The Complement System in Ischemic Heart Disease

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The mechanisms by which tissue injury after acute myocardial infarction (AMI) occurs has not been fully elucidated. Recent evidence in experimental models has suggested involvement of the complement system in microvascular and macrovascular injury subsequent to AMI. With respect to angina pectoris, whether or not the complement system is activated is not clear. The present study assessed the role of complement as a mediator of myocardial inflammation by quantifying products of complement activation, including C3d, C4d, Bb, and SC5b-9 complexes, in 31 patients with AMI, 17 patients with unstable angina pectoris, 19 patients with stable angina pectoris, and 20 normal volunteers. The plasma C3d levels increased in patients with AMI and in those with unstable angina pectoris (p < 0.01). The plasma levels of C4d, Bb, and SC5b-9 increased only in patients with AMI (p < 0.01). The plasma SC5b-9 level was related to peak creatine phosphokinase (r = 0.71) and inversely related to the ejection fraction (r = -0.71). The plasma SC5b-9 level of patients with congestive heart failure was higher than that of patients without congestive heart failure in AMI. These results show that activation of complement system occurs after AMI and show an association of myocardial damage with complement activation. With respect to angina pectoris, the complement system is mildly activated in patients with unstable angina pectoris; however, the cardiac function of patients with unstable angina pectoris is not damaged. The complement system of patients with stable angina pectoris is not activated. (*Circulation* 1990;81:156–163)

Recent studies in experimental models suggest that acute myocardial ischemia is associated with activation of the complement system. First, studies in the baboon have established that C3, C4, and C5 are deposited in most infarction myocardial fibers.1,2 Second, by inactivating the complement system in vivo with cobra venom factor, a significant reduction in myocardial damage was achieved in an animal model.3 Also, a few studies report the complement activation in vivo in patients with acute myocardial infarction (AMI).4–6 However, it is not clear whether the activated complement system affects the myocardial necrotic size and cardiac function in human myocardial infarction. It is also unknown whether or not the complement system is activated in patients with angina pectoris (AP).

This study assessed complement activation in patients with AMI and AP by directly measuring by-products of the reaction. The degree of activation of the complement system was compared with the hemodynamic manifestations and peak creatine phosphokinase (CPK) in patients with AMI.

**Methods**

The study group consisted of 31 patients with AMI, 17 patients with unstable angina pectoris (UAP), 19 patients with stable angina pectoris (SAP), and 20 normal volunteers (Table 1). AMI was defined by at least two of the following: 1) angina lasting longer than 30 minutes, unrelied by rest, 2) serum creatine kinase–MB fraction greater than 5%, 3) inversion or elevation of the ST-T waves, and 4) positive results from either the technetium 99m–pyrophosphate or thallium 201 scan.7–9 UAP was diagnosed according to American Heart Association criteria.10

Patients with AMI were admitted to the hospital within 24 hours after the onset of myocardial infarction. Hemodynamics were monitored with a Swan-Ganz catheter. With a DELTRAN IITM transducer
TABLE 1. Demographic Characteristics of the Patient Group

<table>
<thead>
<tr>
<th>Patient group</th>
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<th>Female</th>
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<tr>
<td></td>
<td></td>
<td>(21–94)</td>
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<tr>
<td>UAP</td>
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<td>57±2</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(38–72)</td>
<td></td>
<td></td>
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<tr>
<td>SAP</td>
<td>19</td>
<td>60±2</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(46–70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>44±3</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(26–77)</td>
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</table>

Age values are mean±SEM; age values in parentheses are ranges.
AMI, acute myocardial infarction; UAP, unstable angina pectoris; SAP, stable angina pectoris; N, normal controls.

(Utah Medical Products Inc, Midvale, Utah) leveled at the midchest, calibrated measurements of mean pulmonary arterial capillary wedge pressure were recorded. Cardiac output (CO) was determined by the thermodilution technique. Cardiac index (CI) was calculated as follows:

\[ CI = \frac{CO}{BSA}, \text{where BSA is body surface area.} \]

Congestive heart failure was defined as the existence of pulmonary congestion by chest radiography, by Killip classes II, III, and IV,11 or by Forrester classes II and IV,12

The patients were not given any specific drug therapy, such as glucocorticoids. All the patients were treated by conventional therapy, and none of the patients was treated by percutaneous transluminal coronary recanalization or percutaneous transluminal coronary angioplasty. The onset time of the myocardial infarction was estimated from the time that the characteristic chest pain appeared.

Venous blood samples were obtained from the antecubital vein on the fifth day after admission in the patients with AMI and at admission in the patients with UAP and SAP. Previously, we assayed C3a serially from day 1 through day 10 in the patients with AMI, using the Radioimmunoassay Kit (Upjohn, Kalamazoo, Michigan).13,14 The level of C3a on the fifth day was the highest of the 10 days after admission. For this reason, plasma samples were obtained on the fifth day after admission from the patients with AMI. Plasma samples from the patients and normal volunteers were assayed for iC3b, C3d, C4d, Ba, Bb, and SC5b-9 by the enzyme-linked immunoassay (ELISA) method, using ELISA Kits (Quidel, San Diego, California) and for C3a by the radioimmunoassay method, using the Upjohn Radioimmunoassay Kit (Figure 1).15-17

Venous blood, which was used for the measurement of complement products, was drawn into a cooled syringe and transferred to a tube containing ethylenediaminetetraacetic acid–disodium salt in

![Classical pathway](image)

**FIGURE 1. Schematic representation of complement activation. The proteins enclosed in boxes indicate the complement fragments measured in this study.**
crushed ice. The blood was centrifuged at 2,000 rpm for 15 minutes at 4°C and stored at −80°C within 30 minutes of phlebotomy. In addition, blood samples used for photometric measurement of CPK were obtained every 3 hours during the first 24 hours and every 6 hours during the second and third 24 hours in the patients with AMI. Peak CPK levels were determined from these measurements.

Left ventricular ejection fraction was calculated by the area-length method from the left ventriculogram in the patients with AMI 3 weeks after hospital admission.

The plasma level of by-products of the complement system was compared with the peak CPK level and ejection fraction in the patients with AMI.

After all patients were told the objective of this study, they gave informed consent.

Statistical Analysis

All values are expressed as mean±SEM. The data were analyzed using Duncan’s multiple range test for multiple comparisons preceded by a one-way analysis of variance. A p value of less than 0.05 was considered to be significant. When the relation between

Variables was considered to be continuous, a linear regression analysis was performed.

Results

The plasma C3d levels of patients with AMI and UAP (Figure 2) were significantly higher than those of normal donors (AMI, 5.94±0.50; UAP, 3.88±0.24; SAP, 3.68±0.24; and normal, 2.90±0.16 ug/ml). The plasma C3d level of patients with AMI was significantly higher than that of patients with UAP. Also, the plasma C4d levels followed patterns of significance similar to those already described for the plasma C3d level (AMI, 145±103; UAP, 14.6±1.0 and 402±20; and normal, 11.6±1.1 and 178±10 µg/ml, respectively). The plasma C4d level of patients with AMI (Figure 3) was significantly higher than that of normal donors (AMI, 5.00±0.97; UAP, 2.88±0.48; SAP, 3.32±0.61; and normal, 2.05±0.51 µg/ml). The plasma Bb level of patients with AMI (Figure 4) was significantly higher than that of normal donors (AMI, 0.70±0.10; UAP, 0.34±0.05; SAP, 0.23±0.04; and normal, 0.20±0.04 µg/ml). The plasma Ba level
followed a pattern of significance similar to that already described for the plasma Bb level (AMI, 1.15±0.10; UAP, 0.59±0.05; SAP, 0.62±0.05; and normal, 0.50±0.07 µg/ml). The plasma SC5b-9 level of patients with AMI (Figure 5) was significantly higher than that of normal donors (AMI, 0.81±0.08; UAP, 0.15±0.05; SAP, 0.09±0.06; and normal, 0.06±0.03 µg/ml).

After determining the complement levels in the four groups of subjects, we investigated the relation between the complement components. The plasma levels of iC3b, C3d, Bb, and C4d were related to the plasma level of SC5b-9 in AMI (r=0.58, p<0.01; r=0.54, p<0.01; r=0.43, p<0.05; and r=0.43, p<0.05, respectively).

The possible role of complement in myocardial necrotic size and hemodynamic manifestations after AMI was assessed by comparing levels of complement activation products with peak CPK and ejection fraction (Table 2). As shown in Figures 6 and 7, the plasma SC5b-9 level was directly related to peak CPK and inversely related to ejection fraction in the patients with AMI (r=0.71, p<0.01; and r=-0.71, p<0.01). The correlations of complement components other than SC5b-9 with peak CPK and ejection fraction in AMI were as follows. Bb was related to peak CPK (r=0.50, p<0.01). Ba, iC3b, and C4d were related to ejection fraction (r=−0.39, p<0.05; r=−0.43, p<0.05; and r=−0.48, p<0.01). By considering these results, we found that the plasma level of SC5b-9 was most closely related to peak CPK and ejection fraction. The plasma SC5b-9 level of patients with congestive heart failure was significantly higher than that of patients without congestive heart failure in AMI (1.10±0.31 vs. 0.66±0.41 µg/ml, p<0.01) (Figure 8).

Discussion
The mechanisms by which tissue injury after myocardial infarction occurs have not been fully elucidated. An activated complement system has been implicated as a causal or contributory factor in the cell injury of myocardial ischemia in experimental models.2,19 The first studies that implicated the complement system in myocardial infarction were performed by Hill and Ward,20,21 who reported that neutrophil chemotactic activity was generated when homogenates of rat heart muscle were incubated with rat serum. The chemotactic activity was also detected in extracts of ischemic myocardium, and it was related quantitatively to the duration of ischemia. More
recently, neutrophil chemotactic activity was detected in blood samples from the coronary sinus of dogs 30 minutes after ligation of a coronary artery. In this case, the chemotactic activity increased to a maximum level at 1 hour, and that level was maintained for the remaining 5 hours of the study.22

Complement activation has also been shown in human myocardial infarction. Schafer et al23 indicated that there were C5b-9 deposits in myocardial cells located within the zones of infarction in the autopsy material derived from patients with AMI. Langlois and Gawrylé6 showed that the plasma SC5b-9 level increased in the patients with AMI. However, there has been no convincing evidence that associates the degree of activation of the complement system with the myocardial necrotic size or cardiac function in vivo in patients with AMI, nor is there compelling evidence that the complement system is activated in the patients with AP.

In our study, the plasma levels of iC3b, C3d, C3a, C4d, Ba, Bb, and SC5b-9 increased in the patients with AMI. Further, plasma levels of Bb and C4d were related to those of SC5b-9. These results show that the complement system is activated through the classic and alternative pathways, and they suggest that the cytolytic membrane complexes are also produced. Immunocytochemically, massive C5b-9 deposition occurs selectively in the areas of infarction, especially in the marginal zones of infarctions where supply of complement components for complex formation may be provided for by residual fluxes from the surroundings into the ischemic areas.23 Mayer et al24 and Bhakdi and Tranum-Jensen25 have shown that C5b-9 is the cytolytic membrane complex, comprising transmembrane pores. C5b, when not directly involved in the formation of cytolytic membrane lesions, can react with C6, C7, and the S-protein to ultimately form the soluble, lytically inactive complex

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<th>Forrester class</th>
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<th>CI (/min/m²)</th>
<th>Pulmonary congestion</th>
<th>EF (%)</th>
<th>Peak CPK (units)</th>
<th>SC5b-9 (µg/ml)</th>
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PCWP, pulmonary capillary wedge pressure; CI, cardiac index; Pulmonary congestion, as detected by chest radiography; EF, ejection fraction.
SC5b-9. Detection of SC5b-9 is evidence of activation of the common terminal pathway of the complement system and the generation of the inflammatory mediator C5a. In this manner, SC5b-9 is one of the most important markers for the cytolytic component, and for this reason, we compared the SC5b-9 level to the peak CPK and ejection fraction in the patients with AMI. The plasma SC5b-9 level was significantly related to peak CPK and inversely, but significantly, related to ejection fraction. The plasma SC5b-9 level of patients with congestive heart failure was significantly higher than that of patients without congestive heart failure in AMI. In addition, except for SC5b-9, the complement components were not so closely related to peak CPK and ejection fraction. These results suggest that the activated complement system, in particular, the common terminal pathway, is associated with the myocardial necrotic size and cardiac dysfunction in AMI and that SC5b-9 may become the best marker for insightful follow-up of the patients with AMI.

Recently, Martin et al.\textsuperscript{26} reported that C5a decreased regional coronary blood flow and myocardial function in pigs. Their data are consistent with our own results.

On the other hand, the plasma iC3b and C3d levels did not increase in the patients with SAP, but the levels did increase in the patients with UAP. The plasma SC5b-9 level did not increase in either of these groups. These results suggest that the complement system is not activated appreciably in the patients with SAP but that it is activated in the patients with UAP. Previous studies suggest that cardiac cells become permeable and release intracytoplasmic enzymes as early as 10 minutes after onset of ischemia in dogs\textsuperscript{27} and 15 minutes after onset of

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Regression plot correlating plasma SC5b-9 level with peak creatine phosphokinase (CPK). The plasma SC5b-9 level was related to peak CPK.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7.png}
\caption{Regression plot correlating plasma SC5b-9 level with ejection fraction (EF). The plasma SC5b-9 level was inversely related to EF.}
\end{figure}
ischemia in baboons. Severe ischemia in UAP may be associated with the activation of complement system, possibly by released cytoplasmic enzymes, in a manner similar to that seen in the experimental models. Kruskal et al suggested the presence of an active thrombotic process and the secondary increased plasmin activity in the patients with UAP. Plasmin cleaves C1 and C3, and it activates the complement system. Therefore, an active thrombotic process may also activate the complement system partially in UAP. The reason the plasma SC5b-9 level did not increase in the patients with UAP may be due to the factors that relate to the production and clearance of these cleavage products. Namely, the C5b-dependent activation of C5 is inhibited in plasma by control proteins, such as β1H-globulin (factor H), C4-binding protein, and the C3b/4b inactivator (factor I), and it is inhibited on cell surfaces by the C3b receptor (CR1).

The mechanisms by which the complement system is activated after myocardial infarction remain unclear. One study suggests that subcellular fractions rich in heart muscle mitochondria can activate the classic and alternative pathways of the complement system in vitro, and another recent study shows that the subcellular constituents of cardiac muscle bound to Clq may activate the complement cascade not only in dogs, but also in the patients with AMI. On the other hand, Pinckard et al and Kelley et al reported that a complement-fixing autoantibody of the IgM class binding to heart mitochondria developed after experimental myocardial infarction in dogs. Such antibodies probably do not contribute to a primary myocardial infarction, but they may be important as a trigger mechanism for the complement system in patients who have multiple myocardial infarctions.

In conclusion, we have shown that complement activation occurs to completion with generation of SC5b-9 complexes in patients with AMI and that there is a strong correlation of the levels of SC5b-9 with the peak CPK and ejection fraction. Consequently, the degree of activated complement system may be associated with the myocardial necrotic size and cardiac dysfunction in patients with AMI. With a better understanding of the inflammatory process subsequent to AMI, we may be able to decrease the ischemic damage and increase the survival rate for patients with AMI. With respect to AP, the complement system is mildly activated only in patients with UAP; however, the cardiac function is not damaged.

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KEY WORDS • inflammation • cardiac function • myocardial infarction • angina pectoris
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