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Tumor Necrosis Factor Alpha Plays a Role in the Acceleration of Atherosclerosis by *Chlamydia pneumoniae* in Mice

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The role of tumor necrosis factor alpha (TNF-α) in *Chlamydia pneumoniae* atherogenesis was evaluated in TNF-α p55 receptor-deficient C57BL/6J mice fed a high-fat/high-cholesterol diet. No acceleration of atherosclerotic lesion development was observed in infected mice compared to uninfected mice, indicating that TNF-α plays a role in the acceleration of atherosclerosis by *C. pneumoniae*.

There has been strong evidence indicating an association between *Chlamydia pneumoniae* and atherosclerosis by seroepidemiological studies, detection of the organism in atherosclerotic lesions, and animal models of atherosclerosis (3). The studies using animal models have also indicated that *C. pneumoniae* is a corisk factor of hyperlipidemia for atherosclerosis (2). However, the immunopathogenic mechanisms by which *C. pneumoniae* accelerates atherosclerosis have not been defined. It has been shown that *C. pneumoniae* can establish chronic infection in atheromatous lesions in hyperlipidemic mice and accelerate lesion formation (4). Since inflammatory processes are essential components of atherogenesis (9), induction of chronic inflammatory reactions by chlamydial infection may promote the progression of atherosclerosis.

Tumor necrosis factor alpha (TNF-α), a proinflammatory cytokine, has been shown to play an important role in immunity to bacterial infections (11), including chlamydiae (13). TNF-α is also an important modulator in the chronic inflammatory process of atherosclerosis (10). TNF-α elicits responses predominantly through the TNF-R1 (p55) receptor, including mediators of inflammatory processes (6, 7, 9). Therefore, p55 receptor knockout mice were used to determine whether signaling through this receptor contributes to the acceleration of atherosclerosis by *C. pneumoniae*.

Male p55 knockout mice on a C57BL/6J background (12) were inoculated intranasally with 10⁷ inclusion-forming units of *C. pneumoniae* AR-39 three times at 9, 11, and 13 weeks of age (1). Control animals were inoculated with buffer. Mice were fed a high-fat/high-cholesterol diet containing 15% fat, 1.25% cholesterol, and 0.5% sodium cholate (Harlan Teklad, Madison, WI) starting at the day of the first inoculation throughout the duration of the experiment. Mice were sacrificed at 21 weeks of age (12 weeks on the diet) and 25 weeks of age (16 weeks on the diet). Mice were sedated, and blood was collected by exsanguination from the femoral artery at necropsy. The heart and aorta were perfusion fixed with 10% buffered formalin and removed in toto. The aorta was separated from the heart, and the base of the heart was frozen in optimal-cutting-temperature compound (OTC; Sakura Finetek, Torrance, CA) and sectioned on a cryostat at the level of the aortic sinus. Once the atrioventricular valves were identified, 8-μm-thick sections were taken and mounted on gelatin-coated slides. Sections of the aortic sinus were collected up to the point where the valves disappeared. Every other section throughout the sinus was used for lesion analysis.

The cross-sectional area of atherosclerotic lesion was determined in 15 oil red O-stained sections per animal by computer-assisted morphometry (Optimas 5.2 software; Optimas Corp., Bothell, WA) and averaged. Measurements were done in a blind fashion with the investigators unaware of the treatment groups.

Serum antibody against *C. pneumoniae* was measured by the microimmunofluorescent test, and total plasma cholesterol was measured by a commercial enzymatic kit (Sigma, St. Louis, MO).

All infected mice developed immunoglobulin G antibody against *C. pneumoniae*, while all sham-inoculated controls were antibody negative. No differences in total plasma cholesterol values were observed between infected and sham-inoculated animals. Total cholesterol levels for noninfected and infected mice were 249 mg/dl (n = 14) versus 262 mg/dl (n = 16) at 21 weeks of age, respectively, and 243 mg/dl (n = 17) versus 253 mg/dl (n = 10) at 25 weeks, respectively. No differences were observed in the numbers of animals which developed foam cell lesions or in the mean areas of lesions in those mice which developed foam cell lesions at both time points between noninfected and infected animals (Table 1).

In our previous studies, C57BL/6J mice were fed a high-fat/high-cholesterol diet for 10, 16, and 18 weeks. All uninfected and infected (three times with 2.4 × 10⁷ to 3.0 × 10⁷ inclusion-forming units of *C. pneumoniae* AR-39) mice developed atherosclerotic lesions in the aortic sinus, and the lesion size was significantly enlarged by 2.5- to 3.3-fold in infected mice in comparison to uninfected mice (1, 5). This is in contrast to the present study, in which only 27% and 47% of uninfected mice and 40% to 44% of infected mice developed atherosclerotic lesions following 12 and 16 weeks of a high-fat/high-cholesterol diet, respectively (Table 1). These findings indicate that TNF-α...
plays an essential role in both diet- and chlamydia-accelerated atherosclerosis.

The role of TNF-α in atherogenesis has been studied by Schreyer et al. (12) using C57BL/6J female TNF-α p55 receptor knockout mice. They reported a 2.3-fold increase in lesions compared to C57BL/6J wild-type mice following 14 weeks of an atherogenic diet, suggesting a protective effect of the p55 receptor in female mice. In the present study, male mice were used, as in our previous studies, because of gender differences observed in the lipid profiles in response to a high-fat/high-cholesterol diet (8).

In summary, the present study shows C. pneumoniae infection does not accelerate foam cell lesion development in the aortic sinus in hyperlipidemic TNF-α p55 receptor knockout mice. These findings suggest that signaling through the p55 receptor may play a role in the atherogenic effects of C. pneumoniae in hyperlipidemic mice.

REFERENCEs


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TABLE 1. Development of foam cell lesions in the aortic sinuses of TNF-α p55 receptor deficient mice fed a high-fat/high-cholesterol diet and infected with Chlamydia pneumoniae

<table>
<thead>
<tr>
<th>Parameter and mouse group</th>
<th>Value for mice on diet for:</th>
<th>12 wk</th>
<th>16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of mice that developed lesions&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Uninfected</td>
<td>27 (11)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47 (17)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>40 (15)</td>
<td>44 (18)</td>
</tr>
<tr>
<td>Avg lesion area (μm&lt;sup&gt;2&lt;/sup&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Uninfected</td>
<td>855 ± 454 (3)</td>
<td>3,129 ± 2,994 (8)</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>1,054 ± 198 (6)</td>
<td>2,755 ± 1,796 (8)</td>
</tr>
</tbody>
</table>

<sup>a</sup> P values for infected versus uninfected mice were not significant by the χ<sup>2</sup> test.
<sup>b</sup> Values in parentheses are numbers of mice.
<sup>c</sup> P values for infected versus uninfected mice were not significant by Student’s t test.

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