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SHORT COMMUNICATION

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The role of chlamydia in the pathogenesis of pulmonary emphysema

Electron microscopy and immunofluorescence reveal corresponding findings as in atherosclerosis

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Abstract *Chlamydia pneumoniae* has been detected in atherosclerotic plaques by various means. Chlamydiae are able to cause persistent infections. Serologically elevated antibody titers are found in severe chronic obstructive pulmonary disease. In atherosclerosis and pulmonary emphysema, inflammatory reactions can be seen by means of light microscopy. Specimens from patients with obliterative arteriosclerosis undergoing thrombendarteriectomy and with advanced emphysema undergoing lung volume reduction surgery were examined using scanning (SEM) and transmission (TEM) electron microscopy, and using immunofluorescence with monoclonal antibodies and antiserum against chlamydiae. SEM shows spherical bodies (SBs) with a diameter from 0.3 µm to 0.6 µm on the surface of the alveoli and bronchioles, as well as in atherosclerotic plaques. In atherosclerosis and emphysema, SBs reveal a double membrane, adherence to collagen fibers, tissue destruction, as well as intracellular and interstitial localization in TEM. They show in parts a densely packed central structure. SBs are seen both in alpha-1-antitrypsin deficiency emphysema and smoker's emphysema. Using immunofluorescence microscopy, spots are seen in corresponding distributions to the SBs. Morphological findings are typical for aberrant chlamydiae seen in persistent infections. Chronic infection and bacterial colonization associated with progressive disease seems to be relevant not only in atherosclerosis but also in pulmonary emphysema.

Key words Chlamydiae · Persisting infection · Atherosclerosis · Pulmonary emphysema · Alpha-1-antitrypsin deficiency

Introduction

Current evidence indicates that chronic *Chlamydia pneumoniae* (Cp) infection may play a causal role in atherogenesis [1, 4, 5, 7, 9]. Cp was detected in atherosclerotic plaques by transmission electron microscopy (TEM), immunofluorescence, polymerase chain reaction (PCR) and culture [1, 6, 7, 9, 11]. Cp is a well-known pathogen in acute and chronic respiratory infections [10]. Serological evidence of infection is especially found in severe cases of chronic obstructive pulmonary disease [10, 19]. In these patients, emphysema is dominant [18]. Recent findings using scanning electron microscopy (SEM) and TEM indicate bacterial colonization of the alveolar space and the bronchioles in pulmonary emphysema [17]. Variable inflammatory changes are found in specimens from patients undergoing lung volume reduction surgery; these proved to be relevant for postoperative outcome [14, 15, 16]. Specimens from atherosclerotic plaques and pulmonary emphysema were investigated for corresponding findings.

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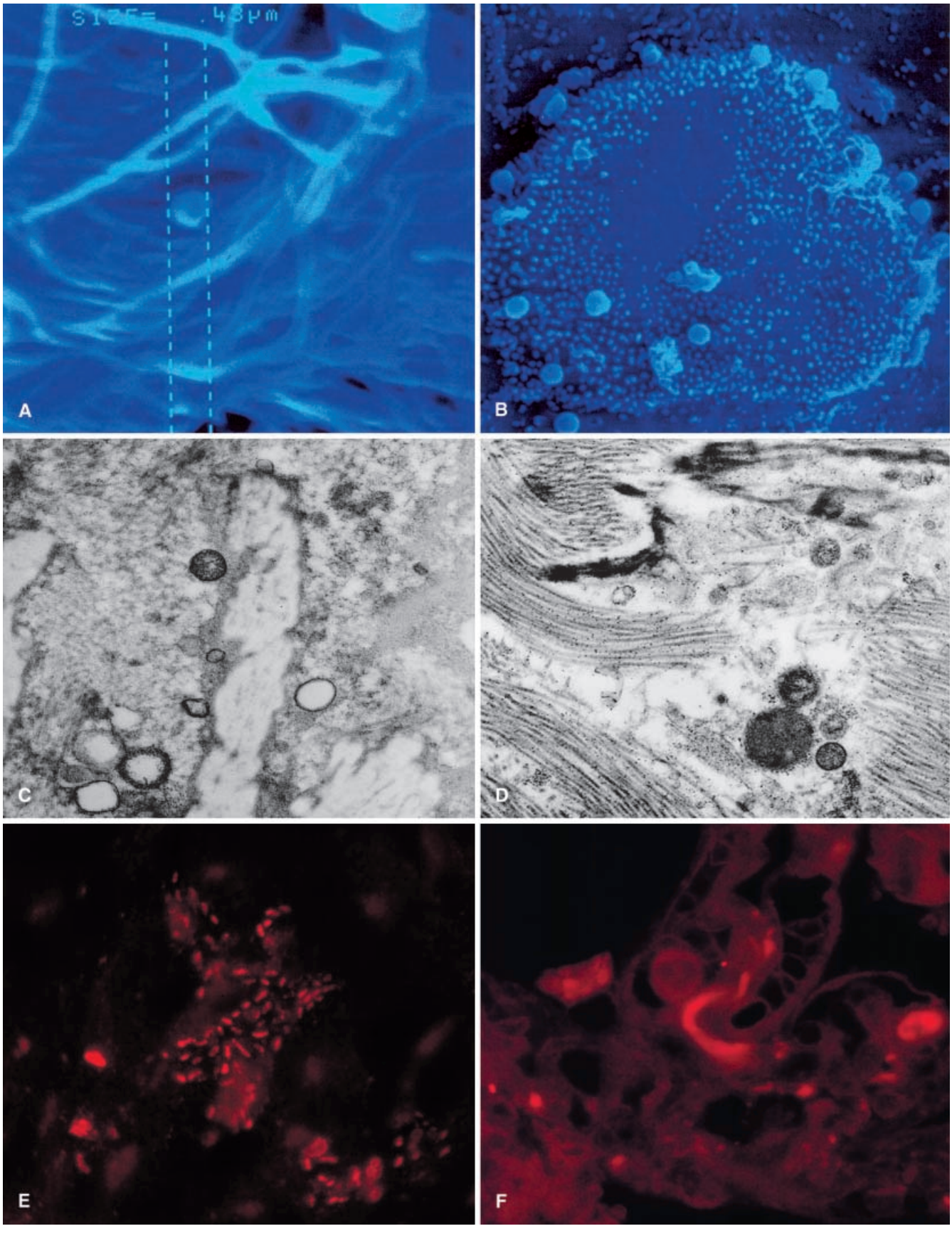
Fig. 1 Corresponding findings in atherosclerosis (*left*) and pulmonary emphysema (*right*). In scanning electron microscopy, spherical bodies are seen in destroyed areas of the arterial walls (A, original magnification ×15,000) and on type-II pneumocytes (B, original magnification ×5600). Transmission electron microscopy reveals bodies with diameters between 0.2 µm and 0.8 µm and corresponding ultrastructure; no differences are seen between atherosclerosis (C, original magnification ×15,000) and emphysema (D, original magnification ×13,000). In immunofluorescence microscopy, similar spots are found with monoclonal antibodies and antiserum against chlamydiae in atherosclerotic plaques (E, original magnification ×1000) and in lung tissue (F, original magnification ×1000) ▶


SIZE= .43μm
A
B
C
D
E
F

Materials and methods

Tissue from four patients undergoing thrombendarteriectomy for obliterative atherosclerosis of the Arteria carotis or femoralis was provided for electron and immunofluorescence microscopy. Of over 120 patients undergoing lung volume reduction surgery for advanced pulmonary emphysema (alpha-1-antitrypsin levels in normal range), specimens from 12 cases were taken for SEM. Tissue from ten persons was fixed for TEM and from four patients for immunofluorescence microscopy. Additionally, formalin-fixed specimens from three cases with alpha-1-antitrypsin deficiency emphysema were investigated using SEM and TEM.

Generally, fixation for SEM was carried out in 3.5% formaldehyde, and material was dried using the critical point method and sputtered with gold after mounting. For TEM, tissue was fixed in 2.5% buffered glutaraldehyde and embedded in epon after post-fixation with osmium tetroxide. Specimens were first viewed using semi-thin cuts, stained with basic fuchsin and methylene blue. Two to five blocks of adequate quality were chosen for further investigation as ultra-thin cuts. For immunofluorescence, specimens were fixed in methanol acetone (1:1), and four different blocks were used in each case. Triple staining with primary genus-specific monoclonal antibodies from the mouse (Progen Biotechnik, Heidelberg, Germany, dilution 1:10) and rabbit (Biodesign, German distributor: Dunn Labortechnik Ansbach, dilution 1:500) antiserum against chlamydiae as well as with 4',6-diamidino-2-phenylindol (DAPI, dilution 1:10,000) was carried out. Alexa Fluor 488 [F(ab')$_2$-fragments of goat anti-mouse, dilution 1:200] and Alexa Fluor 594 [F(ab')$_2$-fragments of goat anti-rabbit, dilution 1:300] were used as secondary antibodies (Molecular Probes Europe, Leiden).

Results

Differently advanced phases of destruction of the arterial walls were seen using SEM. Altered collagen and elastic fibers and fibrin were found. Sometimes, aggregates of spherical bodies (SBs) with a diameter of 0.2–0.6 µm were detected in all samples (Fig. 1A). In pulmonary emphysema, the alveolar walls showed sieve-like destruction. Type-II pneumocytes were multiplied and enlarged. On the surface of the pneumocytes, particularly on type-II pneumocytes, and also on some macrophages, SBs were seen in variable densities in all patients (Fig. 1B). SBs stayed in contact with the top of one or more microvilli. Inflammatory cells were found in arterial and alveolar walls.

Using TEM, SBs were also detectable. They were found in contact with the collagen fibers of the arterial or alveolar wall, on the surface of the alveolar epithelium, or the bronchioles. In macrophages and type-II pneumocytes, bodies were also seen, but in differential diagnosis lysosomes cannot be ruled out. SBs possess a contrast-rich outer double membrane. In the center, different granular and dense structures were seen. The diameter of the bodies changed between 0.2 µm and 0.8 µm, at which point they were found lying in small groups. These SBs were seen in all patients with atherosclerosis (Fig. 1C) and in eight of ten patients with pulmonary emphysema (Fig. 1D). In the remaining two cases of emphysema, bodies were not clearly found, were not typically arranged in groups, and remained sparse.

Emphysema in the three cases of alpha-1-antitrypsin deficiency revealed similar findings to typical SBs using both SEM and TEM. In the arterial walls, areas with degeneration, apoptotic cells, and some small spots were seen using the DAPI stain. Similar spots were easily detected on the alveolar surface. Immunofluorescence with antibodies and antiserum against chlamydiae revealed arranged spots in different densities in those areas of the arteries that showed myxoid degeneration (Fig. 1E). They are also found in and on the alveolar walls, the bronchioles, and also macrophages (Fig. 1F). A co-localization with the DAPI spots could be seen. Positive reactions were detected in at least one of the blocks of each patient.

Discussion

Several theories regarding atherogenesis exist. A large number of studies have been reported regarding associations of atherosclerosis and persistent bacterial or viral infections [4], especially with Cp [1, 6, 7, 9]. Despite of detection of Cp using various means, its role in the pathogenesis of atherosclerosis is still under debate [4, 6]. Chlamydiae are obligate intracellular parasites. Persistent infections are possible and well known [2]. This is associated with the development of aberrant chlamydia forms, which reveal greater diameters than normal elementary or reticulate bodies [2, 12, 13]. SBs found in this study were within this range. SBs could be seen using TEM only in eight of ten cases of emphysema. This corresponds with published findings in atherosclerosis, which describe Cp detection using different methods in 2–100% of the cases [1, 7, 9, 11]. Using SEM, SBs were found in every patient, but this method allows inspection of a greater surface, and clear-cut diagnosis of bacterial colonization is only possible in combination with other methods. Release of bacteria has to be postulated because SBs are detected on the surface of the bronchioles and alveoli. Bacteria and inflammatory cells produce proteases and oxidants that seem to be relevant in pathogenesis of emphysema [5].

Arterial walls in arteriosclerosis and alveolar walls in pulmonary emphysema show degenerative lesions of collagen and elastic fibers. Areas of most severe destruction in both diseases are colonized by chlamydiae. In addition, inflammation is seen. It is known that *Chlamydia trachomatis* possesses a binding affinity to collagen [8]. In coronary plaque tissue, Cp was found particularly in patients with unstable angina, that is, in cases of progression [1].

No fundamental differences were found between emphysema in alpha-1-antitrypsin deficiency and smokers. This reveals that inflammation and bacteria are relevant in both cases, but in alpha-1-antitrypsin deficiency changes are accelerated and more evenly distributed [3]. Unfortunately, adequately fixed tissue was not available from all patients with pulmonary emphysema to allow us to perform all morphological methods in the same patients. Investigations still have to be done to correlate the results using different methods. Chlamydiae in pulmonary emphysema must be cultured and classified by micro-

biological means. Therapeutic studies are also necessary, but morphological findings already show clear-cut similarities to the findings in atherosclerosis.

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Note added in proof

In the meanwhile, *Chlamydia pneumoniae,* DNA has been detected in pulmonary emphysema by PCR as well*.

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