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Chlamydiae as Pathogens: Newer Perspectives

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ABSTRACT

Chlamydiae are a group of intracellular organisms which have been associated with wide spectrum of clinical infections such as trachoma, pelvic inflammatory disease, infertility, psittacosis, respiratory infections, atherosclerosis, asthma and arthritis. In this review, the newer perspectives regarding the microbiology of the Chlamydiae and their association with various clinical conditions are reviewed.

Key words: Chlamydiae, microbiology, pathogenesis

INTRODUCTION

Chlamydiae are a group of intracellular organisms which have been associated with a wide spectrum of clinical infection ranging from trachoma, pelvic inflammatory disease, infertility, psittacosis, respiratory infections, atherosclerosis, asthma and arthritis [Table 1] (1). In addition to the three recognised species *Chlamydia trachomatis*, *Chlamydia penumomiae* and *Chlamydial psittaci*, a fourth species *Chlamydia pecorum*, a pathogen of ruminants has recently been proposed (2). Species were grouped according to their biologic and biochemical properties and a greater than 95% homology is their 16S ribosomal RNA sequence (3). *C. pneumoniae* is a human pathogen recognised as an important cause of respiratory illness. About 40%-60% of adult population have antibodies to *C. pneumoniae* world over, suggesting that the infection is prevalent and re-infection common. Current interest centers on the emerging role of *C. pneumoniae* infection in the pathogenesis of atherosclerosis and asthma (4). This article reviews the newer perspectives regarding the microbiology of Chlamydia and its association with various clinical conditions.

BIOLOGY OF CHLAMYDIAE: AN UPDATE

Increased awareness of the clinical significance of human Chlamydial infection in all parts of the world has been paralleled by interesting basic observations on the biology of these organism. Chlamydia have a unique biphasic life cycle with dimorphic forms that are functionally and morphologi-

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Table 1. *Spectrum of human diseases caused by Chlamydiae*

Species	Acute Diseases	Sequelae/Chronic Diseases
C. trachomatis		
Serovars A-C	conjunctivitis	trachoma
Serovars D-K	urethritis	proctitis, epididymoorchitis, Reiter's syndrome pelvic inflammatory disease, ectopic pregnancy, tubal infertility, Fitz-Hugh-Curtis syndrome
	ophthalmia neonatorum	
	neonatal pneumonia	
LGV serovars	lymphogranuloma venereum	
C. pneumoniae	pharyngitis	Cardiovascular disease
	sinusitis	pasthma
	bronchitis	
	community acquired pneumonia	
C. psittaci		
parrot	atypical pneumonia	
canaries	hepatic and renal dysfunction	
pigeons		
turkeys	endocardits	
ducks		
chickens		
cats	conjunctivitis	
ewes	abortion	

cally distinct. Life cycle is initiated when an infectious but metabolically inactive elementary body [EB] attaches to the host epithelial cell surface. The precise mechanism by which EB's attach and gain entry into the host cells is unknown (5). Recent work suggest that Chlamydia uses heparan sulfate as a bridge to attach to glycosaminoglycan [GAG] receptors on eukaryotic cell surfaces (6). Once endocytosed, the EB differentiates into a larger pleomorphic form called the reticulate body [RB], which replicates by binary fission (2). The intracellular regulatory signal that control EB to RB conversion and vice-versa are not known but the relative concentration of cAMP and cGMP appears to be important (7). Once inside the host cell, Chlamydia resides in a membrane bound vacuole that can evade phagolysosomal fusion. The endosome is transported to the distal regions of Golgi apparatus and incorporates host derived sphingolipids into the inclusion membrane (8,9). Thus it appears that Chlamydia are able to intercept host vesicular traffic bound for the plasma membrane to sequester lipids and possibly other host substances synthesized in the Golgi apparatus. Subversion of host vesicular traffic may represent a dual advantage for Chlamydia in obtaining materials form the host for its metabolism as

well as in modifying the inclusion membrane to evade lysosomal fusion and immune detection. Chlamydia lack the ability to synthesise high energy compounds such as ATP and GTP essential for replication and metabolism leading Moulder in 1974 to coin the term 'energy parasites'. Chlamydia are incapable of de-novo nucleotide biosynthesis and are dependent on host nucleotide pools (10).

ANTIGENEIC RELATIONSHIP

Chlamydia are antigenically complex organisms possessing antigens of genus, species and serotype specificity (11). The group complements fixation [CF] antigen, shared by all the members is the lipopolysaccharide [LPS], located on the outer membrane of both EB and RB (12). The major outer membrane protein [MOMP] contains both species and serotype specific antigens (13). The 15 serovars of *C. trachomatis* are best reognised by micro-immunofluorescence [micro IF] technique. MOMP is responsible for most of the reactivity seen in micro IF test. A 60 kilodalton [kDa] Cysteine rich structural protein [CHSP 60] has a highly immunogenic species - specific epitope (1). A genus specific 57 kDa protein plays an important role in immunopathology (14). Serovars of *C. psittaci* can be demonstrated by neutralisation test and by micro IF (15). Only one serovar of *C. pneumoniae* has been demonstrated.

CLINICAL SPECTRUM OF HUMAN INFECTION

C. trachomatis is almost exclusively a human pathogen known to cause trachoma, conjunctivitis, urethritis - cervicitis, proctitis, epididymoorchitis. Reiter's Syndrome, pelvic inflammatory disease, ectopic pregnancy, tubal infertility, Fitz-Hugh Curtis Syndrome (1). For Chalmydial infections, recent advances in diagnosis and screening technology and single dose antimicrobial therapy will likely have a significant impact on the efficacy of disease control programs and the opportunity for eventual disease eradication.

Human psittacosis is a zoonosis caused by exposure to infected birds or poultry and manifests as a flu-like illness or atypical pneumonia in more severe cases. Infection is characterised by multiorgan involvement often resulting in hepatic and renal dysfunction and endocarditis (16).

C. pneumoniae is a common cause of acute repiratory tract infection, accounts for 6%-10% of community acquired pneumonia (4). Infection is usually mild or asymptomatic but can be severe, especially in the elderly, probably as a result of underlying illness, impaired mucociliary clearance and immune status (17). Seroepidemiologic studies show that most primary infections occur during school age and early teenage years, among adult seroprevalence is 40%-70%. Reinfection are common, and serum antibodies do not appear to be protective.

The association of *C. pneumoniae* infection with coronary heart disease and acute myocardial infarction has been studied by many workers (18-20). The association of *C. pneumoniae* infection with coronary heart disease and acute myocardial infarction was first made on the basis of elevated IgG and IgA antibodies and LPS containing immune complexes in 50% to 60% in patients with coronary heart disease or acute myocardial

infarction compared with 7%-12% in the controls (18). Electron microscopy, polymerase chain reaction [PCR] and immunochemical evidence of *C. pneumoniae* in coronary arterial fatty streaks and atheromatous plaques have also been described (18,19).

The sustained IgA and IgG antibody levels against *C. pneumoniae* in persons with atherosclerosis suggest that chronic infection may be frequent after infection. The site of colonisation for a chronic *C. pneumoniae* infection may be in the alveolar macrophage of the lung. Thus the initial event in atherogenesis may be the formation of the fatty streak. Fatty streaks consist of lipid laden macrophages derived from blood monocytes and T-lymphocytes attracted to the arterial subintima. Conversion of the fatty streak to atheroma depends on many factors, e.g., the proliferation and differentiation of smooth muscle cells and fibroblasts. Chronic infection with *C. pneumoniae* may result form organisms harboured in macrophages trapped in the arterial wall. Growth of *C. pneumoniae* in endothelial, smooth muscle cells, and macrophages from peripheral blood monocytes has been reported (21). The idea that an infectious agent is involved in the atherogenic process is not new, but the role of *C. pneumoniae* in this process needs to be defined.

The first observations on the association of *C. pneumoniae* infection with the exacerbation of asthma were made in 1986 when wheezing was associated with acute bronchitis due to *C. pneumoniae* infection (22). Subsequent studies showed that exacerbation of asthma due to *C. pneumoniae* infection may occur in 1%-11% of respiratory infections in adults as well as children. The mechanism underlying the association is unclear. Preliminary results in animal models suggest that *C. pnemoniae* can produce persistent infection and cause pulmonary inflammation, and production of Chlamydia-specific IgE antibodies in children with reactive airway disease (23). Activated T-lymphocytes and cytokines appear to play a critical role as mediators of persistent inflammation in asthma. Interleukin-4 [IL-4] is essential for B-lymphocyte class switching form IgG to IgE. The role of persistent infection in the pathogenesis of asthma merits further study because unlike viral infections, *C. pneumoniae* infections can be eradicated through appropriate antimicrobial therapy (1). Immunopathology may also be the result of a hit-and-run mechanism in which immune response to CHSP60 breaks self-tolerance to human HSP 60 and leads to an autoimmune reaction that results in tissue damage which needs further exploration. Clinical manifestation associated with *C. pneumoniae* infection continue to emerge. Possible links to chronic conditions, such as atherosclerosis and asthma remain to be elucidated and with the recent discovery of the involvement of infectious agents in other chronic conditions, it seems reasonable to apply molecular tools for Chlamydial detection to identify their potential involvement in other aetiologically undefined chronic inflammatory conditions such as inflammatory bowel disease and rheumatoid arthritis (1).

LABORATORY DIAGNOSIS

Since curative antibiotic therapy for Chlamydial infections is readily available,

early diagnosis is an essential component to control these infections. The goals of early identification are to interrupt the chain of transmission in the community and prevent long-term sequelae (1). The earliest method of isolation in cell culture and embryonated hens eggs has been replaced by antigen detection methods, such as enzyme immunoassay [EIA] and direct fluorescence assay [DFA]. EIA's are suitable for public health laboratories serving large geographic areas because of simplcity in technique. However, EIA lacks the sensitivity as a screening assay because of lower detection limit of 10,000 EB's (24). Monoclonal antibodies specific for *C. pneumoniae* are now commercially available for DGA and culture confirmation (25). Nucleic acid amplification test based on PCR. Ligase chain reaction [LCR] and transcription mediated amplification technology are now commercially available. However, PCR assay have detection limit of 10-100 EBs (26,27).

The micro-IF assay the standard method used for Chlamydial serology was first developed to serotype strains of *C. trachomatis* but soon adapted for detection of antichlamydial antibody which detects type specific antibodies against individual chlamydial serotypes (28). Indian study by Rai and Mahajan (29) showed a strong correlation between micro-IF and ELISA with sensitivity of 97% and 91% respectively. However ELISA has an advantage in being simple to perform and interpret. Radioimmunoassay [RIA] has a very high sensitivity but its routine diagnotic value needs to be evaluated (30). Other tests such indirect haemagglutination [IHA] (31), neutralisation test (32) and immunoelectophoresis (33) have been used. However their sensitivity and specificity are too low and are thus unsuitable for routine diagnostic use. Availability of monoclonal antibodies and EIA for antigen and antibody detection has contributed in early and specific diagnosis of these infections.

TREATMENT

Although doxycycline and erythromycin are effective drugs used in Chlamydial infections the newer macrolides azithromycin and clarithromycin has become the drug of choice. Compared with conventional therapy, azithromycin has excellent pharmacokinetic characteristics, such as increased bio-availability, lower incidence of gastrointestinal tract side effect and increased concentration in mucus, macrophages and tissues with a half life of five to seven days (1). Although the higher cost of azithromycin may be a limiting factor the single dose regimen make it more acceptable to patients (34). Studies are needed to determine if these regimens achieved clinical and microbiological cure.

CHLAMYDIA: INDIAN SCENARIO

Indian scenario regarding the role of Chlamydia in various clinical condition is ambiguous because of limited availability of literature. Bhujwala et al (35) carried out a study on 65 patients of pelvic inflammatory disease and infertility to detect presence of antibodies against *C. trachomatis* and *N. gonorrhoea* using indirect immunoperoxidase test and ELISA. Chlamydial antibody was detected in 62.9% of cases of PID and 60% of cases infertility respectively (35-37).

In another study carried out in cases of ALRTI in children <5 years in India

Chlamydia sp was detected in 11% of cases in association with Mycoplasma or bacteria however it was a sole pathogen in 2.9% cases (38). *C. pneumoniae* infection was seen in 6.4% of cases in the first 2 years of life (39) which is contrary to the observations from previous studies, where it was observed that *C. pneumoniae* infection is uncommon in children under 5 years of age (40). A single IgG antibody titer of >1:512 is considered as diagnostic for acute *C. pneumoniae* inections. Using this criteria more number of cases were detected due to *C. pneumoniae* (38,40).

With the recent availbility of effective single dose oral antimicrobial therapy and sensitive molecular amplification tests that allow the use of non-invasive specimens for diagnosis and screening, it is expected to have a major impact in reducing the prevalence of disease in the next decade (1).

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