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Chemotactic activity of serum obtained from patients with idiopathic dilated cardiomyopathy

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Elevated circulating levels of α- and β-chemokines in heart failure have been reported. The objective of this study was to investigate the interrelation of chemotactic activity of serum and circulating chemokine levels in patients suffering from idiopathic dilated cardiomyopathy (IDCM). Chemokine serum levels (MCP-1, MIP1-α, RANTES, IL-8 and TNF-α) were determined in patients with IDCM ($n = 10$), patients with coronary artery disease with normal (CAD-1; $n = 10$) or depressed (CAD-2; $n = 10$) left ventricular function and healthy controls ($n = 10$). The chemotactic effect of sera obtained from these groups was measured using an *in vitro* chemotaxis assay. Sera obtained from IDCM (5475 ± 681 cells) showed the highest chemotactic activity when compared to controls (1850 ± 215 cells), CAD-1 (3325 ± 275 cells) and CAD-2 (2800 ± 275 cells, $P < 0.05$) associated with significantly higher circulating MCP-1 levels. Sera obtained from IDCM patients show a high chemotactic activity associated with significantly elevated circulating MCP-1.

1. Introduction

Hence the role of cytokines in the context of heart failure is extensively documented, little data exist regarding the importance of chemotactic substances. The finding of lymphocytic and monocytic myocardial infiltration in idiopathic dilated cardiomyopathy (IDCM) raises the question how this infiltration is regulated and whether chemokines play a pathophysiologically important part (Aukrust et al. 1999).

Migration of leukocytes is regulated by chemotactic cytokines (chemokines). In contrast to well known unspecific chemotactic factors the recently described chemokines direct the movement of distinct leukocyte populations with a partly overlapping spectrum (Luster 1998).

Aukrust et al. (1998) for the first time reported elevated circulating levels of CC chemokines in heart failure. They found significantly elevated serum concentrations of monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α(MIP-1α) and RANTES (regulated on activation normally T-cell expressed and secreted) in heart failure irrespective of the etiology of heart failure and inversely correlated with left ventricular ejection fraction (MCP-1 and MIP-1α; Aukrust et al. 1998). Furthermore, the same group reported about elevated levels of CXC-chemokines in heart failure (Damas et al. 2000). IL-8 concentrations showed a gradual increase along with increasing NYHA class. Release of these chemokines from monocytes and platelets has been suggested by the same group to be the cause of elevated circulating levels in congestive heart failure (Damas et al. 2000).

Seino et al. for the first time reported that the MCP-1 mRNA is expressed in the myocardium of patients with IDCM (Seino et al. 1995). In a transgenic murine model of α-cardiac myosin promotor driven MCP-1 over expression it has been demonstrated that MCP-1 leads to increased myocardial leukocyte infiltration and a phenotype of dilated cardiomyopathy (Kolattukudy et al. 1998).

In the light of these reports, the objective of this study was to investigate whether increased circulating levels of chemokines are linked to higher chemotactic activity of sera obtained from patients with heart failure. Different aetiologies (IDCM vs. ischemic) should be compared to determine disease specific effects.

2. Investigations and results

2.1. Influence of serum of heart failure subjects and healthy controls on chemotaxis in vitro

We wanted to analyze the effect of serum of patients suffering from heart failure in comparison to serum of healthy controls on the direction of leukocyte movement *in vitro* (chemotaxis). The baseline characteristics of patients and controls are given in Tables 1 (demographic and routine laboratory parameters) and 2 (echocardiographic parameters and parameters obtained by cardiac catheterization). As indicated the CAD-2 group (impaired left ventricular function) and the IDCM group show a comparable severity of heart failure (NYHA 2.5 ± 0.3 vs. 2.3 ± 0.2). Hence left ventricular function is more depressed in the IDCM group as in the CAD-2 group (24 ± 2.0 vs.

Table: 1: Clinical characteristics of all groups (mean ± SEM)

	Controls	CAD-1	CAD-2	IDCM	P
Female/male	5/5	3/7	2/8	2/8	n.s.
Age (yrs)	27 ± 5.2	64.2 ± 3.5	65.2 ± 2.5	55.6 ± 3.8	n.s.
Height (cm)	182 ± 8.4	167 ± 3.3	176 ± 2.8	172 ± 1.9	n.s.
Body Weight (kg)	73 ± 7.6	81 ± 4.9	87.8 ± 6.4	79.3 ± 2.0	n.s.
ESR 1. Hour (mm)	6 ± 3.3	19 ± 13.5	61 ± 5.5	37 ± 10.0	n.s.
Leukocyte Count (Gpt/l)	6.8 ± 0.7	7.1 ± 0.3	8.6 ± 0.6	8.0 ± 0.4	n.s.
CrP (mg/l)	2.1 ± 0.3	7.7 ± 1.4	24.6 ± 6,3	9.5 ± 2.0	0.5^{+*}
Duration of Symptoms (Months)	–	10 ± 6	39 ± 23	91 ± 57	n.s.
NYHA Class	–	1.0 ± 0.3	2.5 ± 0.3	2.3 ± 0.2	$0.05^{*\#}$
ACEI Therapy	0/10	4/10	8/10	7/10	n.s.
Betablocker Therapy	0/10	9/10	8/10	3/10	n.s.
Diuretics	0/10	2/10	7/10	8/10	0.05
Digitalis	0/10	0/10	2/10	7/10	0.05

CAD-1 = Patients with coronary artery disease and normal left ventricular function; CAD-2 = Patients with coronary artery disease and depressed left ventricular function; ESR = Erythrocyte sedimentation rate; CrP = C-reactive Protein; NYHA = New York Heart Association; ACEI = Angiotensin I Converting Enzyme Inhibitor. For comparison of variables with a steady distribution the t-test for multiple comparison with α-correction was used.($^+$ = Controls vs. CAD-2; * = CAD-1 vs. CAD-2; $^\#$ = CAD-1 vs. IDCM). For comparison of discrete distributed variables the χ^2-test was used. Only groups CAD-1, CAD-2 and IDCM were compared

39 ± 4.5%). The Fig., panel A, shows the results of the *in vitro* chemotaxis assay. Serum obtained from patients with IDCM had the highest chemotactic effect in our *in vitro* chemotaxis assay with significant differences to all other groups studied.

2.2. Measurement of chemokine/cytokine serum concentrations

Serum concentration of MCP-1, RANTES, MIP-1α, Interleukin-8 and TNF-α were measured in patients with IDCM, coronary artery disease (normal vs. impaired LV function) and healthy controls. The results are given in the Fig., panels B–F. In comparison to CAD with reduced left ventricular function and to healthy controls, serum obtained from IDCM patients showed by 84 and by 77% statistically significant higher MCP-1 concentrations. Serum concentrations of RANTES in both groups of patients with coronary artery disease were significantly higher than those determined in the control and IDCM group. The Interleukin-8 serum concentration in the IDCM group was significantly lower than in all other groups. The IDCM and CAD-2 groups showed a comparable clinical severity of heart failure. Hence, compared to the controls only in the IDCM group a significantly elevated level of TNF-α was found.

3. Discussion

In contrast to a host of different cytokines, e.g. the interleukins and tumour necrosis factor – α, the role of chemokines has been studied less extensive (Aukrust et al. 1998; Levine et al. 1990; Lommi et al. 1997; Blum and Miller 1998). Aukrust et al. have first reported elevated serum concentrations of C–C chemokines in patients suffering from heart failure (Aukrust et al. 1998). They found elevated levels of MCP-1, MIP-1α and RANTES in patients with heart failure independent of the underlying disease. In patients with severe NYHA class IV symptomatic heart failure under resting condition they measured the highest chemokine concentrations. The serum concentrations of MCP-1 and MIP-1α were significantly correlated to left ventricular ejection fraction. The authors suggested following studies of isolated peripheral blood cells that CD3 positive lymphocytes and macrophages are responsible for the elevated chemokine serum concentrations.
Therefore, this study should clarify the question whether sera obtained from patients with heart failure and different underlying diseases direct the movement of monocytic cells. We established a chemotaxis assay and studied the following groups: CAD with normal or depressed LV systolic function, DCM and healthy controls. The two heart failure groups were comparable with regard of the clinical severity of heart failure (NYHA class). However, the LVEF was lower and the left ventricular end diastolic diameter were higher in the DCM group compared to the CAD group with left ventricular dysfunction. The chemotactic activity was highest in the group of patients with DCM when compared to controls and both CAD groups. This finding was coincident with higher MCP-1 serum concentrations found in the DCM group. In comparison to controls and patients with CAD and depressed LV function MCP-1 was significantly higher in DCM patients. An increased chemotactic activity might indicate an in heart

Table 2: Echocardiographic and hemodynamic parameter of the groups (mean ± SEM)

	Controls	CAD-1	CAD-2	IDCM	P
LVEDD (mm)	46 ± 1.7	47 ± 1.2	58 ± 3.5	67 ± 2.7	$0.05^{+\S*\#}$
IVSD (mm)	9 ± 0.3	12 ± 0.3	12 ± 0.8	11 ± 0.7	$0.05^{\$+}$
LVPWD (mm)	9 ± 0.1	12 ± 0.5	12 ± 0.5	11 ± 0.5	$0.05^{\$+}$
LVEF (%)	66 ± 1.0	74 ± 2.0	39 ± 4.5	24 ± 2.0	$0.05^{*\#+}$
LVEDP	–	10 ± 0.8	15 ± 3.4	24 ± 4.3	$0.05^{\#}$

LVEDD = Left ventricular enddiastolic diameter, IVSD = Interventricular septum diameter, LVPWD = Left ventricular posterior wall diameter – all determined by echocardiography; LVEF = Left ventricular ejection fraction, LVEDP = Left ventricular enddiastolic pressure – Parameter assessed by ventriculography and invasive pressure measurement. For comparison of variables with a steady distribution the t-test for multiple comparison with α-correction was used. ($^\$$ = Controls vs. CAD-1; $^+$ = Controls vs. CAD-2; § = controls vs. IDCM; * = CAD-1 vs. CAD-2; $^\#$ = CAD-1 vs. IDCM, $^+$ = CAD-2 vs. IDC)

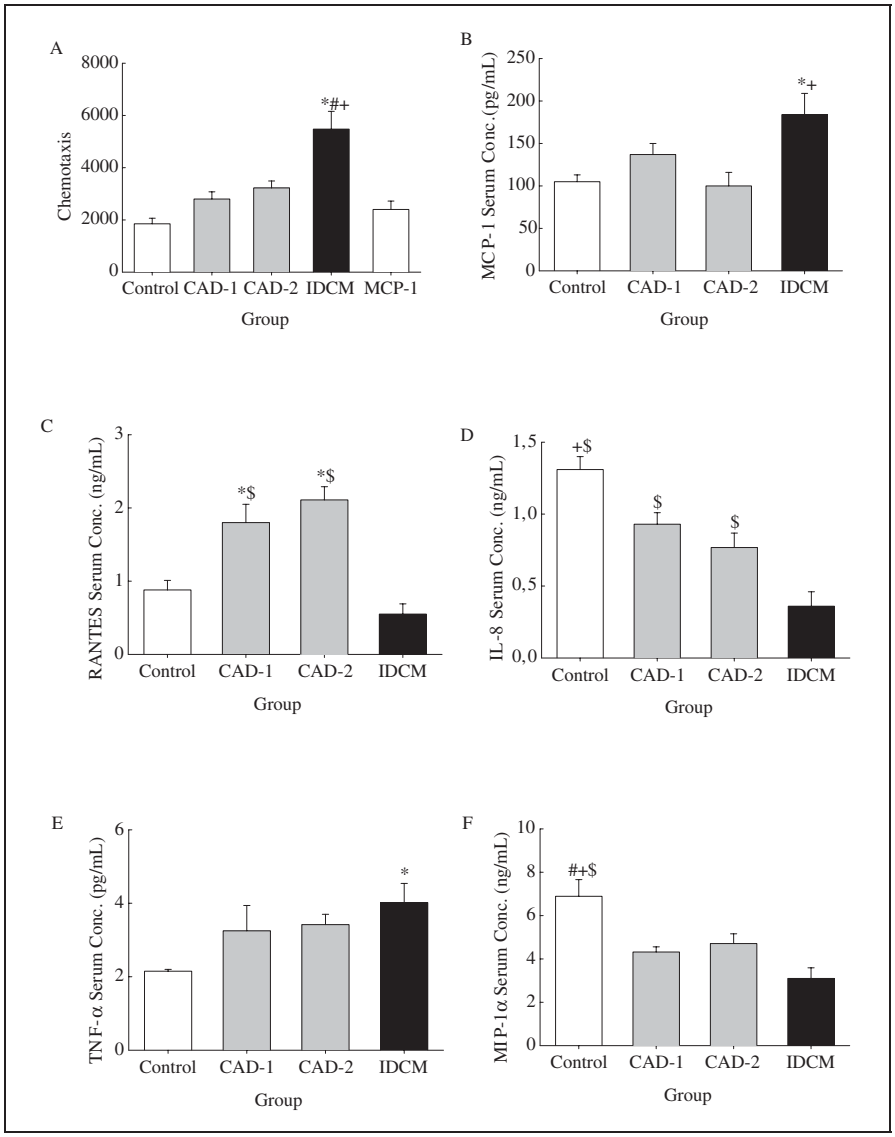

Fig.:
Chemotactic activity and serum concentrations of cytokines/chemokines in patients with heart failure (IDCM vs. CAD) and healthy controls.
Panel A: Chemotactic activity of sera obtained from patients with IDCM (n = 10), CAD and normal left ventricular ejection fraction (LVEF, CAD-1, n = 10), CAD and impaired LVEF (n = 10, CAD-2), healthy controls (n = 10) on myelomonocytic cells (THP-1) in comparison to recombinant MCP-1.
Panels B-F summarize the serum concentrations of MCP-1 (pg/mL), RANTES (ng/mL), Interleukin-8 (ng(mL), TNF-& (pg/mL) and MIP-1& (ng/mL) measured by ELISA in patients with IDCM (n = 10), CAD and regular LVEF (n = 10), CAD and impaired LVEF (n = 10) and healthy controls (n = 10).
* $P < 0.05$ in comparison to control, # $P < 0.05$ in comparison to CAD in comparison to CAD-1, + $P < 0.05$ in comparison to CAD-2, \$ $P < 0.05$ in comparison to IDCM-t-test for multiple comparison with α-correction, respectively

failure generally elevated level of immune activation (Yndestad et al. 2003). However, the presented data seem to indicate that this is an IDCM specific feature. In a myocardial infarction model in mice it has been shown that the blockade of MCP-1 leads to favourable remodelling of the infarcted left ventricle (Hayashidani et al. 2003). In IDCM chemokine activation and increased chemotactic activity might contribute to the formation of mural thrombi found in the left ventricle of these patients (Yamamoto et al. 1995).

In contrast to the data reported by Aukrust et al. (1998), MIP-1α concentrations in the heart failure groups were not found elevated, but were, in contrast, in all patient groups lower when compared to healthy controls. The serum concentrations of RANTES in the two CAD groups were significantly higher as in DCM and controls. RANTES and MIP-1α are chemotactic for monocytes, natural killer cells, dendritic cells, activated T-cells and eosinophilic granulocytes by activating the chemokine receptors CCR5 and CCR1 (Luster et al. 1998).

In conclusion, we found that the pattern of chemokines here assessed in IDCM is dominated by MCP-1. This chemokine presumably is responsible for the strong chemotactic activity found to be a feature of sera obtained from DCM patients.

4. Experimental

4.1. Subjects

The protocol of this study has been approved by the ethics committee of the medical faculty at the University of Jena. Patients and healthy volunteers gave their informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Sera drawn from forty subjects/patients with or without heart failure and healthy volunteers were investigated regarding their properties to direct movement of leukocytes through a membrane (chemotaxis). In detail, we studied ten patients with idiopathic dilated cardiomyopathy, ten patients with coronary artery disease but preserved left ventricular function, ten patients with coronary artery disease and depressed left ventricular function and ten healthy controls. Demographic, routine laboratory, hemodynamic and echocardiographic data are given in Tables 1 and 2. For use in the chemotaxis assay and for chemokine measurement by ELISA (see below) blood was immediately placed on ice and within 10 min centrifuged at 4 °C for 7 min at 3000 rpm to separate corpuscular components. The resulting sera were stored at −80 °C until use. Serum collection and recording of hemodynamics and clinical severity of heart failure were done at the same time.

4.2. In vitro chemotaxis assay

The myelomonocytic cell line THP-1 (Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSM, Braunschweig, Germany) was obtained for this assay. Cells were maintained in RPMI 1640 medium, low endotoxin (BIOCHROM, Berlin, Germany) supplemented with 10% fetal calf serum (GIBCO Life Science, Eggenstein, Germany), 100 μg/ml streptomycin and 100 U/ml of penicillin (both SIGMA CHEMICALS, St. Louis, MO, U.S.A.) at 37 °C under a 5% CO_2 atmosphere with 90% humidity. THP-1

cells were grown until saturation and subsequently 50% of the suspension have been replaced with fresh medium. For adjustment of cell density 100 µl of the suspension were diluted with 200 µl of PBS and 100 µl of Trypan blue to count the number of cells in a Neubauer chamber. Accordingly, the cell suspension was adjusted to one million cells per one mL. All forty samples were studied at the same time under identical conditions to exclude day to day variation of the cell culture. For the assay Falcon 3504 cell culture plates (companion plates for membrane inserts) were used and 800 µl of RPMI 1640 cell culture medium and 10 µl serum were added. As negative control the medium alone and for positive control 100 ng of human recombinant MCP-1 (Bioconcept, Umkirch, Germany) were used. Then, the membrane inserts (Falcon membrane inserts 3097 with 8 µm pores) were brought in placed and 200 µl of the adjusted cell suspension (200,000 cells) were added. After an incubation period of 5 h and 30 min the membrane inserts were removed and the number of cells in the sample was determined by counting in a Neubauer chamber.

4.3. Chemokine/cytokine analysis by ELISA

Serum concentration of MCP-1 has been determined by a competitive ELISA of BIOCONCEPT (Umkirch, Germany). RANTES, Interleukin-8 and MIP-1α serum concentrations were analyzed using kits of CYTIMMUNE SCIENCES INC (Maryland, USA). The concentration of TNF-α was determined by Quantikine HS ELISA of R&D Systems (Wiesbaden, Germany).

4.5. Statistics

Results are given as mean ± SEM. A t-test for multiple comparison with α-correction (Bonferroni-Test) has been used for comparison of more than two groups. The comparison of discrete variables was done using χ^2 testing. A P value of ≤ 0.05 indicated a significant difference. SPPS for Windows, version 6.1 was used for all calculations.

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