Relationship Between Interleukin-6 Production in the Lungs and Pulmonary Vascular Resistance in Patients With Congestive Heart Failure*

Naoko Mabuchi, MD; Takayoshi Tsutamoto, MD; Atsuyuki Wada, MD; Masato Ohnishi, MD; Keiko Maeda, MD; Masaru Hayashi, MD; and Masahiko Kinoshita, MD

Study objectives: We evaluated whether interleukin (IL)-6 is produced in the pulmonary circulation and investigated the relationship between IL-6 spillover in the lung and pulmonary vascular resistance (PVR) in patients with congestive heart failure (CHF).

Patients and interventions: Blood samples were obtained from the main pulmonary artery and pulmonary capillary wedge region in 50 patients with symptomatic left ventricular dysfunction, who had undergone cardiac catheterization, and 9 age-matched control subjects. Plasma IL-6, tumor necrosis factor-α, norepinephrine (NE), endothelin-1, atrial and brain natriuretic peptide, and cyclic guanosine monophosphate (cGMP) levels were determined.

Measurements and results: Plasma IL-6 concentrations were significantly higher in the pulmonary capillary wedge region than in the main pulmonary artery in both control subjects and patients with CHF. IL-6 production in the lung increased markedly in patients with severe CHF compared with control subjects and patients with mild CHF. Among hemodynamic variables, neurohumoral factors, and medications, plasma NE levels (p < 0.0001) showed an independent and significant positive relationship with IL-6 production in the lung, and treatment with β-blockers (p = 0.004) showed an independent and significant negative relationship with IL-6 production in the lung.

There was a significant positive correlation between IL-6 production in the lung and both PVR (r = 0.43; p = 0.001) and cGMP production in the lung (r = 0.498; p < 0.0001).

Conclusion: IL-6 production in the pulmonary circulation increases with the severity of CHF and is mainly associated with the activation of the sympathetic nervous system. The local production of IL-6 in the lung may modify PVR in patients with CHF.

Key words: congestive heart failure; cyclic guanosine monophosphate; interleukin-6; pulmonary vascular resistance

Abbreviations: ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; cGMP = cyclic guanosine monophosphate; CHF = congestive heart failure; ET = endothelin; IL = interleukin; iNOS = inducible nitric oxide synthase; NE = norepinephrine; NO = nitric oxide; NYHA = New York Heart Association; PVR = pulmonary vascular resistance; TNF = tumor necrosis factor

Immune mechanisms are reported to play an important pathophysiologic role in the development of congestive heart failure (CHF). Interleukin (IL)-6, a multifunctional proinflammatory cytokine that mediates both immune and inflammatory responses, increases in patients with CHF. IL-6 and the IL-6 receptor complex, including gp-130, play an important role in ventricular hypertrophy and left ventricular remodeling, and the high concentration of IL-6 may exert endocrine effects such as a decrease of left ventricular contractility through nitric oxide (NO) production. Although the level of plasma IL-6 has been reported to increase in patients with CHF, the origin and the stimulator of the increase in patients with CHF have not been fully clarified.

We previously reported that there is IL-6 production in the peripheral circulation of healthy subjects, that it increases in relation to the severity of CHF, and that the increase in plasma IL-6 level is mainly associated with the activation of the sympathetic

*From First Department of Internal Medicine, Shiga University of Medical Science, Otsu, Japan. This study was supported by a Grant-in-Aid for Scientific Research, Japan. Manuscript received October 10, 2000; revision accepted August 23, 2001.

Correspondence to: Takayoshi Tsutamoto, MD, First Department of Internal Medicine, Shiga University of Medical Science, Taikanova, Otsu 520-2192, Japan; e-mail: tutamoto@belle.shiga-med.ac.jp
vascular resistance (PVR) in patients with CHF. Moreover, a high plasma level of IL-6 was a significant independent prognostic predictor in patients with CHF, suggesting an important role for IL-6 in the pathophysiology of CHF. IL-6 is produced not only in leukocytes, but also in endothelial cells, vascular smooth muscle cells, myocytes, and fibroblasts in various organs, including the lung. Numerous investigators have shown that the plasma IL-6 level is significantly elevated in patients who have pulmonary hypertension. IL-6 has the ability to induce fibroblast and smooth muscle cell proliferation and may have an important pathophysiologic role in the progression of pulmonary hypertension. On the other hand, IL-6 has the potential to increase inducible nitric oxide synthase (iNOS) along with the release of NO, which leads to vasodilatation mediated through cyclic guanosine monophosphate (cGMP) in the vascular bed, and may modulate the pulmonary vascular tone. However, whether IL-6 is produced in the pulmonary circulation in patients with CHF has not been clarified. Therefore, we evaluated whether IL-6 is produced in the pulmonary circulation and the nature of the relationship between IL-6 production in the lung and pulmonary vascular resistance (PVR) in patients with CHF.

**Materials and Methods**

**Patients**

Fifty patients with symptomatic left ventricular dysfunction (ie, left ventricular ejection fraction < 45%) who had undergone cardiac catheterization because of clinical indications were studied. We also selected nine age-matched control subjects, including two women, who had chest pain but normal coronary arteries. Written informed consent was obtained from all patients before their participation in the study, and the protocol was approved by the Human Investigations Committee of our institution. The subjects included 41 men and 9 women (age range, 28 to 77 years; mean age, 57.6 years). Twenty-five patients had dilated cardiomyopathy, and of these patients 19 had experienced a myocardial infarction > 3 months before the study, 3 had hypertensive heart disease, and 3 had valvular heart disease. Thirty-five patients were classified according to the standards of the New York Heart Association (NYHA) as functional class II, 7 patients were classified as class III, and 8 patients were classified as class IV. Thirty-two patients were treated with diuretics, 20 with digitalis, 20 with angiotensin-converting enzyme inhibitors, 20 with nitrates, 8 with β-blockers, 5 with angiotensin II receptor blocker, and 4 with calcium antagonists. Most drugs had been administered for > 2 months. Patients treated with cathelamines were excluded because we recently reported that administration of catecholamine significantly increased plasma IL-6 in patients with CHF.

**Protocol**

After the measurement of hemodynamic parameters, blood samples for the measurement of plasma IL-6, tumor necrosis factor (TNF)-α, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and cGMP levels were collected from the main pulmonary artery and the site of pulmonary capillary wedge pressure determination, as described previously. Blood samples for measuring the levels of plasma norepinephrine (NE) and endothelin (ET)-1 were also collected from the main pulmonary artery. Left ventriculography was performed with a contrast medium or radioscopy within 1 week of the hemodynamic measurements and blood sampling.

**Measurement of IL-6 and TNF-α**

Both IL-6 and TNF-α measurements were performed using a commercially available immunoassay kit (Quantikine HS; R&D Systems; Minneapolis, MN) as reported previously. The intra-assay coefficients of variation for IL-6 and TNF-α were 3.6% and 6.1%, respectively. The interassay coefficients of variation for IL-6 and TNF-α were 3.8% and 7.8%, respectively.

**Measurement of Neurohumoral Factors**

The plasma ET-1 levels were determined by using an antibody directed against synthetic ET-1 (Peninsula Laboratories, Inc; San Carlos, CA) and iodine-125 ET-1 (Amersham Japan; Tokyo, Japan), as previously reported. Plasma NE concentration was measured by high-performance liquid chromatography. Plasma levels of ANP and BNP were measured with a specific immuno-radiometric assay for human ANP and human BNP (Shionogi; Osaka, Japan), as reported previously. Plasma cGMP concentrations were measured by radioimmunoassay with a commercial kit (Yamasu Shoyu Co. Ltd; Choshi, Japan), as previously reported. The sensitivity of the assay was 0.5 pg/mL, and the intra-assay and interassay coefficients of variation were 2.4% and 8%, respectively.

**Calculations**

The mean BP and PVR were calculated using the standard formulas. The index of the amount of IL-6 and cGMP production in the lungs was determined with the following formula: (IL-6 or cGMP level in the main pulmonary artery) × cardiac output × (1 - hematocrit/100). The extraction levels of the natriuretic peptides in the lung were calculated as follows: (ANP or BNP levels in the main pulmonary artery – ANP or BNP levels at the site of pulmonary capillary wedge pressure determination) × cardiac output × (1 - hematocrit/100).

**Statistical Analysis**

All results were expressed as the mean ± SEM. The comparison of the plasma levels of IL-6 and TNF-α in the main pulmonary artery and those at the site of pulmonary capillary wedge pressure determination was done using the paired Student’s t test. Comparisons between multiple groups were determined by one-way analysis of variance with the Scheffé F test. Linear regression analysis was used to determine the correlations among IL-6 spillover in the lungs, PVR, and cGMP production in the lung. Stepwise multivariate regression analysis was used to identify independent predictors of IL-6 spillover in the pulmonary vascular bed and of cGMP production in the pulmonary vascular bed. A p value < 0.05 was considered to indicate statistical significance.
Results

Hemodynamic Data

The left ventricular ejection fraction was significantly lower in patients with mild and severe CHF than in control subjects. In contrast, the mean pulmonary arterial pressure, pulmonary capillary wedge pressure, and right atrial pressure increased with the severity of CHF and were significantly higher in patients with severe CHF than in patients with mild CHF. PVR was also significantly higher in patients with severe CHF than in patients with mild CHF (Table 1).

Neurohumoral and Cytokine Data

Plasma levels of NE, ET-1, TNF-α, IL-6, and cGMP were significantly higher in patients with severe CHF than in patients with mild CHF and control subjects. Plasma levels of ANP and BNP were also higher in patients with severe CHF than in patients with mild CHF and control subjects (plasma ANP: control subjects, 16.2 ± 4.7 pg/mL; mild CHF patients, 95 ± 17 pg/mL; severe CHF patients, 191 ± 37 pg/mL; plasma BNP: control subjects, 13.7 ± 5.7 pg/mL; mild CHF patients, 179 ± 57 pg/mL; and severe CHF patients, 680 ± 229 pg/mL) [Table 2].

Plasma IL-6 Concentration and IL-6 Spillover in the Pulmonary Circulation

Figure 1 shows the plasma levels of TNF-α and IL-6 at the main pulmonary artery and pulmonary capillary wedge region in age-matched and gender-matched control subjects and patients with CHF. There was no difference in plasma TNF-α levels between the main pulmonary artery and pulmonary capillary wedge region either in control subjects or patients with CHF. In contrast, plasma IL-6 concentrations were significantly higher in the pulmonary capillary wedge region than in the main pulmonary artery both in control subjects and patients with CHF. IL-6 production in the lung was markedly increased in patients with severe CHF compared with control subjects and patients with mild CHF (Fig 2).

Relationship Between Plasma IL-6 Production in the Pulmonary Circulation and 13 Variables, Including Hemodynamic Data, Clinical Variables, and Neurohumoral Factors

In univariate analysis, there were significant correlations between the plasma IL-6 production in the pulmonary circulation and NYHA functional class, hemodynamic data (such as right atrial pressure, mean pulmonary artery pressure, and pulmonary capillary wedge pressure), and plasma NE level (Table 3). In multivariate analysis, plasma NE levels showed a significant positive relationship, and treatment with β-blockers showed an independent, significant negative relationship with plasma IL-6 production in the pulmonary circulation. Moreover, female gender was an independent significant predictor of IL-6 production. Figure 3 shows that there was a significant positive correlation between plasma IL-6 production in the pulmonary circulation and PVR.

Relationship Between Plasma IL-6 Production and cGMP Production in the Pulmonary Circulation

We also calculated the production of cGMP, which is a second messenger of NO and cardiac natriuretic peptides, in the pulmonary circulation. The plasma cGMP levels increased with the severity of CHF (Table 2), and there was a significant positive correlation between cGMP production and IL-6 production in the pulmonary circulation (Table 4, Fig 4) that was independent of ANP or BNP extraction in the lung or of treatment by nitrates.

Discussion

We showed here for the first time that there is plasma IL-6 production but not plasma TNF-α...
production in the pulmonary circulation of control subjects and patients with CHF, and that the IL-6 production increases with the severity of CHF and is related to the activation of the sympathetic nervous system. This is similar to our previously reported finding of IL-6 production in the peripheral vascular bed. At least a part of the increased plasma IL-6 levels in patients with CHF is secreted into the peripheral and the pulmonary vascular bed. We cannot rule out that the arteriovenous difference in IL-6 is derived from leukocytes adhering to pulmonary vascular endothelial cells. However, there was no arteriovenous difference in TNF-α, suggesting that the main source of IL-6 production in the pulmonary circulation is the vascular endothelial cells or smooth muscle cells.

**Sympathetic Nervous System and IL-6 Spillover in the Lung**

Many investigators have shown that plasma IL-6 levels increase in patients with CHF. Several factors, such as the levels of TNF-α, hypoxia, and ET-1 and the elevation of intracellular cyclic adenosine monophosphate, have been reported to stimulate IL-6 secretion in *in vitro* studies. In the present study, although we included TNF-α and

![Graphs showing plasma levels of IL-6 and TNF-α in the pulmonary artery (PA) and pulmonary capillary wedge region (PC) in control subjects and in patients with CHF.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/)

![Graph showing IL-6 production in the pulmonary circulation in control subjects and in patients with CHF.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/)
ET-1 in the multivariate analysis, these substances did not correlate with IL-6 production in the lung by multivariate analysis, while plasma NE level was independently correlated and treatment with β-blockers was independently and inversely correlated with IL-6 production. This suggests that sympathetic nervous activity is a very important factor for the secretion of IL-6 in patients with CHF. Recently, we reported that the administration of dopamine significantly increases the level of plasma IL-6 in patients with CHF, suggesting that the activation of the sympathetic nervous system directly stimulates the production of IL-6, which is in agreement with our present data. However, we cannot confirm whether the local production of IL-6 in the lung was directly induced by the activation of the sympathetic nervous system. Further studies including serial measurements of IL-6 production in the lung before and after treatment with catecholamine are needed to confirm our hypothesis.

Female Gender and IL-6

Female gender also showed an independent and significant relationship to pulmonary production of IL-6 in patients with CHF in this study. It is known that estrogen loss results in an increased IL-6 activity and leads to increased bone resorption in patients with postmenopausal osteoporosis. In our study, most of the female patients and control subjects were postmenopausal, suggesting that estrogen decrease may increase the pulmonary production of IL-6 in postmenopausal women with CHF. Moreover, increased local IL-6 production may cause the endothelial dysfunction seen in postmenopausal

**Table 3—Univariate and Multivariate Linear Model of IL-6 Production in Pulmonary Circulation of Patients With CHF**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Correlation Coefficient</th>
<th>p Value</th>
<th>Multivariate β Coefficient (SE)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.117</td>
<td>0.42</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Gender†</td>
<td>0.127</td>
<td>0.38</td>
<td>1.299 (0.638)</td>
<td>0.04</td>
</tr>
<tr>
<td>NYHA class</td>
<td>0.314</td>
<td>0.03</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Etiology‡</td>
<td>−0.162</td>
<td>0.26</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>MPAP</td>
<td>0.461</td>
<td>0.0008</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PCWP</td>
<td>0.364</td>
<td>0.009</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>RAP</td>
<td>0.420</td>
<td>0.002</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CI</td>
<td>0.071</td>
<td>0.63</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF</td>
<td>0.008</td>
<td>0.96</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>NE</td>
<td>0.509</td>
<td>0.0002</td>
<td>0.002 (0.0005)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ET-1</td>
<td>0.232</td>
<td>0.11</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.047</td>
<td>0.75</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Diuretics§</td>
<td>0.055</td>
<td>0.70</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Digitalis§</td>
<td>−0.143</td>
<td>0.32</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ACEI§</td>
<td>−0.143</td>
<td>0.32</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ATRB§</td>
<td>0.018</td>
<td>0.90</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrates§</td>
<td>0.083</td>
<td>0.57</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>β-blockers§</td>
<td>−0.229</td>
<td>0.11</td>
<td>−1.986 (0.672)</td>
<td>0.004</td>
</tr>
<tr>
<td>Calcium antagonists§</td>
<td>−0.107</td>
<td>0.45</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*ACEI = angiotensin-converting enzyme inhibitor; ATRB = angiotensin II receptor blocker; NS = not significant. See Table 1 for abbreviations not used with text.

†One woman and no men.

‡Ischemic heart disease, one patient; nonischemic heart disease, no patients.

§Treatment with drugs, one patient; no treatment with drugs, no patients.

Figure 3. Relationship between IL-6 production in the pulmonary circulation and PVR in patients with CHF.

![Graph showing the relationship between IL-6 production and PVR](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21976/ on 04/09/2017)
women through the induction of iNOS. However, further studies that include a large number of patients are needed to clarify our hypothesis.

Relationship Between IL-6 Spillover and cGMP Production in the Lung

We showed that IL-6 production in the lung correlated with PVR and cGMP production in the lung. The NO-cGMP signal transduction pathway plays an important role in the regulation of pulmonary vascular tone and resistance in patients with pulmonary hypertension and in healthy subjects. cGMP is a second messenger of both natriuretic peptides and NO, which stimulate particulate and soluble guanylate cyclase, respectively, and the plasma cGMP level is useful for assessing their bioactivities. Plasma cGMP is mainly regulated by particulate guanylate cyclase, which is activated by natriuretic peptide in heart failure, as we have reported previously. We demonstrated that ANP extraction, BNP extraction, and IL-6 production in the lung are independently correlated with cGMP production in the lung. The correlation of ANP extraction and cGMP production in the lung in patients with CHF is compatible with the results of our previous report, suggesting that ANP has bioactivity as a vasodilator in the pulmonary circulation. In the present study, we demonstrated for the first time that BNP extraction also is correlated with cGMP production in the lung. ANP and BNP share the same receptor (the GC-A receptor), and BNP also may have similar bioactivity in the lung. Moreover, we showed that IL-6 production in the lung is independently correlated with cGMP production in the lung IL-6 has an ability to induce iNOS, and the NO produced activates soluble guanylate cyclase, leading to the increased production of cGMP. Our report confirmed that some part of plasma cGMP is regulated by soluble guanylate cyclase, which is activated by NO, and that IL-6 may act in an autocrine or paracrine manner in the pulmonary circulation.

The Role of IL-6 in the Pulmonary Circulation

According to our present data and previous report, IL-6 may act as a vasodilator in the pulmonary circulation of patients with CHF to attenuate the excessive vasoconstriction that results from the activation of the sympathetic nervous system. IL-6 has the ability to induce fibroblast and smooth muscle cell proliferation and may have an important pathophysiologic role in the progression of pulmonary hypertension. We cannot rule out the possibility that IL-6 produced in the lung induces the proliferation of fibroblasts and smooth muscle cells in patients with CHF. IL-6 may initially act as a compensatory response to vasoconstriction by catecholamine in patients with CHF, and it then may stimulate the proliferation of fibroblasts and smooth muscle cells and may cause the deterioration of pulmonary hemodynamics.

Table 4—Univariate and Multivariate Linear Model of cGMP Production in Pulmonary Circulation of Patients With CHF*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Correlation Coefficient</th>
<th>p Value</th>
<th>Multivariate β Coefficient (SE)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP extraction</td>
<td>0.754</td>
<td>&lt; 0.0001</td>
<td>0.146 (0.015)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BNP extraction</td>
<td>0.360</td>
<td>0.006</td>
<td>0.025 (0.009)</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-6 production</td>
<td>0.498</td>
<td>&lt; 0.0001</td>
<td>2.564 (0.716)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Treatment with nitrates†</td>
<td>0.204</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

*See Tables 1 and 3 for abbreviations not used in the text.
†Treatment with nitrates = 1; no treatment with nitrates = 0.
Study Limitations

We measured the plasma production of IL-6 in the pulmonary circulation before and after treatment in three patients with CHF and found that it decreased after the treatment, which was associated with a decrease in PVR in all patients (data not shown). However, further studies including serial measurements of IL-6 production in the lung before and after the treatment of CHF are needed to confirm the role of IL-6 in CHF.

In the present study, blood samples were collected from the pulmonary capillary wedge region to confirm the production of IL-6 in the pulmonary circulation. Blood samples were obtained in this way because it is not ethical to perform transseptal catheterization through the fossa ovalis to obtain blood from the pulmonary vein. Our preliminary data showed no apparent difference in plasma IL-6 levels in the pulmonary vein and pulmonary capillary wedge region in patients with heart failure who had an atrial septal defect or in patients with mitral valvular stenosis who had undergone percutaneous transvenous mitral commissurotomy. To obtain these data, we and other investigators\textsuperscript{26,28,37} used the same methods as previously reported.

Conclusion

These results indicate that the IL-6 production in the pulmonary circulation increases with the severity of CHF and that the production of IL-6 is associated mainly with the activation of the sympathetic nervous system. The local production of IL-6 in the lung may modify PVR in patients with CHF.

Acknowledgments: We thank Ms. Ikuko Sakaguchi for excellent technical assistance. We also thank Mr. Daniel Mrozek for assistance in preparing the manuscript.

References


33 Huang CL, Ives HE, Cogan MG. In vitro evidence that cGMP is the second messenger for atrial natriuretic factor. Proc Natl Acad Sci U S A 1986; 83:5015–5018


