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# The many faces of chlamydiae

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***Abstract***

The application of modem research tools has broadened our understanding of the chlamydiae and their role in disease. Chlamydial genome analysis showed the presence of genes for ATP and peptidoglycan synthesis, contradicting the common belief that chlamydiae lack the ability to produce these compounds. Phylogenetic tree analysis suggests that chlamydiae could have evolved from an intracellular existence in amoebae. Newly discovered obligate intracellular organisms with chlamydia-like life-cycles have been classified as chlamydiae by rRNA homology with existing chlamydial species. A proposed new classification adds three new families to the order ***Chlamydiales*** as well as creates two genera and nine species within the family ***Chlamydiaceae.***

Chlamydiae are incriminated in an increasingly large spectrum of diseases both in humans and in animals. The emergence of multi-drug resistant ***C. trachomatis*** strains forewarns therapeutic problems with this organism. While C. ***pneumoniae*** remains a significant respiratory pathogen, the role it plays in the pathogenesis of atherosclerosis and ischaemic heart disease awaits definition.

***Key words:*** chlamydiae, molecular classification, disease spectrum

## INTRODUCTION

The chlamydiae are intriquing organisms. Although discovered almost a century ago, their biodiversity and multifarious pathogenicity are still unfolding. The advent of technically advanced research tools has enabled sophisticated studies on the biology and genetics of these organisms and their association with a wide spectrum of disease. A vast body of new information has been gathered in the past decade. This review briefly reports on current knowledge and recent developments in chlarnydiology.

## MOLECULAR BIOLOGY AND CLASSIFICATION

The chlamydiae were first described by Halberstaedter and von Prowazek as intra-cytolasmic inclusions in giemsa-stained conjunctival smears from patients with trachoma.[1] They were first thought to be protozoans and then viruses[2] because of their small size and obligate intracellular existence. Subsequently, they were recognized to be bacteria[3] possessing both DNA and RNA, a complex cell wall and metabolic enzymes. Unlike other bacteria, however, the chlamydiae have a unique, biphasic life-cycle in which the organism alternates as an extracellular, infectious form called the elementary body (EB) and an intracellular, metabolically-active, non-infectious form called the reticulate body (RB). Following attachment to glycosaminoglycan receptors on the host cell surface, the EB is taken in by endocytosis to lie within a vacuole bound by a membrane that can resist phagolysosomal fusion. In this vacuole known as an inclusion, the EB differentiates into the RB that replicates by binary fission. At the end of the developmental cycle, the RBs are reorganized back into EBs before they are released through host cell rupture or fusion of the inclusion membrane with the host plasma membrane, to start fresh cycles of infection."

### *Genome analysis*

The molecular determinants of this complex biology are being studied by the analysis of gene sequencing data. Under the Chlamydia Genome Project funded by the National Institutes of Health, USA, the entire genome of one species, ***Chlamydia trachomatis,*** has been sequenced.[5] This is a circular DNA of 346 mm length, 660 Mdal molecular weight[6] and roughly one million base-pairs encoding several hundred proteins. Sequence data suggest that, like other bacteria, the chlamydiae have their own genetic code for DNA replication, repair, transcription, translation and aerobic respiration. In addition, genes have been identified that probably govern key functions like the initiation of the different stages of the

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developmental cycle, nutrient acquisition from the host, transmission of regulatory signals to the host, virulence and the production of chlamydia-specific outer membrane proteins. On the other hand, several genes thought to be universally present in bacteria are not found in chlamydiae, including those considered absolutely necessary for prokaryotic cell division. Also absent are many genes for the production of important metabolic enzymes and the synthesis of purine and pyrimidine nucleotides. This lack confirms the dependence of chlamydiae on host-derived metabolites and is likely to be the result of the adaptation of the bacteria to an intracellular existence in metabolite-rich host cells. Chlamydial cell division mechanisms are also likely to be different from those in other prokaryotes.

A surprising finding from the sequence data analysis is the presence of genes potentially involved in de novo adenosine triphosphate (ATP) synthesis.[5] It has always been thought that chlamydiae have to acquire ATP from host cells because they lack the ability to synthesize their own. It now appears that chlamydiae are genetically equipped to generate ATP on their own, but it remains to be seen how much and under what circumstances this high-energy compound is produced.

Another unexpected feature is the presence of a full complement of genes for the synthesis of peptidoglycan. By biochemical analysis, the chlamydial cell wall contains less than 0.04% muramic acid[7] and this finding has been taken to imply that chlamydiae lack peptidoglycan that is the backbone of the eubacterial cell wall. The stability of the chlamydial cell wall is believed to be maintained by a disulfide cross-linked protein complex that is unique to the chlamydiae." However, it has also been observed that the production of EBs can be blocked by drugs that inhibit peptidoglycan synthesis, like D-cycloserine,[9] bacitracin and penicillin.[10] In addition, chlamydiae produce three penicillin-binding proteins (PBPs) that are associated with peptidoglycan production." With the detection of peptidoglycan synthesis genes, these paradoxical observations may now have a molecular basis. It is likely that chlamydiae do produce some peptidoglycan or structures very similar to it.

The most interesting finding from the Chlamydial Genome Project comes from phylogenetic tree analysis which suggests horizontal gene transfer to chlamydiae from bacterial ancestors as well as from eukaryotic hosts.[5] There are twenty eukaryotic genes in chlamydiae compared to three to four in other bacteria. Some of these "eukaryotic" genes are plant-like and could have been acquired from amoebae as chlamydia-like organisms have been isolated from *Acanthamoebae*.[12,13] Hence, it is postulated that chlamydiae were parasites or symbionts in plant-like single-cell amoebae before they evolved to be intracellular parasites of vertebrate hosts.[5]

### *Proposed new classification*

DNA sequence information has been effectively applied to the classification of chlamydiae. Until 1998, the order Chlamydiales to which the chlamydiae belong comprised only of one family, Chlamydiaceae, with just one genus Chlamydia and four species C, trachomatis, C. psittaci, C. *pneumoniae* and C. *pecorum*.[14] This classification is based largely on limited genetic and phenotypic characteristics, antigenicity and host range. Recently, however, more obligate intracellular parasites have been found with chlamydia-like replication cycles and 16S rRNA genes that are >80% related to those in typical chlamydiae. These organisms have been included in a new classification scheme that recognizes four families, five genera and twelve species based on 16S and/or 23S rRNA sequence similarity (Table 1). For strains identified as chlamydiae, the rRNA sequence identity is >99% within the same species, >97% within the same genus, >90% within the same family and >80% within the order Chlamydiales.

The oldest family of Chlamydiaceae consists of the genus Chlamydia with three species and Chlamydophila with six species in human, avian and mammalian hosts. All members of the family share common lipopolysaccharide (LPS) antigens but have different outer membrane protein epitopes and vary in their resistance to sulfadiazine. Glycogen is produced by species of Chlamydia but not by Chlamydophila. Extrachromosomal plasmids have been identified in almost all species of Chlamydiaceae but plasmidless variants also occur in all species.[15-17]

The families of Parachlamydiaceae, *Simkaniaceae* and Waddliaceae have so far, only one species each. These chlamydiae are not recognized by *Chlamydiaceae*-specific anti-LPS monoclonal antibodies. Parachlamydia acanthamoebae are endocytobionts of acantharnoebae. Amoebae carrying this organism have been isolated from nasal mucosa,[13] from humans in an outbreak of humidifier fever[1"] and

**TABLE 1: Classification of the chlamydiae proposed by Everett, Bush and Andersen, 1999.[15]**

| Order | Family | Genus | Species |
|---|---|---|---|
| *Chlamydiales* | *Chlamydiaceae* | *Chlamydia* | *trachomatis* |
| | | | *suis* |
| | | | *muridarum* |
| | | *Chlamydophila* | *pneumoniae* |
| | | | *psittasi* |
| | | | *pecorum* |
| | | | *abortus* |
| | | | *caviae* |
| | | | *felis* |
| | *Parachlamydiaceae* | *Parachlamydia* | *acanthamoebae* |
| | *Simkaniaceae* | *Simkania* | *negevensis* |
| | *Waddliaceae* | *Waddlia* | *chondrophila* |

also from asymptomatic women.[15] ***Simkania nevegensis*** was first discovered as a contaminant in cell cultures; its natural host is not yet known. It has been isolated from adults with community-acquired pneumonia and from infants with bronchiolitis.[19] There is also serological evidence that it may be widespread among humans. ***Waddlia chondrophila*** was isolated from an aborted bovine foetus.[20] No human infection by this organism has yet been reported.

## CLINICAL INFECTIONS OF *CHLAMYDIA* AND *CHLAMYDOPHILA* SPECIES

Chlamydiae cause a wide-spectrum of diseases in their vertebrate hosts. They have also been shown to interact synergistically with viruses or with other bacteria, increasing the virulence of these organisms.[21]

### *Chlamydia trachomatis*

In the genus ***Chamydia,*** C. ***trachomatis*** causes human infections, ***C. suis*** infects swine and ***C. muridarum,*** hamster and mouse. ***C. trachomatis*** is grouped into three biovars, trachoma, lymphogranuloma venereum (LGV) and mouse pneumonitis. The former two biovars are pathogenic in humans, the latter, in mice. LGV strains infect a variety of cell types including mononuclear phagocytes and cause invasive disease spread by lymph and blood vessels. Strains of the biovar trachoma parasitize mostly mucous membranes of the eye and the genital tract where different serovars are associated with endemic trachoma and sexually-acquired oculo-genital infections such as inclusion conjunctivitis, non-specific urethritis and mucopurulent cervicitis with attendant complications in the upper genital tract. In addition, these organisms have been implicated as the cause of pneumonia in adults,[22] pneumonia in immunocompromised patients,'' otitis media,[24] meningoencephalitis,[25] reactive arthritis, Reiter's syndrome,[26] myocarditis,[27] culture-negative endocarditis,[28] perihepatitis[29] and peritonitis.[30] They have been detected in cryopreserved semen and thus pose an infection risk in artificial insemination.[31,32] Chlamydial genital infections are associated with a 3-5-fold increased risk of human immunodeficiency virus acquisition[33] and have been identified by Finnish researchers as a risk factor for invasive squamous-cell cervical cancer that is independent from smoking and infection with oncogenic types of the human-papillomavirus.''

Prevalence studies on ***C. trachomatis*** infections have been greatly facilitated by the use of nucleic acid amplification techniques. Mass screening of populations revealed a large silent reservoir of infection in both males and females.[35,36] The detection of chlamydial nucleic acids in culture-negative clinical specimens has also provided evidence for persistent infection in the pathogenesis of sequelae associated with chlamydial infections.[37,38] Under the influence of host factors like hormones, cytokines and antimicrobials that inhibit or modify the chlamydial developmental cycle, the chlamydiae are believed to be able to survive indefinitely as viable but non-cultivatable altered forms until exogenous conditions are favourable for the resumption of active growth and production of infectious particles.[39] These abnormal, persistent forms can be used to explain many features of

chlamydial infection: the prolonged, insidious or asymptomatic course in some infections; difficulty to isolate chlamydiae from diseased tissues; frequent relapse of chlamydial infection following apparently adequate chemotherapy; and different behaviour in different anatomical locations and disease syndromes.

Chlamydial proteins associated with pathogenicity include outer membrane and heat shock proteins. The major outer membrane protein (MOMP) of *C trachomutis*, besides acting as an adhesin,[40] is an immunoprotective antigen that induces neutralizing antibodies and protective T-cell responses. B-cell epitopes on MOMP antigens have been mapped with the use of monoclonal antibodies, DNA sequence data and the Pepscan" epitope mapping technique that allows single amino acid resolution of critical antibody binding sites. Recombinant DNA technology has been used to mass produce these proteins for vaccine studies in animal models.[41, 42] Chlamydial heat shock proteins are believed to be important in the pathogenesis of trachoma, PID, ectopic pregnancy, tubal infertility and some cases of pregnancy failure. The 60 kDa heat shock protein (hsp60) of chlamydia shows high amino acid sequence homology with human hsp60. It has been postulated that tissue damage in chlamydial infections is due to molecular mimicry and the autoimmunity that develops following an infection.[43, 44]

Traditionally, *C. trachomutis* infections have been treated with tetracyclines or macrolides. Although treatment failures are not uncommon, they have always been attributed to re-infection. However, over the past decade, strains with relative resistance to erythromycin and tetracycline have been identified in the U.S.A.[45, 46] In 1997, the first tetracycline-resistant *C. trachomatis* with a MIC and MBC of >64 mg/l was isolated in France, from an asymptomatic woman who failed with tetracycline therapy but was subsequently cured with pristinamycin.[47] The patient's medical history suggested that this was a case of persistent infection and not re-infection. More recently, researchers from the CDC in Atlanta reported three isolates with multi-drug resistance to doxycycline, azithromycin and ofloxacin at concentrations in excess of 4 mg/l. Two of the strains were not eradicated by standard azithromycin therapy.[48] It appears that in time to come, drug resistance may become an important therapeutic problem in the management of chlamydial infections.

### *Chlamydophila psittaci*

*C. psittaci* was isolated by S P Bedson and others in 1930 (19).[49] This species originally included a heterogeneous group of strains found widespread in nature in a variety of hosts, and producing diseases such as psittacosis, ornithosis, feline pneumonitis and abortion among domestic animals.[50] However, these organisms can be separated into four groups that are phenotypically, genetically and pathogenically distinct. In the new classification proposed by Everett, only avian strains are retained in the species *Cpsittaci. C. caviae* is created to include strains causing guinea pig conjunctivitis; strains causing endemic conjunctivitis and rhinitis among house cats are called *C. felis* and those endemic among ruminants and causing abortion in sheep, cattle and goats are renamed *C. abortus*. The latter have also been isolated from horses, rabbits, guinea pigs, mice, as well as from women who work with sheep.[51] *C. psittaci* serovars are endemic among psittacine birds and a wide variety of other avian hosts. Outbreaks have occurred among pet birds as well as in poultry processing plants. Human infections are incidentally acquired by inhalation of bird fecal aerosols or respiratory droplets that contaminate the environment. Infected patients typically acquire a flu-like illness or an atypical pneumonia syndrome, but may occasionally present as a systemic infection with liver and renal dysfunction, conjunctivitis and glomerulonephritis[52] or as potentially fatal culture-negative endocarditis.

### *Chlamydophila pecorum*

*C. pecorum* is a species created in 1992 [53] and consists of strains distinguished from *C. psittaci* by genetical, immunological and biological characteristics. This species was first isolated from the brain of a calf with sporadic bovine encephalomyelitis but has since been isolated from brains, lungs, joints, embryonic kidneys, faeces, and other parts of cattle and sheep for which the organism is a frequent cause of pneumonia, polyarthritis, encephalomyelitis and diarrhoea.[53] No human infections have been reported so far. Besides ruminants, these strains have also been isolated from swine, koala bears and from the faeces of healthy animals. In "stressed" populations of koala bears, they are associated with reproductive disease, infertility and urinary tract infection.

### *Chlamydophila pneumoniue*

Like C. ***pecorum,*** C. ***pneumoniae*** was also classified as C. ***psittaci*** before it was established as a separate species in 1989.[54] Initially thought to be a strict human pathogen, it is now also associated with infection in animals, having been isolated from ocular and urogenital sites in koala bears, from the respiratory tract of a horse and from a giant barred frog from Australia. In an epizootic of fatal chlamydiosis in a commercial colony of African clawed frogs imported to the USA from western Africa, C. ***pneumoniae*** was seen and isolated from inclusions in the livers of the infected frogs.[55]

In humans, C. ***pneumoniae*** is a significant respiratory pathogen with a 50-70% seroprevalence, world-wide.[56] Primary infection is usually acquired in childhood while re-infections are common in adults. The disease spectrum ranges from asymptomatic[57] to serious pulmonary infection requiring mechanical ventilation. In the upper respiratory tract, it is considered an etiologic agent of sinusitis, acute, chronic and recurring pharyngitis[58-60] and otitis media.[61] Persistent cough lasting two weeks to three months is a common symptom in both children[62, 63] and adults.[64] In the lower respiratory tract, it is associated with chronic bronchitis, asthmatic bronchitis,[65-67] acute exacerbation of chronic obstructive pulmonary disease, diffuse pan-bronchiolitis,[68] epidemic [69,70] and endemic pneumonia, community-acquired pneumonia in older persons, pneumonia in hospitalized patients,[71,72] and acute chest syndrome in sickle cell disease.[73]

Besides respiratory diseases, C. ***pneumoniae*** has also been implicated as a cause of culture-negative endocarditis,[74] Guillain-Barre' Syndrome,[75] sarcoidosis,[76,77] erythema nodosum, Reiter's syndrome,[78] Alzheimer's,[79] and coronary artery disease.[80-83]

The role of C. ***pneumoniae*** in the pathogenesis of atherosclerosis and ischaemic heart disease is still controversial although there has been an increasing body of literature suggesting a possible relationship. The presence of C. ***pneumoniae*** has Been documented in atheromatous plaques in coronary, carotid, internal mammary, pulmonary, iliac, femoral and popliteal arteries, as well as in the aorta, and non-rheumatic stenotic aortic valves.[84, 85] Detection methods used include transmission electron microscopy,[86] immunocytochemistry,[87] immunofluorescence antibody staining,[88] DNA amplification by polymerase chain reaction,[89, 90] and isolation in cell culture.[91] Detection rates in pathological specimens range from 0 to almost 100% versus 0-1% in normal vascular tissue.[85, 92, 93] This wide variation in the detection rate has been attributed mostly to the patchy distribution of the organism in lesions and to the use of different detection methods. The organism has been found in subjects as young as 15 years old,[94] in Western and Asian patients,[95] and in association with different clinical manifestations of vascular disease like angina, myocardial infarction, transient ischaemic attack, stroke, claudication and aortic aneurysm and rupture.[88, 96-98] Although the presence of the organism per se does not prove causality, C. ***pneumoniae*** has been demonstrated to grow in vascular endothelium, smooth muscle cells and monocytes, which are all components of the vessel wall.[99] In addition, the organism stimulates endothelial proliferation, causes the appearance of adhesive molecules on the surface of endothelial cells and promotes the formation of foam cells.[100, 101] Hence, it has been postulated that C. ***pneumoniae*** that is phagocytosed by macrophages in the lung may be trapped in sub-endothelial tissues where it stimulates the formation of the fatty streak that is subsequently converted to atheroma.[102]

The association between C. ***pneumoniae*** infection and atherosclerotic disease was first noticed by the finding of elevated antibody titres and immune complexes in patients with coronary heart disease or myocardial infarction.[81,103] Subsequently, many investigators found a higher frequency and level of chlamydial antibodies in patients with chronic CHD than in matched healthy controls. Seropositivity has also been linked to an increased risk of myocardial infarction,[104] ischaemic stroke[105] and increased intima-media thickness in the common carotid artery in hypertensive men.[106] In a study on patients undergoing percutaneous transluminal coronary angioplasty, antibodies to chlamydial lipopolysaccharide rose within one month after the procedure, suggesting that the immune response was induced by chlamydial antigens released from plaques that were ruptured during angioplasty.[107] However, several prospective studies have not demonstrated an association between chlamydial infection and incident myocardial infarction and death from ischaemic heart disease.[108]

Investigations in animal models have shown that, in the presence of elevated serum cholesterol, repeated intranasal inoculation with C. ***pneumoniae*** results in early changes of

atherosclerosis, and treating animals with macrolide antibiotics can either reduce or prevent these changes.[109] In human subjects, however, there'are conflicting reports on the effectiveness of azithromycin or roxithromycin treatment in the reduction of recurrent adverse cardiac events.[110, 111, 112] Similarly, retrospective case-control analyses on the effect of tetracycline exposure in the primary prevention of myocardial infarction have yielded inconsistent findings.[113,114] The controversy over the use of antibiotics for intervention has to be resolved by further evidence from laboratory studies and large-scale, well-controlled human clinical trials.

## CONCLUSION

Although much has been discovered about the chlamydiae, these organisms continue to fascinate as their saga continues with the discovery of new members, further vertebrate hosts and an expanding disease spectrum. The role of these bacteria in human disease is not always apparent as subclinical infections are common. However, as severe sequelae can complicate infection, the development of an effective vaccine is of public health interest. Towards this goal, research efforts are focussed on obtaining a better understanding of chlamydial biology, immunogenicity and mechanisms of pathogenicity. Current vaccine development strategy is to use purified chlamydial antigens selected for their ability to induce protection but not immunological reactions that can give rise to hypersensitivity. Should the causative role of C. ***pneumoniae*** in atherosclerosis and ischaemic heart disease be established, the race will be on for a vaccine that can eradicate these potentially fatal illnesses that affect millions of individuals world-wide.

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