SERIAL CHANGES OF COMPLEMENT TITERS IN THE ACUTE PHASE OF MYOCARDIAL INFARCTION

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Although some studies suggest the involvement of a complement activation in the development of acute myocardial infarction, there has been little convincing evidence of the change of the complement in patients suffering from myocardial infarction. In 11 patients with acute myocardial infarction the serial changes of the total hemolytic complement titer (CH50), C3, C4, total serum protein and C3 conversion were investigated up to 120 hours after its attack. The level of CH50 and C4 increased consistently in the acute phase of myocardial infarction, but C3 did not show any change and total serum protein declined after 96 hours. β_1 A globulin, the split product of C3, could not be detected in the blood stream.

These results suggest that the infarcted or ischemic myocardium would activate complements, and C3 might be consumed continuously by the infarcted or ischemic myocardium. Such consumption of complements should stimulate the production of complement and would give rise of an increase in C4 and CH50. It is suggested that activated complement might play a significant role in the development of myocardial infarction.

THERAPIES for acute myocardial infarction consist of a careful observation of the hemodynamic state and of treating arrhythmia and pump failure. It is apparent that the prognosis in acute myocardial infarction depends directly on the quantity of the remaining viable myocardium, which is functioning normally. Therefore, if the infarct size in acute myocardial infarction could be reduced, a better prognosis might be expected.

Although some therapeutic interventions for preventing the ischemic myocardium from necrosis and for reducing the infarct size have been tried, no established therapeutic procedures have been able to be obtained. The process

involved in the destruction of the myocardial tissue has been the subject of many investigations. An initial autolytic and a subsequent heterolytic destruction of myocardial cells could be expected in the infarct area, and it is suggested that the complement system plays a significant role in the development of myocardial destruction.¹

When the left anterior descending coronary artery was ligated in dogs pretreated by Cobra Venom Factor (CVF), the infarct size was smaller than that observed in non-treated dogs.² When the distal anterior descending coronary artery were ligated in baboons, the localization of C3 was found in the infarct area, and more of creatine phosphokinase (CPK) activity were verified in the infarct muscle treated by CVF.³ These experimental evidences strongly suggest the involvement of the complement activation in the development of myocardial necrosis.

Key Words:

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Fig. 1. Serial changes of total hemolytic complement titer (CH50/ml) in patients suffering from myocardial infarction. Each value was compared to the value obtained within 24 hours after attack.

Fig. 2. Serial changes of the level of C3 in patients suffering from myocardial infarction. Each value was compared to the value obtained within 24 hours after attack.

However, very little is known about the changes of the complement in the patients suffering from myocardial infarction. It is crucial to elucidate how the complement titer changes in the course of acute myocardial infarction, and thus, we investigated its change in the acute phase in patients with myocardial infarction admitted to our Intensive Care Unit (ICU).

METHODS

The serial changes of serum complement titer were investigated in 11 patients admitted to the ICU of Gunma University Hospital within 24 hours after the onset of myocardial infarction. They were 7 males and 4 females with a mean age of 60.9 ± 3.9 (SEM), ranging from 41 to 78. Hemodynamics were monitored in 9 patients using a Swan-Ganz catheter, and the severity of these patients according to Forrester's classification^{4,5} was class I in 5 cases, class II in one, class III in one and class IV in 2. Two patients expired, one due to heart failure caused by extensive infarction and the other due to a reattack of myocardial infarction. All patients had a typical history of myocardial infarction, and showed typical changes both on electrocardiograms and in serum enzymes (CPK, GOT and LDH) for indicating this disease during their stay in the ICU. One patient had right ventricular infarction, and the others had anterior or inferior infarction. Their circulatory status was managed appropriately with the aid of the hemodynamic

Fig. 3. Serial changes of the level of C4 in patients suffering from acute myocardial infarction. Each value was compared to the value obtained within 24 hours after attack.

data obtained from a computer-assisted monitoring system (DMP-6000, Nihon-Koden, Japan). They included a close observation of arrhythmia (administration of lidocaine and other appropriate anti-arrhythmic agents) and the maintenance of the most suitable hemodynamic status in each patient; the lowering of the rate-pressure product, systemic vascular resistance, pulmonary capillary wedge pressure; the maintenance of an adequate pump function. They did not undergo specific therapies such as an administration of massive

Fig.4. Serial changes of the level of serum total protein in patients suffering from myocardial infarction. Each value was compared to the value obtained within 24 hours after attack.

glucocorticoids or hyaluronidase. The onset time of myocardial infarction was designated at the time of the appearance of the typical chest pain. Blood samples were obtained every 24 hours until 120 hours. Heparinized blood were centrifuged as soon as possible and plasma were

refrigerated at -20°C . Measurement of complement titer were made within 4 weeks. Each patient was transferred from the ICU to the High Care Unit located in an ordinary ward as soon as possible after their hemodynamic stabilization, when it became unnecessary to continue an extensive hemodynamic monitoring.

Total hemolytic complement titer was measured by the standard method using sensitized sheep red blood cells as described by Mayer.⁶ C3 and C4 were measured by the single radial immunodiffusion (Behringwerke AG, Marbutg). $\beta_1\text{A}$ globulin was separated from $\beta_1\text{C}$ globulin by the cross-electroimmunodiffusion method using the anti-human C3 globulin obtained from goat (Behringwerk AG, Marburg) as described by Watkins.⁷ The procedures were as followed: Two μl sample was applied to the spot in agarose plate and electrophorated for 150 min at 4°C with 1.5 mA/cm plate. Then the agarose containing anti-C3c globulin was applied and electrophorated for 18 hours at 4°C with 0.6 mA/cm plate at right-angle to the original direction. Serum total protein was measured by the refractance method. These values were compared to the complement titer obtained on admission to the ICU using Student's *t*-test for

Fig.5. An example of serial cross-electroimmunodiffusion to detect $\beta_1\text{A}/\beta_1\text{C}$ globulin in a 78-year-old patient suffering from myocardial infarction. Figures under each picture show the time after attack. Open circles represent the sample application spot. Each picture shows the single peak and the peak of $\beta_1\text{A}$ -globulin cannot be found. See text for details.

the paired data. P value less than 0.05 was considered significant.

RESULTS

Serial changes of total hemolytic complement titer, C3, C4 and serum total protein are shown in Figs. 1, 2, 3 and 4, respectively. Data is expressed as the percentage changes as compared to the initial control values obtained at the first period in the ICU. The values within 24 hours after the onset of the attack were 31.5 ± 1.5 CH50U/ml for total hemolytic complement, 77.4 ± 6.2 mg/dl for C3, 34.6 ± 4.1 mg/dl for C4 and 6.7 ± 0.2 g/dl for total serum protein (mean $\pm$ SEM). As shown in Figs. 1, 2 and 3, the values of the total hemolytic complement and C4 increased significantly during the post-infarction period, but C3 did not show any significant changes. Total serum protein decreased gradually until 96 hours after the onset of the attack. A typical example of the cross-electroimmunodiffusion of β_1 C/ β_1 A globulin is shown in Fig. 5. β_1 A globulin was not detected in all 11 cases, even from the mixed venous blood in one case.

DISCUSSION

We intended to elucidate the changes in some component of complements and its significance in the acute phase of myocardial infarction. Eleven patients in the present study had all the typical symptoms and laboratory data of acute myocardial infarction. Total serum protein gradually decreased 96 hours after the onset of the attack, which was considered to be due to the restricted food intake. On the other hand, total hemolytic complement titer and C4 increased significantly with the course of time during the post-infarction period, and C3 did not change.

The prognosis of acute myocardial infarction depends directly on the quantity of the remaining viable myocardium, which is functioning normally. Many experimental models of myocardial infarction were produced by the complete occlusion of one branch of coronary arteries and using these models the quantity of the infarction could be somewhat easily estimated.⁸ In patients, however, there has been no accurate indirect method to measure the quantity of the infarction as yet.⁹ In the present study, we did not investigate the relationship between the changes of complement titers and the amount of the infarc-

tion, but we intended to discover whether or not there are any changes in the complement titer in the acute phase of myocardial infarction by comparing the serial changes of complement titer with the values obtained within 24 hours after the onset of the attack.

It is well-known that necrosis of the myocardium does not occur uniformly, but there are some ischemic regions, which are likely to develop necrosis, around the necrotic area.⁸ It is suspected that the necrotic or injured muscle activates complements^{10,11} and such activation of complement cascade results in non-specific cell injury.¹² Moreover, the liberation of chemotactic factor induces the accumulation of inflammation cells which liberate lysosomal enzymes and free radicals.^{13,14} These reactions are a natural protective mechanism from microviral invasion. In myocardial infarction, these could be the cause of excessive cell destruction in the ischemic and threatened myocardial cells. To clarify the role of complement activation, some experiments have been attempted. The localization of the complement was studied around the infarction area,³ a decrease of the infarct area was observed by a treatment with CVF.² The activation of the complement by the mitochondrial membrane was obtained from human myocardial cells.¹⁵ On the other hand, there are only few reports about the change of complement titer in patients suffered from myocardial infarction. Pinckard and his associates¹⁶ have reported that C1, C4 and C3 decreased and CH50 and C6 maintained their stable levels within 72 hours after the attack in 6 patients. This difference from our present results may be due to the difference in the patients group studied.

If complement activation plays a significant role in the development of myocardial infarction, the suppression of complement activation may be an effective therapeutic procedure for myocardial infarction. Based on these assumptions, CVF and glucocorticoids were administered, and excellent results were shown in animal experiments.^{2,17} There has been no report on the treatment of patients based on this assumption, and the effect of the administration of massive doses of glucocorticoid is still debated.^{18,19} Recently we have shown that *in vitro* glucocorticoid and its synthetic analogues inhibit the complement activation induced by endotoxic lipopolysaccharide in a dose-dependent manner.²⁰ This is one of the basic reasons why pharmacological doses of glucocorticoid may be

administered effectively. On the other hand, glucocorticoid suppressed the accumulation of inflammation cells to the infarct area as well as effecting the healing process.²¹ Therefore, the administration of glucocorticoids must be done with extreme caution.

Although we have had no experience of the administration of glucocorticoids in treating patients with myocardial infarction, considerable effect might be expected if optimal doses of glucocorticoids, that is, the necessary and sufficient doses of glucocorticoids to suppress the complement activation, are administered.

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