Microbiological physiology, structure, diagnosis and future perspectives of *Chlamydia pneumoniae* infection

Ariati Safiriani

Abstrak

Chlamydia pneumoniae (C. pneumoniae) adalah suatu patogen intraselular obligat yang baru diidentifikasi pada tahun 1989. Bakteri ini digolongkan sebagai spesies baru dari Chlamydia, berdasarkan definisi ultrastruktur dan analisis homologi asam deoksi ribonukleat (DNA). C. pneumoniae memiliki siklus hidup bifasik yang unik, dengan dua bentuk yang berbeda, yaitu badan elementer dan badan retikulat. Patogen ini dapat mensintesis DNA, RNA dan proteinnya sendiri; namun tidak memiliki jalur metabolik untuk mensintesis ATP. Penggolongan C. pneumoniae diantara patogen yang "baru muncul", mungkin disebabkan oleh karena diagnosisnya yang cukup sulit. Diagnosis laboratorium berdasarkan pada kultur, identifikasi spesimen biologis menggunakan antibodi monoklonal, "polymerase chain reaction" (PCR), dan metoda-metoda serologis lainnya; tidak cukup banyak tersedia. Beberapa spesies Chlamydia yang berbeda juga memiliki persamaan antigenik, sehingga dapat menyebabkan spesifisitas yang rendah. Berbagai tes diagnostik mutakhir, dengan beberapa kelebihan dan kekurangannya akan dibahas lebih terinci pada makalah ini. Perspektif masa yang akan datang pada metoda diagnostik; seperti standarisasi PCR, kloning dan sekuensing gen omp-4 dan omp-5, serta penemuan vaksin; sedang dalam proses pengembangan lebih lanjut.

Abstract

Chlamydia pneumoniae (C. pneumoniae) is a unique obligate intracellular pathogen which has been recently identified in 1989. The pathogen was classified as a new species of Chlamydia, based on its ultrastructural definition and deoxyribonucleic acid (DNA) homology analysis. It has a distinctive biphasic life-cycle, with two different forms, the Elementary Body and the Reticulate Body. The pathogen could synthesize its own DNA, ribonucleic acid (RNA) and protein, but it lacks the metabolic pathway of producing adenosine triphosphate (ATP). The classification of C. pneumoniae among "new and emerging" pathogens is probably due to the rather difficult laboratory diagnosis. Laboratory diagnosis based on the culture, identification of biological specimens using monoclonal antibodies, polymerase chain reaction (PCR) and serological methods, are not widely available. Different Chlamydial species, which are very similar genetically, could also cause low specificity in the diagnostic tests. Several advantages and disadvantages of the recently developed diagnostics test are explicated. Some future perspectives in the diagnosis methods, such as the standardization of the PCR assays, the cloning and sequencing of the omp-4 and omp-5 genes, and vaccine development, are currently in progress.

Keywords: *C. pneumoniae, ultrastructure, biphasic life-cycle, laboratory diagnosis*

Chlamydia pneumoniae (C. pneumoniae), which was first described in 1986, is an obligate intracellular pathogen and a common cause of respiratory infection. Many reports have indicated the importance and the relevance of *C. pneumoniae* not only in respiratory tract infection, but also in extrapulmonary diseases.[1,2,3,4,5] Antibody prevalence rates in Western countries reaches 50% in the adult population and remain high in old age, suggesting a high rate of reinfection. Diagnosis is hampered by the requirement for specialized culture techniques and reliance upon expensive serological tests or Polymerase Chain Reaction (PCR). The wealth of knowledge concerning this pathogen has increased dramatically in the last decade, setting up the way to further exciting research lines.

Respiratory Department Hoechst Marion Roussel Indonesia, Jakarta, Indonesia

History of a new pneumonia agent

There are three species of *Chlamydia: C. pneumoniae, C. trachomatis* and *C. psittaci*. The recognition of *C. pneumoniae* as an individual species within the *Chlamydia* genus is relatively recent, but clues in literature for the existence of this agent, date back to at least 50 years ago.[6] In 1943, Smadel *et al.*, described cases of pneumonia with a positive complement fixation test for psittacosis, but without

history of bird contact.[7] In 1965, during a trachoma vaccine trial in Taiwan, an atypical strain of *Chlamydia* was obtained from the conjunctiva of a school child, and identified on the yolk sac chick embryo egg number 183. The strain was named TW-183. A further step was made in 1983, when another isolate antigenically similar to TW-183 was obtained from the pharyngeal swab of a university student in Seattle, suffering from pharyngitis. The strain was named AR-39 (Acute Respiratory).[6]

Between 1985 and 1986, two studies were published on the clinical relevance of respiratory infections sustained by unusual strain of *C. psittaci*.[8,9] Saikku in 1985, reported an epidemic of mild pneumonia discovered during a chest radiographic survey of young adult in Finland. The following year, Grayston described an isolation of a *C. psittaci* strain within a population of university students with respiratory tract infections. Following ultrastructural definition and DNA homology analysis, in 1989, a new classification of a third species of *Chlamydia*, was recognized.[10]

Within the three *Chlamydia* species, there are some different characteristics, such as the host range of each species, the morphology of the Elementary Body (EB) and the inclusion body, their susceptibility to sulfonamide, the DNA homology and the plasmid DNA (table 1).

Microbiological physiology and structure

The modality of replication is a characteristic of the genus of *Chlamydia*, and the three species show the same complex cycle. *Chlamydia* has a biphasic life-cycle. The Elementary Body (EB) is the smaller (300-400 nm), extracellular, infectious form and the Reticulate Body (RB) is the intracellular, non-infectious, metabolically active form. The RB is a bigger form (800-1000 nm) and it is capable of binary division. The Intermediate Body (IB) is an intermediate form between the RB and the EB. It has a condensed DNA, hence it is also called the Condensing Form (Figure 1).[11]

Table 1. Differentiation of *Chlamydia spp*[11]

	C. trachomatis	*C. psittaci*	*C. pneumoniae*
Host	Human, mice	Bird, human, lower mammal	Human
EB morphology	Round	Round	Pear-shaped
Inclusion morphology	Round, vacuolar	Variable, dense	Round, dense
Glycogen in inclusion	Yes	No	No
Susceptibility to sulfonamide	Yes	No	No
DNA homology	10%	10%	10%
Plasmid DNA	Yes	Yes	No

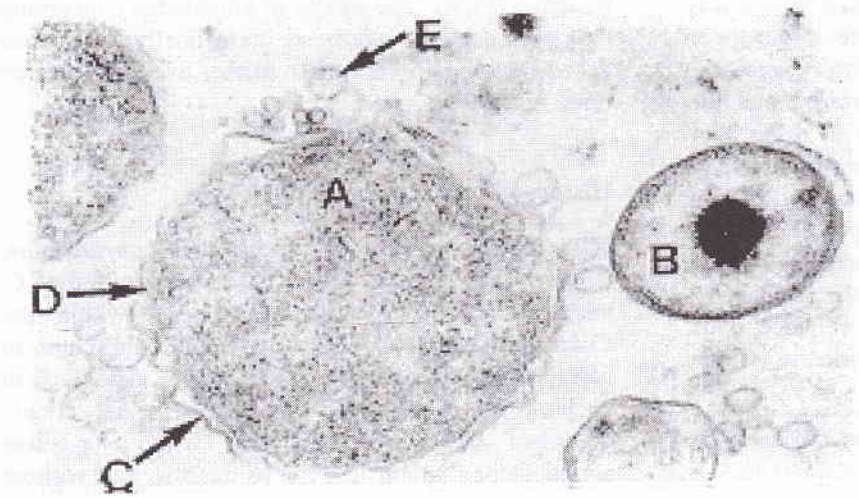

Figure 1. Transmission electron micrograph of Chlamydia.[11]

A transmission electron micro-graph of Chlamydia, showing a Reticulate Body (A) and an Intermediate Body (B), which has nearly completed its transition to an Elementary Body. Note the electron-dense nucleic acid core in the condensing form and the separation between the outer membrane (C) and inner membrane (D) of the Reticulate Body; the smaller form (E) are membrane blebs.

Chlamydia is a nonmotile and nonpiliated micro-organism. It has surface projections which allow nutrient uptake from the host cytoplasm. *In vivo*, the infectious EB attaches to microvilli of susceptible host cells and actively penetrates the host cells. EB then reorganizes into metabolically active, dividing RBs which are able to synthesize DNA, RNA and protein, but could not produce ATP. The reason why *Chlamydia* is called as an energy parasites is, it lacks the metabolic pathway of producing ATP, and therefore it is dependent on the host cell for its ATP.[12] Ninety two hours after infection, the host cells release the EB which could further infect other host cells.[13]

Chlamydia pneumoniae infection

C. pneumoniae infection is usually asymptomatic or minimally symptomatic. *C. pneumoniae* commonly causes upper (pharyngitis, sinusitis, and otitis) and lower respiratory tract infection (pneumonia, acute bronchitis, exacerbations of chronic obstructive pulmonary disease /COPD). *C. pneumoniae* is also linked to some other chronic diseases, such as atherosclerosis, coronary heart disease, arthritis, Guillain-Barre syndrome, and erythema nodosum.[5,14,15,16]

C. pneumoniae could be found worldwide, and it is more common in tropical countries. The sero-prevalence peak is in adults over 70 years. Male is more commonly infected than female. There is no seasonal periodicity for *C. pneumoniae* infection. Its infection seems to be both endemic and epidemic. Epidemic infection seems to occur in cycles with a periodicity of 3 to 4 years, and they generally last 6 to 8 months.[17]

Laboratory Diagnosis of *C. pneumoniae*

The first isolate of *C. pneumoniae* was obtained from egg yolk sac culture of the conjunctival swab of a Taiwanese child.[3] However, the egg yolk sac culture method showed a low sensitivity for *C. pneumoniae* isolation. Several cell lines have been used, such as the HeLa 229 and McCoy cells, and showed better sensitivity than the egg yolk sac.[18] Other cell lines, the human respiratory cell lines, HL, Hep-2 and H-292 cells present a high sensitivity for *C. pneumoniae* isolation and propagation. The culture could be confirmed by fluorescent-antibody staining with species-specific monoclonal anti-body.[19] Specimen for *C. pneumoniae* culture can be obtained from many sources, including pharyngeal swab, sputum, bronchial aspirate or bronchoalveolar lavage and pleural fluid. The handling and storage of specimen, require particular care due to the thermolability of the organism. For transportation, specimen should be placed in appropriate transport media, stored at 4 ^{0} C within 24 hours or frozen at -70 ^{0} C.

Direct antigen detection using monoclonal antibody immunofluorescence test has also been developed to detect *C. pneumoniae* from the specimen. Pharyngeal swab, as well as gargle specimen, has been used as respiratory specimen.[20] In acute respiratory infection, sensitivity of the test is around 20% with relatively high specificity. Non-specific background staining of the mucous and other secretion may cause false positive results.[19] Recent reports indicate the possible use of PCR in the diagnosis of *C. pneumoniae* infection. PCR method is now more commonly used in research.[19, 21]

Many serologic tests measuring specific antibody titer have been developed. The first serological test developed for diagnosis of *chlamydial* infection was complement fixation (CF) test, based on the detection of the lipopolysaccharide antigen. Nevertheless, CF test are not really species-specific. The test can not distinguish different antibodies of the three *Chlamydial* species.[22] The microimmuno-fluorescence test (MIF) has become the serological "gold standard" for *C. pneumoniae* infections. This test is highly specific and sensitive when compared with culture. It allows the determination of specific immunoglobulin G, M and A (IgG, IgM, and IgA) serum fractions.[19] The antibody pattern in response to the primary and secondary infection is shown in Figure 2 and 3.[19]

Grayston *et al.* have proposed a criteria for serological diagnosis of *C. pneumoniae* infection that have been used by many clinicians (Table 2).[23] For acute infection, the patient should have a four-fold increase in the IgG titer, a single IgM titer $\geq$ 1:16, a single IgG titer / 1:512 or a single IgA titer $\geq$ 1:256. Past or pre-existing infection is defined as an IgG $\geq$ 1:16 and $\leq$ 1: 512; or an IgA $\geq$ 1:16 and $\leq$ 1:256. Routine absorption of IgG is recommended before IgM testing, to prevent false positive results due to rheumatoid factor, particularly in older patients.[24]

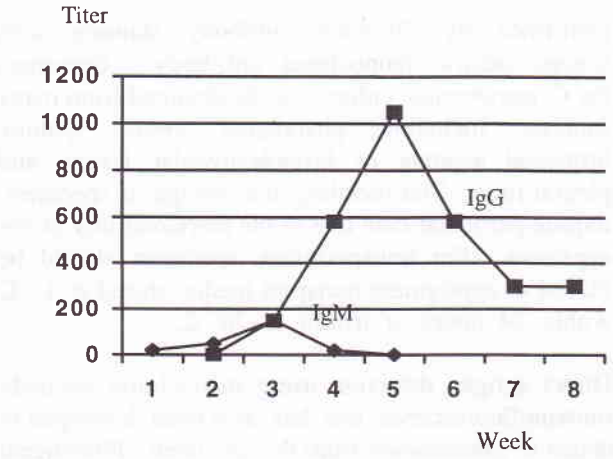

Figure 2. Antibody response to C. pneumoniae primary infection.[19]

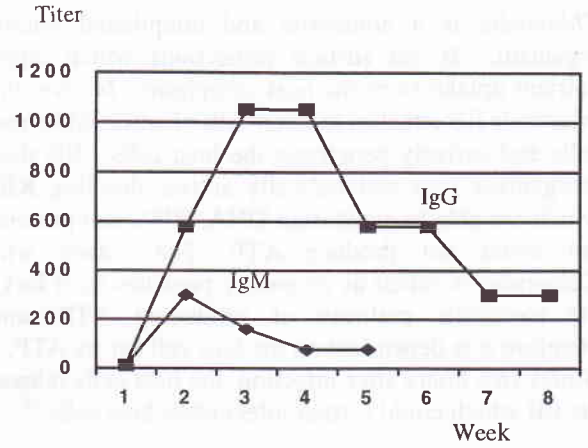

Figure 3. Antibody response to C. pneumoniae secondary infection IgG.[19]

Table 2. Serological tests (MIF and CF) for detection of *C. pneumoniae* infection[23]

	MIF	CF
Acute infection	4x rise IgG/ IgA	4x titer rise
	IgM ≥ 1:16	Titer > 1:64
	IgG ≥ 1:512	Non-specific
	IgA ≥ 1:256	Positive in < 1/3 of infected subjects
Past/chronic infection	IgA ≥1:16 ≤1:256	Not available
	IgG ≥1:16 ≤1:512	

There are still many problems in the diagnosis of *C.pneumoniae* infection. The infection that would produce adequate antibody response, is only the severe, deep and localized one. Diagnostic tools are not widely available; and different *Chlamydial* species, which are very similar genetically, cause low specificity in the diagnostic tests. Furthermore, the presence of lipopolysaccharida and the detection of DNA by PCR, could not differentiate between acute, persistence or chronic active infection. The development of a rapid and specific method for identification of *C. pneumoniae* will provide the means for an accurate diagnosis and an appropriate therapy.

C. pneumoniae treatment

C. pneumoniae is an obligate intracellular parasite. To inhibit growth, antimicrobial agents need to penetrate the cells and interfere with protein synthesis of the microorganism. An antibiotic that would be effective for *chlamydial* infection must have a good intracellular, tissue or secretion penetration, and low minimal inhibitory concen-tration (MIC). The active antibiotic classes for *Chlamydia* infection are macrolides (erythromycin, roxithromycin, clarithromycin and azithromycin), tetracyclines, quinolones (levofloxacin, sparfloxacin), and chloramphenicol (used mainly for *Salmonella typhi* treatment). Treatment with penicillin (β-lactam), amynoglycosides and cephalosporin are not adequate for the infection; because those antibiotics do not enter the host cells adequately, and their target is the bacterial cell wall (a structure lacking in *Chlamydiae*).[25]

Roxithromycin is a newer macrolide generation which has been developed by its prototype, erythromycin. The addition of the ether oxime ring gives some advantages to roxithromycin. Its gastric stability

bioavailability, and spectrum of activity increases. Roxithromycin has a broader antibacterial spectrum which covers both typical and atypical pathogens like *C. pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella spp.* The antibiotic has a high tissue penetration and also a high intracellular concentration, which is important to eradicate the intracellular pathogens.[26] Treatment studies done by many researchers showed good efficacy.[27,28,29]

Future perspectives

Future development in diagnosis, especially the finding of better diagnostic markers for chronic infection, is essential. Even though the development of the PCR test has made a better detection of the organism in the specimen, it does not always correlate with the culture and serological diagnosis. Although PCR assays have been used for different target genes, but neither the DNA extraction method nor the assay are standardized. A commercially standardized available DNA amplification test is being developed and urgently needed. The omp-4 and omp-5 genes, which code for surface exposed immunogenic 96-98 kDa proteins, are specific for *C. pneumoniae*. The cloning and sequencing of those genes, may lead to new insight for the development of the new diagnostic tests.[30]

A possibility of a vaccine development could be the ideal answer to the *C. pneumoniae* infection. A major outer membrane protein (MOMP) of *Chlamydia* has been studied for developing vaccines. The MOMP is found on the outer membrane of both EB and RB, and induces neutralizing antibody. The MOMP seems to be partly immunogenic and no specific differences in the amino acid sequences between different *C. pneumoniae* isolates have been found. However, despite years of effort, an effective vaccine for *Chlamydia* has not existed.[31]

CONCLUSION

Currently, there are no highly specific and sensitive diagnostic tools for *C. pneumoniae* infection that are widely available. In addition, there is no standard consensus on serological criteria for defining the *C. pneumoniae* infection. The development of diagnosis and treatment will lead to more accurate use of antimicrobial agents. Further studies in the development of diagnosis, treatment and prevention of *C. pneumoniae* infection, are therefore, urgently needed.

Acknowledgements

The author would like to thank Galatia Chandra and George Kiongdo, MD (Hoechst Marion Roussel Indonesia) and Septelia Inawati Wanandi, MD, PhD (Biochemistry Department, Faculty of Medicine, University of Indonesia) for their support during the preparation of the manuscript.

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