Complement Activation in Patients With Congestive Heart Failure

Effect of High-Dose Intravenous Immunoglobulin Treatment

Pål Aukrust, MD, PhD; Lars Gullesstad, MD, PhD; Knut T. Lappégård, MD; Thor Ueland, BS; Halfdan Aass, MD, PhD; Lisbeth Wikeby, RN; Svein Simonsen, MD, PhD; Stig S. Frøland, MD, PhD; Tom E. Mollnes, MD, PhD

Background—Increasing evidence implicates innate immunity in the pathogenesis of congestive heart failure (CHF). In the present study, we examined the possible role of complement, an important part of innate immunity, in CHF.

Methods and Results—Complement activation was analyzed in systemic and coronary circulation in 39 patients with CHF and 20 healthy control subjects. In a double-blind, placebo-controlled study, we have recently reported that high-dose intravenous immunoglobulin (IVIG) improves left ventricular ejection fraction (LVEF) in these patients. To examine if this improvement was related to IVIG-induced effects on complement, we also examined complement activation during induction (first week) and maintenance therapy (6 months) with IVIG or placebo. Our main findings were: (1) We found enhanced systemic complement activation involving classic, alternative, as well as terminal pathway in patients with CHF compared with healthy control subjects. (2) Particularly enhanced complement activation was found in coronary sinus, representing venous drainage from the heart. (3) The systemic complement activation was further enhanced during IVIG but not during placebo therapy, particularly during induction therapy. (4) Although IVIG improved LVEF in patients with CHF, the degree of IVIG-mediated complement activation was negatively correlated with this improvement of LVEF.

Conclusions—This study further supports the involvement of innate immunity in the pathogenesis of CHF. Our findings suggest that complement may be added to the list of possible therapeutic targets in CHF and that future studies with specific complement inhibitors may be of interest in this disorder. (Circulation. 2001;104:1494-1500.)

Key Words: heart failure ■ inflammation ■ leukocytes ■ immunology
Thirty-nine patients with chronic stable CHF for

Patients

Methods

Statistical Analyses

Results
C1inh complexes, reflecting activation of the classic pathway, were 27 (22 to 32) AU/mL in patients with CHF and 16 (12 to 18) AU/mL in control subjects ($P$, 0.001). C3bBbP, reflecting activation of the alternative pathway, was 16 (12 to 21) AU/mL in patients with CHF and 10 (6 to 12) AU/mL in control subjects ($P$, 0.001). C3bc, reflecting any initial activation mechanism, was 17 (14 to 24) AU/mL in patients with CHF and 10 (6 to 13) AU/mL in control subjects ($P$, 0.001), indicating that the complement cascade is activated to the very end in patients with CHF. There were no significant differences between ischemic and idiopathic dilated cardiomyopathy with regard to complement activation.

Complement Activation in Coronary Sinus

We also examined complement activation products in paired plasma samples from pulmonary artery (PA), representing mixed venous blood from the total systemic circulation, and coronary sinus (CS), representing venous drainage from the heart, in 14 patients with CHF (Figure 2). C3bc, C3bBbP, and C1rs-C1inh were significantly raised in CS compared with PA, although the rise in C1rs-C1inh complexes was very modest. In contrast, TCC levels showed a significant decrease in CS compared with plasma obtained from PA.

**Effect of IVIG on Complement Activation in CHF During Induction Therapy**

We next examined if IVIG could modulate the enhanced complement activation in CHF. During induction therapy, we found that IVIG but not placebo induced a substantial and
continuous increase in C3bBbP, C3bc, and TCC levels when analyzing peripheral blood samples before and 1 hour after infusion on days 1, 3, and 5 (Figure 3). In 28 patients (14 in the IVIG and 14 in the placebo group), we also measured C1rs-C1 inhibitor complexes before the first and 1 hour after the fifth infusion during induction therapy. IVIG [30 (22 to 34) AU/mL versus 38 (34 to 45) AU/mL, \( P < 0.05 \)] but not placebo [25 (22 to 33) AU/mL versus 27 (21 to 32) AU/mL] induced a marked increase also in this complement product consistent with classic activation, resulting in a significant difference in changes between the two treatment groups (\( P < 0.05 \)).

**Effect of IVIG on Complement Activation in CHF During Maintenance Therapy**

The differences in C3bBbP, C3bc, and TCC between the two treatment groups, as measured in peripheral venous blood, persisted throughout the study, although the differences in C3bc and particularly in TCC levels were reduced at the end of the study (Figure 4). Also, when we analyzed C1rs-C1 inhibitor complexes, C3bBbP, C3bc, and TCC in plasma from PA before and at the end of the study, different patterns of complement activation were observed between the two treatment groups, with an increase in the IVIG and a decrease in the placebo group (Figure 5).

**Effect of IVIG on CRP**

CRP is a well-known complement activator\(^1\); we therefore measured hsCRP at baseline, 1 hour after the fifth infusion during induction therapy, and at the end of the study. Although no significant changes were seen during induction therapy in the placebo group [2.73 (1.67 to 5.57) mg/L versus 2.11 (1.44 to 4.80) mg/L], hsCRP increased in the IVIG group [1.60 (0.93 to 2.58) mg/L versus 3.55 (2.51 to 4.78) mg/L, \( P < 0.005 \)], resulting in a significant difference in changes between the treatment groups (\( P < 0.001 \)). However, during maintenance therapy, hsCRP returned to baseline levels in the IVIG group and remained unchanged in the placebo group (data not shown).

**Changes in Complement Activation in Relation to Changes in LVEF During IVIG Therapy**

We have previously shown a significant increase in LVEF (5 EF units) after IVIG but not after placebo treatment in these patients with CHF.\(^9\) Interestingly, in the IVIG group, those with the most marked increase in C3bBbP, C3bc, and C1rs-C1inh complexes had only a slight increase or a decrease in LVEF during the study, resulting in a significant inverse correlation between changes in these parameter of complement activation and changes in LVEF (Figure 6). No such correlation was found in the placebo group (Figure 6).

**Discussion**

Although a number of reports have shown increased levels of inflammatory mediators in CHF, the literature is virtually devoid of data on complement activation in this disorder. One previous report demonstrated increased TCC levels in pa-
tients who had heart failure during acute myocardial infarction, and similar findings have also recently been reported in CHF. However, the present study is to our knowledge the first to demonstrate markedly enhanced complement activation involving both classic and alternative pathway in patients with chronic stable CHF, with particularly enhanced activation in CS representing venous drainage from the heart.

Complement involvement in myocardial infarction has been suggested, based on reports of complement deposition in the myocardium and activation of complement by intracellular cardiomyocyte structures. Moreover, Weisman et al showed that inhibition of complement activation markedly reduced the area of damage in an experimental model of myocardial infarction. This finding has later been confirmed by several studies suggesting a pathogenic role of complement activation in acute ischemic myocardial damage, as also illustrated by the cardioprotective effect of total complement depletion. Notably, recent evidence suggests that myocardial damage from complement activation may be chronically sustained, implying a role for these mediators also in chronic heart failure. Moreover, it has recently been demonstrated that complement proteins are endogenously produced by the human heart, further supporting a role for complement activation in the pathogenesis of myocardial damage. Thus, the marked complement activation in CHF may not only be an epiphenomenon but may represent important pathogenic processes in these patients, possibly contributing to myocardial damage and ventricular dysfunction. Moreover, although most studies have focused on ischemic disorders, deposition of complement has also been found in IDCM. In the present study we found no difference in the degree of complement activation in patients with or without ischemic cardiomyopathy, indicating that a primary ischemic disorder is not a prerequisite for such activation.

Evidence is accumulating that a variety of extrahapatic tissues produce complement components. Of particular interest is the recent demonstration of locally produced complement proteins in the human heart, particularly during ischemia and reperfusion. In patients with CHF, we found higher concentrations of C3bBbP, C3bc, and to a lesser degree, C1rs-C1inh complexes, in CS than in PA, suggesting local complement activation in the myocardium also in CHF. Endothelial cell dysfunction has been reported within the failing myocardium. Such dysfunction may lead to disintegration of the endothelial cell lining exposing subendothelial structures to the blood stream, which in turn may lead to enhanced complement activation. Moreover, complement activation of the endothelium may per se induce gap formation between endothelial cells, leading to further exposure of subendothelial structures to the blood stream, which in turn may lead to enhanced complement activation. Moreover, complement activation of the endothelium may per se induce gap formation between endothelial cells, leading to further exposure of subendothelial structures, possibly representing a vicious circle in CHF. In contrast to C3bBbP and C3bc, the concentration of TCC was lower in CS, possibly reflecting trapping of TCC within the myocardium, that is, by binding through vitronectin to the vitronectin receptor.

Herein we show raised levels of specific markers for activation of both classic and alternative complement pathways in CHF, that is, C1rs-C1inh and C3bBbP complexes, respectively. Moreover, although methods are not available to
specifically detect activation of the third complement pathway in vivo (ie, the mannose binding lectin pathway), recent in vitro studies suggest the involvement of this pathway in endothelial damage secondary to oxidative stress, possibly playing a pathogenic role in ischemia/reperfusion injury. Thus, the whole complement cascade including C3C appears to be activated in CHF, generating a number of potent inflammatory mediators possibly contributing to damage of endothelial cells and cardiomyocytes and to the formation of cytokines and other inflammatory mediators in patients with CHF.

In the present study we show that complement activation in CHF was further enhanced during IVIG therapy, as assessed by activation products in peripheral blood. IVIG-induced complement activation in vitro through the classic pathway has previously been reported, and in a non-placebo-controlled study in women with recurrent spontaneous abortion, we have shown that one dosage of IVIG activates complement in vivo. However, the present study is, to our knowledge, the first placebo-controlled study demonstrating that intermittent, long-term IVIG administration induces a marked systemic complement activation involving both the classic and alternative pathways. CRP may induce complement activation and notably, during induction therapy, the IVIG-induced complement activation was associated with a marked rise in hsCRP. However, although complement activation in some degree persisted also during IVIG maintenance therapy, hsCRP returned to baseline level at the end of the study, suggesting that complement activation during IVIG does not merely reflect enhanced CRP levels.

Deviation of complement activation and deposition from the target tissue toward the fluid phase have been suggested to contribute to the beneficial effects of IVIG in inflammatory disorders, involving mechanisms such as binding of C1q and activated C3 and C4 as well as C3b inactivation. However, this “diverting complement deposition” hypothesis was challenged by the present data. Thus, although IVIG improved LVEF in patients with CHF, those with the most marked increase in complement activation in the fluid phase had only a slight increase or a decrease in LVEF during such therapy. A reasonable interpretation could be that although a certain complement activation deviating C1q, C3, or C4 from the target may be beneficial, a fluid phase activation over a certain limit may by itself generate inflammatory products that may overcome the protecting effect of a modest activation. Thus, although IVIG appears to have beneficial effects on LVEF in patients with CHF possibly mediated by anti-inflammatory mechanisms, this effect may to some degree be counteracted if complement is activated beyond a certain level.

The present study shows that patients with CHF are characterized by enhanced complement activation, further supporting the involvement of innate immunity mechanisms in the pathogenesis of CHF. Moreover, although it has been suggested that some of the beneficial effects of IVIG in inflammatory disorders may involve complement modulation, this appears not to be the case in patients with CHF. Our findings suggest that complement may be added to the list of possible therapeutic targets in CHF and that future studies with specific complement inhibitors may be of interest in this disorder.

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References


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