Jpn. J. Infect. Dis., 52, 223-227, 1999

Invited Minireview

Chlamydia pneumoniae and Atherosclerosis

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(Received November 29, 1999)

CONTENTS:

SUMMARY: *Chlamydia pneumoniae* is the third species of the genus *Chlamydia* and has been known to cause respiratory tract infections. Since the association between the seropositivity of *C. pneumoniae* and ischemic heart diseases was reported in 1988, the association between *C. pneumoniae* and atherosclerosis has been noteworthy. Positive findings of the association between *C. pneumoniae* and atherosclerosis have been reported as the result of seroepidemiological surveys, histological studies to detect *C. pneumoniae* in human atherosclerotic tissues, and animal infection models. These data supported that *C. pneumoniae* infection occurs in human vascular walls and may accelerate the foam cell formation of macrophage and smooth muscle cells, and may play a causative role in atherosclerosis. Several large-scale studies of the antimicrobial prevention of secondary cardiac events are in progress. The genome projects for *C. pneumoniae* have recently been reported. A number of issues remain unclear, however, and further intensive research is necessary.

1. Introduction

Chlamydia is a small intracellular energy parasite of host cells and has a unique life cycle between an elementary body and a reticulated body. *Chlamydia* is different from a virus because it has both DNA and RNA and is sensitive to antimicrobial agents. Three species in the genus *Chlamydia* are known to cause human infections. *Chlamydia trachomatis* causes trachoma, sexually transmitted diseases, neonatal conjunctivitis, and infantile pneumonia. *Chlamydia psittaci* causes psittacosis. *Chlamydia pneumoniae* is the third species of the genus *Chlamydia* and has been known to cause respiratory tract infections such as pharyngitis, bronchitis, and atypical pneumonia since 1985 (1). Since Saikku et al. (2) reported the association between the seropositivity of *C. pneumoniae* and ischemic heart diseases in 1988, the association between this unique bacteria and atherosclerosis has received a great deal of attention. Although there are many well-known risk factors such as smoking, hypertension, diabetes mellitus, hyperlipidemia, and so on, more than 30% of patients with acute myocardial infarction do not have any known risk factors. We need to find another risk factors such as *C. pneumoniae*. In this paper, I review recent reports that demonstrated this association.

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This article is an Invited Minireview based on a lecture presented at the 9th Symposium of the National Institute of Infectious Diseases, Tokyo, 21 May 1999.

2. Seroepidemiological studies

C. pneumoniae infection is a very common disease at any age. Seropositive rates against *C. pneumoniae* in adult are over 50% and reach to around 80% in older populations all over the world (3,4). Atherosclerosis is also a very common disease in older people, so it seems to us to be very difficult to find the association between *C. pneumoniae* infection and atherosclerosis. However, odds ratios of the seropositivity to *C. pneumoniae* in atherosclerotic diseases such as ischemic heart disease, stroke, and hypertension were almost the same, around 2. 0 (1.6-4.4) in many populalions in the world (2,5-17). These positive findings were reported in more than 20 papers published in English journals, while only two papers reported negative relation (18,19). Although almost all of these studies were carried out in western countries, Miyashita et al. (14) have recently reported the same positive association in Japan.

3. Demonstration of *C. pneumoniae* in atherosclerotic and non-atherosclerotic tissues

Since Shor et al. (20) reported the presence of *C. pneumoniae* in atherosclerotic tissues of the coronary artery by an electron microscopy, many researchers have succeeded

in demonstrating *C. pneumoniae* by immunohistochemistry, polymerase chain reaction, and isolation in culture (21-35). The positive rates of *C. pneumoniae* in atherosclerotic tissues vary, but average approximately 60% (range 33-73%), including ours (25,26). These positive findings were appeared in more than 20 reports published in English journals, while only two published papers reported that no *C. pneumoniae* was found in atherosclerotic tissues (18,36). So far, only three groups succeeded in isolating *C. pneumoniae* by cell culture, although many researchers have attempted. Two groups isolated only one strain, while Maass et al. (35) isolated 11 strains (16%) out of 70 atherosclerotic plaques by more than 10 passages, and concluded that the presence of viable *C. pneumoniae* in atherosclerotic tissues is a common phenomenon. *C. pneumoniae* was also found in non-atherosclerotic tissues; the positive rate, however, was quite low (37).

4. In vitro infection model

The natural hosts of *C. pneumoniae* are originally believed to be the epithelial cells of the human respiratory tract. However, recent in vitro data have shown that *C. pneumoniae* also infects macrophages, vascular endothelial cells, and smooth muscle cells, which are the main players in human atherosclerosis (38-41). *C. pneumoniae* increased cholesterol accumulations in infected macrophages (42,43). These data supported that *C. pneumoniae* infection might occur in human vascular walls and accelerate the foam cell formation of macrophages and smooth muscle cells.

5. Animal infection model

So far two animal models were used to examine the association between *C. pneumoniae* infection and atherosclerosis. One is an ApoE-deficient transgenic mouse (44,45) and the other a New Zealand White rabbit (46-49). Several researchers independently reported that *C. pneumoniae* was found in atherosclerotic tissues at the initial stage of atherosclerosis after intranasal innoculation of *C. pneumoniae* to these mice and rabbits. Atherosclerosis was accelerated by intranasal infection, and then progression of atherosclerosis was inhibited by the early use of antibiotics. These data supported the causative role of *C. pneumoniae* in atherosclerosis in these animals. However the more appropriate animal model, such as non-human primates, should be developed.

6. Antimicrobial prevention of secondary cardiac events

If *C. pneumoniae* plays a causative role in atherosclerosis in humans, antibiotics may have a favorable effect in patients with atherosclerosis, and prevent the secondary attack of ischemic heart disease. Two groups independently reported that macrolide antibiotics significantly reduced the secondary cardiac attacks in patients with unstable angina or in male survivors of myocardial infarction, although the number of patients studied in these reports were very small (50,51). Further researches are requested in order to answer the following questions;

i) Can macrolide antibiotics really reduce the incidence of secondary cardiac attacks, even in patients with a low risk of cardiac attacks?

ii) What is the optimal dose and duration of antimicrobial treatment?

iii) What is a reliable marker for selecting patients for antimicrobial treatment and for evaluating the *C. pneumoniae*-specific response to the treatment?

iv) Do macrolide antibiotics prevent secondary cardiac attack by acting against bacteria other than *C. pneumoniae* or by acting mechanisms other than anti-*C. pneumoniae* activity?

Several large-scale studies have recently begun in western countries to answer the question i). In ACES (Azithromycin for Prevention of Cardiac Endpoints Study) sponsored by NIH in USA, 4,000 patients with ischemic heart disease are expected to paticipate in this study. Patients in the treated group will take azithromycin weekly for 1 year and be monitored regarding the incidence of cardiac events for the following 4 years. The results will appear in 2003. If azithromycin can prevent secondary cardiac events, we will obtain another tool for preventing one of the most common causes of human mortality in developed countries.

7. Pathogenesis

There is little data about the pathogenesis of *C. pneumoniae* infection in either respiratory tract infection or in human atherosclerosis. Although the method for establishing clones of *C. pneumoniae* has been reported, we cannot yet produce stable recombinant mutants of *C. pneumoniae*. Chlamydial heat shock protein and antigen-mimicity have recently been targeted (32,52). To confirm the role of these mechanisms in the pathogenesis of *C. pneumoniae*, we need to develop a reliable method for establishing stable recombinant mutants. Further intensive research in this field is necessary.

8. Genome project

Two groups independently finished genome projects of *C. pneumoniae*. One is the US group of University of California, Berkeley, and used the strain CWL-029 isolated from a patient with acute pneumonia in Atlanta (53). The other is the Japanese group of Yamaguchi University School of Medicine and so on, which used the strain J-138 isolated from a patient with pneumonia in Shimonoseki (54). The genome sizes of CWL-029 and J-138 are 1,230,230 bp and 1,226,570 bp, respectively. A comparative study of the two strains revealed that the nucleotide sequence identity was 96-100% and the deduced amino acid sequence identity was 88-100% between the two strains. Generally speaking, the identification of genes of the two strains is highly similar; there were, however, some differences in the pmp, transporter, and glucose metabolism-related genes. These data from the genome projects will contribute to elucidate the pathogenesis of *C. pneumoniae* and provide a possible tool for preventing and treating *C. pneumoniae* infection, such as antimicrobial agents and vaccines. Several research institutes and companies have already started to develop vaccines to *C. pneumoniae* by using the data of the genome projects.

9. Conclusion

Only 10 years have passed since the association between *C. pneumoniae* and atherosclerosis was first reported. Many things remain unclear. The odds ratios between hepatitis B antigen seropositivity and hepatic cancer were more than 100, while the odds ratios between *C. pneumoniae* seropositivity and coronary artery diseases were approximately 2-4. Therefore, it is very difficult to confirm based only on the seroepidemiological data that *C. pneumoniae* plays a causative or accelerating role

in the development of atherosclerosis. We need further information in addition to the detection of this organism in atherosclerotic tissues, animal infection models, and antimicrobial prevention of secondary cardiac events. We can use the whole genome information from two strains isolated in the US and Japan. However, in order to determine the role of *C. pneumoniae* in the pathogenesis of atherosclerosis by using the genome information, we need to develop a method for cloning stable recombinant mutants of *C. pneumoniae*. We need further intensive researches in the future.

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