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Identification of *Chlamydia pneumoniae* DNA in Carotid Plaques

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ABSTRACT

Chlamydia pneumoniae (CP) is a bacterium that in recent years has been investigated as an etiologic agent for atherosclerosis. It is a ubiquitous microorganism that has been isolated in various regions of the vascular system and its prevalence is about 10% in the patient population. This study involved a group of 43 patients (27 men, 16 women, mean age 68 years) who underwent carotid endarterectomy. About 9.3% of the patients yielded plaques that tested positive for the DNA genome of *Chlamydia pneumoniae*.

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Introduction

Chlamydia pneuomoniae (CP) was first isolated about 10 years ago. This bacterium is considered a common cause of pneumonitis, bronchitis, pharyngitis, and sinusitis both in adults[1] and children,[2] although infections in the latter tend to be less severe than in the former. *Chlamydia pneumoniae* belong to a genetically diverse group of bacteria that also includes *Chlamydia trachomatis* and *Chlamydia psittaci.* These microorganisms have a unique, dimorphic, intracellular life cycle. As obligate intracellular parasites they are dependent on the intracellular environment to supply the metabolite(s) adenosine triphosphate (ATP) and they replicate within the cytoplasm of the cells. *C. pneumoniae* go through several phases leading to intracellular infection. First there is a primitive sporoform existing in the extracellular environment that enters the cell, fuses with a primary liposome, and then changes to a metabolically active reticular form, which is released into the intracellular environment.

In common with many intracellular bacteria, CP can survive and even replicate within phagocytes, which not only fail to combat their diffusion and localization but also assist CP to resist destruction. These intracellular bacteria cause a chronic inflammation, activating killer cells either directly or via interleukin 12, and thus induce cell-mediated immunity by two mechanisms: (1) activation of macrophages through release of cytokines derived from T lymphocytes; (2) lysis of infected cells by cytotoxic T cells CD8+.[3]

In the 1980s Grayston et al[4] identified specific antigenic components of CP in atherosclerotic plaques, thus supporting an infective hypothesis in the multifactorial field of atherosclerotic pathogenesis. The association of CP with atherosclerotic disease followed with seroepidemiologic studies and with discovery of the bacteria in plaques of various vascular sites by means of electron microscopy, immunocytochemistry, and the polymerase chain reaction [PRC].[5-8]

Initially CP was isolated from coronary arteries, but it is now revealed that the organism may reside in plaques located almost anywhere in the vascular system. At present the prevalent hypothesis is that CP infestation plays a role in the progression and friability of plaques rather than in their etiology.[9] In light of the increasing volume of literature on this subject we decided to investigate the incidence of CP in the atherosclerotic carotid plaques of a group of patients attending this institution.

Materials and Methods

There was a total of 43 patients, 27 men and 16 women, mean age 68 years (range 52–84), all of whom underwent carotid thromboendarterectomy for hemodynamically significant atheromatous stenoses. Risk factors for atherosclerosis included smoking (27 patients), hypertension (34), diabetes (15), and cardiac ischemia (12 patients). The carotid lesions were bilateral in three patients and one had diffuse atheromatous plaques throughout the peripheral vascular system. In seven patients there was apparent mild to moderate ulceration in association with the thrombi in the carotid plaques. The plaque specimens removed at surgery were placed in sterile containers without any fixatives and taken to the laboratory where they were either processed immediately or stored frozen at –25°C.

DNA was extracted from all plaque specimens (fresh and frozen) and then we sought the genome of CP by using PCR. Two regions of the CP genome were studied: (1) in the gene Pst 11, of 437 base pairs, adding nested PCR for confirmation of 229 base pairs and; (2) in the gene 16 SrRNA of 119 base pairs. PCR products then underwent direct automatic sequencing (automated DNA Sequencers 373A and 377 ABI, Perkin Elmer, and Sequencer TM 3.0 Software, Gene Codes Corporation, Ann Arbor, MI, USA).

CP, TWAR (ATCC) were used as positive controls (the sequencing of the CP genome has recently been completed), and DNA from histologically normal coronary and carotid arteries was used as a negative control.

Results

The CP genome was detected in four tissue samples, which is 9.3% of patients examined. Two were from men and two were from women with age range 69–77 years. All were hypertensive, hypercholesterolemic, and smokers or ex-smokers but showed no evidence of myocardial ischemia or marked respiratory problems. There were two patients with abdominal aortic aneurysms, one of whom underwent previous surgical resection of this lesion.

Two of the detached, infected plaques appeared ulcerated and two showed fibrous calcification and histologic evidence of inflammation with the presence of macrophages, lymphocytes, and fibroblasts in varying degrees.

Discussion

In recent years *C. pneomaniae* has caused increasing interest among investigators because of its possible role in atherosclerosis as an etiologic agent, or a pathogenetic agent, or both.[10] Some early uncertainties have been partly resolved by the absence of any type of infection in the vascular tissues of healthy subjects and the presence of CP revealed only in vascular specimens involved in atherosclerotic processes. These findings are more consistent with the concept that CP contributes to the progression of atherosclerotic lesions rather than actually initiating them.[7,9,11] Although autopsy studies have demonstrated a preference of CP for vascular (as compared to other) tissues, this does not necessarily indicate that the organism is involved in the development of atheroma.[11,12] In light of these data, Jackson et al[8] advanced the hypothesis that CP develops its role from a simple contaminant of atherosclerosis to an agent involved in the pathology of the lesions.

If CP is one of the potential risk factors in atherosclerotic disease, its wide distribution throughout the cardiovascular system has many implications, including systemic administration of macrolide or tetracycline antibiotics. Naturally, this is of major interest, especially in the industrialized countries where cardiovascular disease is so prevalent.[13] The consensus in available literature is that CP can plausibly favor progression of atherosclerosis but is probably not involved in its genesis. This tentative conclusion is supported by the fact that there are no distinctive histologic criteria associated with specimens of plaque containing CP as compared to noninfected plaques. Therefore, there is a need for further research into possible specific factors that will distinguish the effects of CP on atherosclerotic plaques and, thus, define individual treatments.

In the course of our research we were made aware of the fundamental importance of the diagnostic technique selected because immunofluorescent tests provide a higher incidence of positive results compared to isolation and complete sequencing of DNA. The nested PCR uses two couples of genes called "primer." The first one is complementary to an internal portion of the researched gene and the second to the whole gene. After the amplification of the internal gene, it is possible to amplify the whole gene. In this way the reaction is more specific and sensitive.

Our data are consistent with those in the literature: the genome of CP was detected in approximately 10% of the carotid plaques examined. The significance of this reported incidence notwithstanding, there have been numerous hypotheses as to how atherogenic mechanisms are linked to CP infection. Our hypothesis is that macrophages, infected with CP, attract inflammatory cells (particularly T lymphocytes) into the plaque, stimulating biologic activity and tissue damage.

In those whose lipid profile is a risk factor, foci of antigenic bacteria could accelerate the atherogenic process by invoking a cellular inflammatory response; also there may occur a chronic endothelial infection with associated hypercoagulability. Given the known relations among inflammation, plaque rupture, intima damage, and thromboses, any condition favoring inflammation is an obvious factor favoring instability, playing a possible role in the genesis of the acute complications of the plaque.

Conclusion

According to the literature and our personal experience we can draw the following conclusions.
Among the several studies on atherosclerosis and its clinical effects, the investigations on *Clamydia pneumoniae* are interesting, both for the premises and the results, even if at present no definite answer is possible. Recently, the improvement in technique (use of positive controls of the CP genome, whose complete sequence was obtained in November 1997) provides more reliable results, by reducing false positives and negatives. Immunohistochemical studies made in parallel may complete the diagnostic picture and help solve the existing doubts: Is CP a contaminant that is associated with sick tissues or has it a real connection with genesis or progression of atherosclerotic disease?

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