**Chlamydia pneumoniae** induces neointima formation in coronary arteries of normal pigs

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**Abstract**

**Objectives:** We evaluated the role of intracoronary, intrapulmonary and macrophage-mediated delivery of *C. pneumoniae* (*Cp*) on coronary lesion formation. **Methods:** Pigs were allocated to one of three coronary protocols (intracoronary, macrophage or control groups) or to a fourth—a pulmonary group. In the intracoronary group *Cp* was injected into the wall of the left anterior descending (LAD) and right coronary arteries (RCA) and vehicle into the circumflex (CX). In the macrophage group autologous macrophages preincubated with *Cp* or not were injected into the LAD and CX wall, respectively. Animals in the control group received vehicle in LAD and CX. In the pulmonary group aerosolised *Cp* was given intrabronchially, after a single injection of vehicle into the LAD wall. Delivery into the coronary artery wall was performed with a balloon catheter with low-profile injector ports. **Results:** Seroconversion occurred in the following proportions: 5/6 (intracoronary group), 4/5 (macrophage group), 0/6 (control group), and 1/6 (intrapulmonary group). Significantly higher maximal intimal thickness (MIT) was observed in LADs of intracoronary and pulmonary groups when compared to corresponding CXs. The presence of *Cp* antigen was associated with higher MIT (*r* = 0.73; *P* < 0.0001). Injection of macrophages into the coronary artery wall did not induce proliferation. Arteries without coronary interventions were morphologically normal. **Conclusions:** Intracoronary and intrapulmonary but not macrophage-mediated *Cp* inoculation were associated with moderate intimal proliferation in the absence of a lipid-rich diet. Pre-existing coronary lesions seem a prerequisite for *Cp*-induced proliferation.

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**Keywords:** Atherosclerosis; Coronary disease; Infection/inflammation; Macrophages

1. Introduction

The hypothesis of an infectious aetiology of atherosclerosis emerged after demonstration of seropositive reactions to *Chlamydia pneumoniae* (*Cp*) in 70% of patients with an acute myocardial infarction [1]. Subsequent studies have shown the presence of increased levels of anti-*Cp* antibodies in patients with ischaemic heart disease [2,3]. Furthermore, the ability of *Cp* to infect cells involved in the process of atherosclerosis (macrophages, endothelial cells, and smooth muscle cells) has been demonstrated [4–7], and its presence in atherosclerotic lesions has been widely documented [8].

Several pathophysiological mechanisms linking *Cp* and other infectious agents to atherosclerosis have been proposed. Among these, direct infection of the vessel wall, effects on coagulation and fibrinolysis, ability to alter the growth of smooth muscle cells, changes in local lipid metabolism, and induction of adhesion molecules (such as VCAM-1 and ICAM-1) seem to be more important [2,3,8,9].

Recent technological developments allow highly effective local delivery of various substances into the coronary artery wall [10]. These advances offer the unique oppor-
tunity to study the direct effects of *Cp* on the coronary artery wall. The aim of the present study was to evaluate whether presence of *Cp* in coronary arteries of domestic pigs leads to formation of coronary lesions. The roles of direct infection (i.e. injection of *Cp* into the coronary artery wall), mediated infection (i.e. injection of macrophages preincubated with *Cp*) and pulmonary infection (intrabronchial injection of *Cp*) were studied.

2. Materials and methods

2.1. Study design

Due to technical constraints this was an open, nonrandomised study. All data were analysed blinded to the allocated treatment. Domestic pigs weighing 20–25 kg were assigned to one of three coronary treatment groups or to a fourth pulmonary group. The coronary procedures consisted of injection of *Cp* (intracoronary group), of macrophages (preincubated with *Cp* or not; macrophage group), or of vehicle (control group) into the coronary artery wall (Table 1). Animals assigned to the pulmonary group received *Cp* suspension intrabronchially. The delivery method and the isolation and labelling of macrophages were refined during a pilot phase; additional experiments were performed to validate the results observed in the macrophage group (see below). All animal studies were performed in accordance with the position of the American Heart Association on laboratory animal use and were approved by the Institutional Animal Care and Use Committee of the Catholic University of Leuven.

2.2. *C. pneumoniae* suspension

*Cp* strain AR39 was grown at the National Institute of Public Health and The Environment, Bilthoven, The Netherlands according to a previously described method [11]. Upon arrival in the laboratory, the *Cp* batch was aliquoted and stored at −80 °C until the day of experiment.

2.3. Isolation of blood monocytes/macrophages

This procedure was carried out only in animals assigned to the macrophage group. Macrophages were isolated on three different occasions for every animal: cells were collected 2 weeks prior to each experimental procedure. The time interval between two consecutive inoculations was, on average, 2 weeks. We used a density gradient followed by a plastic-adherence method for the isolation of blood monocytes/macrophages. After this period the adhering macrophages were lifted off the plates, washed in phosphate-buffered saline (PBS) and recounted. Part of the macrophages were then incubated with *Cp* at a 1:25 cell:inclusion forming units (IFU) ratio for 45 min at 37 °C. Cells used for the third inoculation were simultaneously labelled by adding 2.0 μm fluorescent microspheres (Molecular Probes, Eugene, OR, USA) at a 1:25 cell:microsphere ratio during the incubation process. The cells where then washed and kept on ice until the time of intracoronary delivery. In order to enhance the uptake into the macrophages, both *Cp* and fluorescent microspheres were preponsonised by incubation with porcine serum for 75 min at 37 °C. Macrophages not exposed to *Cp* were used as a control in the same animal (see below).

2.4. Experimental protocol

Animals were sedated with azaperone (0.1 mg kg⁻¹, i.m.), anaesthetised with sodium pentobarbital (15 mg kg⁻¹, i.v. bolus) and ketamine (5 mg kg⁻¹, i.v. bolus), and mechanically ventilated. Vascular access was obtained by canulating one of the carotid arteries. All animals received

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LAD, left anterior descending coronary artery; RCA, right coronary artery; CX, Circumflex coronary artery; *Cp*, *Chlamydia pneumoniae*; Vehicle, phosphate-buffered saline; MΦ, autologous macrophages. See text for details.
aspirin (250 mg, i.v. bolus; Aspegic, Synthelabo) and heparin (10,000 I.U., i.v. bolus; Heparine Rohrer, Novo Nordisk). The coronary artery diameter was measured by intravascular ultrasound (EndoSonics, Rancho Cordova, CA, USA) before and after intracoronary nitroglycerine injection. Arterial segments approximately 3.0 mm in diameter and without visible side branches were selected for targeting. A 3.0 Infiltrator® catheter (InterVentional Technology, San Diego, CA, USA) was used for delivery of the various suspensions. The Infiltrator is a triple-lumen balloon catheter allowing intramural delivery via three longitudinal strips of low-profile injection ports capable of penetrating the internal elastic lamina [12]. The balloon was positioned in the targeted segment, inflated at low pressure (1.5–2 atm.) and the suspensions were injected over a period of 30–60 s. The position of the balloon at the time of delivery was recorded on coronary angiograms. In this way we were able to target the same segment during the second and third interventions and to sample the correct region of the coronary artery for pathologic examinations.

The suspensions used in this study and the delivery times are summarised in Table 1. In the coronary group, $C_p$ ($5 \times 10^6$ IFUs in 250 $\mu$l PBS) was injected into the LAD and RCA and vehicle (250 $\mu$l PBS) into the CX. In the macrophage group, macrophages (2$\times$10$^5$ cells in 250 $\mu$l PBS) preincubated with $C_p$ were injected into the LAD and RCA, and nonincubated, control macrophages (2$\times$10$^5$ cells in 250 $\mu$l PBS) into the CX. In the control group, PBS alone (250 $\mu$l) was injected into the LAD, RCA and CX. In all these groups, coronary delivery was performed at baseline and after 2 and 4 weeks.

Animals in the pulmonary group were instrumented as follows. A single injection of vehicle into the LAD and RCA was performed at baseline; there was no CX intervention in this group. The intrabronchial inoculation was performed immediately after the baseline coronary procedure, and without a coronary intervention during the second and third deliveries. $C_p$ (1$\times$10$^7$ IFUs) was suspended in 20 ml PBS. A catheter was advanced in the bronchial tree into the wedge position. Half of the suspension was injected and then aerosolised with 50 ml air. The catheter was repositioned under fluoroscopic control into a different pulmonary segment and the delivery was repeated.

Venous blood samples were obtained during every intervention. After the procedure, the animals were allowed to recover and were kept on a normal diet. Six weeks after the first delivery the animals were sacrificed with an overdose of pentobarbital (Nembutal, Sanofi). The targeted segment of the RCA was carefully dissected from the epicardial surface, excised, cut into 2–3 mm rings, mounted, and frozen in liquid nitrogen. These samples were further used for detection of tracer microspheres and immunocytochemistry (V-CAM, I-CAM and tissue factor). The LAD and CX were then perfusion fixed with 4% paraformaldehyde (100 mm Hg for 60 min). The targeted segments were dissected free, sectioned transversely into 2–3 mm long arterial rings, and embedded in paraffin. These samples were used for morphometric analysis and immunocytochemistry. Additional biopsies were taken from the aorta, lungs, spleen and liver.

2.5. Morphometric analysis

Morphometric analysis was performed on 5-µm thick transverse sections obtained every 100 µm of the targeted segment spanning 15 mm. The cross-sectional areas of the intima and media were measured on haematoxylin–eosin and elastin-stained sections with a computerised morphometric analysis system (TCI Image, C.N. Rood NV, Belgium). The maximal intimal thickness (MIT; defined as the maximal measurement from the luminal surface to the internal elastic lamina), the neointimal area, the intima to media ratio (I/M) and the perimeter of the external elastic lamina were calculated. The analysis was performed blinded to the allocated treatment. A second investigator (ZS) examined the sections. An expert pathologist (EV) supervised the results of the pathological examinations.

2.6. Immunocytochemistry

The presence of the following antigens was tested: $C_p$ antigen (mouse monoclonal antibody RR-402; Washington Research Foundation, Seattle, WA, USA), the monocyte–macrophage CD 68 antigen (Kp1 antibody; Novoceastra Labs.), proliferating cell nuclear antigen (PCNA; Dako), $\alpha$-actin (ICN Biomedicals), the adhesion molecules V-CAM (Novoceastra Labs.) and I-CAM (Dako), and tissue factor (American Diagnostica). Three to five representative slices from each artery were evaluated. Immunostaining was performed with the avidin–biotin–peroxidase method. The slices were counterstained with Maeyer’s haematoxylin. Negative controls were obtained by omitting the first antibody. Presence of nonspecific immunostaining with the anti-$C_p$ antibody was tested on random samples by preincubation with mouse serum.

2.7. Blood samples

Presence of circulating anti-$C_p$ antibodies was tested by complement fixation. Seroconversion was defined as a fourfold increase to a titre of at least 1:16. All animals were seronegative at baseline. The plasma triglycerides, total cholesterol, HDL-cholesterol (direct) and LDL-cholesterol (difference) levels were measured.

2.8. Statistical analysis

Statistical analysis was performed using the SAS software. The normal distribution was tested with the Shapiro–Wilk statistic, and transformations were performed when
appropriate. The treatment effect on morphometric parameters was tested with one-way ANOVA (overall effect) and REGWQ t-test (pairwise comparisons) for the normal distributions, and with the Kruskal–Wallis test (overall effect) and the Wilcoxon rank-sum test (pairwise comparisons) for non-normal distributions. A P value of <0.05 was considered significant. All calculations were performed with the SAS procedures GLM, UNIVARIATE, NP1WAY [13]. Data are presented as mean±SD.

3. Results

A total of thirty-six animals were used. Eight animals were instrumented during the pilot \( n=6 \) and validation \( n=2 \) phase, another five (intracoronary, two; macrophage, two; pulmonary, one) died before completion of the protocol (ventricular fibrillation, four; unknown cause—normal coronary arteries at autopsy, one). The remaining twenty-three animals were allocated to the coronary \( n=6 \), macrophage \( n=5 \), pulmonary \( n=6 \), and control \( n=6 \) protocols.

3.1. Intracoronary group

Histologic examination revealed the presence of four types of coronary artery morphology (see Table 2 and Fig. 1). The first type, a mild cellular proliferation with eccentric neointima formation was typical for the arteries directly injected with \( Cp \) (i.e. the LADs: five out of six). The proliferation occurred in the absence of major damage to the vessel wall as shown by intact internal elastic lamina. We consistently identified the presence of \( Cp \) antigen by immunostaining in the areas of proliferation. Most of the cells were fusiform, and showed positive staining for the smooth muscle cell marker \( \alpha \)-actin as well as for PCNA. Neither the regions with positive \( Cp \) staining nor the adventitia showed presence of inflammatory infiltrate.

The second type, a minimal lesion (2–3 rows of endothelial cells with intact internal elastic lamina and normal media) was mostly observed in the arteries receiving only vehicle (i.e. the CXs: three out of six). Although only vehicle was injected in the CXs, two of these arteries showed endothelial cells positive for \( Cp \) antigen. These findings, together with the absence of CD-68 positive cells in the lesions suggest that (1) recirculating \( Cp \) (from the LAD or RCA sites of delivery) could in certain conditions infect endothelial cells and (2) the macrophages are not always a necessary carrier of \( Cp \).

The third lesion type was related to catheter-induced damage of the vessel wall and was observed in only one animal in this group. The internal elastic lamina and the media were disrupted on significant portions and showed pronounced cellular proliferation; the external elastic lamina was intact. Cells in the damaged area were positive for the \( Cp \) antigen and PCNA at immunostaining. However, we did not observe the presence of inflammatory infiltrate or CD-68 positive cells. Finally, a normal arterial structure with negative immunostaining for \( Cp \), CD-68, PCNA, was observed in one CX in the intracoronary group.

Thus, more proliferation was observed in those arteries directly exposed to \( Cp \) suspension (the LADs) when compared to the control vessels (CX). These findings were confirmed by morphometric analysis, which showed significantly higher MIT, neointimal area and I/M ratio in the LADs. However, cells positive for \( Cp \) were also observed on three counts in the CX and were associated with either minimal lesion (two CXs) or mild neointima formation (one CX). Five out of six animals in the intracoronary group showed seroconversion during the experiment.

3.2. Macrophage group

Neither the arteries exposed to macrophages preincubated with \( Cp \) nor those injected with control macrophages showed an inflammatory reaction after 6 weeks. One animal showed mild neointima formation, one minimal lesions, and all other three a normal morphology. There were no significant differences between LADs and CXs in this group at morphometric analysis. We also noted a discrepancy between the detection of \( Cp \) by immunostaining (one of five pigs) and the immunologic response.
Fig. 1. Morphology and immunostaining. (A–C) Lesion types; HE staining, 25× magnification. (A) Mild proliferation; (B) minor lesions; (C) catheter-induced damage. (D–I) Immunostainings in the LAD of the animal shown in (A), magnification 400×. (D) Cp antigen staining; (E) negative control for Cp; (F) PCNA staining; (G) α-actin staining; (H) CD68 staining; (I) V-CAM staining in the RCA of the animal shown in (A). (J–L) Pilot and validation experiments; (J) macrophages labelled with fluorescent microspheres are present in the media 1 week after intracoronary delivery; (K) CD68 staining in a section consecutive to that shown in (J) note the presence of cells positive for CD68 in the media (arrows); (L) electronic microscopy image of a macrophage incubated with Cp. Note the cellular inclusions containing Cp (arrows; 4900× magnification). See text for details.
(seroconversion in four out of five animals). Furthermore, we found CD68 positive cells in only one animal. Fluorescent-labelled microspheres were detected in minimal numbers (up to three per field).

We performed additional experiments in order to confirm the validity of the delivery method. We first tested in vitro for a possible decrease in the number of macrophages actually injected due to destruction by shear-forces and/or adherence to the catheter. Macrophages were counted prior to and immediately after injection through the catheter in four separate experiments. There was an average loss of 33% (range 20–44%). Microscopic examination of fresh-mounted cells did not reveal morphological alterations upon passage through the delivery device. The effectiveness of Cp uptake by the macrophages was evaluated by electronic microscopy in the pilot phase (Fig. 1).

The in vivo delivery of macrophages was tested in two additional experiments. The cells were isolated, labelled, and injected according to the same protocol used in the macrophage group, and the animals were sacrificed after 1 and 7 days. We were able to show the presence of significant numbers of labelled microspheres in the media 1 week after the coronary delivery. We also found positive CD-68 cells in the same region of the artery.

3.3. Control group

The most-encountered histological picture was that of normal arteries (LAD, four; CX, three). Catheter-induced damage was observed in three CX and in one LAD, and minimal lesions in one LAD. After exclusion of those arteries with catheter-induced damage, there were no significant differences between LAD and CX sections at morphometric analysis. We did not find positive cells for Cp in this group, and none of the animals showed seroconversion throughout the experiment.

3.4. Pulmonary group

The CXs in the pulmonary group (i.e. those arteries without coronary interventions) uniformly showed normal morphology and absence of Cp antigen. On the contrary, those arteries in which a single delivery of PBS was carried out at baseline showed mild neointima formation (one animal), minimal lesions (two animals), and catheter-induced damage (three animals). Despite the fact that Cp was delivered intrapulmonary in these animals, five out of six LADs showed C.p positive cells at immunostaining. The morphometric analysis showed significantly higher neointima, MIT and I/M ratios in the LADs when compared to the corresponding CXs (Table 3). Seroconversion occurred in only one animal in this group. Again, CD-68 cells were absent from the lesions, suggesting that macrophages are not always a necessary carrier for Cp.

<table>
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<th>Morphometric analysis, immunostaining, and serology results</th>
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<td><strong>Table 3</strong></td>
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<td>Seroconversion</td>
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EEL, external elastic lamina; MIT, maximal intimal thickness; I/M, intima/media ratio.

*Significantly different from the corresponding CX.

†Significantly different at pairwise comparisons from the CXs in the vehicle and pulmonary groups.
3.5. Inter-group analysis

The highest values of proliferation indexes (MIT, neointima and I/M ratio) were observed in the LADs of the intracoronary and intrapulmonary groups. The differences between the LADs and corresponding CXs remained statistically significant after exclusion of those animals with catheter-induced lesions (neointimal area in LAD vs. CX: 0.18±0.12 vs. 0.06±0.04 and 0.10±0.07 vs. 0.0±0.0 in the intracoronary and pulmonary group, respectively). There was a good overall correlation between the presence of \( Cp \) as assessed by immunostaining and the degree of proliferation (Spearman’s rank correlation coefficients of 0.74, 0.73 and 0.64 between positive staining and neointimal area, MIT, and I/M ratio, respectively; \( P<0.0001 \) for all). The fact that proliferation was primarily due to the presence of \( Cp \) and not to the coronary intervention per se is further suggested by the significantly higher values of the morphometric indexes in the LADs of the intracoronary and intrapulmonary groups in comparison to the control group. The targeted arteries were of comparable dimension across the different groups, as shown by the similar perimeter of the external elastic lamina. There were no significant differences between groups with regard to serum concentrations of total cholesterol and triglycerides.

4. Discussion

4.1. Role of direct infection with \( C. pneumoniae \)

The role of \( Cp \) in the genesis of atherosclerotic lesions remains controversial. Despite the large body of experimental and epidemiological evidence suggesting a link between \( Cp \) and atherogenesis, data obtained in clinical trials do not support so far a direct, causative role of \( Cp \) in atherogenesis. Neither the randomised trial of roxithromycin in non-Q-wave coronary syndromes (ROXIS) [14] nor the azithromycin in coronary artery disease: elimination of myocardial infection with \( Chlamydia \) (ACA-DEMIE) trial [15] were able to show a protective effect of antibiotic therapy against the development of coronary events. Furthermore, results of the intracoronary stenting and antibiotic regimen trial (ISAR) did not support a beneficial effect of roxithromycin in the prevention of restenosis. We believe that the negative results of the clinical trials (in contrast to the positive experimental and epidemiological data) do not immediately dismiss the infectious hypothesis. Indeed, recent data suggest that \( Cp \) present in macrophages of patients with coronary disease is resistant to therapy with azithromycin or rifampin [16].

This is the first study of the direct effects of \( Cp \) on the coronary artery wall, as well as the first one to evaluate the relationship between chlamydial infection and lesion formation in the arterial wall in a large animal model. We were able to show that \( Cp \) can infect in vivo medial smooth muscle cells upon direct injection into the artery wall, and induce a mild proliferative reaction in the absence of major vascular trauma or inflammation. To what extent this type of lesion is significant for the genesis of atherosclerosis remains unknown. We were not able to demonstrate the presence of types I or II atherosclerotic lesions in our experiment. However, proliferation of smooth muscle cells is an important step in atherosclerosis [17,18], and, thus, any process that hastens such a reaction might play a role in the development of vascular lesions. Our results are in accordance with the finding of atherosclerotic lesions in animal models of \( Cp \) infection by several other groups [19–22].

4.2. Route of infection and the importance of pre-existing lesions

The next issue addressed in our study was the comparative role of various routes of infection. As could have been expected, mild proliferation was more often observed in the arteries directly exposed to \( Cp \) (the LADs in the intracoronary group). An interesting finding of our study was the presence of \( Cp \) antigen in association with various degrees of proliferation in arteries that were not directly exposed (i.e. CXs in the coronary group and LADs in the pulmonary group). The morphological changes occurred in the absence of inflammatory infiltrate or CD68-positive cells. Therefore, we conclude that in our animal model, infection of smooth muscle cells and/or endothelial cells may have occurred independently of the intervention of macrophages, possibly by direct uptake of circulating \( Cp \) bodies.

The presence of \( Cp \) antigen was never detected in those arteries in which coronary interventions were not carried out (i.e. the CXs of the pulmonary group). These arteries showed a normal morphology despite the presence of proliferation (either mild or associated with catheter damage) and positive \( Cp \) staining in the corresponding LADs in 5 out of 6 animals. These results suggest that minimal arterial lesions are a prerequisite for \( Cp \)-induced proliferation.

4.3. Role of the macrophage in \( C. pneumoniae \) infection

The macrophage was suggested as a possible link between pulmonary \( Cp \) infections and the development of atherosclerosis [2]. In this hypothesis, alveolar macrophages play the role of a Trojan horse, picking up \( Cp \) during the course of pulmonary infection and delivering it to the site of vascular inflammation or injury, where it can induce chronic inflammation and eventually lead to formation of atherosclerotic lesions. Indeed, it is known that \( Cp \) can proliferate in human macrophages [23,24].

The most intriguing results were observed in the macrophage group. In our model, we have injected macrophages...
preincubated with *Cp* and virgin macrophages directly into the vessel wall. We were not able to detect an inflammatory reaction after three inoculations over a 6-week period. The vessels targeted with either infected or with control macrophages showed practically normal morphology; mild proliferation being observed in only one animal in this group. The explanation for these findings remains unknown. We have checked the various steps at which procedural failure could have occurred: uptake of *Cp* was demonstrated by electron microscopy, the decrease of cell numbers at the time of delivery due to shear-stress induced destruction or clogging in the catheter was quantified. We were able to demonstrate the presence of cells in significant numbers 1 week after a single delivery in the validation studies, but only in very small numbers 2 weeks after the final delivery in the macrophage group. Finally, seroconversion occurred in four out of five animals in this group, showing that *Cp* antigen was successfully presented to the immune system. Two possible mechanisms could independently or in association explain our findings. First, uptake into the macrophages. However, successful *Cp* infection of cultured macrophages in comparable settings was reported [23]. Furthermore, it is not entirely clear whether intact *Cp* or *Cp* antigens are responsible for the possible effect in atherosclerosis. Indeed, Meijer et al. detected the presence of *Cp* antigen but not *Cp* DNA or mRNA in atherosclerotic plaques [26]. This is an open question that deserves further investigation.

Secondly, we did not test whether *Cp* could survive after uptake into the macrophages. However, successful *Cp* infection of cultured macrophages in comparable settings was reported [23]. Furthermore, it is not entirely clear whether intact *Cp* or *Cp* antigens are responsible for the possible effect in atherosclerosis. Indeed, Meijer et al. detected the presence of *Cp* antigen but not *Cp* DNA or mRNA in atherosclerotic plaques [26]. This is an open question that deserves further investigation.

Presence of circulating anti-*Cp* antibodies was tested with complement fixation. This method is known to be less sensitive and, therefore, our results might have underestimated the true occurrence of seroconversion.

Finally, we did not evaluate the long-term effects of chlamydial infection on the coronary artery wall. The role of infection in conjunction with other demonstrated risk factors for atherosclerosis as well as the role of infection of pre-existing atherosclerotic plaques were not assessed in the present study and deserves further attention.

4.6. Study limitations

Our study has several limitations. First, due to practical constraints the study was open, nonrandomised. We were able to analyse the data blinded to the allocated treatment by giving second codes for all the samples collected. The code was broken only after completion of the analysis. Furthermore, for the morphometric analysis a second measurement was carried out on random samples by another blinded investigator with good interobserver agreement.

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4.7. Clinical implications

In the coronary group we have used a delivery method less relevant for the clinical setting in order to isolate the direct effect of *Cp* on the vessel wall. On the other hand, in the pulmonary group we mimicked the clinical situation of patients undergoing coronary interventions at the time of pulmonary infection with *Cp*. In these animals *Cp* was administered intrabronchially after a single coronary intervention in the LAD. Our findings suggest that *Cp* can infect those arterial segments that are damaged at the time of coronary intervention and subsequently induce a proliferative reaction. The source of *Cp* might be a pulmonary infection, or *Cp* existing in the atherosclerotic plaque itself. Indeed it was recently suggested that *Cp* can be released in the circulation during coronary angioplasty [27].

In the pulmonary group we have also tested the effect of
repeated Cp infection on normal coronary arteries. Our findings that normal vessels were not infected suggest that Cp might be involved in the acceleration or amplification of atherosclerosis, but cannot be the only cause of this disease.

5. Conclusions

In conclusion, our results show that administration of Cp can induce a mild proliferative reaction in the coronary arteries in pigs. These changes were not related to inflammation or high-cholesterol diet. Pre-existing coronary lesions seem to be a prerequisite for Cp-induced proliferation. There is no obvious relationship between the presence of Cp antigen in the tissue and the presence of circulating anti-Cp antibodies. In our model, administration of macrophages infected with Cp directly into the coronary artery wall was not associated with the development of coronary lesions. Future studies to completely elucidate the role of Chlamydia pneumoniae are needed.

References