Interleukin-8 in Takayasu's arteritis: Plasma levels and relationship with disease activity

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ABSTRACT

Objective. *To investigate the plasma levels of interleukin-8 (IL-8) in Takayasu's arteritis (TA) and their relationship with disease activity.*

Methods. *IL-8 levels were detected by quantitative enzyme-linked immunosorbent assay (ELISA) in the plasma of 53 TA patients, 25 age/sex-matched healthy controls and of 10 serially followed up active TA patients on immunosuppressive therapy.*

Results. *Significantly increased levels of IL-8 were observed in TA patients (26.32±48.96 pg/ml) compared to controls (6.0±2.45 pg/ml) ($p = 0.0006$) and in patients with active TA (55.0±71.43 pg/ml) compared to those with an inactive disease (8.94±6.35 pg/ml) ($p = 0.0001$). The increased levels of the chemokine were present in 37% (20/53) of the patients compared to 8% (2/25) of the controls ($p < 0.01$) and in 80% (16/20) of patients with active TA compared to 12% (4/33) of those with an inactive disease ($p < 0.0001$). In the follow-up study, the plasma levels of IL-8 were normalized in 6/10 of the patients and the disease in 5 of these 6 patients was also observed to undergo remission.*

Conclusion. *These results suggest that IL-8 may play an important role in the pathogenesis of TA.*

Introduction

Takayasu's arteritis (TA) is a chronic granulomatous pan-arteritis characterized by stenosis, occlusion or sometimes aneurysm of the large elastic arteries – mainly the aorta and its major branches, including the pulmonary and coronary arteries. It is an autoimmune disease and both cellular and humoral immune mechanisms are involved in its pathogenesis (1).

A key initial event in the development of TA is vascular infiltration of different inflammatory cell types including mainly lymphocytes, monocytes/ macrophages and neutrophils, which are considered to cause tissue damage that eventually culminates in stenosis or aneurysm of the blood vessels, leading to different clinical manifestations of the disease (2,3). Chemotactic cytokines produced at the site of inflammation by vascular cells and or inflammatory cells may play a crucial role in mediating the migration of circulating leukocytes into the vascular wall and thereby in the initiation and perpetuation of the disease. Interleukin-8 (IL-8) is a potent chemoattractant and activating factor for neutrophils (4), lymphocytes (5) and monocytes/macrophages (6), the main cell types that constitute the vascular infiltrate in TA and thus may be fundamentally involved in the pathogenesis of the disese. However, the role of IL-8 in TA has not been elucidated till now.

Therefore, we undertook this study to investigate the plasma levels of IL-8 in patients with TA and healthy controls and in follow-up TA patients in order to determine the role of this chemotactic cytokine in TA and its relationship with disease activity.

Patients and methods

Subjects

Fifty-three patients with TA (14 males, 39 females; mean age 29 ± 13 years) were included in the study. All the patients fulfilled the American College of Rheumatology Criteria for TA and had an angiographically proven disease (7). Disease activity in the patients was determined by the following criteria: (i) systemic features such as fever, arthralgias, myalgias or weight loss of unknown cause; (ii) carotidynia (painful arteries); (iii) elevated erythrocyte sedimentation rate (ESR) (>30 mm/hr); and (iv) elevated C-reactive protein (CRP) (> 0.6 mg/dl) level. A patient was considered to be in the active stage

if 2 or more of these criteria were present along with other features of the disease (8). Accordingly 20 patients had active and 33 had inactive disease. Control subjects consisted of 25 age- and sex-matched healthy individuals. Most of the controls were individuals residing in the same area, as of the patients while some were paramedical staff of the Institute. After informed consent 2.5 ml of heparinized blood was obtained from each individual, plasma isolated within one hour of sample collection as per standard procedure and was stored at –80ºC until analysis.

All patients with active disease were put on an immunosuppressive therapy consisting of prednisolone and azathioprine, which we generally give for two years with tapering of their doses as the disease become less active. Plasma samples of 10 of these patients who regularly visited our clinic were obtained as above at every 3-month interval and stored at –80ºC. A follow-up study was carried out in these samples to evaluate the relationship of plasma levels of IL-8 with disease activity.

Quantitative IL-8 Assay by ELISA

The plasma levels of IL-8 were detected by quantitative enzyme linked immunosorbent assay (ELISA) using commercially available kit (Human IL-8 OptEIA™, Pharmingen, San Diego, CA, USA) as per the manufacturer's instructions.

The mean zero standard absorbance was subtracted from the mean of each set of duplicate standards and test samples to obtain the specific optical density (OD) for each sample. The quantity of IL-8 present in each test sample was estimated from standard curves drawn by plotting known concentrations of IL-8 versus their OD values using SPSS 9.0 software. Results were presented as the concentration of IL-8 in ρg/ml. A test sample was considered to contain an increased level of IL-8 if its value exceeded the mean + 2SD of the IL-8 concentrations of the normal controls. The lowest detection limit of the assay was 2.0 pg/ml with an intra-assay and inter-assay coefficient of variation of < 5% and < 8.5%, respectively.

Statistical analysis

Statistical analysis was performed using Z statistics for parametric data and Man Whitney U-test for non-parametric data. A p value of <0.05 was considered to be statistically significant.

Results

Significantly increased plasma levels of IL-8 were observed in patients with TA (26.32 ± 48.96; range: 4-280 pg/ml) compared to controls (6.0 ± 2.45; range: 4-26 pg/ml) ($p = 0.0006$) and in patients with active disease (55.0 ± 71.43; range: 6-280 pg/ml) compared to those with an inactive disease (8.94 ± 6.35; range: 4-38 pg/ml) ($p = 0.0001$) (Fig. 1).

The increased plasma levels of the chemokine were present in 37% (20/53) of TA patients and in 8% (2/25) of the healthy controls ($p < 0.01$). In patient group 80% (16/20) of the patients with active TA as compared to 12% (4/33) of those with an inactive disease ($p < 0.0001$) had increased levels of the IL-8.

The follow-up study showed that IL-8 levels in 6 out of 10 patients (60%) returned to normal levels in 6 to 12 months period of follow-up. In 5 of these six patients with normalized levels of IL-8, the disease was also observed to undergo remission. The remaining 4 patients who had elevated IL-8 during the given follow-up period, also continued to have an active disease (Table I).

Discussion

We have observed increased levels of IL-8 in patients with TA compared to controls and in patients with active TA compared to inactive TA, showing a relationship of this chemokine with disease activity. To the best of our knowledge this is the first report on IL-8 in the disease.

IL-8 either at the protein or mRNA levels has not previously been studied in TA, but a recent report by Noris *et al.* (9) showing increased serum level of RANTES (regulated on activation, nor-

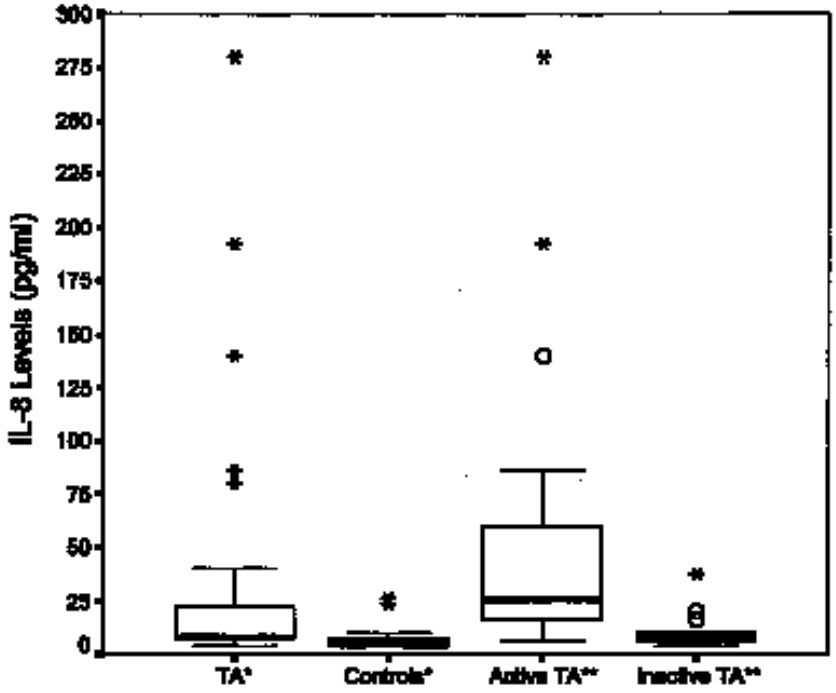

Fig. 1. Box plots showing plasma levels of IL-8 in Takayasu's arteritis (TA) patients (n = 53), healthy controls (n = 25), patients with active TA (n = 20) and patients with inactive TA (n = 33). The box includes observations from the 25th to the 75th percentiles. The horizontal line within the box represents the median value. The upper and lower lines outside the box represent the highest and lowest values, respectively. The circles and asterisks represent outlier and extreme values of IL-8 in different subject groups. The plasma levels of IL-8 were significantly higher in patients with TA as compared to controls (*p = 0.0006), and in patients with active TA as compared to those with inactive TA (**p = 0.0001).

Table I. Relationship of IL-8 levels with laboratory measures of disease activity *viz.* C-reactive protein (CRP; normal value: < 0.6 mg/dl) and erythrocyte sedimentation rate (ESR; normal value; < 30 mm/h) in 10 follow up patients treated with immunosuppressive therapy.
(The cut off value for IL-8 quantity: 10.9; calculated as the mean + 2 SD of the controls)

Pt.	Pre-treatment	On last follow-up				
	IL-8 levels (pg/ml)	IL-8 levels (pg/ml)	CRP (mg/dl)	ESR (mm/h)	Disease activity	Follow-up period (mos.)
1	320	8	0.522	22	Inactive	12
2	128	10	< 0.5	21	Inactive	14
3	72	8	< 0.5	14	Inactive	6
4	35	8	0.529	18	Inactive	16
5	18	8	0.5	12	Inactive	12
6	18	8	1.22	36	Active	18
7	16	38	1.5	54	Active	13
8	38	24	3.02	39	Active	6
9	108	24	0.8	40	Active	6
10	16	310	2.9	55	Active	12

mal T-cell expressed and secreted) and its correlation with disease activity suggests the role chemotactic cytokines in the disease. Among the other vasculitides, increased levels of IL-8 have previously been reported in Kawasaki disease (KD) (10) and Behçet's disease (BD) (11), supporting the involvement of IL-8 in the pathogenesis of vasculitis.

In KD patients with coronary aneurysm, the levels of IL-8 during the acute stage of the disease have been shown to highly correlate with those of CRP and ESR (10). In Behçet's disease, similar to our observation, its prevalence and levels were shown to be significantly higher in patients with active disease as compared to those with quiescent disease (11). In a follow-up study of patients with BD, it has been shown that the levels of IL-8 decline with the therapy and were considered to be a more reliable marker than ESR and CRP (12). We have also prospectively investigated IL-8 levels in 10 active TA patients who were on immunosuppressive therapy. It was observed that in 6 of these patients, IL-8 levels became normal within the 6-12 month period of follow-up and all patients except one with normalized levels of the chemokine also showed a regression of disease activity. The remaining 4 patients, in whom IL-8 levels remained elevated during follow-up, also continued to have active disease.

There are reports in the literature that clinical and laboratory measures of disease activity do not always reflect the true activity of the disease and patients considered to be clinically in remission may have active/progressive disease (13,14). These studies have demonstrated that the "gold standard" for inactive disease is the absence of new vascular lesions when patients are clinically in remission, and conversely the most definitive proof for an active disease is the appearance of new lesions in the involved vaculature. We have previously demonstrated that systemic symptoms and laboratory parameters of disease activity are resolved within a 3-month period and, as evidenced by a follow-up angiogram of the patients, disease progression is halted with no appearance of new lesions after a one-year immunosuppressive regimen consisting of prednisolone and azathioprine (15). Thus, although we have not evaluated a direct relationship of IL-8 levels with vascular imaging in the follow-up patients in this study, in view of the above data our classification of active and inactive disease appears to be correct. However, on the basis of the present data we do not claim that IL-8 is a strict marker of disease activity in TA, for which a study of larger cohort of follow-up patients demonstrating a relationship of this chemokine with angiographic as well as histologic findings of the disease will be required.

The increased levels of IL-8 in the disease could be derived from inflamed endothelium of the affected vessels or from systemic activation of circulating inflammatory cells, particularly the monocyte/macrophages, both of which are important sources of this chemokine. The expression of heat shock protein-65, HLA II and adhesion molecules (VCAM-1 and ICAM-1) in the aortic tissues and increased levels of soluble VCAM-1 in TA (2, 16), together indicate that the arterial endothelium is in an activated state in the disease and thus could be an important source of IL-8. The accumulation of different inflammatory cells in the vascular wall (2,3) itself implies that from this site the mediators, which are chemotactic to such cells, are being produced. The infiltrating monocytes/macrophages, following their autocrine activation by IL-8 present in the local milieu, may also contribute to the elevated pool of this chemokine. An increased *in vitro* production of IL-8 by endothelial cells has been shown following treatment with sera of Behcet's disease, a vasculitis demonstrated to increase circulating levels of this chemokine (17). The important stimuli involved in the induction of IL-8 in TA may include anti-endothelial cell antibodies and anti-monocyte antibodies, which have been reported to induce *in vitro* production of different cytokines and chemokines by activating endothelial cells and monocytes, respectively (18, 8)

IL-8 could have several important implications in TA. Its local production by endothelial cells may be an initial event in the recruitment of neutrophils (4), T-cells (5) and monocyte/macrophages (6), the main cell types that constitute the inflammatory infiltrate in TA (2, 3). Furthermore, since IL-8 is also a potent angiogenic factor, it may have a critical role in the development of stenotic lesions by inducing proliferation and migration of different vascular cell types (19). However, the precise role of IL-8 in TA is presently unknown and need to be determined.

In conclusion, our observation of significantly increased levels of IL-8 in TA particularly during the active stage of the disease and its normalization in most follow-up patients after immunosuppressive therapy suggests an important role of this chemokine in the pathogenesis of the disease. Future studies

on the role of IL-8 *in vivo* and the stimuli that induce its production in the disease would be of paramount importance and may provide a new basis for the development of immunotherapeutic interventions for TA and other related vasculopathies.

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