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In vitro production of cytokines by monocytes/macrophages in patients with chronic heart failure

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Abstract
Many studies showed an important role of inflammation in heart failure (HF). Monocytes/macrophages are main cells take part in immune response. The purpose of study was to investigate spontaneous cytokines production by monocytes/macrophages in patient with chronic heart failure of ischemic origin.
Materials and Methods: 96 patients with HF of ischemic genesis were observed. The spontaneous production of interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10) by monocytes/macrophages *in vitro* was detected by ELISA method.
Results: The *in vitro* spontaneous production of proinflammatory cytokines IL-1β and IL-6 by monocytes/macrophages in patients with HF was significant higher and anti-inflammatory IL-10 was lower than in control group. The progression of HF caused to increase of spontaneous production by monocytes/macrophages of IL-1β and IL-6 but decrease of IL-10.
Conclusion: The monocytes/macrophages in patients with ischemic HF are in condition of chronic activation which manifests of overproduction of proinflammatory cytokines and poor secretion of anti-inflammatory IL-10.

Keywords: Heart failure, monocytes/macrophages, interleukin 1β, interleukin 6, interleukin 10

Introduction
Heart failure (HF) is a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress [1]. It is a major health issue in society today, because it is associated with health, death and consumption of healthcare resources. The prevalence of HF depends on the definition applied, but is approximately 1–2% of the adult population in developed countries, rising to ≥10% among people >70 years of age [2]. A US study has predicted that the prevalence of HF will increase by 3 million (25%) in the next 20 years; a similar study based on data from Scotland predicts a 21% increase in the number of admissions for HF by year 2020 [3, 4]. It is estimated that 1-2% of all healthcare expenditure is devoted to HF in developed countries.
Myocardial dysfunction, which take place in HF, leads to activation of several neurohormonal compensatory mechanisms aimed at improving the mechanical environment of heart. In addition to causing further myocardial injury, the neurohormonal responses have detrimental effects on the blood vessels and organs of human body, and create a pathophysiological "vicious circle", accounting for many of the clinical features of the HF syndrome, including electrical instability of myocardium [5].
Different models indicate a role of innate immunity independent of HF etiology. Innate immunity is activated in the myocardium early by recognition of rather unspecific stimuli, summarized as danger-associated molecular patterns. This form of sterile inflammation is prototypically initiated by engagement of innate pattern recognition receptors, like toll-like receptors [6]. It is likely that inflammation is also initiated in human myocardium by innate recognition of pathogen-associated molecular pattern even well before the heart failure becomes symptomatic/diagnosed. However, clinical data to corroborate findings made in animal studies are widely limited to the demonstration of increased circulating levels of soluble mediators, mainly cytokines, in a variety of patient cohorts with heart failure [7].
Monocytes play an important role in immune defence, inflammation, and tissue remodelling and they do so by phagocytosis, antigen processing and presentation, and by cytokine production. Activated, monocytes/macrophages produce many cytokines, chemokines, and

growth factors, including IL-1α and -β, IL-6, tumor necrosis factor-α, macrophage inflammatory proteins 1 α/β etc. [8]. More recent studies reported a strong association between peripheral monocytosis, left ventricle (LV) dysfunction, and LV aneurysm formation after myocardial infarction (MI) [9]. Consequently, inhibition of monocytes activation is a tempting therapeutic target in the prevention of ischaemia-related HF. The present experimental data about spontaneous production of cytokines by macrophages in HF patients are contradictory [10, 11, 12].

The purpose of study was to investigate spontaneous cytokines production by monocytes/macrophages in patient with ischemic heart failure.

Materials and methods

The study was performed in Ivano-Frankivsk Central City Hospital (Ukraine) in accordance with the Helsinki Declaration and Good Clinical Practice Guideline. All patients gave written informed consent and the local ethics committee approved the study protocol. 96 patients with HF of ischemic genesis were observed. The diagnosis was verified by clinical, laboratory and instrumental methods according to European Society of Cardiology recommendations (2016, 2019). Patients were divided into 3 subgroups (according New-York Heart Association (NYHA) functional class (FC) classification of HF): FC II (NYHA) – 27 patients, FC III (NYHA) – 39 patients and FC IV (NYHA) – 30 patients. Control group consist of 19 practically healthy persons. Suspension of monocytes from blood obtained by Recalde H. method [13]. The isolated cells were labeled with a monoclonal antibody (Daco, Glostrup, Denmark) against the monocyte specific positive antigen CD14. The procedure yielded a population of 89-96% CD14-positive cells in the isolated fraction. Cell viability was confirmed by trypan blue test and was 89-93%. Monocytes were suspended in 199 medium supplemented with 30% blood autoserum, 100 U/ml penicillin, 100 µg/ml streptomycin and 10 µg/ml fungizone (Gibco, Grand Island, NY, USA). The cells were counted and the monocyte concentration was adjusted to 1×10^6 cells/ml. A constant number of monocytes (1×10^6 monocytes per well) was placed in a plastic 24-well microtiter plate (Becton-Dickinson, Franklin Lakes, NJ, USA) and left intact for 2 h to allow them to adhere. The medium was then changed, and the cultures were incubated for additional 24 h. Incubations were performed in triplicate at 37 °C in a humidified atmosphere containing 5% CO_2 in air. Interleukin 1β (IL-1β), interleukin 6 (IL-6), and interleukin 10 (IL-10) levels in culture supernatant were determined using commercial ELISA kits (ProCon, Russia; Amersham Pharmacia Biotech, UK) according to the manufacturer's instructions. Statistical analyses were performed using the Statistica 12.0 (StatSoft, Tulsa, OK, USA). Statistical significance was assumed at $p<0.05$.

Results and Discussion

The average age of observed patients with HF was (68.24±9,87) years. In this cohort 22 (22.9%) were females. 68 (70.8 %) persons had history of myocardial infarction (MI). As concomitant diseases more frequent were: arterial hypertension, permanent atrial fibrillation, type 2 diabetes mellitus and chronic kidney diseases.

The *in vitro* spontaneous production of proinflammatory cytokines IL-1β and IL-6 by monocytes/macrophages in patients with HF was significant higher than in control group: (119.50±4.12) pg/10^6 cells vs (51.39±3.71) pg/10^6 cells ($p<0.001$) and (6.62±0.41) pg/10^6 cells vs (2.79±0.28) pg/10^6 cells ($p<0.001$) respectively. Instead the spontaneous production of anti-inflammatory IL-10 was lower in HF: (3.79±0.41) pg/10^6 cells vs (5.86±0.76) pg/10^6 cells ($p<0.01$). The severity of HF was associated with increased production by monocytes/macrophages of IL-1β and IL-6 and with decreased production of IL-10 (see table).

It's known, *in vitro* macrophages can be generated from bone marrow precursors by various means. Macrophages generated in the presence of interferon-gamma (IFNg) or lipopolysaccharide (LPS) have been termed M1, or classically-activated, inflammatory, macrophages. Macrophages generated in the presence of IL-4 or IL-10, however, have been called M2, or alternatively activated macrophages, and carry a pro-resolution profile [14]. In our case we can allow about M1 (proinflammatory) pathway of monocytes activation which could leads to HF destabilization. Some recent studies showed the similar results. In particular, CD14 expression and monocyte cytokine production (IL-1β, IL-6, TNF-α), both unstimulated and after LPS stimulation, are increased in moderate-severe CHF when compared with mild CHF [15]. Another study showed that IL-10, as strong anti-inflammatory cytokine, profoundly inhibits TNF-α release from monocytes/macrophages isolated from patients with chronic HF [16]. These data suggest that circulating monocytes, possibly via overproduction of proinflammatory cytokines, may play a significant role in the immunologic dysbalance observed in advanced CHF.

Table 1: The in vitro spontaneous production of cytokines by monocytes/macrophages in HF patients (M±SE)

Parameter	HF patients, n=96			Control group, n=19
	FC II, n=27	FC III, n=39	FC IV, n=30	
IL-1β, pg/10^6 cells	96.27±3,14 $p_1<0.01$	115.12±4.75 $p_1<0.001$ $p_2<0.05$	147.11±4.34 $p_1<0.001$ $p_2<0.001$ $p_3<0.001$	51.39±3.71
IL-6, pg/10^6 cells	5.11±0.45 $p_1<0.001$	6.41±0.37 $p_1<0.001$ $p_2<0.05$	8.34±0.44 $p_1<0.001$ $p_2<0.01$ $p_3<0.01$	2.79±0.28
IL-10, pg/10^6 cells	5.21±0.45 $p_1>0.05$	3.21±0.47 $p_1<0.05$ $p_2<0.05$	2.96±0.33 $p_1<0.01$ $p_2<0.01$ $p_3<0.05$	5.86±0.76

Remarks: HF – heart failure; FC – functional class of heart failure (NYHA); p_1 – difference with control; p_2 – difference with FC II group; p_3 – difference with FC III group.

Conclusion

The monocytes/macrophages in patients with ischemic HF are in condition of chronic activation which manifests of overproduction of proinflammatory cytokines and poor secretion of anti-inflammatory IL-10.

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