Antibody Response to Chlamydial Heat Shock Protein 60 Is Strongly Associated With Acute Coronary Syndromes

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Background—Heat shock proteins (HSPs) are a family of proteins with immunogenic and proinflammatory properties. Human and Chlamydia pneumoniae (Cp) HSP60 were found in patients with stable coronary disease.

Methods and Results—We measured the levels of anti–Cp-HSP60 and anti-Cp immunoglobulin G (IgG) in 179 patients with unstable angina, 40 with acute myocardial infarction, and 40 with stable angina (SA), as well as 100 control subjects. Forty-one patients with acute coronary syndromes (ACS) were also studied at follow-up. We also measured plasma levels of high-sensitivity C-reactive protein (hs-CRP) and troponin T (TnT). Seropositivity to Cp-HSP60 was found in 99% of ACS patients but in only 20% of SA patients and none of the control subjects. Seropositivity to Cp was detected in 67% of ACS patients, 60% of SA patients, and 30% of the control subjects. No differences in Cp-HSP60 IgG and in Cp IgG were observed between patients with myocardial infarction and patients with unstable angina. No correlation was found between Cp-HSP60 IgG, TnT, and hs-CRP or between IgG against Cp and hs-CRP. In ACS patients at follow-up, Cp-HSP60 IgG decreased from 0.88±0.25 to 0.45±0.14 arbitrary units (P<0.0001), becoming negative in 12 patients.

Conclusions—Seropositivity for Cp-HSP60 appears to be a very sensitive and specific marker of ACS, unrelated to Cp IgG antibody titers or hs-CRP and TnT levels. Its causal involvement in instability and its diagnostic role in ACS deserve further study. (Circulation. 2003;107:3015-3017.)

Key Words: angina • infarction • infection • inflammation

Heat shock proteins (HSPs), a family of chaperone proteins with strong antigenic properties, may represent a plausible link between infection, inflammation, and acute coronary syndromes (ACS). Antibodies to mycobacterial HSP 65 correlate with carotid thickening,1 and human (hu) HSP60 was reported to be associated with ischemic heart disease.2–5 The HSP60 produced by Chlamydia pneumoniae (Cp-HSP60) is of particular interest because of its proinflammatory and immunogenic properties.6,7 In a recent methodological study, we have observed a strikingly high prevalence of elevated Cp-HSP60 immunoglobulin G (IgG) antibody titers in patients with ACS compared with zero prevalence in control subjects.8 Therefore, we investigated whether this high prevalence of Cp-HSP60 IgG antibody titers was related to the presence of coronary artery disease, myocardial necrosis, and levels of high-sensitivity C-reactive protein (hs-CRP) and whether it decreases during follow-up, which would suggest a specific link with the acute phase of instability.

Methods

We studied 219 patients admitted to the Coronary Care Unit: 179 with unstable angina (UA) Braunwald class IIIB and 40 with acute myocardial infarction (AMI). The clinical characteristics of the patients are reported in the Table. We also studied 40 patients with stable angina (SA), who had been free from angina for at least 2 weeks, and 100 control subjects, who were matched for age, sex, and risk factors. To investigate the time course of Cp-HSP60 and Cp IgG levels in ACS, we reassessed these parameters in 41 patients at an average of 350±260 days’ follow-up. Cp-HSP60 IgG was measured by an in-house ELISA, as described elsewhere.8 All sera were diluted 1:50, and seropositivity was defined as an ELISA reading of >0.40, corresponding to twice the maximum value of antigen-only–coated wells. This value also corresponds to the upper limit of the mean±2SD of the control subjects. Anti-Cp IgG antibody levels were measured by a commercially available microimmunofluorescence assay (Labsystems). IgG titers ≥1:32 were regarded as positive. hs-CRP was measured by latex-nephelometry (Dade-Behring) and troponin T (TnT) by Elecsis (Roche).

Statistical Analysis

Percentages were analyzed by χ2 test. Because anti-Cp IgG levels were not normally distributed but IgG Cp-HSP60 levels were normally distributed, the Spearman rank correlation or the linear correlation and the Wilcoxon test or the paired t test were used, as appropriate. A 2-tailed probability value <0.05 was considered significant.
Demographic and Serological Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>ACS Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UA + AMI (n=219)</td>
<td>UA (n=179)</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>65±14</td>
<td>64±12</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>55</td>
<td>53</td>
</tr>
<tr>
<td>Family history of ischemic heart disease, %</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>Cp seropositivity, %</td>
<td>67†</td>
<td>68</td>
</tr>
<tr>
<td>HSP60 seropositivity, %</td>
<td>99*†</td>
<td>98</td>
</tr>
<tr>
<td>Mean HSP60 ELISA reading</td>
<td>0.9±0.3†‡</td>
<td>0.92±0.3</td>
</tr>
<tr>
<td>Median CRP, mg/L (range)</td>
<td>5.1 (0.1–117) †</td>
<td>5.05 (0.1–65.3)</td>
</tr>
</tbody>
</table>

Values are mean±SD, percentage, or median (range). Family history of ischemic heart disease is defined as history of ischemic heart disease in siblings, parents, or first cousins ≥65 years of age. Hypertension is defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or need for antihypertensive medication. Diabetes is defined as fasting glucose levels ≥140 mg/dL or need for antidiabetic medications. Smoking is defined as current smokers. Hypercholesterolemia is defined as total cholesterol ≥200 mg/dL or need for lipid-lowering therapy.

Cp seropositivity: *ACS patients vs control subjects, P=0.001; †ACS patients vs SA patients, P=NS; ‡SA patients vs control subjects, P=0.001.

HSP60 seropositivity: *ACS patients vs SA patients, P=0.0001; †ACS patients vs SA patients, P=0.003; ‡SA patients vs control subjects, P=0.0003; ‡SA patients vs control subjects, P=0.05.

HSP60 ELISA readings: *ACS patients vs control subjects, P=0.0001; †ACS patients vs SA patients, P=0.001.

CRP >3 mg/L: *ACS patients vs control subjects, P=0.0001; †ACS patients vs SA patients, P=0.013; ‡SA patients vs control subjects, P=NS.

CRP levels: *ACS patients vs control subjects, P=0.001; †ACS patients vs SA patients, P=0.001; ‡SA patients vs control subjects, P=NS.

Discussion

Our study demonstrates that anti–Cp-HSP60 IgG antibody titers are above the detection threshold in 99% of patients with ACS but in only 20% of SA patients and in none of the control subjects. In addition, they become weakly positive or negative after about 1 year from the acute event and therefore appear to be a strikingly sensitive and specific marker of ACS. Anti–Cp-HSP60 IgG clearly diverged from anti-Cp IgG, suggesting that they probably reflect an immune response not directly related to Chlamydia pneumoniae infection, possibly involving the patient’s host immune response. As described in the previous methodological study, recombinant proteins (in particular HSP60) of Chlamydia pneumoniae were generated to assess antibody responses in subjects with ACS. In the present study, we specifically addressed the question of whether elevated anti–Cp-HSP60 IgG is a specific finding of ACS or more generally a marker of coronary artery disease.

Previous Studies

Several authors have assessed the relationship between hu- or Cp-HSP and atherosclerotic disease; however, patients with ACS seldom have been considered, and different antigenic preparations of HSP60 were used. We used a nondegenerated protein capable of finely detecting antibodies against the native Cp-HSP60, compatible with a good conformational epitope preservation, and our data, consistent with previous reports, do not support a direct association between anti-HSP60 response and infection.
Potential Mechanisms Involved in the Response to Cp-HSP60

The high degree of sequence similarity between Cp- and hu-HSP60 suggests the possibility that antibodies formally measured against a preparation of Cp-HSP60 were in fact generated against hu-HSP60, which is compatible with the lack of correlation between Cp-HSP60 and Cp seropositivity. Priming with Cp-HSP60 may lead in some patients to anti-self immune response and raised titers of hu-HSP60 antibodies.3,9,10 Alternatively, the seropositivity to Cp-HSP60 may result from an anti-self immune response related to release of hu-HSP60 caused by cellular stress.3 The progressive reduction seen in Cp-HSP60 IgG levels and the very low levels found in SA patients argue for a mechanism tightly linked to ACS. However, no differences were observed in Cp-HSP60 IgG antibody titers levels between UA and AMI patients or between TnT-positive and TnT-negative UA patients, suggesting that the process is not likely to be related to myocardial necrosis. Evidence of colocalization of hu-HSP60 and Cp-HSP60 in human atheromatous plaques11,12 suggests the possible production of these proteins at the plaque level, where they can regulate tumor necrosis factor-α and matrix metalloproteinase expression and activate endothelium and smooth muscle cells. Independently of its origin, Cp-HSP60 may exacerbate the inflammatory cascade by stimulating innate immunity.13 The absence of correlation between Cp-HSP60 with hs-CRP in our study is consistent with the possibility that anti–Cp-HSP60 antibodies may develop as a result of an anti-self immune response and may be explained by the variable increase in CRP resulting from such a process.14

Conclusions

Antibodies against Cp-HSP60 appear to be a strikingly sensitive and specific marker of ACS. Their levels are unrelated to anti-chlamydial antibodies, hs-CRP, or TnT levels, and markedly decrease after the unstable phase. The anti–Cp-HSP60 antibody response might be caused or enhanced by an anti-self response related to antigenic mimicry between Cp- and hu-HSP60. This immune component of ACS may account for the widespread coronary inflammation reported in UA.15 The pathogenetic role of Cp-HSP60 in ACS remains to be elucidated, and its possible significance as a diagnostic marker of instability awaits confirmation in larger studies.

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References

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