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 responses 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 $10^7$ 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. 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 infected 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 infected mice (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 $2.4 \times 10^7$ to $3.0 \times 10^7$ 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 and infected 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.

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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>
</tr>
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<tbody>
<tr>
<td></td>
<td>12 wk</td>
</tr>
<tr>
<td></td>
<td>16 wk</td>
</tr>
<tr>
<td>% of mice that developed lesions(^a)</td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>27 (11)(^b)</td>
</tr>
<tr>
<td>Infected</td>
<td>40 (15)</td>
</tr>
<tr>
<td>Avg lesion area (μm(^2))(^c)</td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>855 ± 454 (3)</td>
</tr>
<tr>
<td>Infected</td>
<td>1,054 ± 198 (6)</td>
</tr>
</tbody>
</table>

\(^a\) P values for infected versus uninfected mice were not significant by the χ\(^2\) test.

\(^b\) Values in parentheses are numbers of mice.

\(^c\) P values for infected versus uninfected mice were not significant by Student’s t test.

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**REFERENCES**