Complement Activation in Patients With Congestive Heart Failure

Effect of High-Dose Intravenous Immunoglobulin Treatment

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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

Increasing evidence implicates effectors of innate immunity in the pathogenesis of congestive heart failure (CHF). Thus, increased plasma levels and myocardial expression of inflammatory mediators, for example, tumor necrosis factor-α, interleukin-6, and Toll-like receptor 4, occur in experimental and clinical heart failure.1–3 Moreover, there is growing consensus that such molecules also may contribute to maladaptive cardiac remodeling.4,5

The complement system, comprising a protein cascade of more than 30 proteins, represents an important part of the innate immune system playing a central role in the host defense against microbes. However, enhanced complement activation may also induce tissue damage and inflammation, and there is some evidence suggesting that complement may play a role in the pathogenesis of myocardial damage secondary to ischemia and reperfusion,6,7 but the role of complement in chronic myocardial failure is at present unclear.

Intravenous immunoglobulins (IVIG) in high dosages are used as treatment for an increasing number of inflammatory disorders,8 and we have recently demonstrated that IVIG also improves left ventricular ejection fraction (LVEF) in CHF secondary to both ischemic and idiopathic dilated cardiomyopathy (IDCM).9 How CHF and other inflammatory disorders are modulated by IVIG are, however, poorly understood but may involve several non–mutually exclusive modes of action such as Fc receptor blockade, modulation of the idiotypic–anti-idiotypic antibody network, and modulation of cytokine production.9–11 IVIG has also been found to attenuate complement-mediated tissue damage, possibly by binding...
The study design has previously been described. Briefly, the 39 patients with chronic stable CHF for a total period of 26 weeks. IVIG or an equal volume of placebo was given as induction therapy [1 daily infusion (0.4 g/kg) for 5 days] and thereafter as monthly infusions (0.4 g/kg) for a total of 5 months as maintenance therapy. At baseline and at the end of study (26 weeks, ie, 4 weeks after the last infusion), LVEF was assessed by ECG-synchronized, gated radionuclide ventriculography at rest, and mixed venous blood samples were collected from the pulmonary artery and in some patients also from coronary sinus during right-sided heart catheterization. In addition, peripheral venous blood was collected before and 1 hour after IVIG or placebo infusion on days 1, 3, and 5 during induction therapy and before IVIG or placebo infusion 1, 2, 3, 4, and 5 months after baseline during maintenance therapy. Blood samples were collected into pyrogen-free EDTA tubes (Becton Dickinson), immediately immersed in melting ice, and centrifuged within 15 minutes at 1000g for 15 minutes; plasma was stored at −80°C.

Data are given as mean±SEM.

activated C3 and C4, by deviating C1q from the target to the fluid-phase, and by enhancing C3b inactivation. In the present study, we wanted to further examine the possible role of complement in CHF by 3 different experimental approaches. First, we examined the degree of systemic complement activation in patients with CHF and healthy control subjects. Second, we evaluated complement activation locally in the myocardial circulation in patients with CHF by analyzing blood samples from the pulmonary artery and coronary sinus. Finally, we examined complement activation in patients with CHF who were randomly assigned in a double-blind fashion to receive therapy with IVIG or placebo for a total period of 26 weeks.

**Methods**

**Patients**

Thirty-nine patients with chronic stable CHF for >6 months were included in the study (Table). The patients were included if they (1) were in New York Heart Association functional class II to III, (2) had LVEF <40%, and (3) were receiving optimal medical treatment with no changes in medication during the last 3 months. Patients were excluded if they had (1) evidence of myocardial infarction or unstable angina during the last 6 months or (2) significant concomitant diseases such as infections or connective tissue disease. None of the patients changed the conventional cardiovascular treatment regimen during the study. The underlying cause of CHF was classified as coronary artery disease (n=22) or IDCm (n=17), based on disease history and coronary angiography. For comparison, blood samples were also collected from 20 sex- and age-matched healthy control subjects. The study was approved by the regional ethics committee. Written informed consent was obtained from each patient.

**Study Design**

The study design has previously been described. Briefly, the patients were stratified according to cause (ie, coronary artery disease and IDCm), and, after baseline measurements, were randomly assigned to IVIG [Octagam, Octapharma; IgG (final concentration, 50 g/L) dispensed in sterile water containing 10% maltose] or placebo (5% glucose) in a double-blind fashion. IVIG or an equal

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**Complement Activation in Chronic Heart Failure**

**Measurements of Complement Activation and High-Sensitivity C-Reactive Protein**

The following activation products were measured: C1rs-C1-inhibitor complexes (classic pathway), C3bBbP (alternative pathway), C3bc (final common pathway), and TCC (terminal complement complex). The analyses were performed with double-antibody enzyme immunoassays. The results are given in arbitrary units (AU) per milliliter, related to a standard of activated serum defined to contain 1000 AU/mL. Standards were made by zymosan-activated serum except for the C1rs-C1inh assay, in which heat-aggregated IgG activated serum was used. The general performance of these assays is given in detail elsewhere. Briefly, C1rs-C1-inhibitor complexes (C1rs-C1inh) were measured by using the KOK-12 monoclonal antibody (mAb; a kind gift from Prof C.E. Hack, Amsterdam, The Netherlands) specific for a neoepitope in C1-inhibitor when it is in complex with the protease. Plates were coated with the KOK-12 antibody, reacted with plasma and control samples, and the complex was detected with a cocktail of anti-C1r and anti-C1s antibodies. C3bBbP, the alternative pathway convertase composed of C3b, Bb, and properdin (P), was detected with mouse monoclonal anti-human properdin (Quidel) as capture antibody. Detection was made by polyclonal rabbit anti-human C3c (Behringwerke A/G) and peroxidase-conjugated anti-rabbit Ig (Amersham International). Activation of the final common pathway (C3bc) was quantified by enzyme immunoassays with the monoclonal antibody bH6 specific for a neoepitope exposed in C3b, C3b, and C3c. The sum of C3b, C3b, and C3c is collectively termed C3bc. The terminal SC5b-9 complement complex (TCC) was quantified with the neoepitope-specific mAb aE11 as capture antibody, recognizing an epitope reacted with plasma and control samples, and the complex was detected with a cocktail of anti-C1r and anti-C1s antibodies. The analyses were performed with double-antibody enzyme immunoassays. The results are given in arbitrary units (AU) per milliliter, related to a standard of activated serum defined to contain 1000 AU/mL. Standards were made by zymosan-activated serum except for the C1rs-C1inh assay, in which heat-aggregated IgG activated serum was used. The general performance of these assays is given in detail elsewhere.

**Statistical Analyses**

Differences between groups were calculated by Mann-Whitney U rank-sum test for unpaired data. The Wilcoxon signed-rank test was used for paired data. When analyzing variables from more than 2 time points, repeated-measures ANOVA was used. Relations between variables were tested with Spearman’s rank correlation test. Data are given as medians and 25th to 75th percentiles if not otherwise stated. Probability values are 2-sided and taken as statistically significant at <0.05.

**Results**

**Systemic Complement Activation in Patients With CHF and Healthy Control Subjects**

All four complement activation products were markedly increased in peripheral venous blood in 39 patients with CHF compared with 20 healthy control subjects (Figure 1). C1rs-
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 \[30 (22 to 34) \text{ AU/mL versus } 38 (34 to 45) \text{ AU/mL}, P = 0.05\] but not placebo \[25 (22 to 33) \text{ AU/mL versus } 27 (21 to 32) \text{ AU/mL}\] 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\(^{19}\); 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 \[2.73 (1.67 to 5.57) \text{ mg/L versus } 2.11 (1.44 to 4.80) \text{ mg/L}\], hsCRP increased in the IVIG group \[1.60 (0.93 to 2.58) \text{ mg/L versus } 3.55 (2.51 to 4.78) \text{ mg/L}, P = 0.005\], 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.\(^7\) 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 DCM. 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 extrahepatic 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, 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 (i.e., 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 TCC 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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