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 disease. 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 −80°C 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 −80°C. 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 pg/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.

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-
Interleukin-8 in Takayasu’s arteritis / N.K. Tripathy et al.

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)

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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.

References