Clinical Significance of Antiheart Antibodies after Myocardial Infarction

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SUMMARY

We used one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis of myocardial proteins followed by Western blotting to study the formation of antiheart antibodies during three months after myocardial infarction and the relationship between the appearance of antibodies and clinical and laboratory findings. Fifty-four percent of the 66 patients with infarction had different types of antiheart antibodies. The autoantibodies detected most frequently were against 35 and 42 kDa cardiac proteins. Immunoblottings with purified proteins showed that these autoantibodies reacted against myocardial tropomyosin and actin, which have been detected after acute myocardial infarction and can have immunogenetic activity through a humoral immune response. However, only the presence of autoantibody against myocardial tropomyosin correlated significantly with the presence of clinical and laboratory findings. Our results suggest that autoantibody against myocardial tropomyosin may play an immunopathogenic role in the development of symptoms in these patients. (Jpn Heart J 1997; 38: 779–786)

Key words: Acute myocardial infarction, Autoantibodies, Myocardial proteins, Western blotting

W E have recently shown that one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by Western blotting is a sensitive method for the detection of circulating heart antigens after myocardial infarction.1) This technique has also been used with electrophoresis of myocardial protein after cardiac injury to detect circulating autoantibodies to heart tissue.2) Several authors have documented the importance of autoimmunity in different
kinds of cardiac surgery.\textsuperscript{3-5}) The humoral immune response against myocardial proteins appears to play a role in the appearance of symptomatology after valve surgery, coronary bypass surgery and peripheral vascular surgery.\textsuperscript{6} However, the role of antiheart antibodies in the clinical findings seen after acute myocardial infarction remains questionable, although it is supported by the effectiveness of corticosteroid therapy and immunosuppressor drugs.\textsuperscript{7} The aims of the present study were to use Western blotting techniques to investigate the prevalence of autoantibodies against cardiac proteins in serum from patients with myocardial infarction, to identify the most frequent antiheart antibodies in these patients, and to evaluate the correlation between these circulating antibodies and the development of clinical and laboratory findings.

\textbf{METHODS}

\textbf{Patients and clinical and laboratory findings:} We studied 66 patients diagnosed as having had acute myocardial infarction on the basis of clinical, electrocardiographic and laboratory criteria at the Cardiology Service of the Virgen de las Nieves Hospital in Granada, Spain. Control assays were done in 29 serum samples obtained from the Granada Provincial Blood Bank (Table). All control samples were from patients with no history of angina pectoris or myocardial infarction and without clinical findings. All patients were studied during their hospital stay (10 days) and 1 and 3 months post-episode. As clinical findings we recorded fever, pain, myalgia and arthralgia. Laboratory studies included total

\begin{table}
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\caption{Demographic and Clinical Characteristics of the Patients Studied}
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Patients & Control group & Patients with AMI* \\
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Sex & 29 & 66 \\
Female & 9 (31.1\%) & 35 (53\%) \\
Male & 20 (68.9\%) & 31 (46.9\%) \\
Age (years) & 46–60 & 50–56 \\
Clinical and laboratory data after AMI & & & \\
Fever & No & 8 (12.1\%) \\
Pain & No & 8 (12.1\%) \\
Myalgia & No & 5 (7.5\%) \\
Arthralgia & No & No \\
Leukocyte > 12000 & No & No \\
High sedimentation rate & No & No \\
Chest X-ray & No & 1 (1.5\%) \\
Low voltage ECG & No & No \\
Infarction site & & & \\
Anterosepcial & 30 & & \\
Anterolateral & 19 & & \\
Posterior & 17 & & \\
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\end{tabular}
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*AMI = acute myocardial infarction.
leukocyte count, sedimentation rate, electrocardiogram and X-ray to detect abnormalities indicating pleural effusion with or without pulmonary infiltrate.

**Sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting:** Blood samples were collected 10, 30 and 90 days post-episode. Samples were centrifuged at 500 g for 5 min to obtain approximately 1 ml of serum, to which was added 15 μl thimerosal (0.02%) to prevent contamination. The serum samples were frozen at −20°C. Total human cardiac proteins were obtained from cardiac biopsies with a modified version of the method of Lewis et al.8) Protein concentration in each sample was determined with the dye-binding procedure of Bradford.9) The proteins were precipitated with 1:6 (vol/vol) acetone at −20°C for 1 h, and collected by centrifugation at 3000 g for 15 min at 4°C. Total proteins were resuspended in Laemmli sample buffer10) at a concentration of 1 μg/μl and separated in triplicate by sodium dodecyl sulfate polyacrylamide gel electrophoresis in a Mini Protean II cell (BioRad, Irvine, CA, USA) at 60 mA for 1 h at room temperature. The gels were transferred to nitrocellulose paper by applying a current of 30 V at room temperature for 12 h in 25 mM Tris HCl (pH 8.3), 192 mM glycine, and 20% (vol/vol) methanol.

**Detection of antiheart antibodies:** Nitrocellulose strips containing separated myocardial proteins were blocked for 3 h with a blocking solution (20 mM Tris, 0.9% NaCl, 10% non-fat milk), washed with 10 mM TBS (pH 7.4) in 0.05% Tween 20, and then incubated with the patients’ serum diluted 1:50 in blocking solution for 12 h. The strips were washed thoroughly in Tris-buffered saline-Tween, and positivity was detected with horseradish peroxidase-conjugated rabbit antibodies to mouse IgG and IgM (Dako) at a dilution of 1:200, and developed with 4-chloronaphthol as described in the protocol of the immunoblot assay kit (BioRad Immunoblot AGR-HRP Assay Kit). Purified actin and tropomyosin (Sigma, St. Louis, MO) were used in separate immunoblottings to identify the most frequent autoantibodies in serum samples. Fisher’s exact test (4F BMDD) was used to compare the presence of autoantibodies between controls and patients diagnosed with acute myocardial infarction, between patients with and without clinical and laboratory findings, and between groups of patients with infarction at different sites. The chi-squared test (4F BMDP) was used to analyze the significance of the differences in age and sex between patients with and without clinical and laboratory findings.

**Results**

**Clinical data in patients with acute myocardial infarction:** The Table shows the demographic and clinical characteristics of the patients in the study. Among the 66 patients with acute myocardial infarction, 20 showed clinical and/
Figure 1. Western blot analysis of serum from patients with acute myocardial infarction. A: Representative blot of a serum sample showing a single band of approximately 35 kDa. a, Negative control (serum from patients with no history of cardiac surgery); b, 10 days postinfarction; c, One month postinfarction; d, Three months postinfarction. B: Representative blot of a serum sample showing a band of approximately 42 kDa. a, Negative control; b, 10 days postinfarction; c, One month postinfarction; d, Three months postinfarction. C: Representative blot of serum samples showing bands approximately 51 and 55 kDa. a, Negative control; b, Serum sample yielding bands at 42, 51 and 55 kDa (10 days postinfarction); c, Serum sample yielding bands at 51, 55 and 66 kDa (10 days postinfarction); d, Serum sample yielding bands at 51, 55, 66 and 97 kDa (1 month postinfarction).
or laboratory findings post-episode which were detected during the hospital stay. The most frequent clinical findings were fever and pain. Eight patients had fever, and two of these patients also had pain. Six patients showed only pain, five patients showed myalgia and only one showed chest X-ray abnormalities indicating pleural effusion.

**Correlation between humoral immune response and clinical data:** Western blot analysis of 66 postmyocardial infarction serum samples showed that 36 patients (54.5%) had bands of IgM or IgG reactivity with myocardial proteins. All control samples were negative to antiheart antibodies. Significant differences were found in the release of antiheart antibodies between controls and patients with acute myocardial infarction ($p < 0.001$), although not between the autoantibodies detected in patients and infarction sites.

Among the 20 patients with acute myocardial infarction and positive clinical and/or laboratory findings, 17 had antiheart antibody to a 35 kDa protein. This was the only band detected in these patients, and the band became more intense with time after the episode (Figure 1A).

Among the 46 patients without clinical and/or laboratory findings, 19 showed antiheart antibodies. Sera from 13 patients showed an intense 42 kDa band (Figure 1B, lane b). This was the only band detected in 8 patients, and this band was more intense one month post-episode (Figure 1B, lane c). In 1 of these 13 patients we found faint antibody reactivity to myocardial proteins of 35 and 38 kDa, which appeared 3 months post-episode (Figure 1B, lane d). In addition,
two faint bands indicating proteins of 51 and 55 kDa were detected 3 months post-episode in 4 patients (data not shown). Sera from 6 other patients showed intense 51 and 55 kDa bands which did not vary in intensity during the study. Among these patients, 5 had a faint band at 42 kDa (Figure 1C, lane b). In the remaining patient we noted increased reactivity with a band located at 66 kDa, which was detected 10 days after acute myocardial infarction (Figure 1C, lane c). One month post-episode we also detected a 97 kDa band, which did not vary in intensity during the rest of the follow-up period (Figure 1C, lane d). Figure 2 shows the antiheart antibodies detected in patients with myocardial infarction.

Immunoblottings with purified proteins detected the presence of antiheart antibodies to a 35 kDa (myocardial actin) and a 42 kDa protein (myocardial tropomyosin), the most frequent autoantibodies in these patients (data not shown). However, statistical studies showed that only the antiheart antibody against tropomyosin correlated significantly \((p < 0.001)\) with the presence of positive clinical and/or laboratory findings in patients with acute myocardial infarction. Statistical analyses showed no significant differences in age and sex between patients with and without clinical and laboratory findings.

**DISCUSSION**

Antibodies against cardiac proteins are frequently produced in different processes of cardiac injury.\(^3\)-\(^5\) These autoantibodies may be formed in response to cardiac proteins released after myocardial damage during surgery, or as a result of the cardiac pathology itself. In this connection, cardiac tropomyosin,\(^1\) myosin light chain\(^2\) and troponin I\(^3\) have been detected in the serum of patients with acute myocardial infarction. We recently reported that the contractile protein \(\alpha\)-actin was released into the circulation during myocardial cell distress.\(^1\)\(^,\)\(^14\) These myocardial proteins may act as circulating antigens able to cause a humoral immune response.

The use of sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by Western blotting is a more sensitive method than immunofluorescence for the detection of antiheart antibodies.\(^2\)\(^,\)\(^15\) With this approach we have detected autoantibodies against myocardial proteins in the serum of patients with acute myocardial infarction. Our results show a significant correlation between cardiac injury by myocardial infarction and the presence of antiheart antibodies. These antibodies were formed mainly against proteins of approximately 35 and 42 kDa, and less frequently against molecules of approximately 97, 66, 55, 51 and 38 kDa. Antibody production increased steadily throughout the first two weeks postinfarction, with elevated levels persisting for at least three months thereafter. This pattern was similar to that described by Engle et al.,\(^16\) although in our study
some patients developed new autoantibodies after the first month post-episode. The reason why different antibodies were produced at different times is not clear, although it may be related to different release patterns of myocardial proteins (depending on intracellular location) after acute myocardial infarction.\textsuperscript{1,12} Our results showed that actin (42 kDa) and tropomyosin (35 kDa) were the two principal cardiac proteins responsible for the development of antiheart antibodies in patients with acute myocardial infarction. These findings are supported by Cummins et al.\textsuperscript{11} who found circulating tropomyosin in patients with acute myocardial infarction, and by De Scheer er et al.,\textsuperscript{6} who found a high proportion of autoantibodies against actin in the serum of 28 patients with acute myocardial infarction. We showed that in these patients, only the antiheart antibody against tropomyosin was significantly correlated with the presence of positive clinical and laboratory findings. Our results support an auto-antibody-mediated immunopathogenic role in the clinical and laboratory findings after acute myocardial infarction, and suggest that autoantibodies to tropomyosin play a role in the appearance of these symptoms. However, the absence of a relation between clinical and laboratory findings and the immune response against cardiac proteins other than tropomyosin requires further study.

In conclusion, we have shown that there is a significant relationship between the development of antiheart antibodies and myocardial infarction. The autoantibodies found most frequently in these patients were those against 35 and 42 kDa proteins. However, only the presence of antiheart antibody against tropomyosin in patients with acute myocardial infarction correlated with the appearance of positive clinical and laboratory findings. Our results suggest that this autoantibody may be an important factor in the development of symptoms after acute myocardial infarction, although the clinical relevance of antiheart antibodies in these patients requires further elucidation.

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**REFERENCES**