Congestive Heart Failure

Serum complement activation in congestive heart failure

David J. Clark, MBBS, FRACP, Michael W. Cleman, MD, Steven E. Pfau, MD, Scott A. Rollins, PhD, Tarik M. Ramahi, MD, Craig Mayer, BS, Teresa Caulin-Glaser, MD, Edouard Daher, MD, Mikhail Kosiborod, MD, Leonard Bell, MD, and John F. Setaro, MD

From the *Section of Cardiovascular Medicine, Yale University School of Medicine, and †Alexion Pharmaceuticals, Inc, New Haven, Conn.


Laboratory analysis of samples was supported by Alexion Pharmaceuticals, Inc, New Haven, Conn.

Submitted July 8, 2000; accepted December 22, 2000

Reprint requests: John F. Setaro, MD, Section of Cardiovascular Medicine, Yale University School of Medicine, 333 Cedar St, Box 208017, New Haven, CT 06520-8017.

Email: john.setaro@yale.edu

The complement system is composed of >20 serum proteins that act in concert to generate products that have both immunoprotective and proinflammatory properties. In particular, C5a and C5b-9 may mediate cardiac tissue injury through leukocyte activation, cytokine production, myocyte lysis, endothelial cell activation, vasoconstriction, and thrombosis. Complement has been implicated in myocardial injury in acute myocardial infarction and reperfusion injury after cardiopulmonary bypass. In experimental models, blockade of terminal complement activation in these settings has proven beneficial through a decrease in myocardial injury.

Recently, activation of the immune system has been implicated in the pathogenesis of both ischemic and nonischemic congestive heart failure (CHF). As a measure of this activation, cytokines such as tumor necrosis factor (TNF-α) and interleukin-6 are significantly elevated, and the levels of these cytokines may be negatively associated with prognosis. Significant activation of the complement system in patients with CHF has not been previously described.

The purpose of this study was to evaluate complement activation in congestive heart failure and to examine the relation between the degree of complement activation and clinical outcome. We chose to use serum C5b-9 levels as a measure of complement activation for two reasons. First, many of the deleterious effects of complement are mediated through C5b-9 or C5a, and second, it can be reliably measured in the peripheral circulation.

**Methods**

**Patient population**

Thirty-six patients with CHF were enrolled from inpatient cardiology services at Yale–New Haven Hospital and Yale Uni-
versity School of Medicine outpatient cardiology clinics. Patients were included if they had New York Heart Association (NYHA) class II to IV symptoms of heart failure at time of enrollment and a left ventricular ejection fraction (LVEF) of <40%. Patients were excluded if there was severe comorbidity, myocardial infarction, or unstable angina in the 6 weeks before enrollment or inflammatory illnesses such as sepsis, malignancy, arthritis, and connective tissue disease. A sampling of 36 patients meeting these inclusion and exclusion criteria was chosen.

An age-matched group of 12 patients provided blood samples for use as control. Of these, 7 had stable coronary artery disease, 5 had no known coronary disease, and all had a LVEF of >50% with no history of CHF. All patients gave informed consent at the time of initial evaluation. This study was approved by the Human Investigation Committee of Yale University School of Medicine.

**Blood sampling and baseline clinical evaluation**

Baseline clinical evaluation was performed on 36 patients with CHF. At the time of enrollment, 18 of the patients also underwent right heart catheterization for clinical (noninvestigational) evaluation of new-onset or worsened CHF symptoms. Venous blood was collected into vacuum tubes containing EDTA and placed at 4°C. All samples were centrifuged within 2 hours, and serum was frozen at −70°C until analysis. Enzyme immunoassay was used to measure serum C5b-9 (Quidel, San Diego, Calif) and TNF-α (Quantikine HS, R & D Systems, Minneapolis, Minn).

**Clinical follow-up**

All patients were followed in-hospital or through regular outpatient visits. At 6 months, clinical information regarding the combined end point of death, admission to the hospital with a primary diagnosis of worsening heart failure, or requirement for urgent status 1 (intravenous ionotrope–dependent and inflammatory mediator, TNF-α) admission to the hospital inpatients with decompensated CHF were provided by the treating cardiologist without knowledge of C5b-9 or TNF-α levels.

**Data analysis**

Because the distribution of the biological marker in these 36 patients did not follow a normal distribution, serum C5b-9 levels were expressed by a median (25th to 75th percentiles). The Wilcoxon rank-sum test was used to determine whether patients in the CHF population had a higher serum C5b-9 level than the control patients.

To analyze the C5b-9 levels in these 36 patients with CHF, we used a method previously reported to evaluate another inflammatory mediator, TNF-α in CHF. Patients in the CHF group were divided into 2 groups, based on their level of serum C5b-9. Group A patients were in the lowest 50th percentile (C5b-9 levels ≤100 ng/mL); group B patients were in the highest 50th percentile (C5b-9 levels >100 ng/mL). A serum level of C5b-9 of >100 ng/mL was >2 SD greater than the mean level of the control patients. Univariate comparisons of clinical, biochemical, and hemodynamic characteristics between these two groups were made with the Wilcoxon rank-sum test for quantitative data, Fisher exact test for qualitative data, and the log-rank test for time to the various events.

The 36 patients with CHF were also divided into two groups, based on those who had clinical events by 6 months versus those who were event free. Serum C5b-9 levels were compared between these two groups by the Wilcoxon rank-sum test. The same method was used to compare the TNF-α levels in these two groups. Values of \( P < .05 \) were considered significant.

**Results**

The baseline characteristics of the 36 patients with CHF are shown in Table I. The study population was representative of a cross section of general cardiovascular medicine practice. There were more men than women in this sample. Patients were included with both new and established diagnoses and ischemic as well as nonischemic causes.

The median LVEF was severely depressed. Half of the patients had NYHA class IV symptoms, and nearly all of these were hospital inpatients with decompensated heart failure.

The serum C5b-9 levels [median (25th to 75th)] in the 36 patients with CHF [101.5 ng/mL (40 to 164 ng/mL)] were significantly \( (P = .003) \) higher than in the 12 control patients [36.5 ng/mL (22 to 50 ng/mL)] (Figure 1). Not all of the 36 patients with CHF had elevated serum C5b-9 levels. The 18 patients in the lowest 50th percentile (group A, serum C5b-9 ≤100 ng/mL) had levels similar to the control group. All of the 18 patients in the highest 50th percentile (group B, serum C5b-9 >100 ng/mL) had levels >2 SD greater than the control patients.
Comparison between C5b-9 levels and patient characteristics

A comparison of the characteristics between the group of patients with CHF in group A (serum C5b-9 ≤100 ng/mL) versus group B (serum C5b-9 >100 ng/mL) is shown in Table II. Of the patients with class IV NYHA symptoms, significantly (P = .04) more were in the group with the higher C5b-9 levels. No significant differences in age, sex, duration of CHF, cause of CHF, LVEF, hematocrit, renal function, medical therapy, or hemodynamics were detected between the two groups.

Comparison between C5b-9 levels and outcome

There was a 36% (13 of 36) overall event rate in the CHF population at 6 months (6 deaths, 4 hospital admissions with worsening CHF, and 3 urgent heart transplants). There were significantly (P = .02) more
clinical events during the 6-month follow-up period in group B (serum C5b-9 >100 ng/mL) than in group A (serum C5b-9 ≤100 ng/mL) (Table II). This is further illustrated by a Kaplan-Meier analysis of event-free survival of the two groups in Figure 2. The exact C5b-9 levels of the CHF population of patients who were event free during the 6 months versus those who had clinical events are shown in Figure 3. The 13 patients who had adverse events had significantly ($P < .01$) higher levels of serum C5b-9 than the 23 patients who were event free.

**Comparison between C5b-9, TNF-α levels, and outcome**

The median (25th to 75th) TNF-α levels in the 36 patients with CHF were 4.2 (2.5 to 7.9) pg/mL. As shown in Table II, there were no significant differences between the TNF-α levels of the patients in group B (serum C5b-9 >100 ng/mL) versus group A (serum C5b-9 ≤100 ng/mL). However, the TNF-α levels of the 13 patients with CHF who had clinical events were significantly higher ($P = .02$) when com-
pared with the 23 patients with CHF without events (Figure 4).

**Discussion**

**Main findings**

This study describes complement activation associated with symptomatic congestive heart failure. We found that circulating C5b-9 levels were significantly elevated in CHF. Not all the patients with CHF had elevated levels of C5b-9, but interestingly, we found that the patients with CHF with the highest levels were more likely to have NYHA class IV symptoms and to have an adverse clinical outcome by 6 months than the patients with CHF with lower levels.

In this study, no obvious relations between the level of C5b-9 and age, sex, cause of CHF (ischemic vs nonischemic), specific medications, hemodynamics, or degree of left ventricular dysfunction were detected. Studies of other inflammatory mediators such as TNF-α and interleukin-6 in CHF similarly have not demonstrated a correlation with these clinical and hemodynamic parameters.11,13,14

**Comparison with previous studies of complement activation in CHF**

No other study has described significant complement activation in heart failure. However, in a previous study7 of complement in acute myocardial infarction, the subgroup of patients who had CHF as a complication had significantly higher serum C5b-9 levels compared with those without CHF. In another series,6 patients with a primary diagnosis of CHF had insignificant C5b-9 elevation compared with healthy control patients. Unfortunately, the clinical characteristics of their patients with CHF are not described in detail, making comparison to our study difficult.

A relation between inflammation and adverse outcome in CHF is beginning to emerge. The Studies of Left Ventricular Function (SOLVD) Investigators12 found that TNF-α was progressively elevated in relation to deteriorating functional class and described a trend toward increased CHF mortality rates with increasing levels of TNF-α. Similarly, an analysis from the Prospective Randomized Amlodipine Survival Evaluation (PRAISE) trial13 showed a trend toward adverse outcomes in CHF (death or readmission to hospital with CHF) with higher levels of interleukin-6. Our TNF-α levels were higher in the patients with CHF who had adverse events. In addition, this study suggests that there is a relation between a measure of complement activation, C5b-9 levels, and clinical outcome in CHF.

**Potential role of complement in the pathogenesis of CHF**

Previous preclinical and clinical studies suggest possible mechanisms by which complement activation may cause ventricular damage or dysfunction. Termi-
nal complement causes myocardial cell apoptosis in vivo, and preclinical studies have demonstrated that inhibition of terminal complement activation during coronary ischemia prevents apoptosis. Apoptosis has been implicated in the progression of CHF. Furthermore, terminal complement activation may directly cause coronary vasoconstriction and ischemia because administration of C5a results in coronary vasoconstriction in preclinical models in vivo. Finally, terminal complement activation is associated with prolonged decreases in left ventricular function in preclinical studies.

In addition to the potentially direct contribution of complement activation to chronic cardiac damage, complement activation might also contribute to ongoing cardiac injury through an interaction with cytokines, particularly TNF-α. Although the role of TNF-α in the pathophysiology of CHF is uncertain, it may act through a negative inotropic effect as well as a deleterious influence on ventricular remodeling. Although TNF-α production may be elicited by a variety of stimuli, preclinical studies suggest that terminal complement activation is a physiologically important contributor to the production of TNF-α. For instance, in studies in which inflammation through terminal complement activation has been inhibited, TNF-α levels are reduced by more than half. Furthermore, complement activation might also play a permissive role for TNF-α-induced inflammation and cardiac damage because in certain inflammatory models, the presence of complement activation is required for TNF-α-mediated disease.

Potential mechanisms of complement activation in CHF

Although the mechanisms of complement activation in CHF are unknown, there are several possibilities. Autoantibodies have been identified in patients with cardiomyopathy that could directly activate the classic complement pathway. C-reactive protein, a direct activator of complement, has been shown to be elevated in states of heart failure. In addition, damaged or necrotic tissue can serve as an activating source for both the classic and alternative pathways and may promote bystander effects on surrounding tissue. Finally, clinical or subclinical tissue ischemia during heart failure may contribute to complement activation, because tissue ischemia has recently been shown to activate the complement system through the lectin pathway by direct action of mannose-binding lectin to hypoxic cells.

Study limitations

The current study is limited by its modest sample size and nonconsecutive patient enrollment. The ability to fully explore whether the relation between C5b-9 levels and subsequent clinical events is independent of differences in the multiple covariates is therefore reduced. Because of its observational design, the findings are hypothesis-generating rather than conclusive. Further larger studies will be required to confirm and refine these findings and address specific pathological mechanisms. Whether C5b-9 is a marker or mediator of adverse outcome in CHF will require studies with pharmacologic terminal complement inhibition.

Conclusions

Our study suggests that circulating C5b-9 is elevated in symptomatic CHF. In our population, high levels of C5b-9 were associated with NYHA class IV symptoms and adverse clinical outcomes by 6 months.

References

15. Bing OHL. Hypothesis: apoptosis may be a mechanism for the transition to heart failure with chronic pressure overload. J Mol Cell Cardiol 1994;26:943-8.