THE COMPLEMENT SYSTEM IN THE ACUTE PHASE OF MYOCARDIAL INFARCTION

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Although some studies suggest that complement activation is involved in the development of acute myocardial infarction, there has been little convincing evidence of a change in the complement system in patients suffering from myocardial infarction. In this study circulating levels of C3a, C3, C4 and the total hemolytic complement titer (CH50) were serially measured in 12 patients with acute myocardial infarction up to 10 days after an attack. The plasma C3a level was greatly elevated throughout the post-attack observation period. The C3, C4 and CH50 levels were significantly increased above those controls on days 8, 9 and 10 after infarction.

These findings indicate that there is complement activation in patients with acute myocardial infarction, and suggest a pathogenetic role for complement activation in the development of myocardial damage after infarction.

THE mechanism by which myocardial tissue is destroyed in acute myocardial infarction (AMI) is not clear. An initial autolytic and subsequent heterolytic destruction of myocardial cells may occur in the infarcted area, and it has been suggested that the complement system plays a significant role in myocardial degradation. Histological evidence of inflammation is frequently seen in ischemic tissue, and the complement system appears to have a role in inducing this inflammation. In addition, it seems likely that complement-dependent inflammation is responsible for the myocardial injury occurring when heart muscle is temporarily deprived of oxygenated blood. A significant reduction in myocardial damage can be achieved in coronary occlusion experiments by prior inactivation of the complement system, suggesting that complement activation is involved in the development of myocardial necrosis.

However, there are few reports concerning complement activation in patients with AMI, even though it is crucial to elucidate how the complement titer changes in the course of such an infarction. In this study we measured C3a levels in addition to those of C3, C4 and CH50. Because C3a is normally present in small quantities, and is produced after the cleavage of C3 component when the complement cascade is activated, measurement of its concentration may be a more sensitive marker of complement activation than measurement of C3, C4 and CH50 levels.

MATERIALS AND METHODS

Circulating complement levels were measured...
TABLE 1 CLINICAL CHARACTERISTICS OF THE PATIENTS AT THE TIME OF ADMISSION, AND THEIR C3a LEVELS ON DAY 5

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bp (mmHg)</th>
<th>HR (beats/min)</th>
<th>PCWP (mmHg)</th>
<th>CI (l/min/m²)</th>
<th>Forrester's Subset</th>
<th>Peak CPK (U)</th>
<th>C3a (Day 5) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>120/70</td>
<td>86</td>
<td>11</td>
<td>3.25</td>
<td>I</td>
<td>1801</td>
<td>1699</td>
</tr>
<tr>
<td>2.</td>
<td>132/90</td>
<td>78</td>
<td>7</td>
<td>2.75</td>
<td>I</td>
<td>2020</td>
<td>1756</td>
</tr>
<tr>
<td>3.</td>
<td>114/74</td>
<td>76</td>
<td>20</td>
<td>2.67</td>
<td>II</td>
<td>4300</td>
<td>1387</td>
</tr>
<tr>
<td>4.</td>
<td>112/80</td>
<td>68</td>
<td>9</td>
<td>2.55</td>
<td>I</td>
<td>2322</td>
<td>2444</td>
</tr>
<tr>
<td>5.</td>
<td>134/90</td>
<td>78</td>
<td>16</td>
<td>2.69</td>
<td>I</td>
<td>6610</td>
<td>1419</td>
</tr>
<tr>
<td>6.</td>
<td>80/50</td>
<td>48</td>
<td>20</td>
<td>2.40</td>
<td>II</td>
<td>2088</td>
<td>1991</td>
</tr>
<tr>
<td>7.</td>
<td>86/56</td>
<td>42</td>
<td>9</td>
<td>1.50</td>
<td>III</td>
<td>2450</td>
<td>2094</td>
</tr>
<tr>
<td>8.</td>
<td>80/60</td>
<td>70</td>
<td>13</td>
<td>1.97</td>
<td>III</td>
<td>2899</td>
<td>2328</td>
</tr>
<tr>
<td>9.</td>
<td>106/72</td>
<td>72</td>
<td>6</td>
<td>2.78</td>
<td>I</td>
<td>1531</td>
<td>1564</td>
</tr>
<tr>
<td>10.</td>
<td>140/72</td>
<td>90</td>
<td>4</td>
<td>2.56</td>
<td>I</td>
<td>1531</td>
<td>1564</td>
</tr>
<tr>
<td>11.</td>
<td>116/74</td>
<td>90</td>
<td>11</td>
<td>3.26</td>
<td>I</td>
<td>1531</td>
<td>1564</td>
</tr>
<tr>
<td>12.</td>
<td>140/60</td>
<td>66</td>
<td>22</td>
<td>2.22</td>
<td>II</td>
<td>4030</td>
<td>1477</td>
</tr>
</tbody>
</table>

BP = blood pressure; HR = heart rate; PCWP = pulmonary capillary wedge pressure; CI = cardiac index

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in 12 patients with AMI. The diagnosis of AMI was based on at least 2 of the following; (1) typical angina-like pain lasting longer than 30 min, unrelieved by rest; (2) serum CK-MB fraction greater than 5%; (3) inversion or elevation of the ST-T waves; and (4) positive results from either a technetium $^{99m}$Tc pyrophosphate or thallium $^{201}$Tl scan$^{6–8}$ The patients were admitted within 24 hours after the onset of symptoms of myocardial infarction, and consisted of 9 males and 3 females with a mean age of 69, and a range of 49 to 77. Hemodynamics were monitored using a Swan-Ganz catheter. They were not given any specific therapeutic regimens, such as glucocorticoids, anticoagulants or platelet inhibitors, and none received treatment by percutaneous transluminal coronary recanalization. The time of onset of AMI was estimated by the time of appearance of the typical chest pain.

Blood samples were taken every day until the 10th day after admission. Plasma samples from 13 normal volunteers, as controls, as well as from the patients, were assayed for C3a by radioimmunoassay, using an Upjohn Radioimmunoassay Kit.$^9$ Using this kit human complement C3a could be measured in the range of 40–3000 ng/ml. Both C3a and C3 contain two tyrosyl residues, and in each molecule at least one of these side chains is available for radioiodination. As C3 cross-reacts with C3a, the ethylene diamine tetraacetic acid-disodium (EDTA)-plasma solution is mixed with an equal volume of 0.9% NaCl and then 10N HCl is added to a final concentration of 1M HCl in the plasma. This acidification procedure denatures and precipitates the C3, while leaving the acid-stable anaphylatoxin, C3a, in solution. C3b, C3c and C3d do not cross-react with C3a, because their molecular structures do not have tyrosyl residues. Five milliliters of venous blood were drawn into a cooled syringe.

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Fig.1. Serial changes in C3a levels in patients with myocardial infarction.
and transferred to a tube on crushed ice containing 7.5 mg of EDTA. The blood was centrifuged at 900g for 15 min at 4°C and stored at -80°C within 30 min after sampling.

The total hemolytic complement titer (CH50) was measured by a standard method, using sensitized sheep red blood cells, as described by Mayer. Serum levels of C3 and C4 were measured by a single radial immunodiffusion method. To assess changes in the complement system, the results of the following three periods were compared; from the first to second day (Days 1, 2), from the fifth to the sixth day (Days 5, 6) and from the eighth to the tenth day (Days 8, 9, 10) after onset of the attack.

STATISTICAL ANALYSIS

The values were expressed as the mean ± standard error (SE). The data were analyzed statistically using Duncan’s multiple range test for multiple comparisons preceded by a one-way analysis of variance. A p value less than 0.05 was considered to be significant.

RESULTS

The 12 AMI patients consisted of 7 with anterior AMI and 5 with inferior AMI. The severity of their congestive heart failure was graded according to Forrester’s Subsets: Subset I (7 cases), Subset II (3 cases), and Subset III (2 cases) (Table I). The patients’ plasma C3a levels during all periods were much higher than those in normal controls (Days 1, 2; 1288 ± 104, Days 5, 6; 1732 ± 88; Days 8, 9, 10; 1614 ± 146, and in controls; 95 ± 10 ng/ml, respectively) (Fig. 1). The patients’ C3a levels during both Days 5, 6 and Days 8, 9, 10 were significantly higher than they were during Days 1, 2. The mean C3 level on Days 8, 9, 10 was significantly higher in the patients than in the normal controls (Days 1, 2; 80.3 ± 6.1, Days 5, 6; 82.8 ± 4.7, Days 8, 9, 10; 93.1 ± 1.3, and in controls; 85.0 ± 2.0 mg/dl, respectively) (Fig. 2). The mean C4 levels during all periods were significantly higher in the patients than in the normal controls. The patients’ mean C4 level during Days 8, 9, 10 was significantly higher than that during Days 1, 2 (Days 1, 2; 45.0 ± 0.6, Days 5, 6; 50.6 ± 2.6, Days 8, 9, 10; 57.5 ± 2.3, and in controls; 30.0 ± 1.0 mg/dl, respectively) (Fig. 3). The mean CH50 levels during Days 5, 6 and Days 8, 9, 10 were significantly higher in patients than in either the normal controls or in the
patients during Days 1, 2 (Days 1, 2: 36.0 ± 1.1, Days 5, 6; 42.9 ± 2.0, Days 8, 9, 10; 46.9 ± 2.0, and in controls; 36.5 ± 1.0, respectively) (Fig. 4). Patients’ C3a levels on Day 5 were not related to peak CPK or Forrester’s Subset values.

Finally, the serial C3a changes in a 49 year-old woman with AMI are shown (Fig. 5). Her C3a level was higher than that of normal controls throughout the observation period following infarction. Her maximum C3a value occurred on Day 5 (1699 ng/ml).

DISCUSSION

The mechanisms causing tissue injury after myocardial infarction have not as yet been fully elucidated. Histological studies of infarcted myocardium have suggested an important pathological role for inflammatory mechanisms. In experimental models a pathogenetic role for the complement system in myocardial damage has been suggested by a study demonstrating the presence of C1q, C3, C4 and C5 in infarcted myocardial fibers and by a study demonstrating that the size of myocardial infarcts can be reduced by prior administration of cobra venom factor, a substance known to cause decomplementation. However, there are only a few reports describing changes in complement levels in patients suffering from myocardial infarction.

In our study, a striking elevation of circulating C3a was observed as early as Days 1 and 2 after infarction, and continued until Day 10, with a peak at Days 5 to 6. This result indicates that the complement system was activated during the 10-day period following infarction. Serum levels of C3, C4 and CH50 increased significantly during Days 8, 9 and 10. Since the serum levels of C3, C4 and CH50 reflect both synthesis and degradation, it is difficult to detect activation of the complement system after myocardial infarction by measuring only these indices. Elevated levels of C3, C4 and CH50 will indicate increased synthesis of these components only after their synthesis exceeds concurrent complement degradation following complement activation.

In order to evaluate the clinical importance of C3a values, C3a levels on Day 5 were compared to peak CPK and Forrester’s Subset values. Unfortunately, there was no correlation. The reason why C3a was not related to these clinical parameters might be due to the following reasons. Firstly, C3a might be expected to induce either acute myocardial edema due to increased vascular permeability or decreased regional myocardial perfusion due to constriction of vascular smooth muscle. While C5a not only has properties similar to those of C3a, it may also be able to attract neutrophils that can produce superoxides and initiate oxygen-free radial injury in the myocardium. Secondly, the terminal complement complex, C5b-9, may be a more important
component in the complement system causing tissue damage than C3a. Thirdly, the number of patients used in this study may have been too small to evaluate the clinical importance of C3a. Thus, it may be necessary to measure plasma C5a and C5b-9 levels in a large number of AMI patients in order to evaluate the role of the complement system in AMI.

The mechanisms of complement activation following myocardial infarction remain unclear. Substances present in mitochondria-rich subcellular fractions of heart muscle can activate both the classical and alternative complement pathways in vitro. Lysozomal proteases may be some of these substances while in addition, Pinckard has identified an IgM complement-fixing, anti-heart mitochondria autoantibody that developed in dogs after experimental myocardial infarction. Kruskal et al. suggested the presence of an active thrombotic process and secondarily increased plasmin activity in patients with AMI. Ratnoff et al showed that plasmin would cleave C1 and C3, and thus activate the complement system. Therefore, the active thrombotic process may activate the complement system partially in AMI.

In conclusion, our study has demonstrated that the complement system is activated during the acute phase of human myocardial infarction.

Acknowledgment

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