Telithromycin Treatment of Chronic *Chlamydia pneumoniae* Infection in C57BL/6J mice

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Chronic *Chlamydia pneumoniae* infections have been associated with atherosclerosis, but clear knowledge about how these infections should be treated is lacking. We studied the effect of a new ketolide antibiotic, telithromycin, on chronic *C. pneumoniae* lung infection. Female C57BL/6J mice on a 0.2% cholesterol diet were inoculated intranasally with *C. pneumoniae* either two or three times every fourth week. Telithromycin was given to the mice subcutaneously at 75 mg/kg of body weight once daily for 5 or 10 days, starting at 3 days after the last inoculation. Samples were taken at 4 and 12 weeks after the last inoculation. The presence of *C. pneumoniae* DNA in lung tissue was demonstrated by PCR and the detection of lipid accumulation in the aortic sinus by Oil-Red-O staining. *C. pneumoniae* DNA positivity and inflammatory reactions in the lung tissue of the mice inoculated twice were significantly affected by treatment after both inoculations or only after the second inoculation at 12 weeks. Intimal lipid accumulation in the aortic sinus was also slightly but significantly less abundant in the mice treated after both inoculations compared to the levels in those treated only after the second inoculation for 10 days (geometric means, 823 and 4,324 μm², respectively; *P* = 0.033). No differences between the infected, untreated controls and the group inoculated three times and treated for 5 days were seen.

We conclude that telithromycin is effective in preventing the development of chronic *C. pneumoniae* infection and intimal lipid accumulation in C56BL/6J mice when the treatment is given after each inoculation.

*Chlamydia pneumoniae* is an obligate intracellular bacterium that causes infections of both the upper and the lower respiratory tracts. Similar to the other chlamydial species, it has a tendency to cause persistent infections. Persistent *C. pneumoniae* infection has been associated with several chronic diseases, such as asthma (24), chronic obstructive pulmonary disease (53), and coronary heart disease (CHD) (13), in several studies. *C. pneumoniae* is susceptible to macrolides and tetracyclines (12, 29), which are commonly used for the treatment of acute chlamydial infections. Fluoroquinolones (25), rifampin (21), and most recently, ketolides (34, 45) have also been found to be effective. At present, knowledge of the successful therapies for the treatment of latent and persistent infections is limited. The definition and development of appropriate and effective treatments and means of eradication of chronic *C. pneumoniae* infection would, however, be very important, especially if such treatments turned out to have an effect on the development of the associated chronic diseases.

Previous animal models have shown that repeated inoculations of chlamydia increase the presence and persistence of chlamydial DNA in several mouse tissues (10, 36). *C. pneumoniae* also causes atherosclerotic changes in rabbits (18, 31) and hyperlipidemic mice (27, 36), although controversial findings have been published from studies with murine models (1, 9). In addition, early treatment of *C. pneumoniae*-infected rabbits with macrolide antibiotics has been shown to prevent the development of atherosclerosis (17, 39), while in apolipoprotein E-deficient mice, azithromycin did not decrease the progression of lipid lesions (47). The first two small-scale antibiotic treatment trials with CHD patients (22, 23) gave promising results concerning the decreased incidence of cardiovascular events. In a more recent study, patients with acute unstable angina or non-Q-wave myocardial infarction were treated with clarithromycin for 3 months, which led to a significant reduction in cardiovascular events during monitoring for an average of 555 days (49). In the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) study, however, 3 months of azithromycin treatment of patients with stable coronary artery disease improved inflammatory markers but did not decrease *C. pneumoniae* antibody levels at 6 months (3) and did not reduce ischemic events during the 2-year period of monitoring (38).

Very recently, two large-scale azithromycin treatment trials with adequate patient series reported negative results for the whole patient population as well: the WIZARD trial included 7,747 patients with previous myocardial infarction and elevated *C. pneumoniae* antibody levels (40), and the Azithromycin in Acute Coronary Syndrome (AZACS) trial included 1,439 patients with unstable angina or myocardial infarction (11). The ongoing large trials of antibiotics and CHD patients may yield new information on the possible preventive effects of antibiotics on cardiovascular events. On the other hand, more animal models are needed to determine whether antimicrobial drugs can eradicate chronic *C. pneumoniae* infection, to establish the effect of antibiotic treatment on atherosclerosis, and to determine the optimal drug or combination of drugs as well as the dosage and timing of treatment.

In previous studies with mice, multiple *C. pneumoniae* inoc-
ulations caused only inflammatory reactions and no atherosclerotic changes in the aortas or aortic sinuses of C57BL/6J mice fed normal chow (7). Mice of this strain, however, have been shown to be susceptible to intimal lipid accumulation when they are fed a high-fat, high-cholesterol diet (41), and with this kind of diet C. pneumoniae inoculations have been shown to increase atherosclerotic changes significantly (6). In the present study, we investigated the development of chronic infection and inflammatory reactions in mice without the possible confounding effects of a high-fat diet and genetic modifications. Therefore, we used normal C57BL/6J mice fed a 0.2% cholesterol diet to study the effects of a ketolide antibiotic, telithromycin, on the treatment and eradication of chronic C. pneumoniae infections and on the development of lipid lesions in the aortic sinuses of these mice.

MATERIALS AND METHODS

Organism and inoculum. C. pneumoniae isolate Kajaani 7 (K7), a Finnish epidemic strain (15) grown in HL cells, was used in the study. Infected cells were harvested with sterile glass beads and disrupted by ultrasonication. The cell debris was separated by low-speed centrifugation, followed by sonication and two cycles of high-speed centrifugation, to purify the chlamydial particles. Finally, the pellet obtained was resuspended in sucrose-phosphate-glutamic acid buffer for storage. The number of inclusion-forming units (IFU) of viable organisms per milliliter was determined by culture in HL cells. Briefly, HL cells were infected with 10-fold dilutions of the chlamydia stock culture by centrifugation and incubated at 35°C in 5% CO2 for 72 h. Culture medium (RPMI 1640) contained 7% fetal bovine serum, 1% l-glutamate, 20 μg of streptomycin per ml, and 0.5 μg of cycloheximide per ml. The inoculum dose was estimated on this basis, and the infectious dose given to the animals was confirmed by culture in HL cells, as described above.

Animal model and treatment. Six-week-old female inbred C57BL/6J mice were purchased from M&B A/S, Ry, Denmark. Normal feeding (1324; Altromin, Lage, Denmark) with 0.2% cholesterol supplement was started when the animals arrived at the facilities. After 2 weeks on a cholesterol-supplemented diet, each mouse was inoculated intranasally with 1.05 × 106 IFU of C. pneumoniae K7 while the mouse was under inhaled methylxanthine (Metofane; Schering-Plough, Bloomfield, N.J.) anesthesia. One or two reinfections were given similarly 4 weeks later. The mouse was inoculated intranasally with 1.05 × 106 IFU/ml. The inoculum dose was estimated on this basis, and the infectious dose given to the animals was confirmed by culture in HL cells, as described above.

Telithromycin was dissolved with glacial acetic acid and further diluted with phosphate-buffered saline (PBS) solution (final acetic acid concentration, 0.1%). The telithromycin dose used was chosen on the basis of preliminary studies (52), in which doses of 25, 50, and 100 mg/kg for the treatment of acute C. pneumoniae lung infection were tested. Telithromycin doses of 50 and 100 mg/kg have been used in other mouse models as well (43, 51), and such high doses are apparently needed in mice with high metabolic and elimination rates to achieve good treatment responses. Previously, Bonnefoy et al. (8) was able to measure telithromycin levels for 8 h after intravenous or oral administration of 10 mg of the drug per kg to Swiss mice, and the half-life was reported to be 1.2 h by the intravenous route of administration.

The mice were divided into three treatment groups and two placebo-control groups, with 20 mice in each group. Groups 1 and 2 were inoculated with chlamydia twice. Group 1 was treated for 10 days after both inoculations, and group 2 was treated for 10 days after the last inoculation, i.e., postinfection (p.i.). The placebo group (group 3) was given diluent after both inoculations. Group 4 was inoculated three times and was treated only after the last inoculation for 5 days (although the intent had been to treat them for 10 days). The other placebo group (group 5) was inoculated three times and received diluent after the last inoculation for 5 days, similar to group 4. Samples were taken at 4 and 12 weeks p.i. The inoculation and treatment regimens are also presented in Fig. 1. The Animal Care and Use Committee of the National Public Health Institute, Helsinki, Finland, approved all procedures involving animals.

Measurement of C. pneumoniae antibody titers. Antibody titers were measured by the microimmunofluorescence test (originally described elsewhere [55]) by using purified, formalin-fixed whole elementary bodies of K7 as the antigen. Immunoglobulin G (IgG) antibodies were detected in serum by using fluorescein isothiocyanate-conjugated anti-mouse IgG [Fab′(a′), fragments; Serotec].

Culture of lung tissue. Lobes from the right lung were mechanically homogenized in 2 ml of sucrose-phosphate-glutamic acid buffer. The tissue suspensions were centrifuged to remove the debris, and the supernatant was collected and frozen at −70°C. The remaining lung tissue debris was stored for C. pneumoniae DNA detection. The lungs of the mice were assayed at 4 weeks p.i. for the presence of viable C. pneumoniae organisms. All analyses were performed in duplicate. HL cells infected with different dilutions of the homogenates were centrifuged at 490 × g for 1 h and incubated at 35°C under 5% CO2 for 72 h after which the plates were centrifuged again as described above, new culture media were added, and the cultures were grown for another 72 h. Finally, the cells were washed with PBS solution, fixed with methanol, and stained with a Chlamydia genus-specific monoclonal antibody conjugated to fluorescein isothiocyanate (Pathfinder; Sanofi Diagnostics Pasteur, Redmond, Wash.).

Detection of C. pneumoniae DNA in lung tissues. Lung tissue debris (50 mg) was lysed with proteinase K (Qiagen) in tissue lysis buffer (Qiagen). After incubation at 56°C overnight, DNA was purified with a commercially available QIamp tissue kit, according to the instructions of the manufacturer (Qiagen). The purified DNA was kept frozen at −20°C. The PCR primers were synthesized at the Institute of Biotechnology, Helsinki, Finland. The sequences for the primers used for DNA amplification (135-bp product), primer HB1 (5′-ATAG TCTCCGTAAACTTCCAGCAGC-3′) and biotinylated primer HB2 (5′-CCTGT AGGGAAACCTTCTGTAGT-3′), were derived from the C. pneumoniae omp1 gene. PCR was performed with a mixture of 400 μM each deoxynucleoside triphosphate, 4.0 mM MgCl2, 50 mM Tris-HCl (pH 8), 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol, 1% Triton X-100, 1.0 U of Taq polymerase (PromegaTag), 50 pmol of each primer, and 10 μl of DNA isolated...
from clinical samples. The total reaction volume was 50 μl. After 5 min of denaturation at 94°C, the samples were subjected to 50 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 30 s) with a PerkinElmer Cetus GeneAmp 9600 thermocycler. A time-resolved fluorescence-based hybridization assay with an Eu-labeled hybridization probe (5'-CCATATTGTA-3') (2 ng/100 μl) was used to test for the presence of the C. pneumoniae-specific PCR product. The probe was incubated overnight at 37°C, and the reaction was stopped by washing the reaction mixture 10 times. Otherwise, the assay was done as described previously (44). The detection limit of the PCR assay is 0.8 genome equivalents. All samples positive in the first run were assayed twice, and the result was considered positive only if the result was the same in both runs.

**Lung histopathology.** The left lung of each mouse was removed and fixed in 10% buffered formalin. Specimens were embedded in paraffin, and 4-μm sections were cut and stained with hematoxylin-eosin (HE). The inflammation detected by HE staining was evaluated for the severity of bronchointerstitial pneumonia and was graded on a scale that ranged from 0 to 4. Grade 0 indicated no inflammatory reaction is visible. Grade 1 was considered mild inflammation. Grade 2 was considered moderate inflammation, grade 3 was considered marked inflammation, and grade 4 was considered severe inflammation. Grade 4 also included patchy consolidation of lung tissue with alveolar macrophages and plasma cells, whereas grade 2 and grade 3 indicated a small number of alveolar macrophages and plasma cells, respectively.

**Aorta and aortic sinus histopathologies.** After the lungs had been dissected, the heart and the adjoining aorta were perfused with 1 ml of PBS, removed, and fixed in 10% buffered formalin. The aorta and the heart were cut apart, and the sections were embedded in paraffin. Transversal 4-μm sections were cut from the branching sites of the aortic arch and stained with HE. The upper half of the heart from five mice per group was also embedded in paraffin, and 4-μm sections from the area of the aortic sinus valve cups (as described below) were collected and stained with HE. All sections were evaluated histopathologically for lesions and inflammation.

**Quantitative analysis of lipid accumulation in aortic sinus.** Lipid lesions from 8 to 10 mice in each of the treatment groups were analyzed. The upper half of the formalin-fixed heart was embedded in gelatin and frozen. Sections (thickness, 5 μm) were collected from the area of the aortic sinus in which valve cups and valves were clearly present by the method described by Paigen et al. (42) and were stained with Oil-Red-O. Every other section was placed on a slide, for an average total of 24 to 30 sections per mouse, and every third section collected was analyzed. Of these, the area of lipid accumulation from six consecutive sections per mouse was quantified by computer-assisted image analysis.

**Statistics.** The nonparametric Mann-Whitney U test was used for statistical analyses of the lipid accumulation areas. PCR positivity was tested by Pearson’s chi-square test or Fisher’s exact test, as appropriate, and the lung histopathology findings were tested by the chi-square test for trend (SPSS for Windows, version 10.0.5). Exact P values were reported as appropriate.

**RESULTS**

**Clinical observations.** No symptoms of respiratory infection were detected in the mice inoculated with chlamydia, and the mice in all groups gained weight steadily throughout the study. For an unknown reason, the mice inoculated three times and treated only after the last inoculation had skin irritation and developed sores at the injection sites on their necks. The skin irritation and sores were detected mostly in the telithromycin-treated groups, but they were also detected to some extent in the placebo groups. For this reason, the treatment for these groups had to be terminated after 5 days, after which the sores healed rapidly. Consequently, the treatment time for these mice (group 4) after the last inoculation was only 5 days, whereas it was 10 days in the groups of mice (groups 1 and 2) inoculated twice.

**Serology.** All mice had antibody titers >128, as measured by the microimmunofluorescence method. At 4 weeks p.i., the mice inoculated twice and treated after both inoculations had significantly higher antibody titers (geometric mean of titers per study group, 987) than the placebo group (mean titer, 530) (P = 0.025, Mann-Whitney U test). At 12 weeks p.i., the corresponding titers in these two groups were 630 and 461, but the difference was no longer statistically significant.

**C. pneumoniae culture and detection of DNA from lung tissue.** All lung tissue homogenates of the samples taken at 4 weeks p.i. were culture negative. The hybridization assay method that we used with PCR gave a quantitative value for the amount of amplified DNA in the PCR product but not directly for the amount of original DNA in the sample. For this reason, we compared the groups only on the basis of positive and negative results. The positive values therefore refer to the number of DNA-positive mice per group, expressed as a percentage. The C. pneumoniae DNA positivity values at 4 and 12 weeks p.i. for the mice receiving different treatments are shown in Fig. 2. In the mice inoculated twice, the rates of DNA positivity decreased significantly from 4 to 12 weeks p.i. in both treatment groups, whereas the decrease in the placebo-treated group was not significant. The treatment given after both inoculations was most effective, with only 10% (2 of 20) of the mice remaining PCR positive. In the mice inoculated three times, the treatment after the last inoculation did not significantly decrease the rate of DNA positivity, and in the placebo-treated control group, the rate of positivity even increased slightly between 4 and 12 weeks p.i. (42 and 59%, respectively). The number of samples with discrepant results (samples positive in the first round of analysis and negative in the second round) was 6 of 60 (10%) samples (excluding samples with negative results) at 4 weeks p.i. and, similarly, 19 of 54 (35%) samples at 12 weeks p.i.

**Lung histopathology.** No marked or severe inflammatory changes (grades 3 and 4, respectively), which are frequently present during acute C. pneumoniae infection in mice, were detected in this study. There were no significant differences between the groups at 4 weeks p.i. (results not shown), but at 12 weeks p.i., earlier treatment with telithromycin clearly decreased the level of inflammation in the lungs of the mice: a moderate inflammatory reaction (grade 2) was present in 50%
of the untreated mice, 11% of those treated only after the last inoculation, and 5% of the mice treated after both inoculations (P = 0.009, chi-square test for trend) (Table 1). The treatments given after all three inoculations had no effect on the inflammatory reactions in the lungs. When the lung histopathology scores were compared to the rates of PCR positivity for the telithromycin-treated mice, more DNA-positive mice were seen among the mice with histology grades 1 and 2 at both time points, and the trends were statistically significant (P = 0.017 at 4 weeks p.i. and P = 0.031 at 12 weeks p.i., chi-square test for trend) (Fig. 3B). For the placebo-treated control mice, a significant trend was seen only at 4 weeks p.i. (P = 0.043, chi-square test for trend) (Fig. 3A).

Heart and aortic arch histopathologies. The histopathologies of the aortic arch and the aortic sinus showed no abnormal inflammatory reactions on HE staining, nor were any lesions visible in the aortic arches of these mice.

Lipid accumulation in aortic sinus. The lipid lesion areas in the mice at 12 weeks p.i. (age 24 weeks) are shown in Fig. 4. In the mice inoculated twice, the lipid lesion areas were significantly smaller in group 1 (treated twice) than in group 2 (treated only once) (P = 0.033, Mann-Whitney U test). No difference in the lesion areas between the group treated once and the placebo group was detected, whereas the group treated twice showed decreased lipid lesion areas compared to those for the placebo group, but this difference was not significant. The telithromycin-treated mice inoculated three times showed a trend toward decreased lipid accumulation compared to the level of lipid accumulation in the placebo-treated groups (at age 28 weeks), but no statistically significant differences were detected. The lipid lesion areas detected at the earlier time point, i.e., at 4 weeks p.i. (ages 16 and 20 weeks), were very small, and no differences between any of the groups were

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**TABLE 1.** Percentages of mice with different inflammation grades within a treatment group

<table>
<thead>
<tr>
<th>Treatment groupa</th>
<th>% Mice with the following severity of inflammationb</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 inoc. + 2 treatm.</td>
<td>20</td>
<td>75</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2 inoc. + 1 treatm.</td>
<td>27.8</td>
<td>61.1</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>2 inoc. + placebo</td>
<td>10</td>
<td>40</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>3 inoc. + 1 treatm.</td>
<td>5.3</td>
<td>57.9</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>3 inoc. + placebo</td>
<td>5.3</td>
<td>52.6</td>
<td>42.1</td>
<td></td>
</tr>
</tbody>
</table>

a inoc., inoculations; treatm., treatments.
b Inflammatory changes are according to the lung histopathologies for the telithromycin-treated and the control groups. 0, no changes; 1, mild lymphocyte and plasma cell infiltration; 2, moderate changes with perivascular and peribronchial lymphoid reactions. The results were obtained at 12 weeks p.i.
c Significant trend (P = 0.009) for an increase in the severity of inflammation when only one treatment or no treatment (placebo) was given (chi-square test for trend).
A) Example of an Oil-Red-O-stained section of the aortic sinus of a mouse inoculated three times and treated after the last inoculation. (B) More detail of the intimal lipid accumulation analyzed in this study. The cartilage-like formation with fat staining visible in the bottom left part of panel B was not included in the analyses.

FIG. 5. (A) Example of an Oil-Red-O-stained section of the aortic sinus of a mouse inoculated three times and treated after the last inoculation. (B) More detail of the intimal lipid accumulation analyzed in this study. The cartilage-like formation with fat staining visible in the bottom left part of panel B was not included in the analyses.

detected (data not shown). An example of an analyzed section with intimal lipid accumulation is shown in Fig. 5.

**DISCUSSION**

In this study we showed that telithromycin treatment suppresses the pulmonary inflammatory reaction, reduces the number of *C. pneumoniae* DNA-positive mice (lungs), and affects the lipid accumulation in the aortic sinus in C57BL/6J mice with chronic chlamydial infections when the treatment is given after each inoculation. Treatment after both inoculations decreased the level of *C. pneumoniae* DNA in mice significantly at 12 weeks p.i., but DNA remained detectable in lung tissue, despite the treatment: at 3 months p.i., the lung tissues of 10% of the mice inoculated and treated twice, 28% of those inoculated twice and treated once after the last inoculation, and 60% of those inoculated three times and treated only after the last inoculation were PCR positive. The proportions of discrepant results detected by PCR analysis were 10% at 4 weeks p.i. and 35% at 12 weeks p.i. The high percentage of inconsistency at 12 weeks may partly be due to the very small amount of chlamydial DNA present, thus leading to problems with sensitivity. Repeats of all analyses more than once would possibly have given even more accurate findings.

In agreement with our results, it was shown in a previous study (5) that chlamydial DNA persists for 2 months in 38% of the lung tissues of NMRI mice inoculated once and treated for 7 days with a combination of azithromycin and rifampin early after the infection. In another study, chlamydial DNA remained detectable for 26 weeks in half of ApoE-deficient mice inoculated twice and given delayed treatment with two doses of azithromycin (47). A recent study with humans showed that the persistence of *C. pneumoniae* DNA in nasopharyngeal swab specimens correlated with the persistence of symptoms (35). Some patients may display persistent infections and prolonged symptoms or relapses with positive culture and/or PCR findings and may not be cured by conventional treatment for 7 to 21 days (16, 26, 35, 46); even prolonged treatment has been ineffective in some cases (16).

The C57BL/6J mice used in the study have been shown to be susceptible to chlamydial infection, which results in a self-restricted pneumonia (54, 57). After secondary inoculation, most of the chlamydiae are eradicated within 3 to 4 weeks in these mice (54), but as shown above, the persistence of bacterial DNA is detected in some animals. In accordance with this, we found no viable chlamydia in the lung tissue by culture at 4 weeks p.i., and successful infection was confirmed by high *C. pneumoniae* IgG antibody titers. It has been questioned whether the presence of chlamydial DNA can be considered an indicator of a persistent, chronic infection. Previously, culture-negative, persistent *C. pneumoniae* infection in mice, demonstrated as PCR positivity of lung tissue after the primary inoculation, has been shown to be reactivated by immunosuppressive cortisone treatment (32, 33). In the present study, untreated mice inoculated three times showed a slight increase in PCR positivity during 8 weeks of monitoring (between 4 and 12 weeks p.i.), and only a marginal decrease was seen in a telithromycin-treated group. The persistence of PCR positivity was seen less often in mice that received only two inoculations. This is in accordance with the findings of previous studies (36), which showed that the persistence of DNA increases with repeated inoculations. Lung histopathology detected a moderate inflammatory reaction (grade 2) in 50 and 42% of the mice in the groups inoculated with *C. pneumoniae* two and three times, respectively, and treated with placebo at 12 weeks p.i. In our preliminary studies, grade 2 inflammation was not seen in mock-inoculated C57BL/6J mice of the same age (L. Törmäkangas, M. Leinonen, and P. Saikku, unpublished data). In addition, both the telithromycin-treated and the untreated groups, PCR positivity was clearly more common in the mice with grade 1 and 2 lung inflammation than in those with no inflammation. These findings suggest the continuous presence of a chronic, nonculturable form of chlamydia that maintains inflammation in the lung tissue. In vitro studies have shown that *C. pneumoniae* is able to persist in a metabolically active and viable state in human monocytes and macrophages with the restricted development of infectious progeny (2) and to induce nonreplicating but viable persistent infection when it is exposed to gamma interferon stimulation (4). In vivo, alveolar...
and peritoneal macrophages from inoculated mice were shown to be able to transport the infection to uninfected mice (37), and *C. pneumoniae* is frequently found in circulating mononuclear cells in human peripheral blood samples (50). Furthermore, Gieffers et al. (20) and Yamaguchi et al. (56) recently showed that *C. pneumoniae* isolates inoculated into circulating human monocytes (20) and mouse primary lymphocytes (56) are resistant to antibiotic treatment.

The treatment timing seems to have an influence on the development of chronic infection and atherosclerotic lesions. In a previous study, Rothstein et al. (47) treated ApoE-deficient mice that had been inoculated twice with an oral dose of azithromycin at 2 and 3 weeks p.i. At 26 weeks of age, i.e., 12 weeks p.i., the *C. pneumoniae* infection increased the sizes of the aortic Oil-Red O-stained lesions in the infected mice, but azithromycin did not reduce their sizes (47). The delay in the beginning of treatment seen in the study by Rothstein et al. (47) may have caused the negative result. This effect was also demonstrated in rabbit models by Muhlestein et al. (39) and Fong et al., who inoculated the rabbits three times and treated them with azithromycin (17) or clarithromycin (17, 19). In the last two studies (17, 19), delayed treatment with clarithromycin slightly diminished the area of atherosclerotic lesion development, whereas all the early treatments in the three studies were significantly effective in decreasing the lesion areas.

To our knowledge, the murine model described here is the first to demonstrate that antimicrobial treatment affects the formation of aortic sinus lipid lesions in *C. pneumoniae*-infected mice. The results must be interpreted with caution because of the wide range of lesion areas in each group. Despite the variability, the effect of the treatment was statistically significant when telithromycin was given after each inoculation compared to the effect obtained when the mice were treated only after the second inoculation in the groups inoculated twice. No statistical difference, however, was found when the results for the group treated twice were compared to those for the placebo-treated control group. It was unfortunate that the treatment of the mice inoculated three times had to be terminated after 5 days due to skin irritation and that the effects of a 10-day treatment on lipid accumulation could not be assessed for this group. The drawbacks of our study are that we were not able to assess the aortic sinuses, where the lesions were detected, for the presence of chlamydial antigen, nor did we have an infected control group with which to compare the results for the inoculated, untreated animals. We therefore cannot estimate the direct influence of chlamydiae on the development of atherosclerotic lesions. The repeated inoculations clearly increased the persistence of the pathogen in lung tissue, and treatment decreased this development in the group treated twice, but additional studies with more mice are needed to verify the lipid accumulation results and determine whether the decreased levels of lipid accumulation in our mouse model were really due to the antichlamydial effect of telithromycin.

Macrolide antibiotics, especially semisynthetic erythromycin A derivatives (like clarithromycin and roxithromycin), have been shown to possess several anti-inflammatory activities (28, 30, 48). Structurally, telithromycin is derived from erythromycin A (14), but there are no reports on the potential anti-inflammatory effects of telithromycin. In our study, some mice were found to be PCR positive even when no inflammation was detected in lung tissue from the telithromycin-treated groups, whereas no chlamydial DNA was detected in lung tissue from the untreated mice without inflammation. This may be due to some anti-inflammatory effect of telithromycin. We cannot exclude the possibility that the decreased lipid accumulation in the treatment groups was partly due to the anti-inflammatory effect as well, although the treatment actually intensified the IgG antibody response to *C. pneumoniae*. Fong et al. (19) have suggested that the anti-inflammatory action of clarithromycin is not very marked in rabbits, because in noninfected, cholesterol-fed rabbits the decrease in the development of atherosclerotic lesions seen after clarithromycin treatment was minor compared to that seen in chlamydia-infected rabbits.

The fact that only treatment provided early after infection seems to be effective in preventing the development of chronic infection and decreasing infection-accelerated atherosclerosis in animal models should be kept in mind when considering future human treatment trials. As most antimicrobial agents used to treat chlamydial infections affect only replicating bacteria, their potential to cure chronic infections may be modest.

In conclusion, our data suggest that telithromycin has the potential to affect the development of chronic *C. pneumoniae* infection in a mouse model and that telithromycin treatment may have an effect on decreasing atherosclerotic changes in these mice. These effects were seen only in the groups in which the treatment was given after each inoculation, yet some mice in these groups remained DNA positive. On the basis of the present findings and the findings obtained with other animal models, we hypothesize that the conventional antimicrobial treatments may not be effective in totally eradicating chronic and persistent chlamydiae and that the effects of longer treatment regimens and/or combinations of different antibiotics should be further studied.

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