those measured initially

TABLE 1. Characteristics and serum cytokine levels of patients with TA and controls at baseline and after follow-up period

	At baseline			After follow-up
	Active group	Stable group	Controls	
n	34	15	12	19
Age (years)	33.4 ± 13.0	34.1 ± 12.0	33.3 ± 10.0	34.1 ± 13.3
Males/females	2/32	1/14	1/11	1/18
Disease duration (months)	29.4 ± 27.1	31.4 ± 22.9	–	39.9 ± 27.7
Laboratory findings				
ESR (mm/h)	44.4 ± 19.0*¶††	12.5 ± 8.8¶	9.1 ± 4.3*	17.2 ± 9.6††
CRP level (mg/dl)	1.4 ± 1.0	0.5 ± 0.5	0.3 ± 0.2	0.8 ± 0.5
WBC count (/mm^3)	$11\,500 \pm 5990$	8880 ± 4410	6900 ± 2110	9270 ± 5020
Angiographic classification				
Type I	11 (32.6%)	5 (33.3%)	–	–
Type IIa	1 (2.9%)	0 (0%)	–	–
Type IIb	1 (2.9%)	1 (6.7%)	–	–
Type III	3 (8.8%)	2 (13.3%)	–	–
Type IV	7 (23.5%)	3 (20.0%)	–	–
Type V	10 (29.4%)	5 (33.3%)	–	–
Serum cytokine levels				
TNF-α (pg/ml)	21.0 ± 10.3*	14.5 ± 9.9‡	2.2 ± 1.1*‡	17.5 ± 9.1
IFN-γ (pg/ml)	7.4 ± 4.6	7.7 ± 4.1	2.6 ± 1.0	5.9 ± 3.2
IL-6 (pg/ml)	54.3 ± 21.2†¶	14.7 ± 5.5‡¶	3.21 ± 1.0†‡	39.3 ± 21.8
IL-12 (pg/ml)	10.6 ± 6.1	9.3 ± 5.1	3.0 ± 0.6	7.7 ± 4.8
IL-18 (pg/ml)	850.0 ± 211.1†**††	378.7 ± 154.1§**	98.3 ± 21.4†§	390.0 ± 116.5††

Data are mean $\pm$ s.d. and comparisons between baseline measurements and follow-up measurement were made only in 19 patients with paired samples. ESR, normal value <15 mm/h in men and <20 mm/h in women; CRP, normal value: <0.8 mg/dl; WBC count, normal value $<10\,800$/mm^3. *$P < 0.05$ active group *vs* controls; †$P < 0.001$ active group *vs* controls; ‡$P < 0.05$ stable group *vs* controls; §$P < 0.001$ stable group *vs* controls; ¶$P < 0.05$ active group *vs* stable group; **$P < 0.001$ active group *vs* stable group; ††$P < 0.001$ initial measurement *vs* follow-up measurement.

($P < 0.001$), but CRP levels and WBC counts were not different between initial measurements and follow-up measurements.

Cytokine levels in patients with TA and controls at baseline

Mean TNF-α, IL-6 and IL-18 levels of patients with TA were significantly higher than those of controls ($P < 0.05$), but mean IL-12 and IFN-γ levels were not (Table 1). At baseline, IL-18 levels of patients with TA correlated well with ESR levels ($P < 0.05$), but not with age, disease duration, CRP level and WBC count. Baseline TNF-α, IFN-γ, IL-6 and IL-12 levels of patients with TA did not correlate with any of the demographic and laboratory parameters. The differences in TNF-α, IL-6 and IL-18 levels according to the angiographic class were not significant. However, although statistical significances were not found, IL-6 and IL-18 levels of patients with TA who had angiographic class V, which represent more diffuse disease, showed a tendency to increments compared with those of patients with TA who had angiographic class I, which represents a localized disease ($P = 0.083$ for IL-6 and $P = 0.069$ for IL-18). No significant correlation was found among levels of each cytokine at baseline.

Cytokine levels and disease activity

Mean IL-6 and IL-18 levels of the patients with active disease were significantly higher than those of the patients with stable disease ($P < 0.05$), but mean TNF-α, IFN-γ and IL-12 levels were not different between them (Table 1).

In 19 patients who had been given glucocorticoids and/or an immunosuppressive agent, IL-18 levels at the remitted stage after treatment were significantly decreased compared with those at the active stage before treatment ($P < 0.001$). However, the decreases in IL-18 levels were not significantly different between those who had been given glucocorticoids alone and those who had been given glucocorticoids and an immunosuppressive agent. TNF-α, IFN-γ, IL-6 and IL-12 levels were not different between stages (Table 1). The changes in ESRs between the active and remitted stages correlated well with those of serum IL-18 levels ($r = 0.61$, $P < 0.001$), but not with those of serum TNF-α, IFN-γ, IL-6 and IL-12 levels (Table 2).

Discussion

TNF-α, IFN-γ, IL-6, IL-12 and IL-18 are known to be involved in the pathogenesis of several inflammatory diseases that feature granuloma formation [7–12]. In pulmonary tuberculosis, TNF-α, IFN-γ, IL-12 and IL-18 contribute to immunity against myco-bacterial infection and lead to the formation of granulomas [7, 8]. Also, in giant cell arteritis and Wegener's granulomatosis, tissue production of IL-2, IL-6, TNF-α and IFN-γ was increased and serum levels correlated with disease activity [9–12], suggesting their pathogenic roles in the diseases.

In this study, we investigated serum cytokine profiles in TA, a chronic granulomatous vasculitis, and evaluated their potential use in understanding disease pathogenesis and monitoring disease activity. We found that the patients with TA had elevated TNF-α, IL-6 and IL-18 levels compared with controls and those with active disease had further elevated IL-6 and IL-18 levels than those with stable disease, suggesting the possible role of these cytokines in pathogenesis of TA. Our data are in agreement with previous cross-sectional studies that showed an increase in IL-6 in patients with TA and its strong association with disease activity of TA [4, 5]. Moreover, the significant elevation of the TNF-α level in patients with TA observed in the present study is also in agreement with a recent report by Tripathy et al. [6] showing increased constitutional expression of TNF-α in PBMCs of 10 patients with TA. They also found increased stimulated expression of IFN-γ in patients who had apparent stable disease. However, in the present study we could not document the elevation of IFN-γ in patients with TA, even in those with active disease, and our finding is supported by a previous report by Seko et al., which showed a lack of IFN-γ gene expression in aortic tissues from patients with TA [4].

We also found a marked reduction in serum IL-18 levels after treatment, as well as its significant correlation with ESR. These findings indicate that the measurement of IL-18 levels can be used for monitoring disease activity in the clinical setting. IL-18, originally identified as a factor that collaborates with IL-12 to induce IFN-γ production, is a potent inducer of the inflammatory mediators of T lymphocytes. IL-18 and IL-12 may have a synergistic effect to induce increased production of proinflammatory cytokines, including IFN-γ, TNF-α and IL-12 [15, 16]. In contrast, it has also been suggested that IL-18, in the absence of IL-12, enhances the cytotoxic activity of natural killer cells, leading to Fas-mediated apoptosis and tissue damage [17, 18], and the latter phenomenon was found in the pathology of tuberculous granuloma [19]. Our study is the first showing a significant association of IL-18 levels with disease activity of TA. Thus, we sought to determine whether the prominent action of IL-18 in TA is the induction of proinflammatory cytokines by collaboration with IL-12 or the induction of cytotoxic activity in the absence of IL-12, and found a lack of significant elevations of IL-12 and IFN-γ in patients with TA and the absence of their correlations with disease activity or IL-18 levels. This finding supports the idea that the latter action of IL-18, which is exerted through the activation of natural killer cells and triggering apoptosis, might be involved in pathogenesis of TA. However, we can not exclude the role of the Th1 response in TA, because other proinflammatory cytokines, such as TNF-α and IL-6, were up-regulated in patients with TA and the latter cytokine also correlated well with disease activity in a cross-sectional manner, indicating an additional role of these cytokines in TA. Further studies into these associations may prove to be informative for understanding the pathogenesis of TA.

TABLE 2. Correlations of the changes in cytokine levels with the changes in acute-phase reactants levels between at active stage before treatment and at remitted stage after treatment in 19 patients with paired samples

Changes in cytokine levels	Change in ESR		Change in CRP levels		Change in WBC counts	
	r	P	r	P	r	P
TNF-α (pg/ml)	0.132	0.137	0.005	0.232	0.196	0.112
IFN-γ (pg/ml)	0.094	0.171	0.015	0.213	0.088	0.199
IL-6 (pg/ml)	0.213	0.097	0.117	0.150	0.210	0.082
IL-12 (pg/ml)	0.111	0.119	0.254	0.087	0.089	0.201
IL-18 (pg/ml)	0.610	< 0.001	0.186	0.177	0.109	0.162

Results are the correlation coefficients and P values calculated using Pearson's correlation test, and the changes refer to the changes in each parameter measured between the active stage before treatment and the remitted stage after treatment.

Previous attempts to identify the pathogenic role of cytokines in TA have been limited, and therefore there has been little evidence supporting the use of biological agents for the treatment of TA [20]. Our data indicate that IL-18 and IL-6 might be involved in the pathogenesis of TA and that measuring their levels, particularly IL-18 levels, would be a useful marker for following disease activity and monitoring the therapeutic response in TA. Furthermore, our findings provide the possibility that patients with TA, particularly those with active disease, might benefit from biological therapy that blocks these inflammatory cytokines.

Rheumatology	Key messages
	• Serum IL-18 and IL-6 levels were elevated in patients with Takayasu's arteritis, especially in those with active disease.
	• Serum IL-18 levels correlated well with disease activity of Takayasu's arteritis.

There are no competing interests for any of the authors.

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