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The Pathogenesis of *Chlamydia* Species

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Introduction

Chlamydia species have long been recognized as pathogens. However, the recent association of *Chlamydia pneumoniae* with coronary artery disease has returned these microorganisms to the forefront of clinical medicine. Members of the *Chlamydia* species are unusual microorganisms in comparison to either viruses or bacteria; many physicians may be unaware of some of their unique characteristics. Therefore, this brief review of the pathogenesis of *Chlamydia* species is offered as an introduction to this interesting pathogen.

Genus *Chlamydiae*

The genus chlamydiae is unique and therefore has been placed in a separate order (*Chlamydiales*) and family (*Chlamydiaceae*) (Figure 1).

There are four species currently recognized: *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, and *Chlamydia pecorum*.

Pathogenicity of *Chlamydiae*

These four currently-recognized species — *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, and *C. pecorum* — demonstrate considerable overlap in their pathogenesis. *C. psittaci* predominantly infects birds and lower mammals while *C. pecorum* infects horses, cattle, and sheep. Both, however, can infect humans. *C. trachomatis* and *C. pneumoniae* are primarily pathogens of man and account for a

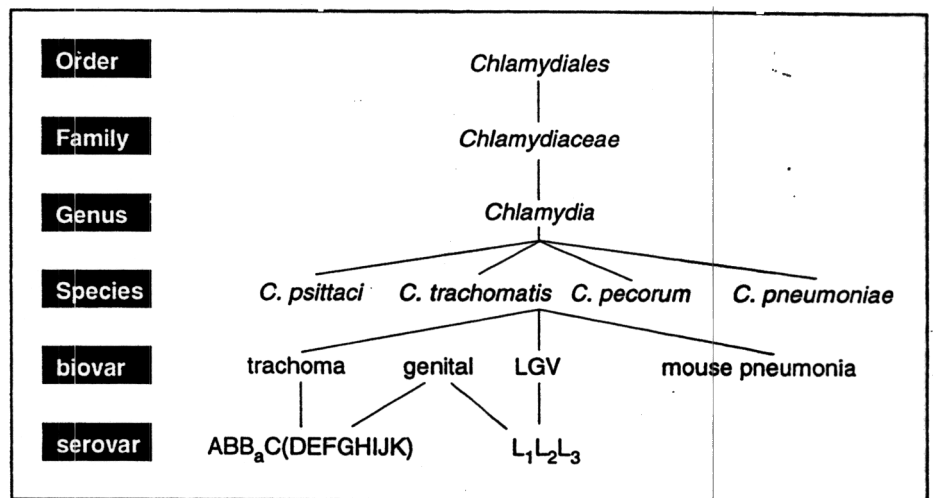


Figure 1. Phylogenetic classification of *Chlamydia*

tremendous burden of human disease.

Within each *Chlamydia* species, there may be differences in the specificity of host cells infected. For example, using a microimmunofluorescence (MIF) assay, Perez-Martinez and Storz differentiated 25 strains of *C. psittaci* of mammalian origin into 9 immunotypes. Correlation between serovars and biovars indicated that strains with unique pathogenic properties exhibit unique antigenic compositions. Table 1 catalogs immunotype (serovars) with species and pathogenicity (biovars). In non-mammalian hosts such as birds, *C. psittaci* is well known to produce both acute and chronic intestinal infections with excretion of chlamydiae in feces or diarrheal fluid that can be infectious for humans. *C. psittaci* in mammalian hosts clearly infects a wide variety of tissues, is wide-spread in occurrence, and is associated with chronic sequelae related to its antigenicity.

Infection in humans by all members of the genus *Chlamydiae* is frequently varied in symptomatology and often may be without any symptoms. Yet, chlamydial infection in humans is well

In This Issue

The Pathogenesis of
Chlamydia Species 83
Charles W. Stratton, MD,
William M. Mitchell, MD

Pneumococcal Endocarditis:
A Report of Two Cases 88
A case report

The United States-Japan
Cooperative Medical Sciences
Program Conference on
Emerging Diseases 90

known to induce a significant inflammatory response at the cellular level. For example, genital lesions produced by *C. trachomatis* frequently have a vigorous influx of lymphocytes, macrophages, and plasma cells suggesting the development of both humoral and cellular immunity. Once fully established, some infections caused by *Chlamydia* species appear difficult to eradicate with frequent relapses being seen following antibiotic therapy. Evidence also indicates that the *Chlamydia* may become dormant and are shed in quantities too few to reliably detect by culture. Moreover, there appears to be a chlamydial immune component that enhances chronic disease. For instance, active trachoma caused by *C. trachomatis* is a disease of childhood. The complications secondary to ocular scarring, however, rarely become evident prior to age 40. During the interval, individuals appear to be protected from recurrent active disease despite exposure to infection and rarely do they shed viable chlamydiae. Early attempts at vaccination thirty years ago demonstrated that any induced protection was short-lived and type-specific while hypersensitivity reactions were long-lived and group-specific. Thus the best recognized

chlamydial infection of humans (*C. trachomatis*) can be characterized as a widely prevalent pathogen that is silent through much of its chronic infectious process and in which the human host demonstrates evidence of aberrant immune response to infection. It is likely that *C. pneumoniae* is the same. The characteristics of *Chlamydia* species that result in this chronic and silent infectious process can be best appreciated by examining the life cycle of this pathogen.

Chlamydia Life Cycle

Chlamydiae are obligate intracellular bacterial parasites of eukaryotic cells. Members of the chlamydial genus have a unique biphasic developmental cycle with distinct morphological and functional forms. This developmental growth cycle alternates between intracellular life forms of which two are currently recognized; 1) an intracellular life form which can exist as a metabolically-active, replicating organism known as the reticulate body (RB) or as a persistent, nonreplicating organism known as the cryptic body, and 2) an extra-cellular life form that is an infectious, metabolically-inactive form known as the elementary body (EB).

EBs are small (300 to 400 nm) infectious, spore-like acellular forms which are metabolically inactive and non-replicating. EBs are resistant to a variety of physical insults such as enzyme degradation, sonication, and osmotic

Members of the chlamydial genus have a unique biphasic developmental cycle with distinct morphological and functional forms.

pressure. This physical stability is thought to be a result of extensive disulfide cross-linking of the cysteine-rich major outer membrane protein (MOMP). Under the oxidizing conditions in the acellular milieu of the host, the outer membrane of EBs is relatively impermeable and indestructible. EBs are thus able to survive long enough outside of their hosts to be transmitted to a new host in the form of a droplet nuclei or a fomite.

Chlamydial EBs appear to possess a receptor site that is a molecular mimic of heparin sulfate and attaches to glycosaminoglycan (gag) receptors on eukaryotic cell surfaces. After attachment to microvilli on the cell surface, the EBs then penetrate into the host cell by a process called "parasite-specified endocytosis" for which a detailed pathogenic mechanism is unknown. Endocytosis is rapid and saturable but limited by the slow rate of chlamydial attachment. The chlamydial vacuole has similarities with recycling endosomes which are the endocytic compartments through which recycled receptors and substrates are trafficked before being transported to the plasma membrane.

Table 1. Mammalian Serovars of *Chlamydia psittaci* and Associated Pathogenicity

		Pathogenicity
1	Ruminants	Abortions, seminal vasculitis, pneumonia, silent intestinal infections
2	Ruminants	Conjunctivitis, polyarthritis, encephalitis, enteritis
	Ruminants	Intestinal flora (?)
4	Porcine	Polyarthritis
	Porcine	Silent intestinal infection
6	Porcine (Bovine)	Abortion, pneumonia (Pneumonia-single isolate)
7	Feline, bovine	Pneumonia
	Guinea pig	Inclusion conjunctivitis
9	Ruminants	Intestinal flora (?)

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Moreover, chlamydial endocytosis appears to require a functional cytoskeleton of the host cell as chlamydial endocytosis is markedly inhibited by cytochalasin, an inhibitor of host cell microfilaments. Chlamydial EB inclusions appear to have no interaction with the classic early or late endocytic pathway once the inclusion membrane is established. Chlamydiae interrupt an exocytic pathway between the *trans*-Golgi and the plasma membrane. Recent work has demonstrated trafficking of Golgi-derived sphingolipids to the chlamydial inclusion via this pathway.

Once endocytosed, initiation of chlamydial growth begins within minutes and can be detected by the presence of mRNA transcripts. Intracellular EBs rapidly transform to larger (500 to 1,000 nm), pleomorphic, metabolically active RBs which can replicate by binary fission. The transformation of EBs to RBs also involves the presence of surface projections on the RB which are identical to those described on EB envelopes. Chlamydiae also appear to modify the inclusion membrane by the insertion of a chlamydiae-derived protein which has a yet unknown function. It is thought that this membrane-specific protein may promote nutrient acquisition from the cytoplasm.

Chlamydiae lack a mechanism for aerobic glycolysis. Thus, RBs require ATP and nutrient resources from the host cell in order to metabolize and replicate. Because chlamydiae are incapable of *de novo* synthesis of nucleotides and also lack the ability to generate ATP, they are considered obligatory intracellular parasites. Chlamydial dependence on host cell energy must necessarily deplete the host cell's existing energy output at the expense of depriving its own biosynthetic pathways. Finally, a high multiplicity of infection of host cells with chlamydiae has been shown to quickly bring host cell division to a halt, whereas lower multiplicities slow, but do not immediately stop, the division of host cells.

As the chlamydial infection of the host cell progresses, the RBs begin an asynchronous condensation to become EBs. This process is thought to utilize histone-like proteins expressed late in the growth cycle to inactivate the expression of chlamydial genes. Approximately two to four days after endocytosis, lysis of

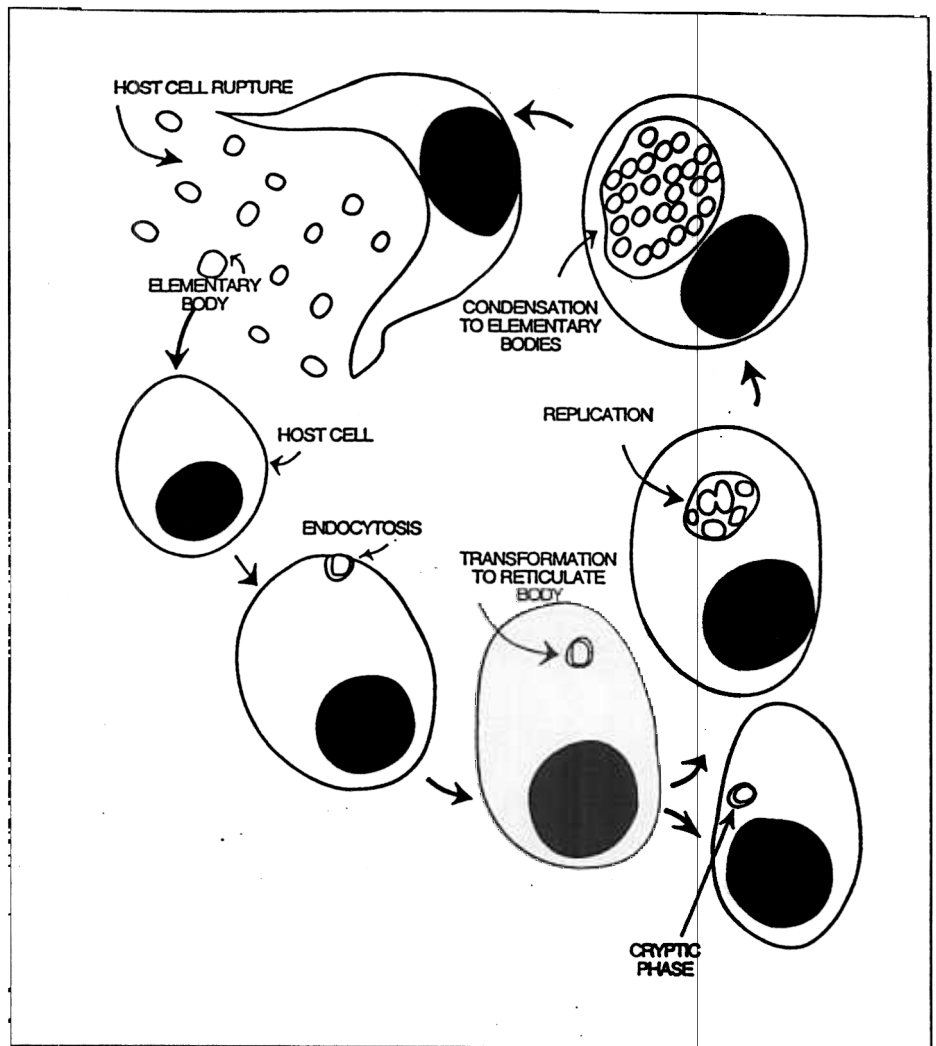


Figure 2. Chlamydia Life Cycle

the host cell may occur. This releases EBs into the extracellular milieu where they may encounter and infect susceptible host cells and thus complete the life cycle (Figure 2).

Chlamydial Persistence

The chlamydial life cycle in infected cells may not progress to completion and lysis of the host cell within the usual two to four days. Instead, persistence of the chlamydial infection in these cells may develop. This intracellular persistence of chlamydiae describes a long-term association between this pathogen and its host cell in which this microorganism remains in a metabolically active and viable state, but is culture-negative. Chlamydiae in this culture-negative state are known as cryptic forms, and their persistence in the host cell has been defined as cryptic infection. Some host cells that are per-

sistently infected may absorb the same or heterologous strains of chlamydial EBs, but their differentiation and replication is arrested. However, most persistently infected host cells do not absorb any additional EBs. Evidence that host cells carry cryptic chlamydial forms is seen by the occasional emergence of new infectious cycles that produce EBs. Moreover, persistent chlamydial infections have been established in cell culture systems. Finally, the perpetuation of chlamydiae within the host cell without overt growth or replication has long been recognized as a major factor in the pathogenesis of chlamydial disease in birds and animals. Chlamydiae, thus, appear to have the innate ability for intracellular persistence in host cells; this ability may represent an important and heretofore poorly appreciated facet of their pathogenesis in human disease.

Arrested Development and Chlamydial Persistence

During the normal developmental cycle of chlamydiae, the arrested growth of the microorganisms is thought to simply correlate to a reduction in metabolic activity which, in turn, delays differentiation. This arrested growth is associated with atypical cryptic forms. Such forms are seen with exposure to antimicrobial agents such as penicillin where large swollen reticulate bodies are formed from which bud small daughter reticulate bodies, termed "miniature" reticulate bodies. Similar pyknotic forms have been observed during active chlamydial replication induced when host cells are treated with cycloheximide. In every case in which the normal development of chlamydiae is arrested, the development of infectious chlamydial forms derived from aberrant organisms is characterized by the budding of "miniature" reticulate bodies.

Antimicrobial Induction of Chlamydial Persistence

A number of studies have clearly demonstrated that many different antibiotics when used as single agents merely inhibit the differentiation of chlamydiae and thus result in cryptic infection. The effects of these different agents seem to be dependent on the concentration of antibiotic present and the stage of the chlamydial developmental cycle during which infected cells are exposed to these substances. Although, the primary target for each of these antimicrobial agents may be different, each agent appears to generate chlamydial persistence.

Immunologic Induction of Chlamydial Persistence

In addition to persistence induced by the inhibition of the developmental cycle of chlamydiae by nutrient limitations and antimicrobial agents, persistence can be induced by immunologic mediators such as gamma-interferon. This effect appears to be related to tryptophan depletion. Although high levels of gamma-interferon have been shown to completely inhibit the active growth of chlamydiae, low levels induce the development of morphologically aberrant intracellular forms similar to those seen with other causes of developmental arrest. These persistent forms not

only exhibit a highly unusual intracellular morphology, but also display differential expression of key chlamydial antigens, some of which may be expressed on the host cell surface.

Clinical Importance of Chlamydial Persistence

Chlamydiae have long been recognized as causing latent infections in birds. In animals, persistent infections is also the rule. Thus, it would appear that an important characteristic of chlamydial infections is the ability of chlamydiae to cause persistence. In vitro cell models and in vivo clinical studies strongly support this view.

Perhaps the most important aspects of the pathogenesis of Chlamydiae is their persistence in host cells.

The potential for persistence of chlamydial infections has recently become an important issue in general for the pathogenesis of these microorganism in humans, and particularly for the pathogenesis of *C. pneumoniae*. *C. pneumoniae* is now recognized as a common and exclusive human pathogen with no known animal reservoir. This pathogen is known to infect the upper and lower respiratory tract of humans of all ages with pneumonia being the most important infection. However, *C. pneumoniae* is now known to infect human pulmonary macrophages and replicate within them. As human pulmonary macrophages appear unable to kill *C. pneumoniae*, it is not surprising to find that this pathogen occasionally causes chronic pulmonary infections.

The disturbing possibility exists that *C. pneumoniae* may have a wider dissemination in humans and cause an untold number of occult chronic infections. This possibility is strongly suggested by the well-studied pathogenesis of *C. pneumoniae* in a murine model where the recently demonstrated ability of *C. pneumoniae* to infect macro-

phages and thus disseminate widely and to infect blood vessels and cause atherosclerosis implies the same possibility in humans. Indeed, the ability of *C. pneumoniae* to infect human macrophages as well as endothelial cells and smooth muscle cells in arteries and veins has been well demonstrated and implies that, in humans, disseminated microorganisms could readily infect any blood vessels within any tissue in the human body. This raises an important question as to the role of persistent chlamydial infections in the vascular system in the etiology of atherosclerosis.

Antimicrobial Therapy and Chlamydial Persistence

Available clinical data teaches that persistence is a characteristic of chlamydial infections. A notable exception would seem to be human genital infections caused by *C. trachomatis*. In these genital infections, several weeks of antimicrobial therapy appear to result in clinical cures. However, one possible explanation is that epithelial cells in the genital tract are constantly being sloughed and renewed which may limit chlamydial persistence. Another possibility is that persistent chlamydial infection exists in deeper tissues as recently has been noted for *C. psittaci* in the reproductive tract of sheep. In either case, it is known that repeated genital infections in the murine model, whether from reinfection or from persistence and relapse, cause an increase in severity due to the host immune response.

If persistence of human chlamydial infections exists as is suggested by serologic data for *C. pneumoniae*, persistence will be a very important issue in terms of antimicrobial therapy. An antimicrobial agent used for 2 to 3 weeks to treat a chlamydial infection may not only fail to completely eradicate a systemic chlamydial infection, but may well induce cryptic forms and promote chronic infections. If commonly used agents for chlamydial infections appear to eradicate the infection, but actually produce cryptic infections, the long term sequelae of such chronic infections such as atherosclerosis might not be prevented, but instead could be potentially enhanced. Appropriate clinical trials to investigate this possible outcome of short-term anti-chlamydial therapy are clearly needed.

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Case Report

Pneumococcal Endocarditis: A Report of Two Cases

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Streptococcus pneumoniae is an uncommon cause of endocarditis in the antibiotic era. It is usually associated with a primary pneumococcal infection, usually pneumonia, presents with an acute and occasionally severe clinical picture, and has a high mortality rate ranging from 28% to 67%. This report describes two cases of pneumococcal endocarditis and their subsequent cure

by a combination of antibiotics and surgery.

Case 1

A 56-year-old female with a history of chronic obstructive pulmonary disease presented to the hospital with a three-week history of dyspnea on exertion, cough, fever, and malaise. She had not responded to an outpatient course of erythromycin for presumed bronchitis. Physical examination revealed a temperature of 38.9°C orally with tachycardia. Her neck was supple and examination of the heart and lungs was unremarkable. A chest X-ray revealed a possible right middle lobe infiltrate; a WBC count was 19,800/mm³ with a left shift. She was admitted with a diagnosis of pneumonia and started on intravenous cefuroxime. Two blood cultures drawn on admission grew *S. pneumoniae* with a MIC to penicillin of 0.19 µg/ml.

On the second day, her shortness of breath worsened and physical examination revealed signs of congestive heart failure. A grade 3/6 systolic murmur at

the cardiac apex with radiation to the axilla was also noted. A transthoracic echocardiogram showed moderately severe mitral regurgitation, a finding confirmed on transesophageal echocardiogram. However, the transesophageal study also revealed a vegetation on the posterior leaflet of the mitral valve. She was switched to intravenous penicillin at a dose of 4 million units every 4 h with subsequent defervescence. Repeat blood cultures were negative. As significant mitral regurgitation persisted, she underwent mitral valve replacement. A vegetation was present on the excised mitral valve, from which cultures were negative. She was discharged home to complete six weeks of i.v. penicillin, and remained well on follow-up six months later.

Case 2

An 80-year-old female presented to the hospital with a one-week history of fever, chills, cough, nausea, and chest pain. Her past medical history was unremarkable; she was allergic to penicillin. Physical