



Interleukin-10 Levels in Heart Failure Syrian Patients

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ABSTRACT

Inflammation plays a significant contributory role in the pathogenesis of chronic heart failure (CHF). IL-10 has potent deactivating properties in macrophages and T-cells and thus acts as a down-regulator of cell-mediated immune responses. The aim of the present study was to assess whether serum concentrations of IL-10 significantly differ between patients with CHF and healthy control subjects. Patients with CHF (n= 60, 61.5± 11.7 years; mean ejection fraction, 33.67± 12.08%; New York Heart Association (NYHA) class II–IV and 28 healthy controls (68.2± 5.2 years) were examined. Serum IL-10 as well as TNF-α concentrations were measured using commercially available immunoassays. Patients with CHF showed significantly lower IL-10 concentrations (2.52± 0.75 compared with control 4.83± 0.59 pg/ml; P <0.001). Patients with advanced CHF (NYHA class III and IV) had the lowest IL-10 serum levels. The ratio of TNF-α to IL-10 was significantly higher in patients with advanced CHF (NYHA class III and IV, ratio (2.26± 0.64 and 17.28± 31.55 respectively, compared with control 0.26± 0.23; P <0.01). Our present study demonstrates significantly decreased serum levels of IL10 in patients with advanced CHF. Since IL-10 is known as a potent anti-inflammatory cytokine, its decrease in advanced CHF may favor the inflammatory milieu in CHF.

Keywords: Chronic heart failure, cytokines, immunology, inflammation.

INTRODUCTION

Over the past decade there has been increasing interest in the potential role of inflammatory mediators in cardiac diseases, such as chronic heart failure (CHF). Elevated circulating levels of cytokines, in particular tumor necrosis factor-α (TNF-α), has consistently been identified in patients with CHF^{1,2}. Several studies^{3,4} suggest that the extent of cytokine production directly correlates with the severity of the disease process.

Interestingly, many aspects of the syndrome of CHF can be explained by the known biological effects of these inflammatory mediators: serum concentrations of TNF-α frequently found in CHF can induce progressive left ventricular (LV) dysfunction, LV remodeling, fetal gene expression and cardiomyopathy⁵. Thus, analogous to the well known elevation of neurohormones in CHF, TNF-α can predict the functional class and the clinical outcome in patients with CHF. The emerging link between inflammatory cytokines in the pathogenesis and progression of CHF has already resulted in the development of anti-cytokine strategies that might be used as adjunctive therapy in patients with CHF. Although inflammatory mediators have moved to the centre of interest in leading heart failure trials, little is known about the role of anti-inflammatory cytokines in this disease setting. IL-10, which is produced by various inflammatory cells, especially macrophages⁶ and T-cells, is a major inhibitor of cytokine synthesis, suppresses macrophage function and inhibits the production of proinflammatory cytokines⁷ as well as matrix metalloproteinases, which

have already been described as playing an important role in CHF⁸. Recently, Bolger et al.⁹ have shown that IL-10 inhibited TNF-α release from peripheral blood mononuclear cells ('PBMC') isolated from patients with CHF. Although, there are some interesting data on the anti-inflammatory potential of IL-10 in chronic inflammatory diseases, only little is known about its role in the patho-physiology of CHF. In the present study, we examined the IL-10 serum concentrations in patients suffering CHF.

SUBJECTS AND METHODS

Patients and controls

Patients (n =60, 42men and 18women; mean age± S.D 61.5± 11.7years) with symptomatic CHF, defined as dyspnea or fatigue at rest or on exertion for more than 3 months, were studied (Table 1). The severity of the CHF ranged from New York Heart Association (NYHA) functional class I to IV (Table1). The underlying cause of CHF was classified as coronary artery disease (n =18), idiopathic dilated cardiomyopathy (dCM; n =21), or hypertension (n =21) based on disease history and coronary angiography. All patients underwent echocardiography. Mean EF± S.D measured was 33.67± 12.08%. None of the patients was acutely decompensated. Their clinical and haemodynamic situations were stable, with no changes in medication during the last month before study entrance.

Blood was taken after admission before any change of medication or any diagnostic or interventional



procedure was done which could have provoked an inflammatory reaction.

Medical treatment consisted of angiotensin converting-enzyme (ACE) inhibitors, β -blockers, diuretics, digitalis and angiotensin-II-receptor-1 blockers.

All patients none had any concomitant diseases such as fever, infection, myocarditis, malignancies, pulmonary disease.

Control subjects were 28 healthy sex and age matched blood donors (15men and 13women; mean age \pm S.D 68.2 \pm 5.2years) without any history of cardiac disease.

Table 1: The clinical characteristics of the study population

Parameters	Controls	CHF patients
Age (years)	68.2 \pm 5.2	61.5 \pm 11.7
Sex (male/female)	15/13	42/18
NYHA functional class (I/II/III/IV)		3/23/28/6
Cause of CHF		
Coronary artery disease		18
Idiopathic dCM		21
hypertension		21
LVEF (%)		33.67 \pm 12.08%

Values are means \pm S.D.

Blood sampling protocol

For serum sampling, blood was drawn into pyrogen-free blood collection tubes. Tubes were immediately immersed in melting ice and centrifuged within 15min (1000 g for 15 min at 4°C). Serum was stored at -80°C. Samples were frozen and thawed only once.

Laboratory analysis

Serum levels of IL-10, TNF- α , soluble TNF- α receptor 2 (sTNF α R2) were measured using commercially available ELISA kits (Komabiotech, korea), according to the manufacturers' instructions.

All kits had the following sandwich ELISA format: microtitre plates already precoated with a murine monoclonal antibody against the human cytokine being measured. Standards of the analyte and serum samples were added in duplicate, along with a second antibody against another epitope of the analyte conjugated to streptavidin-HRP (for TNF- α , sTNF α R2, and IL10). The samples were incubated for 2h. Finally, the chromogen tetramethylbenzidine was added and incubated for 30 min in room temperature. After the addition of 1M H2SO4, the absorbance at 450 nm were read.

Statistical analysis

Statistical analysis was performed using t-student test to compare means. We considered P<0.05 as a significant level. Microsoft Office Excel 2007 was used to process and analyze data. . All values are reported as means \pm S.D.

RESULTS

Circulating levels of IL-10 in patients with CHF

Patients with CHF had significantly decreased levels of IL-10 compared with healthy control subjects (2.52 \pm 0.75 compared with 4.83 \pm 0.59 pg/ml respectively; P <0.01; Table2).

Table 2: serum cytokine levels in CHF patients and control subjects

Cytokine	Controls	CHF patients	P value
IL-10 (pg/ml)	4.83 \pm 0.59	2.52 \pm 0.75	P <0.01
TNF- α (pg/ml)	1.15 \pm 0.92	4.46 \pm 0.73	P <0.01
sTNFR2 (pg/ml)	1383.53 \pm 381.27	3360.34 \pm 945.71	P <0.01
TNF- α /IL-10	0.26 \pm 0.23	1.99 \pm 1.02	P <0.01

Values are means \pm S.D.

In particular, patients suffering advanced CHF, as classified as NYHA class III and IV, showed the lowest IL-10 levels (Figure1).

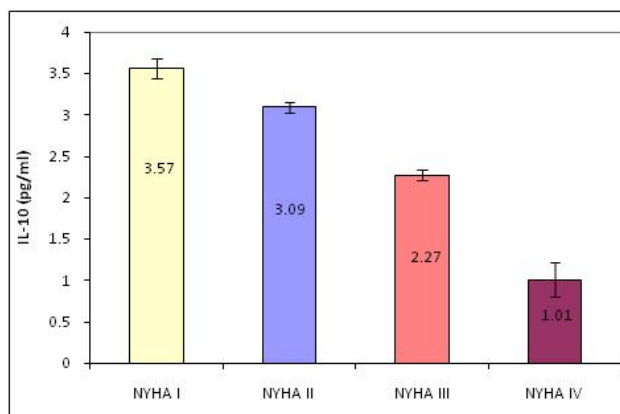


Figure 1: Relation of serum IL-10 levels to NYHA functional class

Values are means \pm S.D.

We next examined whether the IL-10 levels were related to the cause of heart failure and compared the serum levels in patients suffering dCM with those having ischaemic cardiomyopathy (iCM)and those having hypertension. We found significantly decreased IL-10 levels in all patient groups. However, there was no significant difference between iCM, hypertension and dCM patients (2.65 \pm 0.66 compared with 2.47 \pm 0.64 and with 2.47 \pm 0.92 pg/ml respectively; p=0.678).

Circulating levels of inflammatory cytokines in patients with CHF

Serum TNF- α levels were higher in patients with CHF than in healthy control subjects (4.46 \pm 0.73 compared with 1.15 \pm 0.92pg/ml respectively; P <0.01; Table2) and increased as NYHA functional class increased(Figure2A). Levels of the sTNFR2 followed a similar pattern (Table2 and figure2B).

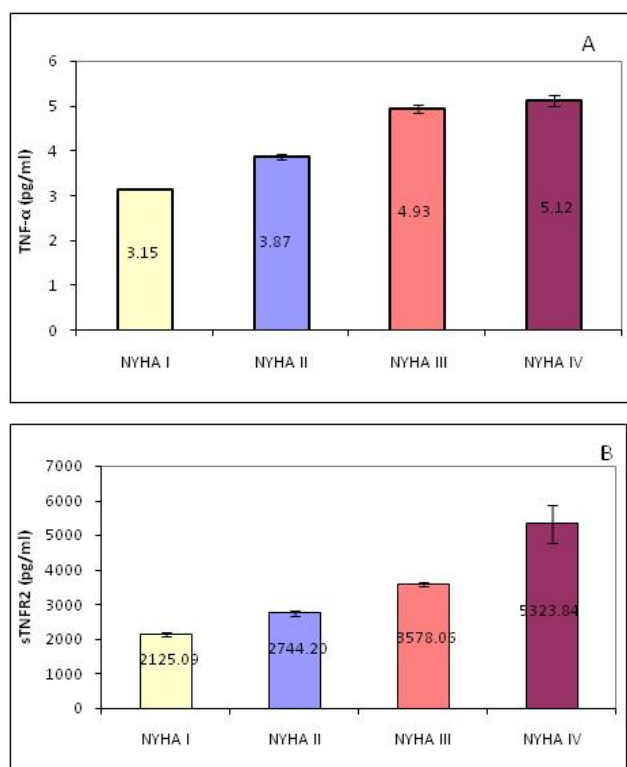


Figure 2: Relation of serum TNF- α (A), sTNFR2 (B) to NYHA functional class

Values are means \pm S.D.

Next, we compared the inflammatory to the anti-inflammatory 'profile' in our group of CHF patients given as the ratio of TNF- α to IL-10. This ratio was found to be significantly higher in the CHF group, especially in NYHA class III and class IV (NYHA class III and IV 2.26 ± 0.64 and 17.28 ± 31.55 respectively; control, 0.26 ± 0.23 ; $P < 0.01$).

DISCUSSION

CHF is one of the major health care problems in developed countries and one of the most frequent reason for patients being admitted to hospital. Despite significant advances in its treatment, the prognosis of CHF remains still poor. Over the past decade there has been increasing interest in the potential role of inflammatory mediators in CHF. This interest was fostered further by the observation that many aspects of the CHF syndrome can be explained by the known biological effects of these molecules¹⁰. Consequently, there is a growing body of evidence that anti-cytokine therapy may represent an additional approach for the treatment of patients with CHF. Despite the growing number of studies examining the role of proinflammatory mediators in the pathogenesis of CHF, there are only a few reports on the potential role of anti-inflammatory cytokines such as IL-10.

IL-10, initially designated cytokine synthesis-inhibitory factor ('CSIF'), was originally identified as a product of macrophages and lymphocytes of the Th2 subtype. It inhibits many cellular processes that have already been described to play important roles in CHF progression such as production of cytokines (e.g. TNF- α)¹¹, activation of

nuclear factor κ B ('NF- κ B')¹² as well as apoptosis and cell death¹³.

In our present study, we observed significantly decreased levels of IL-10 in patients with CHF. In particular, patients suffering advanced CHF, given as NYHA class III and IV, showed the lowest IL-10 levels.

On the other hand, as expected, we found signs of chronic activation of the immune system as mirrored by an activation of proinflammatory cytokines, such as TNF- α and sTNFR2.

Thus, obviously, the 'immunologic balance' in our present study group is changed in favour of a more inflammatory milieu as seen in an increased TNF- α to IL-10 ratio. In particular, patients suffering CHF NYHA class III/IV showed a high TNF α /low IL-10 constellation. The exact mechanism and cause of this disturbed balance still remains unclear. The clinical importance of this constellation, however, is underscored by a report¹⁴ demonstrating a strong association between the combined high TNF- α /low IL-10 constellation and early graft rejection in a population of heart transplant recipients. The clinical importance of IL-10 is fostered further by some encouraging reports on its therapeutical use in chronic inflammatory diseases in which inflammatory mediators, such as TNF- α , are believed to predominate, including psoriasis¹⁵, chronic hepatitis C¹⁶ and Crohn's disease¹⁷. It has furthermore been shown that human recombinant IL-10 can be safely administered and is generally well tolerated. It was only recently that Bolger et al.,⁹ demonstrated the inhibition of TNF- α production by IL-10 in peripheral blood mononuclear cells isolated from CHF patients. Previously, Damas et al.¹⁸ showed that the production of TNF- α stimulated monocyte chemotactic protein-1 ('MCP-1'), a chemotactic cytokine believed to play an important role in the development and progression of CHF, is decreased by IL-10 in rat ventricular cardiomyocytes.

CONCLUSION

On the basis of our present results, it is tempting to hypothesize that decreased levels of IL-10 may favor a more inflammatory milieu and that, in particular, patients with a high TNF- α /IL-10 ratio could benefit from a therapy with recombinant IL-10. However, these preliminary data about the value of IL-10 in chronic heart failure need to be confirmed in larger patient studies and trials of its therapeutical use should be considered.

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