Human soluble tumor necrosis factor receptor I (sTNF-RI) and interleukin-I receptor antagonist (IL-I Ra) in different stages of acute rheumatic fever

Akut romatizmal ateşin farklı evrelerinde solubl tümör nekrosis faktör reseptör I (sTNF-RI) ve interlökin-I reseptör antagonist (IL-I Ra) düzeyleri

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ABSTRACT

Objective: Acute rheumatic fever (ARF) results from an autoimmune response to infection with group A streptococci. Serum concentrations of two anti-inflammatory cytokines, interleukin-I receptor antagonist (IL-IRa) and human soluble tumor necrosis factor receptor I (sTNF-RI) were determined in patients with ARF at the time of admission and 3 months after treatment in order to evaluate changes in cytokine concentrations occurring during different stages of the disease.
Methods: Serum concentrations of two anti-inflammatory cytokines, IL-I Ra and sTNF-RI , were investigated in children with ARF at the time of admission (n=21) and after 3 months following the cessation of treatment (n=15). The sTNF-RI and sIL-IRa were measured quantitatively in serum using enzyme-linked immunosorbent assay (ELISA).
Results: Levels of IL-1Ra and sTNF-RI were found to be significantly higher during acute phase and remission period of ARF when compared to age-matched healthy controls (p=0.001 and p=0.0001, respectively).
Conclusion: Our study demonstrated that two anti-inflammatory cytokines, serum sTNFRI and IL-1Ra, are increased in acute and remission stages of ARF reflecting activation of the cellular immune response. We suggest this increase might probably be generated in an effort to counteract the already increased concentrations of proinflammatory cytokines. *(Anadolu Kardiyol Derg 2008; 8: 139-42)*
Key words: Acute rheumatic fever, interleukin-1 receptor antagonist, soluble tumor necrosis factor receptor I

ÖZET

Amaç: Akut romatizmal ateş (ARA) grup A streptokoklara karşı otoimmun yanıt sonucu gelişir. Bu çalışmada ARA tanılı olgularda, iki antiinflamatuvar sitokin olan interlökin-I reseptör antagonist (IL-IRa) ve solubl tümör nekrosis faktör reseptör I (sTNF-RI) düzeyleri tanı anında ve tedavi başlandıktan 3 ay sonra ölçülerek hastalığın değişik evrelerindeki sitokin konsantrasyonlarındaki değişikliğin saptanması amaçlanmıştır.
Yöntemler: Serum IL-IRa ve sTNF-RI düzeyleri ARA tanılı olgularda tanı anında (n=21) ve tedavi başladıktan 3 ay sonra (n=15) değerlendirilmiştir. Serum sTNF-RI, sIL-IRa konsantrasyonları kantitatif olarak ELISA metodu ile ile ölçülmüştür.
Bulgular: Serum IL-1Ra ve sTNF-RI düzeyleri ARA tanılı olgularda hem akut dönemde hem de remisyonda yaş uyumlu sağlıklı kontrol grubuna göre anlamlı yüksek bulunmuştur (p=0.001 ve p=0.0001).
Sonuç: Akut romatizmal ateşte serum sTNFRI ve IL-1Ra düzeylerindeki hem akut, hem de remisyon dönemlerindeki artış sellüler immün yanıttaki aktivasyonu göstermektedir. Antiinflamatuvar sitokin düzeylerindeki bu artış akut romatizmal ateşte aynı zamanda artmış bulunan proinflamatuvar sitokin konsantrasyonuna karşı dengeyi sağlamak amacı ile olabilir. *(Anadolu Kardiyol Derg 2008; 8: 139-42)*
Anahtar kelimeler: Akut romatizmal ateş, interlökin-1 reseptör antagonist, solubl tümör nekrosis fakör reseptör I

Introduction

Acute rheumatic fever (ARF) results from an autoimmune response to infection with group A streptococci (GAS). Although the acute illness causes considerable morbidity, major clinical and public-health effects arise from long term damage to heart valves leading to chronic rheumatic heart disease (CRHD). Over the past century, ARF and CRHD have become rarer in developed countries with the improvement of socioeconomic status and life quality. The introduction of antibiotics has also helped to reduce the burden of disease (1, 2). Turkey is one of the countries where the incidence of ARF and, consequently,

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the prevalence of CRHD have declined remarkably over the last decades but are still high especially in low socioeconomic groups (3).

Delay in manifestations of ARF following infection and the presence of infiltrates of T- helper lymphocytes and macrophages in acute rheumatic valvulitis and B cells in Aschoff body suggest an important role of the immune system in the pathogenesis of this disease.

However, it is not known whether cellular infiltrates in heart valves or increased components of cellular immune system in the peripheral blood secrete cytokines or other factors which lead to abnormal collagen synthesis and damage to heart valves (4-5).

Increased production of proinflammatory cytokines, such as interleikin (IL)-1, tumor necrosis factor (TNF)-α, by peripheral and heart infiltrating T cells of patients with rheumatic fever and CRHD have been reported in previous studies (6-12). Narin et al. (13) have reported that proinflammatory cytokine IL-1a and IL-2 levels were significantly elevated in patients with ARF while TNF-α concentrations remained unchanged during different stages of the disease. In another study by Kutukculer et al., IL-8 concentration was found to be significantly elevated in acute stage with a tendency to decrease in remission (14). These authors drew attention to excessive production of IL-8; probably by cellular infiltrates in the joint throughout the active period of rheumatic disease. Interleikin-1a is produced by several types of macrophages as well as by normal B lymphocytes, cultured T cell clones, fibroblasts, neutrophils, and endothelial cells (15). Monocyte-macrophages can produce TNF-α and a number of endogenous mediators including IL-1 are also active inducers of TNF-α (9). Involvement of cellular immune system in the pathogenesis of ARF has been reported. Morris et al. (12) have found significantly elevated amounts of IL-1 and IL-2 in ARF and active CRHD patients at all intervals up to 48 weeks. Anti-inflammatory cytokines such as sTNF-a are detectable in normal serum and significantly increased in patients with various infections, malignancies, ARF or autoimmune diseases (9, 10).

Interleikin-I receptor antagonist (IL-IRa) is naturally occurring anti-inflammatory molecule that blocks action of IL-1 (16). The IL-IRa knockout mice spontaneously develop inflammatory arthritis resembling rheumatoid arthritis (17). Anti-inflammatory cytokine concentrations in ARF have not been thoroughly examined.

One of these, tumor growth factor (TGF)-β1 acts as a regulatory cytokine limiting the extent of local inflammation with its inhibitory-suppressive capability. A study by Aksu et al. evaluated the concentrations of TGF-β1 in ARF patients during disease progress (18). They have observed very high TGF-β1 concentrations in their patients as compared to healthy controls and normal individuals, tending to decrease gradually from acute to remission and chronic stages.

As a continuum of our previous studies, serum concentrations of two anti-inflammatory cytokines, IL-IRa and human soluble tumor necrosis factor receptor I (sTNF-RI) were determined in patients with ARF at the time of admission and 3 months after treatment in order to evaluate changes in cytokine concentrations occurring during different stages of the disease.

Methods

Patients characteristics

Twenty-one patients (16 males, 5 females, mean age 11.05 ± 3.36 years) (Group Ia) who were referred to Pediatric Cardiology unit within a 42-month period were included in the study. They were diagnosed as ARF according to modified Jones criteria (19). None of the patients had previous history of ARF. They were found to have major and minor manifestations of ARF.

All patients had pharyngitis or flu like syndromes during the month before the onset of ARF. First blood samples for the measurement of the cytokines were taken on admission before any treatment was initiated. A 10-day course of penicillin, followed by benzathine penicillin G prophylaxis were given every 3 weeks to all patients. The patients who had polyarthritis (17/21) received acetylsalicylic acid 90 mg/kg/day and the patients who had carditis (15/21) received prednisolone 2 mg/kg/day for 3-6 weeks until their acute phase levels returned to normal levels. Six of twenty-one patients who had moved to another city were excluded from the study.

Fifteen patients of 21 patients with ARF, whose clinical findings improved, had no chronic sequela like carditis, and whose acute phase reactants were all within normal ranges at the end of three months, were included in study Group Ib.

Serum sTNF-RI, IL-I Ra concentrations were measured from blood samples taken on admission before beginning any medication (Group Ia, n=21) and at the end of the following 3 months under treatment (Group Ib, n=15). Age and sex matched twenty children (12 males, 8 females, mean age- 9.72±2.34 years) who had not suffered any sore throat or other infection in the past 6 months were included in the study as a healthy control group.

Cytokine analyses

The sTNF-RI, sIL-IRa were measured quantitatively in serum using enzyme-linked immunosorbent assay (ELISA) with commercially available kits (Biosource, Nivelles, Belgium). Minimum detectable concentrations for sTNF-RI and sIL-IRa was 0.05 ng/ml and 4 pg/ml, respectively.

Statistical analyses

Statistical analyses of data were performed using Pearson correlation analysis, Mann-Whitney U test and Kruskal-Wallis test for comparison of variables through groups of patients with ARF and healthy controls using SPSS for Windows 12.0 statistical software (Chicago, IL, USA). P values of less than 0.05 were accepted as statistically significant. The mean ± SD values calculated for each variable in Group I patients reflected the results of the original 21 acute patients, but the paired comparisons were done for the remaining 15 patients who could be followed in remission stage.

Results

Clinical features of Group Ia are presented in Table 1. Migratory polyarthritis (80.9%) and carditis (71.4%) were the most commonly documented major criteria. None of the patients had erythema marginatum, chorea or subcutaneous nodules.

Serum levels of sTNF-RI and sIL-IRa were significantly different between groups Ia, Ib and controls (p=0.001 and p=0.0001, respectively) (Table 2). There were no significant difference between serum levels of sTNF-RI and sIL-IRa on admission (Group Ia) and 3 months after treatment (Group Ib) (p=0.357 and p=0.117, respectively) (Table 2). Pairwise comparisons yielded statistically significant differences between Group Ia and controls for sTNF-RI and sIL-IRa (p=0.0001 and p=0.0001, respectively). This was also true for Group Ib and controls (p=0.010 and p=0.007, respectively for sTNF-RI and sIL-IRa). These levels were significantly higher in Group Ia and Group Ib than in controls. Concentrations of serum sIL-1Ra and sTNF-RI in ARF patients before the initiation of treatment (Group Ia) were not correlated with erythrocyte sedimentation rate (p=0.153 and p=0.093, respectively). This was also true for Group Ib (p=0.315 and p=0.424, respectively). Cytokine levels were not significantly associated with qualitative C-reactive protein values (p=0.574 and p=0.654, for sIL-1Ra and sTNF-RI, respectively) in Group Ia.

Discussion

Although ARF has been known to be related with streptococcal infection for many years, its exact pathogenesis has not been completely clarified. Environmental and genetic factors as well as immune abnormalities are believed to play a role in the epidemiology and pathogenesis of rheumatic fever.

Overproduction of proinflammatory cytokines have been implicated in a variety of human diseases, including sepsis, cerebral malaria, and autoimmune diseases (20, 21). Increased production of TNF-α, IL-1, and IL-6 in peripheral blood and heart infiltrating T cells of patients with rheumatic fever and CRHD have previously been shown. These data suggested that these inflammatory cytokines may play a role in the pathogenesis of these conditions (8, 12, 13). Yegin et al. (8) have found significant increases in TNF-α, IL-8 and IL-6 levels in the acute phase of ARF. Narin et al. have reported that proinflammatory cytokine IL-1 and IL-2 levels in patients with ARF were significantly elevated on admission compared to remission. The TNF-α concentrations remained unchanged during different stages of the disease (13). The results of ARF patients in remission and CRHD patients were not significantly different from the results of healthy controls. It was suggested that the elevation in IL-1 and IL-2 plasma concentrations reduced to normal levels within 3 months. In our study, we couldn't demonstrate proinflammatory cytokine levels simultaneously with anti-inflammatory cytokines.

Anti-inflammatory cytokines such as sTNF-R are significantly increased in patients with various infections, malignancies, ARF and autoimmune diseases (9, 10). In our study, sTNF-RI levels were found to be significantly higher in the peripheral blood of ARF patients on admission and after receiving adequate therapy when compared to healthy controls. These data suggest that elevation of proinflammatory cytokines in acute stage and also on remission probably is representative of an ongoing inflammatory process.

The IL-1Ra is an anti-inflammatory molecule that inhibits IL-1 (16). IL-1Ra knockout mice spontaneously develop inflammatory arthritis resembling rheumatoid arthritis (17). In the present study, IL-1Ra levels were significantly higher in the peripheral blood of ARF patients on admission and after 3 months of adequate medical treatment when compared with healthy controls. No significant difference was observed in sTNF- RI and IL-1Ra levels between these two patient groups.

Significantly elevated levels of antiinflammatory cytokines sTNF- RI and IL-1Ra in respect to controls were important showing the inflammatory process going on in these patients both in acute and remission states, although tending to decrease in remission. These increased levels in patients might also serve to predict the chronic process but we did not record chronic sequela in our follow-up.

Conclusion

In conclusion, our study demonstrated that two anti-inflammatory cytokines, serum sTNFRI and IL-1Ra, are increased in acute and remission stages of ARF reflecting activation of the cellular immune response. We suggest this increase might probably be generated in an effort to counteract the previously reported increased concentrations of proinflammatory cytokines in ARF.

Table 1. Clinical characteristics of patients in Group Ia (n=21)

Age, years	11.05±3.36
Sex, M/F	16/5
Clinical symptoms and signs	
Carditis, n(%)	15 (71.4)
Polyarthritis, n(%)	17 (80.9)
Chorea, n(%)	-
Erythema marginatum, n(%)	-
Subcutaneus nodules, n(%)	-
Fever, n(%)	13 (66.6)
Arthralgia, n(%)	7 (33.3)

Table 2. Serum concentrations of sTNF-RI and IL-1Ra in study and control groups

	Study group		Healthy Control	
	Group Ia	**Group Ib**		**p**
Number of subjects	**21**	**15**	**20**	
sTNF-RI, ng/ml	3.45 ± 1.32***	2.99 ± 0.89*	2.31 ± 0.43	0.001
IL-1Ra, pg/ml	1134.2 ± 515.4***	912.2 ± 615.8**	477.0 ± 372.4	0.0001

Data are represented as mean±SD
Kruskall Wallis test for comparison of 3 groups
*- p=0.01, **- p=0.007 and ***- p=0.0001 for comparison with control group. Mann Whitney U test
IL- 1Ra-interleukin-I receptor antagonist, sTNF-RI- human soluble tumor necrosis factor receptor I

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