Enhanced Gene Expression of Chemokines and Their Corresponding Receptors in Mononuclear Blood Cells in Chronic Heart Failure—Modulatory Effect of Intravenous Immunoglobulin

Jan K. Damås, MD,*† Lars Gullestad, MD, PHD,* Halfdan Aass, MD, Ptd,* Svein Simonsen, MD, Phd,* Jan G. Fjeld, MD, Ptd,‡ Lisbeth Wikeby, RN,† Thor Ulæand, BS,‡ Hans G. Eiken, Ptd,†¶ Stig S. Frøland, MD, Ptd,†|| Pal Aukrust, MD, Ptd†||

Oslo, Norway

OBJECTIVES We sought to study the gene expression of chemokines and their corresponding receptors in mononuclear blood cells (MNCs) from patients with chronic heart failure (CHF), both of which were cross-sectional and longitudinal studies during therapy with intravenous immunoglobulin (IVIg).

BACKGROUND We have recently demonstrated that IVIg improves left ventricular ejection fraction (LVEF) in patients with CHF. Based on the potential pathogenic role of chemokines in CHF, we hypothesized that the beneficial effect of IVIg may be related to a modulatory effect on the expression of chemokines and their receptors in MNCs.

METHODS We examined: 1) the gene expression of C, CC and CXC chemokines and their receptors in MNCs from 20 patients with CHF and 10 healthy blood donors; and 2) the expression of these genes in MNCs from 20 patients with CHF randomized in a double-blind fashion to therapy with IVIg or placebo for 26 weeks.

RESULTS Our main findings in CHF were: 1) markedly raised gene expression of macrophage inflammatory protein (MIP)-1α, MIP-1β and interleukin (IL)-8; 2) enhanced gene expression of their corresponding receptors; 3) modulation in a normal direction of this abnormal chemokine and chemokine receptor gene expression during IVIg, but not during placebo therapy; 4) down-regulation of MIP-1α, MIP-1β and IL-8 during IVIg at the protein level in plasma; and 5) a correlation between down-regulation of MIP-1α gene expression and improved LVEF during IVIg therapy.

CONCLUSIONS Our results further support a pathogenic role for chemokines in CHF and suggest that IVIg may represent a novel therapeutic approach, with the potential to improve LVEF in patients with CHF, possibly by modulatory effects on the chemokine network. (J Am Coll Cardiol 2001;38:187–93) © 2001 by the American College of Cardiology

Increasing evidence supports that inflammation is involved in the pathogenesis of chronic heart failure (CHF). Increased plasma levels and myocardial expression of inflammatory cytokines are found in patients with CHF (1,2) and have been shown to induce myocardial dysfunction in various animal models (3,4). Chemokines are a family of inflammatory cytokines characterized by their ability to cause directed migration of leukocytes into inflamed tissue (5,6), and recent studies suggest that these mediators may be involved in the pathogenesis of CHF (7–9). Thus, myocardial up-regulation of chemokines has been found in animal models of heart failure, possibly inducing myocardial infiltration of mononuclear blood cells (MNCs), with the development of pathologic changes characteristic of CHF (10,11). Chronic, low-grade inflammation with infiltrating leukocytes has also been found in the failing human myocardium (12). By playing a crucial role in the activation and recruitment of these cells, chemokines may indirectly lead to damage and dysfunction of the cardiac muscle.

Intravenous immunoglobulin (IVIg) has been tried in a wide range of immune-mediated disorders (13), and beneficial effects have also been suggested in acute and peripartum cardiomyopathy (14,15). We have recently demonstrated that IVIg improves left ventricular ejection fraction (LVEF) in patients with CHF (16), and we hypothesized that the beneficial effect of IVIg may be partly related to the modulatory effects on the chemokine expression. To further elucidate the possible role of chemokines in the pathogenesis of CHF, we examined: 1) the gene expression of C, CC and CXC chemokines and their corresponding receptors in MNCs isolated from patients with CHF and healthy blood donors; and 2) the expression of these genes in MNCs collected during right-sided heart catheterization in patients with CHF randomized in a double-blind fashion to receive therapy with either IVIg or placebo for a total period of 26 weeks.
Clinical Characteristics of Patients With Congestive Heart Failure Participating in the Study

<table>
<thead>
<tr>
<th></th>
<th>IVIg (n = 10)</th>
<th>Placebo (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>59 ± 1.6</td>
<td>61 ± 2.2</td>
</tr>
<tr>
<td><strong>Gender (male/female)</strong></td>
<td>8/2</td>
<td>7/3</td>
</tr>
<tr>
<td><strong>NYHA functional class (II/III)</strong></td>
<td>4/6</td>
<td>3/7</td>
</tr>
<tr>
<td><strong>Cause of heart failure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IDCM</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Duration of heart failure (yrs)</strong></td>
<td>3.6 ± 0.8</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td><strong>Left ventricular ejection fraction (%)</strong></td>
<td>25 ± 2</td>
<td>27 ± 3</td>
</tr>
<tr>
<td><strong>Exercise data (peak VO₂, l/min)</strong></td>
<td>1.30 ± 0.10</td>
<td>1.16 ± 0.08</td>
</tr>
<tr>
<td><strong>N-terminal-pro-ANP (pmol/l)</strong></td>
<td>2,280 ± 384</td>
<td>2,150 ± 450</td>
</tr>
<tr>
<td><strong>Medications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Angiotensin II blockers</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Diuretics</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Digitalis</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SEM or number of patients or subjects.

**Abbreviations and Acronyms**
- CCR = CC chemokine receptor
- CHF = chronic heart failure
- CXCR = CXC chemokine receptor
- IL-8 = interleukin-8
- IVIg = intravenous immunoglobulin
- LVEF = left ventricular ejection fraction
- MIP-1α or -1β = macrophage inflammatory protein-1α or -1β
- MNCs = mononuclear blood cells
- (m)RNA = (messenger) ribonucleic acid
- RPA = ribonuclease protection assay

**METHODS**

**Patients.** The MNCs were collected from 20 patients participating in the IVIg study (Table 1) (16). Patients were included in the IVIg study if they: 1) had chronic, stable CHF for >6 months; 2) were classified in New York Heart Association functional class II to III; 3) had LVEF <40%; 4) had no changes in medication during the last three months; and 5) were receiving optimal medical treatment. Patients were not included if they had: 1) evidence of myocardial infarction or unstable angina during the last six months, or 2) significant concomitant disease, such as infections, pulmonary disease or connective tissue disease. The conventional cardiovascular treatment was not changed in any of the patients during the study. The etiology of CHF was classified as coronary artery disease (n = 10) or idiopathic dilated cardiomyopathy (n = 10). For comparison, MNCs were isolated from peripheral venous blood drawn from the antecubital vein in 10 healthy gender- and age-matched control subjects. The study was approved by the Regional Ethics Committee and conformed to the Declaration of Helsinki. Written, informed consent was obtained from each patient.

**Preparation of IVIg.** Octagam (Octapharma, Vienna, Austria), produced from fresh-frozen plasma collected from Norwegian blood banks, was dispensed in sterile water containing 10% maltose (final IgG concentration of 50 g/l) (16).

**Design of the IVIg study.** The study design has previously been described (16). Briefly, after baseline measurements, the patients were randomized to either IVIg or placebo (5% glucose) in a double-blind fashion and stratified according to etiology (i.e., coronary artery disease or idiopathic dilated cardiomyopathy). Either IVIg or an equal volume of placebo was given as induction therapy (one daily infusion of 0.4 g/kg body weight for five days), and thereafter as a monthly infusion (0.4 g/kg for five months). Baseline measurements were repeated at the end of the study (26 weeks, four weeks after the last IVIg or placebo infusion). At baseline and at the end of the study, LVEF was assessed by electrocardiographically synchronized, gated radionuclide ventriculography at rest (16), and blood samples from the pulmonary artery and coronary sinus were collected during right-sided heart catheterization. Plasma samples from peripheral venous blood were obtained at baseline and after one, three and five months. In addition, four patients with CHF (not included in the IVIg study) underwent left- and right-sided heart catheterization to examine the differences in chemokine gene expression between the femoral vein, femoral artery and pulmonary artery, and in the femoral vein between the start and end of the catheterization procedure. Collection and storage of plasma were performed as previously described (16).

**Ribonuclease protection assay (RPA).** The MNCs were obtained from heparinized blood by Isopaque–Ficoll (Lymphoprep, Nycomed Pharma AS, Oslo, Norway) through gradient centrifugation within 45 min. Total ribonuclease acid (RNA) was extracted from frozen cells using RNeasy columns (Qiagen, Hilden, Germany) and stored in RNA storage solution (Ambion, Austin, Texas) at −80°C until used. Multiprobe template sets (hCK5, hCR5 and hCR6) were available with reagents for in vitro transcription and RPA (RiboQuant; Pharmingen, San Diego, California). The RPA was used for the detection and quantification of messenger RNA (mRNA) species, as previously described (9).

**Miscellaneous.** Plasma levels of macrophage inflammatory protein (MIP)-1α, MIP-1β and interleukin (IL)-8 were measured by enzyme immunoassays (R&D Systems, Minneapolis, Minnesota). The numbers of CD2⁺, CD4⁺, CD8⁺ and CD19⁺ lymphocytes and monocytes (CD14⁺ cells) were determined by immunomagnetic quantification (17).

**Statistical analysis.** Differences between the groups were compared by using the Mann–Whitney rank-sum test for unpaired data. In the paired situation, two-way repeated measures analysis of variance was performed a priori; if the
outcome was significant, the Wilcoxon signed rank test for paired data was performed a posteriori. Correlations between variables were tested using the Spearman rank test. The p values are two-sided and considered significant at \( p < 0.05 \).

RESULTS

Changes in MNC’s chemokine gene expression in human CHF. Of the eight chemokine genes tested, seven were detected with RPA in patients with CHF and healthy blood donors (Fig. 1). The gene expression of MIP-1\( \alpha \) (~21-fold), MIP-1\( \beta \) (~10-fold) and IL-8 (~36-fold), but not regulated upon activation, normally T cell expressed and secreted (RANTES), was markedly increased in the patients compared with the healthy control subjects (Fig. 1). The other chemokines were detected at very low levels in both patients with CHF and control subjects.

In the patients with CHF, the mRNA level of MIP-1\( \alpha \) was significantly higher in MNCs from arterial blood compared with cells from venous blood (~24% increase, \( p < 0.05 \)). Moreover, gene expression of this chemokine was also significantly increased in cells from the coronary sinus compared with those from the pulmonary artery (~30% increase, \( p < 0.01 \)). Furthermore, there were no differences between peripheral and central venous blood in patients with CHF and no significant induction of chemokine genes during the catheterization procedure (data not shown).

Changes in MNC’s chemokine receptor gene expression in human CHF. Concomitant with the enhanced expression of MIP-1\( \alpha \), MIP-1\( \beta \) and IL-8, the gene expression of their corresponding receptors (CC chemokine receptor [CCR] 1, CCR5, CXC chemokine receptor [CXCR] 1 and CXCR2) was also significantly increased in MNCs from patients with CHF (Fig. 2). In addition, CCR2 (i.e., the MCP-1 receptor) and CX3CR (the fractalkine receptor) showed enhanced gene expression in CHF (Fig. 2). In contrast, gene expression of chemokine receptors involved in constitutive lymphocyte homing (i.e., CXCR5 and CCR7) (6) was significantly reduced in patients with CHF compared with healthy blood donors (Fig. 2).

Effect of IVIg therapy on chemokine and chemokine receptor expression. Next, we examined whether IVIg could modulate the enhanced expression of chemokines and their corresponding receptors in patients with CHF receiving IVIg (\( n = 10 \)) or placebo (\( n = 10 \)) for 26 weeks (see Methods section). The interaction between the time of observation and chemokine gene expression was significant for MIP-1\( \alpha \) (\( p < 0.001 \)) and MIP-1\( \beta \) (\( p < 0.01 \)), but no consistent interaction was seen for IL-8 gene expression (\( p = 0.08 \)). Gene expression of MIP-1\( \alpha \) and MIP-1\( \beta \) significantly decreased during IVIg treatment, but not during placebo; this decrease was seen in all but one patient (Fig. 3). We also measured plasma levels of MIP-1\( \alpha \), MIP-1\( \beta \) and IL-8 at baseline and after one, three and five months of therapy. The interaction between the time of observation and chemokine plasma levels was significant for MIP-1\( \alpha \) (\( p < 0.001 \)) and MIP-1\( \beta \) (\( p < 0.001 \)) and IL-8 (\( p < 0.05 \)). Although these chemokines tended to increase in the placebo group, they significantly decreased during IVIg therapy, resulting in significant differences in changes between the groups for MIP-1\( \alpha \) and MIP-1\( \beta \) (Fig. 4). Moreover, there was also a significant interaction between the time of observation and chemokine receptor gene expression; CCR1 (\( p < 0.001 \)), CCR5 (\( p < 0.01 \)) and CXCR1 (\( p < 0.05 \)). Thus, the decrease in chemokine levels...
was accompanied by a significant decrease in their receptor levels on MNCs (i.e., CCR1, CCR5 and CXCR1) during IVIg, but not placebo, treatment (Fig. 5).

Chemokine and chemokine receptor gene expression in MNCs in relation to LVEF. Gene expression of MIP-1α (r = −0.66, p < 0.01), MIP-1β (r = −0.52, p < 0.05) and their common receptor CCR1 (r = −0.64, p < 0.01) was inversely correlated with LVEF at baseline. We have previously shown a significant increase in LVEF (five ejection fraction units) after IVIg treatment, but not after placebo, in these patients with CHF (16). Importantly, this increase in LVEF was significantly correlated with the decrease in MIP-1α mRNA levels during IVIg (Fig. 6).

DISCUSSION

Increased chemokine expression has been detected during inflammation in most organs, resulting in a selective recruitment of leukocyte subpopulations into inflamed tissue (5,6). To the best of our knowledge, the present study is the first to show markedly altered gene expression of several chemokines and their corresponding receptors in MNCs from patients with CHF, and notably, these abnormalities were significantly modulated in a normal direction during IVIg therapy. As for MIP-1α levels, down-regulation was significantly correlated with improved LVEF during such therapy. These findings support a role for abnormal chemokine activation in the pathogenesis of CHF and suggest that IVIg may modulate such activity.
Enhanced gene expression of chemokines and their receptors in MNCs from patients with CHF. In the present study, we have shown increased gene expression of both CC (i.e., MIP-1α and MIP-1β) and CXC chemokines (i.e., IL-8) in MNCs from patients with CHF, with particularly high expressions of MIP-1α and MIP-1β in patients with a low LVEF. Moreover, chemokine expression was significantly enhanced in MNCs from arterial versus venous blood and in MNCs from the coronary sinus versus the pulmonary artery, suggesting an activation of chemokine expression during passage through the lung and pulmonary vasculature.

**Figure 4.** Plasma levels of macrophage inflammatory protein-1α (MIP-1α) (A), macrophage inflammatory protein-1β (MIP-1β) (B) and interleukin-8 (IL-8) (C), given as the percent change from baseline (MIP-1α: 25.2 ± 2.0 pg/ml; MIP-1β: 93.4 ± 4.5 pg/ml; IL-8: 22.8 ± 1.8 pg/ml), in 20 patients with chronic heart failure before and at different time points after initiating treatment with intravenous immunoglobulin (n = 10; solid circles) or placebo (n = 10; open circles). Data are given as the mean value ± SEM. *p < 0.05; **p < 0.01 versus baseline; #p < 0.05 when comparing changes between intravenous immunoglobulin and placebo.

**Figure 5.** Relative messenger ribonucleic acid (mRNA) levels of chemokine receptor (CCR) 1 (A), CCR5 (B) and CXCR1 (C) in mononuclear blood cells from 20 patients with chronic heart failure before and after six months (mo) of treatment with intravenous immunoglobulin (IVIg) (n = 10) or placebo (n = 10). As for CCR1 (p < 0.001) and CCR5 (p < 0.01), there were also differences in changes between the two groups. Horizontal lines indicate mean values; n.s. = not significant. rpL32 = ribosomal protein L32.
heart circulation, respectively. Recent reports indicate that chemokines are involved in the migration of MNCs into the cardiovascular system in various disorders, such as atherosclerosis and myocarditis (5,6,11). These activated MNCs may, in turn, damage the vessel wall and myocardium through production of reactive oxygen species, proteolytic enzymes and inflammatory cytokines (5,6). Thus, MIP-1α knock-out mice do not develop cardiac lesions after Cox-sackie virus B infection because of attenuated myocardial inflammation (5,11,20). However, although the present study suggests a potential role for chemokines in the pathogenesis of CHF, a small number of patients were studied, and the effect of IVIg on the chemokine network in patients with CHF will have to be confirmed in future studies.

Conclusions. The present study demonstrates markedly altered gene expression of chemokine and chemokine receptor in MNCs from patients with CHF, and as for MIP-1α and MIP-1β and their corresponding receptors, enhanced expression was significantly correlated with depressed LVEF. Notably, these chemokine abnormalities were significantly modulated in a normal direction during IVIg therapy, concomitant with a significant improvement of LVEF. Our findings further support a role for chemokines in the pathogenesis of CHF and suggest that IVIg may represent a novel therapeutic approach, with the potential to improve LVEF in patients with CHF, at least partly by modulatory effects on the chemokine network.

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Reprint requests and correspondence: Dr. Jan K. Damås, Research Institute for Internal Medicine, Rikshospitalet, N-0027 Oslo, Norway. E-mail: j.k.damas@klinmed.uio.no.

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Figure 6. Correlation between absolute change in left ventricular ejection fraction (LVEF) (%), as assessed by electrocardiographically synchronized, gated radionuclide ventriculography at rest, and absolute change in messenger ribonucleic acid (mRNA) levels of macrophage inflammatory protein-1α (MIP-1α) in mononuclear blood cells from 10 patients with chronic heart failure receiving intravenous immunoglobulin for six months (mo). rpL32 = ribosomal protein L32.


