Interleukin-6 and RANTES in Takayasu Arteritis
A Guide for Therapeutic Decisions?

Marina Noris, Chem PharmD; Erica Daina, MD; Sara Gamba, RN; Samantha Bonazzola, Biol ScD; Giuseppe Remuzzi, MD

Background—In patients with Takayasu arteritis, circulating lymphocytes are activated, and histological findings indicate that cell-mediated immunity plays an important role in the pathogenetic sequence leading to vascular lesions.

Methods and Results—To delineate the profile of inflammatory and chemoattractant cytokines involved in T-cell activation in Takayasu arteritis, we measured by ELISA serum levels of interleukin (IL)-6, IL-1β, and RANTES in 18 patients. Subsequently, we wanted to establish whether any of these molecules could be used as a marker to monitor the clinical course of the disease and to predict disease exacerbations. We found that all patients with Takayasu arteritis studied during an active phase of the disease have increased serum concentration of IL-6 compared with healthy control subjects ($P<0.01$). Enhanced IL-6 serum levels paralleled disease activity to the extent that its serum concentrations were comparable to those of control subjects when patients were studied in remission. RANTES concentrations were also higher than normal in the serum of all patients with Takayasu arteritis ($P<0.01$) studied during an active phase of the disease. RANTES serum levels tended to normalize in remission, but values remained higher than those of control subjects ($P<0.05$). In contrast, serum concentrations of IL-1β were below the detection limit of ELISA in both healthy subjects and all patients with Takayasu arteritis. A positive correlation was found between either IL-6 ($\rho=0.705$, $P<0.01$) or RANTES ($\rho=0.607$, $P<0.05$) serum level and disease activity.

Conclusions—The close correlation of serum IL-6 and RANTES levels with disease activity suggests that these cytokines contribute to vasculitic lesions in Takayasu arteritis and raises the possibility that their monitoring in serum helps clinicians find adequate treatment adjustments in individual patients. (*Circulation*. 1999;100:55-60.)

Key Words: Takayasu arteritis ■ immunology ■ lymphocytes ■ interleukins ■ RANTES

Takayasu arteritis is a chronic vasculitis that affects primarily large elastic arteries such as the aorta, its main branches, and the pulmonary arteries. Women of reproductive age are most often affected.$^1$

In most patients, disease onset is insidious; early, systemic manifestations such as fever, arthralgia, and weight loss are often absent or vague and go unrecognized. On the other hand, in some patients, nonspecific inflammatory features can antedate signs and symptoms of vascular involvement by a prolonged period.$^2$ The clinical course of the disease may be fulminant, may progress gradually with periods of inactivity followed by relapses, or may stabilize. It is therefore crucial to find appropriate markers to monitor disease activity and possibly predict exacerbations to aid in early treatment.

The cause of the disease is unknown. Histological findings of inflammatory cell infiltration and necrosis of the arterial vascular cells strongly suggest that cell-mediated immunity plays an important role in the pathogenetic sequence leading to the lesions.$^3$ In Takayasu arteritis, inflammatory cell infiltration tends to be localized in the adventitia and outer part of the media, involving the vasa vaso
tum. Infiltrating cells consist mainly of γδ T lymphocytes, natural killer (NK) cells, macrophages, cytotoxic T lymphocytes, and T helper cells. It has been postulated that the above-mentioned cells, especially NK cells, may recognize a 65-kD heat-shock protein expressed in the aortic tissue and induce vascular cell injury by releasing the cytolytic factor perforin.$^5$

Recent studies have documented quantitative and qualitative alterations in cytokine production in systemic vasculitis other than Takayasu arteritis.$^6$ Thus, elevated plasma levels of interleukin (IL)-6$^7$ and increased expression of IL-6 and IL-1β in both circulating mononuclear cells and tissue-infiltrating macrophages have been observed in patients with giant-cell arteritis.$^8$ In addition, a recent study has shown high expression of IL-6 in the aortic tissue from 4 patients with Takayasu arteritis.$^9$

IL-1β and IL-6 are proinflammatory cytokines synthesized mainly by activated monocytes, macrophages, and T cells. These cytokines have overlapping activities such as B- and T-cell activation, fibroblast proliferation, and acute-phase protein synthesis.$^{10}$ Moreover, IL-1β induces a large number of cytokines and upregulates the expression of adhesion
molecules on endothelial cells, thus promoting leukocyte interactions with the endothelium; IL-6 enhances T-cell cytotoxicity and NK cell activity. Chemokines, a family of small cytokines with chemotactic properties, have been implicated as having a central role in the recruitment of monocytes and lymphocytes within vascular tissue in immune-mediated vasculitis such as Kawasaki disease. One of them, RANTES (regulated on activation, normal T cell expressed and secreted), displays a potent and selective chemoattractant activity for most mononuclear cell types—CD4⁺ T memory lymphocytes, γδ T lymphocytes, macrophages, and NK cells—that predominate in Takayasu arteritis infiltrate. RANTES is synthesized by macrophages, T lymphocytes, and endothelial cells on immune activation. Thus, the possibility that RANTES release may have a role in mononuclear cell recruitment in Takayasu arteritis is worth investigating.

To delineate the profile of inflammatory and chemoattractant cytokines in Takayasu arteritis, we measured serum levels of IL-6, IL-1β, and RANTES in patients with this disease. Subsequently, we wanted to establish whether any of these molecules could be used as a marker to monitor the clinical course of the disease and to predict disease exacerbations.

**Methods**

**Subjects**
We studied 18 consecutive adult Italian patients with Takayasu arteritis (1 man, 17 women; age, 19 to 67 years) referred to the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò” between April 1995 and October 1998. The diagnosis was based on the presence of symptoms and signs of ischemic, inflammatory large-vessel disease, as well as supportive arteriographic findings. All patients also fulfilled >3 of the 1990 American College of Rheumatology criteria for classification of Takayasu arteritis. None of them had cranial symptoms, clinical picture of temporal arteritis, or polymyalgia rheumatica.

Average age at clinical onset was 30 years (range, 14 to 62 years); average age at diagnosis was 33.6 years (range, 16 to 64 years). From these data, we can derive that there was a significant delay between onset of symptoms and the final diagnosis. Indeed, the mean interval between onset and diagnosis was 3.3 years (range, 0 to 19.3 years). Only 2 patients had disease onset at >40 years of age (46 and 62 years).

Before entering the study, all patients were evaluated for NIH criteria of disease activity: (1) presence of systemic features such as fever or musculoskeletal problems (no other cause identified); (2) elevated erythrocyte sedimentation rate (ESR); (3) presence of features of vascular ischemia or inflammation such as claudication, diminished or absent pulse, bruit, vascular pain (carotidynia), or asymmetric blood pressure in either upper or lower limbs (or both); and (4) typical angiographic features.

A complete aortogram was done if active disease was suspected. In the other patients, the absence of new vascular lesions was confirmed by either angiography or ultrasonography. New onset or worsening of each of the above-mentioned features was given a score of 1; a score of ≥2 defined active disease. According to this definition, 6 patients were in an active phase of disease when they entered the study, and 12 were in remission.

Of the 6 patients with active Takayasu arteritis, 4 underwent remission, defined as complete resolution of all clinical features in the setting of fixed vascular lesions, during the study period and were studied again. Clinical details of patients with active and inactive Takayasu arteritis are given in the Table. A group of 16 age- and sex-matched healthy subjects was also studied as control.

**Serum Samples**
Venous blood was collected into sterile tubes, incubated for 30 minutes at 37°C to allow clotting, and centrifuged at 2000g for 10 minutes at room temperature. Serum samples were then frozen and stored at −20°C until the assays were performed.

**IL-6, IL-1β, and RANTES Assays**
Serum levels of IL-6, IL-1β, and RANTES were determined by ELISA with commercially available kits obtained from Biosource International (distributed by Celbio) for IL-6 and from Amersham Life Science (Little Chalfont) for RANTES and IL-1β. The minimum detectable concentration was 2.5 pg/mL for RANTES and IL-6 and 0.06 pg/mL for IL-1β, with an intra-assay variability <5% and an interassay variability <6%.

**Statistical Analysis**
Data were expressed as mean±SD. Differences between groups were analyzed by the Kruskal-Wallis test or by Wilcoxon signed-rank test for paired subjects as appropriate. A Spearman ρ correlation coefficient was determined to evaluate the correlation between serum IL-6 or RANTES and disease activity score and between serum IL-6 or RANTES and ESR.

The association between high levels of IL-6 or RANTES and active disease was univariately analyzed by fitting logistic regression models. Data analysis was performed with the SAS package.

**Results**
Serum-immunoreactive IL-6 levels in healthy subjects are given in Figure 1A. Data showed that levels were below the detection limit of the assay in 8 of 16 subjects screened. In contrast, serum IL-6 levels were remarkably higher in all patients studied during an active phase of Takayasu arteritis (48.13±21.46 versus 4.77±7.19 pg/mL for healthy subjects, P<0.01, Kruskal-Wallis test; Figure 1A) but not in patients who were studied during remission of the disease (8.24±12.88 pg/mL, P<0.01 versus active, Kruskal-Wallis test; Figure 1A), who instead had absolute values comparable to those recorded in healthy subjects, with levels below the detection limit of the assay in 9 of 16 patients (Figure 1A). Of 6 patients with active Takayasu arteritis, 4 were studied again after remission. In all patients, improvement in clinical signs and symptoms was associated with a marked reduction in serum IL-6 levels (P<0.01 versus active, Wilcoxon signed-rank test; Figure 1B) compared with values recorded during active disease.

At variance with IL-6, serum concentrations of IL-1β were below the detection limit of ELISA in both healthy subjects and all patients with Takayasu arteritis and did not change in relation to disease activity.

In active Takayasu arteritis, serum levels of RANTES were higher than those of healthy subjects (75.54±17.28 versus 34.98±7.43 ng/mL, active Takayasu versus healthy subjects, P<0.01, Kruskal-Wallis test; Figure 2A). In the Takayasu arteritis patients studied in remission, serum RANTES levels were lower than those in active patients (46.04±17.05 ng/mL, P<0.01, Kruskal-Wallis test; Figure 2A) but remained significantly higher than the values recorded in healthy subjects (P<0.05, Kruskal-Wallis test; Figure 2A). In the patients studied both during relapse and in remission, RANTES concentration in serum decreased in the inactive phase (P<0.01 versus active, Wilcoxon signed-rank test; Figure 2B).

To evaluate whether IL-6 and RANTES serum concentration values in Takayasu arteritis patients were actually related
to signs of disease activity, we calculated Spearman’s correlation coefficients between either IL-6 or RANTES concentration and disease activity score, which was calculated as described in Methods (see the Table). As shown in Figure 3, a positive correlation was found between either IL-6 or RANTES serum level and disease activity score (IL-6: \( r = 0.705, P = 0.01 \); RANTES: \( r = 0.607, P = 0.05 \)). In addition, serum IL-6 and RANTES correlated significantly, although more weakly, with ESR values (IL-6: \( r = 0.618, P = 0.01 \); RANTES: \( r = 0.559, P < 0.05 \)). In contrast, we have been unable to find any correlation between serum IL-6 or serum RANTES and platelet count, hemoglobin, or plasma C-reactive protein. As expected, ESR values correlated with disease activity score (\( r = 0.692, P < 0.01 \)), whereas the correlation between C-reactive protein and disease activity score was not significant (\( r = 0.388, P = \text{NS} \)).

Results of univariate logistic analysis showed that the value of either IL-6 or RANTES had a significant predictive value of Takayasu arteritis disease activity (IL-6, \( P = 0.014 \); RANTES, \( P = 0.03 \)).

**Discussion**

We found that all patients with Takayasu arteritis studied during an active phase of the disease had increased serum concentration of IL-6 compared with healthy control subjects. Enhanced IL-6 serum levels paralleled disease activity to the extent that their serum concentrations were quite comparable to those of control subjects when patients were studied in remission.

Although the pathogenesis of Takayasu arteritis is far from clarified, data in favor of autoimmunity are now exceedingly solid and imply lymphocyte activation on interaction with a still–ill-defined antigen, secondary endothelial involvement, and large-vessel inflammation.3,4 Accordingly, in the peripheral circulation of patients with Takayasu arteritis, one finds an increased number of HLA-DR
\(^1\) and CD45RO
\(^1\) lymphocytes compared with normal subjects with a functional pattern of cell activation, ie, enhanced basal activity of protein kinase C and high intracellular calcium levels.24,25

It is well known that activated lymphocytes and macrophages release IL-6,\(^10\) which, besides promoting antibody formation in B cells, also triggers T-cell proliferation and differentiation to cytotoxic subtype, in turn promoted by IL-2 receptor induction and IL-2 formation.26 The finding of high serum levels of IL-6 in patients with Takayasu arteritis can be taken as indirect evidence of peripheral mononuclear cell activation whose cytokine product contributes to the full expression of the inflammatory process.

### Clinical Details of Patients with Active and Inactive Takayasu Arteritis

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A indicates angiography; MTX, methotrexate; US, ultrasonography; and CY, cyclophosphamide. Zero signifies absent; 1, present.

*Normal value <1 mg/dL.*
The extent to which the vasculitic lesions that characterize the disease are actually dependent on IL-6 producing cells is open to speculation. Already available data show that lymphocytes taken from peripheral blood of patients with Takayasu arteritis have a higher rate of blast transformation in response to purified human aortal antigen extracts and an increased cytotoxicity on cultured human endothelial cells compared with normal lymphocytes. These findings are consistent with the possibility that Takayasu lymphocytes are sensitized against aortal antigens and are capable of enhancing vascular damage via large-vessel antigen-induced activation and cytokine release. In harmony with this possibility are findings of inflammatory cell infiltration in aortic tissue samples taken from patients with Takayasu arteritis, which consisted mainly of γδ T lymphocytes, NK cells, macrophages, cytotoxic T lymphocytes, and T helper cells.

What is the signal that drives mononuclear leukocytes to infiltrate large vessels in these patients? We attempted to address this issue by measuring circulating concentrations of RANTES, a chemokine that displays potent and selective chemoattractant activities for T lymphocytes, NK cells, and macrophages. RANTES concentrations were remarkably higher than normal in the serum of all patients with Takayasu arteritis studied during an active phase of the disease. RANTES serum levels tended to normalize in remission, but values remained significantly higher than in control subjects.

Relevant to the above sequence of pathogenetic events are findings that RANTES is produced by endothelium when activated by an immune injury such as an immediate early stress response and would indeed create a gradient from endothelium to the circulation that promotes massive T-cell and macrophage adherence to and entrance into the tissue. However, RANTES is also expressed by circulating T cells after activation by antigens, and expression is maintained in terminally differentiated cytotoxic T lymphocytes. Of note, RANTES may function as a costimulatory agent in T-cell proliferation. It is therefore conceivable that in patients with Takayasu arteritis, RANTES of activated T-cell and/or endothelial cell origin contributes to the maintenance of T-cell activation and proliferation and is involved in monocyte/lymphocyte adhesion to endothelial cells and recruitment within inflamed vessel wall. At variance with IL-6, circulating levels of RANTES also were elevated at the time when patients were clinically well, although absolute values were significantly lower than during the active phase of the disease. This would indicate that current therapies, particularly glucocorticoids, do not completely prevent RANTES overexpression, which could possibly favor the development of artery lesions that normally precede clinical recurrence.

Another finding of the present study is that serum concentration of either IL-6 or RANTES positively correlated with disease activity, and univariate logistic analysis showed a significant...
RANTES levels with disease activity, besides the issue of whether these cytokines contribute to vasculitic lesions, raises the possibility that their monitoring in serum helps clinicians find adequate treatment adjustments in individual patients. These measurements can be part of routine hospital laboratory facilities that are easy to perform at low cost. Furthermore, the noninvasive nature of such measurements makes them attractive for minimizing patient discomfort.

Acknowledgments

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References


Figure 3. Correlation of serum IL-6 (top) and serum RANTES (bottom) concentrations with disease activity score in patients with Takayasu arteritis (Spearman’s correlation test).


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