Clin. Lab. 2022;68:XXX-XXX

REVIEW ARTICLE

Systematic Infection of Chlamydia Pneumoniae

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SUMMARY

Background: ***Chlamydia pneumoniae*** **(Cpn) is one of the most common respiratory pathogens in children and adults. It is characterized as an obligate intracellular parasite. Peripheral blood monocytes (PBMC), lymphocytes, and macrophages are involved in spreading chlamydia infection to extrapulmonary organs indicating that Cpn infection can cause systematic symptoms** ***in vivo*** **via blood transmission.**
Methods: **This review summarizes the mechanisms of Cpn infection in host cells, the immune response of the body, and the relationship between Cpn infection and some chronic diseases.**
Results: **Cpn participation in extrapulmonary chronic diseases has been proven owing to the presence of Cpn DNA in AS plaque, nerve tissues, and synovium tissues of the joints.**
Conclusions: **Cpn infection is related to the development of chronic diseases such as atherosclerosis, Alzheimer's Disease (AD), and reactive arthritis through** ***in vivo*** **and** ***in vitro*** **experiments.**
(Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2021.210908)

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KEY WORDS

systematic infection, *Chlamydia pneumonia*, atherosclerosis, Alzheimer Disease, blood transmission, Immune response, chronic diseases

LIST OF ABBREVIATIONS

Cpn - Chlamydia pneumoniae
AD - Alzheimer's Disease
ATP - adenosine triphosphate
PBMC - peripheral blood monocytes
LPS - lipopolysaccharide
OMP - outer membrane protein
PAMP - pathogen-associated pattern molecule
PRR - pattern recognition receptor
NK - natural killer
IDO - indoleamine 2,3-dioxygenase
TNF-α - tumor necrosis factor-alpha
MS - multiple sclerosis
CSF - cerebrospinal fluid
TTS - type III secretion system

Case Report accepted November 22, 2021

INTRODUCTION

Background: *Chlamydia pneumoniae* (Cpn) is a common pathogen that causes acute and chronic respiratory infectious diseases via droplet transmission in adults and children over 5 years of age, mainly targeting the mucosal epithelial cells *in vivo* [1]. The strain of Cpn (TW-183) was isolated from a chicken embryo in Taiwan in 1965 [2] for the first time. As *adenosine triphosphate* (ATP) cannot synthesize itself, it is most likely that Cpn must be parasitic in living cells. Seroepidemiological studies have shown that the positive rate of Cpn-specific antibodies is above 50% in patients with pneumonia [3]. The Cpn infection rate in children under 5 years of age has also been found to increase [4].

Cpn has been reported to be correlated with the development of atherosclerosis, Alzheimer's disease (AD), adult-onset asthma, reactive airway disease in children, and reactive arthritis [5-10]. The Cpn mechanisms responsible for inducing these chronic diseases have attracted the attention of researchers. Cpn affects the human peripheral blood mononuclear cell (PBMC) and the macrophage circulates to the extrapulmonary organs resulting in hidden infections, which might induce chronic diseases, such as atherosclerosis [11,12]. This review summarizes the mechanisms of Cpn infection in host cells, the immune response of the body, and the relationship between Cpn infection and related chronic diseases.

C. pneumoniae infection and immune response

The two developmental stages of Cpn are protoplasm and primordium. The protoplasm enters the cell through phagocytosis and develops into primordium in the cell vacuoles. The primordial division *in vivo* generates the progeny protoplasms [13]. The mature protoplasts are released from the host cell, which then infect the new host cells to begin a developmental cycle. The protoplasm development is dependent on energy and the growth factors provided by the host cells. In suitable conditions, the cells become dormant or inactive [14-15]. The two types of Cpn antigens are lipopolysaccharide (LPS) and protein antigens. LPS is a genus-specific antigen of Chlamydia, which contains the cross-reactive epitope with other microorganisms [16]. Protein antigen is generally linked to the outer membrane protein (OMP), which is the main component of the Chlamydia outer membrane complex and shows strong immunogenicity. The innate immune system and the specific immune response mediated by the T- and B-lymphocytes would be triggered after Cpn infection.

LPS is a pathogen-associated pattern molecule (PAMP), which can be bounded by the pattern recognition receptor (PRR) of innate immune cells to result in a nonspecific immune response and up-regulated PRR such as CD14, toll-like receptor 2, and toll-like receptor-4 [17-21]. It was observed *in vitro* that the effect of dendritic cells increased gradually with age after Cpn infection [22]. Natural killer (NK) cells could also induce different types of cytokine production after Cpn infection and participate in innate immunity after certain chlamydia infections [23].

Although a specific neutralizing antibody can inhibit Cpn absorption in the host cells, its duration is not long-lasting, and it shows poor immunity. The adaptive cellular immune response mediated by the T-cells plays a more significant role compared to the humoral immunity mediated by B lymphocytes. CD8+ T-cells have a specific memory for Cpn, and they play a major role in specific immunity [24,25]. In the early stages of the infection, CD8+ T-cells were demonstrated to play a protective role by changing the CD4+ T cytokine Th2 to Th1 [24]. Th1 and Th2 cytokines, such as interleukin (IL), interferon-γ (IFN-γ), TNF, and other secreted cytokines, could inhibit Cpn proliferation after infection [7, 26]. CD8+ T-cells were activated and differentiated into cytotoxic T-cells, which could specifically secrete Th1 type cytokines and kill the Cpn infected cells. Inf-γ was the most critical cytokine involved in the removal of Cpn from the infected cells. By inducing the production of the enzyme indoleamine 2,3-dioxygenase (IDO), it consumed the essential amino acid tryptophan and inhibited the replication of Cpn in human cells [27]. However, IFN-γ could also induce Cpn persistent infection [28,29]. Apart from Cpn, the other types of chlamydia exhibit a persistent form of infection in certain conditions. Chlamydia can survive for a long time in the host cell with no infection, which makes it more suitable for evading the host immune response [1]. Persistent infection of chlamydia in humans and rodents could be associated with the poor response of CD8+ T-cells [30,31]. Protective cytokines such as tumor necrosis factor-alpha (TNF-α), IFN-α, and IFN-β might have a delayed response towards clearing Cpn [32]. The condition during persistent infection of Cpn may show complete resistance towards antibiotics, complicating its elimination.

Extrapulmonary infection of *C. pneumoniae*

Cpn infection progresses to extrapulmonary organs after the local infection [33]. Cpn was found in the lung, heart aorta, spleen, and the central nervous system (brain and olfactory bulb) of mice after intranasal inoculation [34,35]. Besides, Cpn was observed to diffuse into the heart (ascending aorta) in a dose-dependent manner [35]. Cpn DNA amplification was detected in the macrophage, lung, spleen, liver, and heart tissues of rats that were injected with the Cpn strain intraperitoneally, and the amplification level appeared to decrease in these tissues (macrophage = lung > spleen > liver > heart). However, Cpn specific DNA sequence was not detected in the tissues of these rats that had been inoculated with vector or *Chlamydia trachomatis* in the control group [36].

The local infected Cpn infiltrated the lung tissue of the host by contacting the immune cells, which is an essential condition for Cpn proliferation. Less inflammation and low mortality were observed in the mast-cell deficient mice, the mechanism may be that mast cells can

regulate cell tight junction openings to promote the contact of immune cells with the outside world. Therefore, mast cell deficiency reduces the chance for Cpn to contact lung immune cells.

Detection of *C. pneumoniae* from clinical samples

Cpn can infect the vascular system after local infection [37]. Cpn was found to be present in the vascular cell walls of the coronary artery, carotid artery, aorta, femoral artery, and popliteal artery [38]. The spreading of Cpn to the extrapulmonary organs may be mediated by PBMC, lymphocytes, and macrophages. The presence of Cpn in PBMCs was confirmed by PCR and microorganism isolation [39-41]. Human PBMCs are affected by Cpn, wherein their functionality can be impaired owing to Cpn infection [11,42-44]. Cpn can survive and reproduce in Mono Mac 6 cells *in vitro* [43]. Cpn can also infect the PBMCs isolated from human peripheral blood, thereby promoting the secretion of TNF-α, interleukin, and the release of chemokines [11]. Therefore, Cpn might immigrate by infecting PBMCs. Studies also indicate that lymphocytes may act as mediators when promoting the spread of Cpn to extrapulmonary organs. Haranaga et al. [45] were the first to confirm that Cpn could survive in human peripheral blood lymphocytes or mouse spleen cells. The complete Cpn developmental cycle was observed in human lymphoid Jurkat cells in the presence of IFN–γ [27,46,47]. Webley et al. [48] discovered chlamydia inclusion bodies in the lymphocytes of some blood donors in 2006 and found that lymphocytes may shelter Cpn to avoid the influence of IFN-γ and promote the continuous infection of Cpn. However, the current evidence is limited, and further studies are required to explore the mechanisms of latent infection of Cpn in lymphocytes. Cpn antigen was found to primarily exist in the macrophages of lymphoid in the alveoli and bronchi [49]. Cpn can spread to other organs through macrophages by blood and lymph transmission [39]. Cpn expression was detected in the alveolar macrophages of the mice that were inoculated with Cpn through the nose and abdominal cavity. The Cpn DNA could be detected in the lungs, thymus, spleen, and abdominal lymph nodes of the mice after the infected alveolar macrophages were transferred to uninfected mice, which indicated that Cpn infection in extrapulmonary organs may be mediated by macrophages through blood and lymph transmission. The mechanism of systematic Cpn infection is shown in Table 1.

***C. pneumoniae* infection and chronic diseases**

Atherosclerosis is one of the leading causes of death in the Western world. Recent studies have shown that Cpn infection may be related to certain chronic diseases, including atherosclerosis, AD, and some chronic respiratory diseases [5].

Several animal models have been applied to study Cpn infection. Kishimoto [50] first established a mouse model to analyze Cpn infection in the early 1990s. Twenty weeks after single and multiple intranasal inoculations, Cpn was detected in the lungs, aortas, and spleen of 25% - 100% of the apolipoprotein E-deficient transgenic mice. Further, it was detected in the aorta of 8% of C57BL/6J mice without an atherosclerotic diet two weeks after a single intranasal injection. The persistent presence of Cpn in the atherosclerotic lesions suggests the tendency of Cpn infection [51]. Little et al. [35] found that the infection spread more quickly in aged mice group compared to that in the immature mice group, when Cpn was administered via the nose to BALB/c mice. In addition, the Cpn titer detected in the central nervous system was also higher in aged mice group, which suggested that Cpn infection could be more severe in aged animals. Herrera et al. [36] determined that Cpn could induce the formation of foam cells in rat macrophages and increase the instability of atherosclerosis plaques, which was consistent in the animal models of rabbits and pigs [52,53]. Cpn remained pathogenic despite controlling the risk factors associated with diet and exercise, indicating that Cpn infection may be an independent risk factor for atherosclerosis [36].

Clinical studies have found the potential correlation between Cpn and atherosclerosis. Fatty striae is an early pathological change observed in atherosclerosis. In a Japanese autopsy study involving children, the detection rate of fatty striae in the infant aorta was as high as 29%, while the detection rate in the coronary artery of children aged between 1 - 9 years was 3.1% [54]. The detection rate of fatty striae in the coronary arteries was found to increase from 50% (2 - 15 years old) to 85% (21 - 39 years old), which is consistent with the results reported by Tanaka K et al. [54,55]. Cpn was found to be related to vascular endothelial function impairment and an increase in carotid and aortic intima-media complex thickness in children [56]. Cpn infection may be involved in the early stages of atherosclerosis; however, there is no evidence that it is an independent risk factor for pathological changes of atherosclerosis in children. The impact of the time point of Cpn infection on atherosclerosis in children has not seen any consistent conclusion. Therefore, further study is required on the effect of Cpn infection in children on the arterial system in adulthood.

Several studies on relevant aspects have been performed in adults. Boman et al. [41] detected Cpn DNA in PBMCs of patients and volunteers suspected of coronary heart disease by nPCR (59% vs. 46%). LE Yazouli et al. [57] detected Cpn DNA and atherosclerotic plaques in the PBMCs of 115 patients with cardiovascular disease through real-time PCR, and the detection rate was identified to be more than 60%. Liu et al. [58] proved that Cpn infection could promote monocyte migration through the endothelial cells and early atherosclerosis by increasing tyrosine phosphorylation of human vascular endothelial cells (VEC) and internalization of VE-cadherin in VEC *in vitro*.

Table 1. Mechanism of Systematic Cpn Infection.

Mechanism	Targets	Action
Induce immune response	Improve Th2 inflammation to release IFN-γ	Induce Cpn persistent infection
	A weak immunity of protective cytokines (TNF-α, IFN-α, and IFN-β)	
Invasion of pathogens	Cpn enters the lung and forms the primary infection foci, infecting immune cells	Local infection
	Cpn spreads to the extrapulmonary organs via immune cells (maybe PBMC, lymphocytes, and macrophages)	Multiple organ infections

Animal studies have found that Cpn can be transported to the nervous system through the blood-brain barrier after Cpn infection in PBMCs [35,51]. Gerard et al. [59] cultured Cpn with metabolic activity and viability in the brain tissue of AD patients in 2006. In AD patients, astrocytes, microglia, and neurons have been found to be the host cells of Cpn. These infected cells may secrete inflammatory cytokines and chemokines resulting in AD neurodegeneration [60,61]. In 2015, a meta-analysis revealed that the risk of subjects being infected with Cpn was more than five times compared to those in the control group, which indicated that there was a strong positive correlation between Cpn and AD [62]. Therefore, Cpn can be considered to be one of the microbial factors in the pathogenesis of AD [63].

Cpn infection plays an important role in the pathogenesis of multiple sclerosis (MS) [64]. Cpn can be detected in the cerebrospinal fluid (CSF) of MS patients. The hypothesis of Cpn infection inducing MS is similar to that of AD, which involves the circulation of Cpn to the extrapulmonary organs by PBMCs. PBMCs with Cpn invade the central nervous system when the blood-brain barrier is damaged due to inflammation, promoting the development of MS by infecting the neural tissue or inducing the production of cross antibodies [65].

Reactive arthritis is acute non-suppurative arthritis, which is secondary to infections in the other parts of the body. Studies have indicated that arthritis is elicited by chlamydiae infecting the synovial tissue in an unusual biologic state-designated persistence [66]. The detection of Cpn in the blood or joint fluid of patients indicated that Cpn might infect the synovium of the joint via blood [67,68]. It was also believed that Cpn is involved in the development of chronic arthritis in children [69]. The role of Cpn infection in the pathogenesis of arthritis in children and adults requires more attention.

Cpn has been considered to be one of the pathogens of endocarditis [70]. Gdoura et al. [71] reported the negative test result of an endocarditis patient with Cpn-pathogen culture but the Cpn-DNA was detected positive by using PCR in the aortic valve and the mitral valve tissues. P. Szabo et al. [72] reported a case of infectious endocarditis caused by Cpn infection after liver transplantation.

Four studies were conducted using PCR and RT-PCR to detect Cpn DNA in healthy adult blood samples that were donated for blood transfusion. The study results demonstrated the positive rates of Cpn DNA to be 7.1% (14/196), 8.9% (21/237), 26.9% (31/115), and 18.5% (13/70), respectively [12,48,73-74]. The positive rate of Cpn was determined to be 1.7% after platelet apheresis, suggesting that the blood products after leukocyte clearance may still have the possibility of transmitting Cpn [48]. The presence of Cpn in healthy blood donors may be a potential risk factor for blood transmission, promoting the development of atherosclerotic lesions by directly or indirectly damaging the vascular system [75]. The mechanism may be the initiation of an immune response or vascular wall colonization [48,55,57]. High-quality large sample size research may further explain the role of Cpn in the development of diseases in patients who have undergone blood transfusion.

Bronchial asthma (referred to as asthma) is a common chronic airway disease in children and adults. Allergen-specific Th cells produce cytokines, which can induce many hallmark features of asthma, including bronchial hyperresponsiveness, neutral and eosinophilic inflammation, and airway remodeling. Cytokine-producing Th subsets produced by cytokines including Th1 (IFN-γ), Th2 (IL-4, IL-5, IL-13), Th9 (IL-9), etc., which are thought to play a role in the development of asthma. At present, a large number of studies have confirmed that Cpn plays a potential role in the induction and aggravation of asthma [7-9], the mechanisms may be that Cpn infection induces Th2 response, such as IL-4 and immunoglobulin E (IgE) response in PBMC of asthmatic patients. IFN-γ has been confirmed to be associated with persistent CP infection [28,29]. Recent studies have also found that Cpn is necessary to induce in vitro response of IFN-γ in PBMC of asthmatic patients. In a pediatric study, the level of IFN-γ in the supernatant of PBMC in the asthma group was significantly higher than that in the non-asthma group, suggesting that Cpn-induced IFN-γ production in vitro is more common in asthma patients than in non-asthma patients [7]. The role of Cpn infection in the pathogenesis of asthma is still not entirely clear, which deserves to be further studied.

Treatment and vaccine

Although antibiotics have been used to treat coronary heart diseases, the results are not optimistic. A high-quality randomized double-blinded study found that there were no differences between antibiotics and placebo treatments in the events of secondary prevention of coronary heart diseases [76]. Administering doxycycline and rifampicin for three months reduced the degree of cognitive deterioration in mild to moderate AD patients; however, the effect cannot be attributed to the antibiotic treatment on Cpn. In a meta-analysis published in 2005, Andraws et al. [77] reported results that the effect of clarithromycin, azithromycin, roxithromycin, or gatifloxacin treatment on the secondary prevention of coronary heart disease revealed that antibiotic treatment did not reduce the incidence of cardiovascular events and all-cause mortality of the patients. Gieffers et al. [78] reported the identification of Cpn growth and the development of inclusion bodies in PBMCs of two healthy adult volunteers before and after administration of azithromycin or rifampicin, which indicated that Cpn was resistant to antibiotics in latent infection status. Further, it also partially explained the reason why antibiotic treatment was not effective in patients with cardiovascular and cerebrovascular diseases.

Cpn infections in children are mostly primary infections and their risks of developing chronic infections are lower compared to that of adults [79,80]. However, the effect of macrolides on Cpn infection was also unsatisfactory [81]. Scientists are researching other ways to control Cpn infection. Cpn includes the type III secretion system (TTS) gene, which transfers bacterial proteins to host cells. TTS secretes the corresponding Cpn effector proteins into the cytoplasm of the host cells. These proteins are significant virulence factors of Cpn, which has an infectious pattern similar to few gram-negative bacilli [44,82]. Further, the TTS inhibitor can inhibit the growth of Cpn and reduce the expression level of several TTS genes in exposed cultures [83]. Therefore, the TTS inhibitor may be beneficial to control the chronic infection of Cpn.

Vaccines for Cpn are also in the process of development. LcrE is the putative "lid" of TTS that targets effector molecules into host cells in a calcium ion-dependent manner. Studies have shown that LcrE could be used to induce the activation of CD4+ and CD8+ T cells, and type 1 cytokine secretion to neutralize the antibody in mice, which was found to be effective for eliminating the Cpn infection. The preliminary study of intraperitoneal inoculation of Cpn in the hamster showed that the LcrE vaccine was effective [84]. This vaccine serves as an effective technique to prevent Cpn infection [85]. The structural proteins RVOM1, RVOM2, and RVEC1 of Cpn may also be involved in inducing B cells and T cells, which are potential candidates for the Cpn vaccine [86]. Chlamydial antigens, presented by MHC class Ib molecules, may serve as novel targets for inclusion in the anti-Cpn vaccines [87].

The specific pathogenesis of chronic Cpn infection is not yet clearly understood and requires further exploration. Results in *in vitro* and animal studies supported the hypothesis of the involvement of Cpn in chronic diseases such as atherosclerosis and AD. Cpn infection in children requires additional attention for analysis.

CONCLUSION

When acting as a pathogen transmitted through the respiratory tract, Cpn can induce chronic infections in the blood cells according to the results of the *in vitro*, animal, and serology experiments. The presence of Cpn in the blood vessel wall, atherosclerosis plaque, nerve tissue, cells in cerebrospinal fluid, synovium tissues of the joint, and heart valve membrane indicated that Cpn may be related to some extrapulmonary chronic diseases. Cpn infection may be one of the risk factors for atherosclerosis and Alzheimer's disease in adulthood. However, the role of Cpn infection in the pathogenesis of adult chronic diseases remains unclear. Some disease models can further help in exploring the mechanisms of Cpn pathogenesis when promoting chronic disease development, aiming at guiding appropriate interventions. In the case that current antibiotic treatment may be ineffective or even induce Cpn persistence, a possible treatment method is to manipulate an immune response that can eliminate intracellular Cpn infection. Cpn vaccines are also under development, and the feasibility and efficacy need further investigation [86-89]. Negative events in childhood may influence cardiovascular risk in adulthood. At present, there are relatively few studies on Cpn in children. More attention should be paid to Cpn infection in children, and more basic and clinical studies are needed.

Acknowledgment:

Not applicable.

Declaration of Interest:

The authors declare no conflict of interest and no funding.

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