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Chlamydia pneumoniae (TWAR)

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INTRODUCTION

Chlamydia pneumoniae (TWAR) is a recently recognized third species of the genus *Chlamydia* that causes acute respiratory disease, including pneumonia, bronchitis, sinusitis, and pharyngitis. The organism was first isolated in 1965 from the conjunctiva of a Taiwanese child participating in a trachoma vaccine trial (72). The isolation was in the yolk sac of an embryonated chicken egg, the only method then available for growth of chlamydiae. In 1971, when cell culture methods became available, the organism (TW-183) was observed to form round, dense inclusions in host cells in cell culture which were more similar in morphology to those of *C. psittaci* than to those of *C. trachomatis*. An organism cultured from the eye of a child in Iran in 1968 and isolated in a chicken egg yolk sac (IOL-207) has also proven to be *C. pneumoniae* (25). Despite the conjunctival source of these two isolates, serologic studies suggested that the organism was not related to eye disease.

The organism's role as a human pathogen was not defined until 1983, when the first respiratory isolate (AR-39) was obtained in Seattle, Wash., from a university student with pharyngitis (46). This isolation was accomplished because serologic evidence in our laboratory suggested that the orphan TW-183 organism was associated with pneumonia (116). The strain name TWAR was derived from the laboratory designation of the first conjunctival and respiratory isolates (TW-183 and AR-39). In 1989, TWAR was established as a third species of *Chlamydia*, *C. pneumoniae* (44). Since only one strain or serovar of *C. pneumoniae* has been identified, at this time the strain name, TWAR, is synonymous with the designation *C. pneumoniae.*

In the past decade, considerable research attention has been devoted to *C. pneumoniae*. We estimate, on the basis of a compilation of studies, that it causes an average of 10% of cases of community-acquired pneumonia and 5% of bronchitis and sinusitis cases. Infection is most common among children 5 to 14 years of age, and the majority of adults have serologic evidence of past infection (1, 134). Antibodies have also been found frequently in people in many countries worldwide (32, 64, 89, 95, 133, 134). Much of the knowledge of the epidemiology of *C. pneumoniae* infection has been derived from serologic studies utilizing the *C. pneumoniae*-specific microimmunofluorescence (MIF) test. More recent improvements in isolation techniques and the application of the PCR have also greatly improved the capability to detect the organism in clinical specimens and facilitated more detailed microbiologic studies. Here, we will review the current state of knowledge of the microbiology, epidemiology, clinical manifestations, labo-

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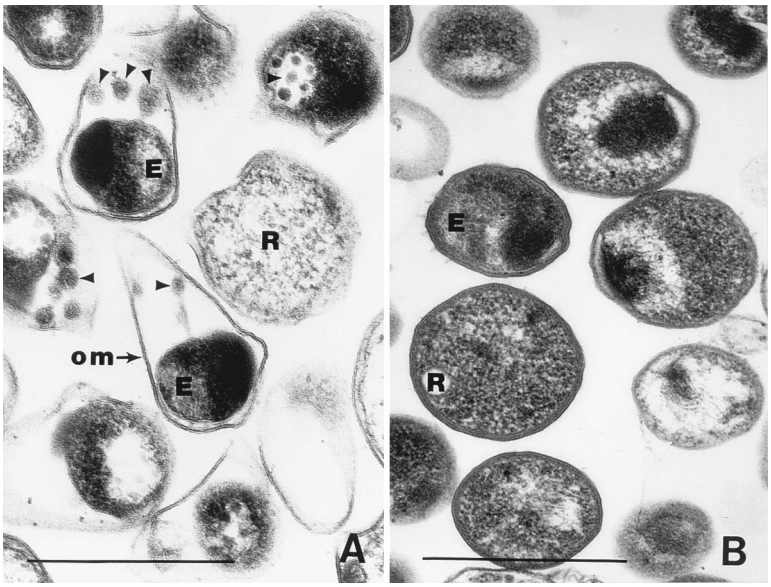

FIG. 1. Electron micrographs of *C. pneumoniae* (A) and *C. trachomatis* (B). E, elementary body; R, reticulate body; om, outer membrane. Arrowheads indicate small electron-dense bodies (minibodies). Bar = 0.5 μm. Reprinted from reference 44.

ratory diagnosis, treatment, and pathogenesis of *C. pneumoniae* infection.

MICROBIOLOGY

Classification

Chlamydiae are obligate intracellular parasites that are classified as bacteria because of the composition of their cell wall and their growth by binary division. They have a unique biphasic life cycle with a smaller extracellular form, the elementary body (EB), and a larger replicating intracellular form, the reticulate body (Fig. 1). The EBs attach to susceptible host cells and are phagocytized. Within the phagosome, they transform into reticulate bodies, which replicate by using the host-cell energy stores and form characteristic cytoplasmic inclusions. The reticulate bodies revert to the EB form prior to cell lysis.

Table 1 shows some of the basic characteristics that distinguish *C. pneumoniae* from the other chlamydial species that infect humans. Different *C. pneumoniae* isolates have 94 to 100% DNA homology with each other but less than 5% DNA homology with *C. trachomatis* and less than 10% homology with *C. psittaci* (23). *C. pneumoniae* organisms have a characteristic pear-shaped EB surrounded by a periplasmic space that is morphologically distinct from the round EBs of *C. trachomatis* and *C. psittaci* (Fig. 1). There have been recent reports of TWAR isolates with round EBs as shown by electron microscopy that are otherwise similar to other TWAR strains by molecular analysis (19, 63, 106). However, the published electron micrographs of these round EBs suggest the presence of a periplasmic space not seen in other chlamydial species. The significance of this morphologic difference is unknown.

No animal reservoir of *C. pneumoniae* has been identified; however, *C. pneumoniae*-like chlamydial strains isolated from a

TABLE 1. Characteristics and properties of the three chlamydial species that infect humans[a]

Characteristic or property	*C. pneumoniae*	*C. trachomatis*	*C. psittaci*
Major diseases	Pneumonia, bronchitis	Trachoma, sexually transmitted diseases, infant pneumonia	Pneumonia, fever of unknown origin
Natural host	Humans	Humans	Birds and lower mammals
No. of serovars	1 (TWAR)	18	Unknown
DNA homology to TWAR (%)	94–100	<5	<10
Morphology of EB on electron microscopy	Pear shaped	Round	Round
MOMP contains species-specific antigens	No	Yes	Yes
Inactivation of specific antigen by methanol	Yes	No	No
Susceptibility to tetracycline and macrolides	Yes	Yes	Yes
Susceptibility to sulfa drugs	No	Yes	No
Estimated annual no. of U.S. cases of pneumonia	300,000	12,000[b]	150[c]

[a] Adapted from reference 39 with permission of the publisher.
[b] In infants.
[c] Reported to the Centers for Disease Control and Prevention.

horse (119) and a koala bear (62) have been reported. These strains have round EBs, and their relation to *C. pneumoniae* was based on the homology in the sequence of the major outer membrane protein (MOMP) gene and cross-reaction with *C. pneumoniae*-specific monoclonal antibodies (MAbs). Unlike *C. pneumoniae*, which does not contain plasmid DNA, the horse isolate contains a plasmid. Whether these isolates represent strains of *C. pneumoniae* has yet to be determined by DNA-DNA hybridization.

Antigenic Structure

The presence of *C. pneumoniae* species-specific antigens was first demonstrated by MAbs in the MIF test (72). This reactivity is destroyed in the MIF test by fixation of EBs with methanol but not acetone (135). Because the antigen is labile to physical and chemical treatment, attempts to characterize the reactive antigen by immunoblotting, immunoaffinity, chromatography, and radioimmunoprecipitation have been unsuccessful (109). These species-specific MAbs were shown to neutralize infectivity in cell culture (109).

In all TWAR isolates examined, protein profiles have been identical, with a prominent 39.5-kDa band analogous to the MOMPs of other chlamydiae (15, 61). In addition to MOMP, cysteine-rich proteins of 15.5, 60, and 98 kDa have been found in the Sarkosyl-insoluble fraction, demonstrating their association with the outer membrane complex (15, 90). While the majority of the proteins (MOMP and 15.5- and 60-kDa proteins) found in the outer membrane complex are similar in molecular mass and structure to those of the other *Chlamydia* species (99, 105), the 98-kDa cysteine-rich protein appears to be present only in the outer membrane complex of *C. pneumoniae*. It was originally postulated that the additional presence of a 98-kDa cysteine-rich protein might provide a more rigid membrane structure to sustain a pear-shaped morphology because cross-linking of the disulfide bonds provides rigidity to the outer membrane (90). Analysis of a Japanese *C. pneumoniae* isolate with round EB morphology showed a protein profile similar to those of the prototype strains, TW-183 and AR-39, but the 98-kDa protein was significantly less concentrated (63). However, metabolic labeling studies of another round isolate from Japan showed that the 98-kDa protein is also cysteine rich (69). Thus, the role of this protein in contributing to the pear-shaped morphology remains unclear.

In contrast to the other chlamydial species, the 39.5-kDa *C. pneumoniae* MOMP is not immunodominant, and reactivity to the MOMP is cross-reactive among chlamydial species (16, 33, 61, 63). Immunoblot analysis with rabbit immune sera and/or sera from patients with *C. pneumoniae* MIF antibody demonstrated reactivities with other proteins, including the MOMP and 30-, 60-, 68-, and 75-kDa proteins, that were shared among chlamydial species (15, 16, 33, 61).

Several *C. pneumoniae* species-specific proteins have been identified by immunoblot. The appearance of antibody against the 98-kDa protein is demonstrated following the onset of seropositivity in acute infection (16). Proteins with other molecular weights with reactivities that are specific for *C. pneumoniae* have been demonstrated with human and animal sera, including the 43-kDa protein and proteins with a molecular mass range of 50 to 60 kDa (33, 61, 63, 81). In a study of MIF-positive sera by Freidank et al., a *C. pneumoniae*-specific reactivity directed against a 54-kDa protein was predominant (33). Iijima et al. reported 43-, 46-, and 53-kDa proteins as immunodominant *C. pneumoniae*-specific antigens recognized during human infection (61). Two proteins of molecular mass similar to the 43- and 53-kDa proteins described by Iijima et al. were also frequently recognized by MIF-positive sera obtained postmortem from South African patients with atherosclerotic lesions in which *C. pneumoniae* was detected and by MIF-positive sera from patients with sarcoidosis (107, 108). In those studies, recognition of the 42- and 52-kDa proteins was found to be species specific. Mouse MAbs against the 53-kDa *C. pneumoniae*-specific protein have been produced (61).

Genetics

Sequence analysis has identified *C. pneumoniae* genes that are markedly similar to those of *C. trachomatis* and *C. psittaci* encoding structural, functional, and immunologically important proteins. Among these are homologs of the *ompA*, *ompB*, *groEL*, and *dnaK* genes. Sequence analysis of the MOMP gene (*ompA*) from several isolates has shown them to be identical (19, 62, 105). Structurally, the *C. pneumoniae* gene is similar to that of other *Chlamydia* species, having regions that are conserved among the species interspersed with four regions, corresponding to variable domains, that share little sequence similarity to the other chlamydial MOMPs. For *C. trachomatis* and *C. psittaci*, the antigenic diversity of the MOMP has been mapped to these domains (4, 118, 146, 147). In contrast, sequence analyses of the VDIV region of *C. pneumoniae*, the largest of the hypervariable regions of the other species, were found to be identical in 13 isolates (36). Cumulatively, these studies demonstrate the genetic homogeneity of the *C. pneumoniae* MOMP gene and validate the inability to isolate MAbs with serological specificity, other than genus reactivity, against the MOMP. All seven cysteine residues involved in the formation of disulfide-linked complexes of the other chlamydial MOMPs are similarly conserved in *C. pneumoniae* (90). Likewise, the cysteine residues within OmpB (60-kDa cysteine-rich protein), are also highly conserved in all chlamydial species (29, 137), suggesting structural function in formation of disulfide-linked complexes.

Two genes encoding heat shock proteins (HSP; GroEL and DnaK homologs) associated with immunopathologic and immunoprotective responses in *C. trachomatis* (24, 83, 98) have been isolated from *C. pneumoniae* (67, 71). For *C. trachomatis*, the Sarkosyl-soluble HSP60 (GroEL) has been associated with a delayed-type hypersensitivity response in chronic infections (98). In contrast, antibodies against the 70-kDa HSP (DnaK) neutralize infectivity (83); however, the antigen elicits no delayed hypersensitivity activity (122). Both HSP60 and HSP70 of *C. pneumoniae* are recognized by sera from patients with *C. pneumoniae* MIF antibody (16, 61, 107). The *C. pneumoniae* GroEL homolog has 95 and 97% amino acid sequence similarity to the *C. trachomatis* and *C. psittaci* homologs, respectively, while the *C. pneumoniae* DnaK homolog shares 87% amino acid similarity with the *C. trachomatis* protein.

The first gene isolated that encodes a *C. pneumoniae*-specific antigen was a 76-kDa protein gene (91). This protein is distinct from the genus-reactive HSP70 described above. The monospecific rabbit hyperimmune serum prepared against the 76-kDa recombinant protein was shown to specifically recognize *C. pneumoniae* inclusions in cell culture and neutralize infectivity of *C. pneumoniae* in cell culture.

Biology

C. pneumoniae requires all amino acids, except lysine, for growth in cell cultures (77). In comparison, *C. trachomatis* requires all amino acids except threonine. Paradoxically, depletion of lysine from 100 to 90% and of methionine from 90 to 70% was shown to enhance the growth of *C. pneumoniae*.

An ultrastructural study of entry of the TWAR organism into HeLa cells demonstrated differences between TWAR and other chlamydiae in the mode of attachment and endocytosis (73). The TWAR EBs first attach to host cells by the pointed end and then secure other binding sites on the host cells by forming cell wall protrusions, enter host cells by invaginating the host cell membrane, and form vacuolated endocytic vesicles.

Immunology

Infection with *C. pneumoniae* induces serum immunoglobulin M (IgM), IgA, and IgG responses. These antibodies against TWAR can be detected by fluorescent-antibody testing or enzyme-linked immunosorbent assay (ELISA), using EBs or infected cells (inclusions). Antibodies against group-specific lipopolysaccharide (LPS) also develop and can be demonstrated by complement fixation (CF), immunoblotting, or ELISA. Although TWAR-specific MAbs have been shown to neutralize the infectivity of TWAR specifically in cell culture (109), how the neutralizing antibodies contribute to immunity against *C. pneumoniae* infection is unknown.

As with *C. trachomatis*, persons infected with *C. pneumoniae* develop a cell-mediated immune response as demonstrated by the lymphocyte transformation assay with peripheral blood (121) or synovial lymphocytes (11). Lymphocyte transformation activity is associated with the number of organisms shed from the cervix in *C. trachomatis* infection (12). However, no studies on the role of cellular immunity in resistance against *C. pneumoniae* have been reported.

EPIDEMIOLOGY

Much of the current information on the epidemiology of *C. pneumoniae* infection is derived from serologic studies with the *C. pneumoniae*-specific MIF test. These studies indicate that *C. pneumoniae* is a common cause of infection throughout the world, with a seroprevalence of over 50% among adults in the United States and many other countries (32, 64, 89, 95, 133, 134). In addition, although *C. pneumoniae* was first recognized as a respiratory pathogen in 1983, testing of banked serum specimens has revealed that it is not a new pathogen but has been a frequent cause of infection since at least 1963 (1, 39).

Age Distribution

Infection appears to be most common among school-aged children, with children under age 5 years affected much less frequently. Figure 2 illustrates the seroprevalence of *C. pneumoniae* antibody by age from pooled serologic studies of the Seattle population (39). A very small percentage of children under 5 years of age have serologic evidence of past infection with *C. pneumoniae*. The prevalence then increases dramatically from ages 5 through 14 years, and by age 20 years approximately 50% of persons have detectable levels of antibody to the organism. The seroprevalence continues to increase among older age groups, but at a slower rate, and reaches approximately 75% in the elderly. These prevalence rates exist despite the fact that first infection induces a time-limited antibody response (3 to 5 years), suggesting that most people are infected and reinfected throughout life (101).

A similar trend in age distribution of acute infection was shown when a series of serum samples from individuals in a long-term study of Seattle families was tested (1). Children 5 through 9 years of age had the highest incidence of acute infection, as evidenced by a fourfold or greater rise in antibody titer (Table 2). The incidence among children 10 to 14 years of age was slightly lower. There was evidence of a higher rate of cough illness among those who had seroconverted; however, many of the children appeared to be asymptomatic (2). A more recent study of Swedish children showed a similar age distribution of acute infection, with annual seroconversion rates of 8.0% in children ages 8 through 12 years and 5.9% in children ages 12 through 16 (51).

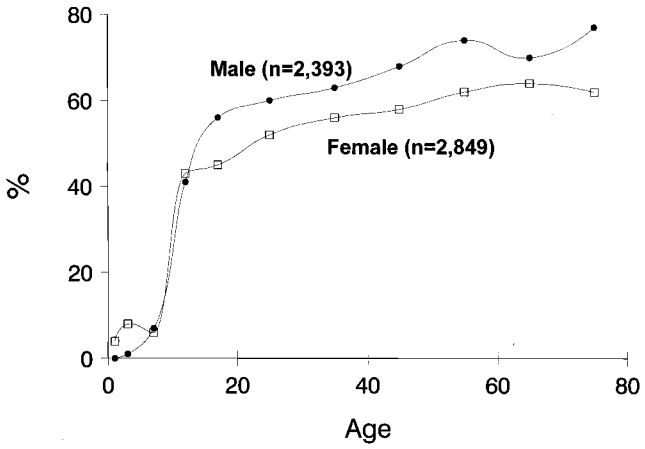

FIG. 2. Prevalence of MIF antibody to *C. pneumoniae*, by age, among 5,242 persons in Seattle. IgG titers ranged from 8 to 256. Reprinted from reference 39 with permission of the publisher.

Sex Distribution

Seroprevalence is approximately equal in both sexes under 15 years of age; however, seroprevalence among adult men is considerably higher than that among adult women (Fig. 2). This sex difference among adults has been demonstrated in all countries from which sera have been tested. To date, no explanation for the increased frequency among males has been found.

Global Distribution

C. pneumoniae appears to have a worldwide distribution, although the prevalence of infection due to this organism may vary by region (32). Studies of adult sera from 10 areas of the world have shown a higher population prevalence in tropical, less developed countries than in more northerly, developed countries, with Canada, Denmark, and Norway having the lowest rates (134). There is some evidence that infection among children less than 5 years of age, uncommon in the United States, may be both more common and more severe in tropical countries (115).

TABLE 2. Incidence of acute TWAR infection with *C. pneumoniae* in subjects in Seattle, 1975–1979[a]

Age (yr)	No. with fourfold MIF antibody titer rises	No. of person-yr	Incidence rate per 100 person-yr at risk
0–4	0	27	
5–9	14	151	9.2
10–14	15	242	6.2
15–19	2	91	2.2
>19	6	394	1.5

[a] Reprinted from reference 1 with permission of the publisher.

Transmission

Humans are the only known reservoir of *C. pneumoniae*, and transmission is believed to be from person to person via respiratory secretions. This transmission appears to be relatively inefficient. When contacts of patients with *C. pneumoniae* infection are investigated, few cases of secondary transmission are detected, as evidenced by follow-up of contacts of University of Washington students with documented *C. pneumoniae* infection (41) and by a longitudinal seroepidemiologic study of Seattle families (1). In addition, investigations of several military outbreaks revealed no direct chain of transmission between cases, and the outbreaks extended over a period of several months, despite the close proximity of susceptible contacts (27, 70). These findings also suggest that infection may be acquired via transmission from asymptomatic carriers. Although it appears that the organism is usually transmitted relatively inefficiently, evidence that some infected persons may be much more efficient transmitters of the organism is shown by several outbreaks in families and groups of families in which all or most members were affected (96, 97).

The incubation period of infection due to *C. pneumoniae* is several weeks, which is longer than that for many other respiratory pathogens. This has been demonstrated by serologic testing of family and household contacts of patients with *C. pneumoniae* infection, showing an interval of 3 weeks between seroconversion for pairs with the closest contact (spousal pairs) (97). A similar interval was demonstrated between exposure and infection during a *C. pneumoniae* outbreak (68).

Laboratory studies have indicated that *C. pneumoniae* can survive in aerosol at room temperature in conditions of high relative humidity. Although there is a rapid decline of infectivity with time, a decrease by half in the first 30 s, survival in these conditions supports the possibility of direct person-to-person transmission in a crowded humid environment (123). The organism can remain viable on Formica countertops for 30 h and on tissue paper for 12 h (30), suggesting that transfer from fomites may occur.

CLINICAL MANIFESTATIONS

Respiratory Infections

Pneumonia and bronchitis are the most frequently recognized illnesses associated with *C. pneumoniae*, although asymptomatic infection or unrecognized, mildly symptomatic illnesses are the most common result of infection. In a series of studies, ~10% of cases of pneumonia and approximately 5% of bronchitis and sinusitis cases in adults have been attributed to the organism (39).

No set of symptoms or signs is unique to pulmonary infections with *C. pneumoniae*; however, several characteristics of the clinical presentation may help distinguish it from other causes (39, 41, 46, 127). A subacute onset is common. Pharyngitis, sometimes with hoarseness, is often present early in the course of the illness. There may be a biphasic pattern to the illness, with resolution of pharyngitis prior to development of a more typical bronchitis or pneumonia syndrome. Cough is very common and is often prolonged. Fever is often not present at examination, but there may be a history of fever. The period from onset to clinic visit is longer for TWAR infections than other acute respiratory infections. Symptoms of sinus infection commonly occur in association with TWAR respiratory infections.

Although the patient's leukocyte count is usually normal, the erythrocyte sedimentation rate is often elevated. A chest radiograph usually demonstrates a single subsegmental pneumonitis in milder, nonhospitalized cases. More extensive or bilateral pneumonitis may be seen in hospitalized patients. Pleural effusions have also been demonstrated in persons with more severe disease. Most cases of pneumonia are relatively mild and do not require hospitalization. Even in mild cases, however, complete recovery is slow, despite appropriate antibiotic therapy, and cough and malaise may persist for many weeks after the acute illness. Older adults appear to have, on average, a more severe clinical course than do young adults. The available evidence also suggests that underlying illnesses and concurrent infection with other bacteria, such as the pneumococci, are associated with more severe disease. Studies of patients hospitalized with *C. pneumoniae* pneumonia have found that the majority had one or more underlying illnesses (43, 85). In addition, most of the fatalities associated with *C. pneumoniae* infection have been in persons with underlying illness and complications such as pneumococcal bacteremia (22, 34, 43, 66).

The role of *C. pneumoniae* as an opportunistic pathogen among immunocompromised persons is not well defined. The organism has been isolated from the lungs of patients infected with the human immunodeficiency virus (3) and has been detected by PCR in bronchoalveolar lavage specimens from human immunodeficiency virus-infected and other immunocompromised patients (35). However, whether immunocompromised persons are at increased risk of infection with *C. pneumoniae*, or more severe disease as a consequence of infection, has not been determined. It has also been suggested that *C. pneumoniae* infection may be more common among persons with chronic obstructive pulmonary disease on the basis of a study which found a higher prevalence of *C. pneumoniae* antibody among persons with that condition (5).

Severe Systemic Infection

Severe systemic infections with *C. pneumoniae*, while uncommon, do occur. We have been aware of several severe adult cases with serologic evidence of TWAR infection only. We have also identified *C. pneumoniae* in autopsy tissue by PCR, suggesting that the organism played at least a part in the infectious process prior to death. Recently, we reported a febrile illness in a 10-year-old boy with pneumonia, pericarditis, pleuritis, and hepatosplenomegaly (40). A commercial laboratory found very high TWAR MIF IgG serum antibody, which led to further studies that resulted in a PCR demonstration of *C. pneumoniae* in stored lymph node and liver biopsy specimens.

Other Syndromes

TWAR has also been associated with other acute illnesses. It has been isolated from patients with purulent sinusitis (56) and otitis media with effusion (100). Primary pharyngitis due to *C. pneumoniae* has been reported; however, the frequency of this infection is unclear. While *C. pneumoniae* infection has been reported in as high as 8% of adults with pharyngitis in Finland (60), it appears to be uncommon (less than 2% of cases) in studies of young adults in the United States (54, 127). Other reported clinical syndromes include endocarditis (86) and lumbosacral meningoradiculitis (93).

Several chronic diseases have also been presumptively associated with *C. pneumoniae* infection. Patients with *C. pneumoniae* respiratory infection have been shown to be more likely to develop asthmatic bronchitis following their respiratory illness, suggesting that TWAR may be a factor in the development of asthma or asthma exacerbations (49). *C. pneumoniae*

has been associated with sarcoidosis by serologic studies (8, 48, 107) and with erythema nodosum (28, 120). A case of Guillain-Barré syndrome following infection with *C. pneumoniae* has been reported (50). *C. pneumoniae* has also been implicated in reactive arthritis or Reiter's syndrome (11).

Coronary Artery Disease

An association between coronary artery disease and other atherosclerotic syndromes and *C. pneumoniae* infection has been suggested by both seroepidemiologic studies and the demonstration of the presence of the organism in atheromatous plaque. The initial study indicating a possible association between *C. pneumoniae* and coronary artery disease was performed in Finland and showed that patients with coronary artery disease were significantly more likely to have serologic evidence of past infection with TWAR than were controls (114). Since that time, serologic studies from the United States and other countries have demonstrated similar findings among patients with coronary artery disease (82, 108, 113, 126, 128) as well as in patients with thickening of the carotid arteries (92).

Morphologic and microbiologic evidence of the presence of *C. pneumoniae* in atheromatous plaques has been obtained by electron microscopic studies of coronary atheroma (80, 117) and immunocytochemical staining and PCR testing of coronary (80), carotid (45), and aortic atheroma (74). The organism has also been demonstrated in atheromatous tissue removed from patients by directional coronary atherectomy and was found more commonly in restenotic lesions than in primary lesions (67 versus 43%) (18). Another study of autopsy specimens from young persons (15 to 35 years of age) has offered the opportunity to study coronary arteries from persons without atherosclerosis, an opportunity for control material not available in older adults (140). The organism was not detected by PCR or immunocytochemical staining in 31 coronary artery specimens that showed no atheroma but was demonstrated in 2 of 11 specimens showing probable early lesions (intimal thickening) and in 6 of 7 specimens with developed atherosclerotic plaques (79). While these studies clearly associate TWAR organisms with atheromatous plaques, the role of TWAR infection in the pathogenesis of atherosclerosis is unknown.

TREATMENT

There are as yet no published controlled trials of treatment for *C. pneumoniae* infection. Erythromycin, tetracycline, and doxycycline demonstrate in vitro activity against the organism (76), and these agents are recommended as first-line therapy. The organism is not susceptible in vitro to penicillin, ampicillin, or sulfa drugs. Clinical experience shows that symptoms of *C. pneumoniae* infection frequently recur after short or conventional courses of appropriate antibiotics, and persistent infection has been documented by culture after treatment (52). Therefore, intensive long-term treatment is recommended: tetracycline, 500 mg four times daily for 14 days; doxycycline, 100 mg twice daily for 14 days; or erythromycin, 500 mg four times daily for 14 days (or 250 mg four times daily for 21 days if the higher dose is not tolerated). If symptoms such as cough or malaise persist after one course of antibiotics, a second course may be useful. Unless the drug is contraindicated, tetracycline or doxycycline is recommended for the second course.

Two newer agents, azithromycin, an azalide, and clarithromycin, a macrolide, have been demonstrated to be effective against *C. pneumoniae* in vitro (54, 139). *C. pneumoniae* is also susceptible to certain fluroquinolones (31, 53). Both azithromycin and clarithromycin are associated with fewer gastrointestinal side effects and are thus better tolerated than erythromycin. Azithromycin achieves a very high intracellular concentration and has a longer duration of action than clarithromycin, which may allow a shorter course of therapy and more convenient dosage schedule. In in vitro studies, we have found it more bactericidal than clarithromycin. Both are also effective against sexually transmitted *C. trachomatis* infections, which can be treated with a single dose of azithromycin (87). Preliminary results of recently completed clinical trials suggest that azithromycin is at least as effective as erythromycin for the treatment of *C. pneumoniae* respiratory infections (20, 42). Similar trials with clarithromycin have been carried out and can be expected to show similar results (112). It is likely that these antibiotics will be recommended for treatment of *C. pneumoniae* after formal evaluation is completed.

LABORATORY DIAGNOSIS

Isolation

C. pneumoniae grows poorly in culture, and the inclusions formed are smaller than those seen with other chlamydiae. Isolation is best performed by cell culture, although like *C. trachomatis*, *C. pneumoniae* can also be cultured by using the yolk sacs of embryonated chicken eggs (72). The most sensitive cell lines for isolation are HL (21, 78) and HEp-2 (141). Cell lines commonly used for isolation of *C. trachomatis*, such as McCoy and HeLa 229 cells, are not sensitive for *C. pneumoniae* (72, 78). As with *C. trachomatis*, centrifugation of the inoculum onto cell monolayers and incorporation of cycloheximide into the culture medium to inhibit host cell metabolism enhance the sensitivity of isolation. The development of a TWAR-specific MAb conjugated with fluorescein has greatly enhanced identification of the few inclusions generally seen in cell culture.

Specimens for isolation are usually obtained from swabs of the oropharynx. Satisfactory techniques for isolation from sputum samples have not been developed. The pharyngeal swab should be placed in chlamydial transport medium, SPG (phosphate buffer containing sucrose and glutamic acid), and refrigerated at 4°C, since the organism has been shown to be inactivated rapidly at room temperature (75). TWAR organisms are also susceptible to inactivation by rapid freezing or freezing and thawing. Specimens should be chilled to 4°C for 1 to 4 h before freezing to below −65°C to preserve viability (75). Low (<5) and high (>8) pH and an NaCl concentration of less than 80 mM have also been shown to be detrimental to the organism (124).

Serology

The MIF test, devised in 1970 for *C. trachomatis* (132), is the only specific and sensitive serological assay for any of the chlamydiae. The test with TWAR antigen is specific for *C. pneumoniae*. It can distinguish between antibodies in the IgM and IgG serum fractions, which is very helpful in distinguishing recent from past infection and reinfection from primary infection. Testing with antigen prepared from *C. pneumoniae* isolated from the geographic region where the patient resides has been suggested to be more sensitive for detection of TWAR antibody (7, 9, 59); however, no significant difference in detection between assays performed with TWAR isolates from geographically diverse areas was found in a recent study (136).

The criteria for serologic diagnosis of *C. pneumoniae* by MIF are shown in Table 3. The appearance of MIF antibody is slow; therefore, a 3- to 4-week interval is recommended for obtain-

TABLE 3. Criteria for serologic diagnosis of *C. pneumoniae*

Test	Positive result
MIF with *C. pneumoniae* antigen[a]	
Acute antibody	Fourfold titer rise; or IgM ≥ 16; or IgG ≥ 512
Preexisting antibody	IgG, 8–256
Chlamydia CF[b]	
Acute antibody	Fourfold titer rise; or ≥64

[a] EB antigen specific for *C. pneumoniae.*
[b] CF test is *Chlamydia* genus specific, not species specific.

ing the second, convalescent, serum sample. For investigational purposes, a third serum sample obtained 2 months after onset may be useful to detect late rises in antibody titer. Failure to detect MIF antibodies in patients from whom *C. pneumoniae* was isolated may occur (52, 111) but is usually due to the slow antibody response. False-positive MIF IgM antibody tests may occur if patients have circulating rheumatoid factor, the prevalence of which increases with age. Therefore, removal of IgG rheumatoid factor for MIF IgM-positive sera is recommended (131).

Two patterns of antibody response to acute TWAR infections have been identified: one is associated with primary infection and the other is associated with reinfection. In primary TWAR infection, a prompt chlamydial CF antibody response is seen. TWAR MIF IgM antibody appears later, about 3 weeks after the onset of illness. Antibody in the IgG fraction may not appear until 6 to 8 weeks after onset. In reinfection, CF and IgM antibody may not appear or may appear only at low titers. The IgG antibody titer rises quickly, often in 1 to 2 weeks, and may reach a value of 512 or more.

Understanding these patterns is important in interpreting serologic studies of TWAR infections. In a first infection, if the second serum is obtained less than 3 weeks after onset, the antibody response can be missed. In reinfections, the absence of CF and IgM antibody can make it difficult to distinguish between acute infection and persistent antibody from past infection. Follow-up serologies on patients with an acute antibody response reveal that, while IgM usually begins to fall within 2 months and usually disappears 4 to 6 months after an acute infection, IgG persists and may be detected for over 3 years after acute infection in some patients. For the serologic diagnosis of *C. pneumoniae* infection, paired serum specimens should be obtained whenever possible. The presence of a high titer of antibody alone provides a much less precise serological diagnosis than a fourfold rise in titer from paired sera. This is especially true for elderly patients, who may have had multiple *C. pneumoniae* infections in the past and may have persistently high IgG titers.

We examined throat swabs from several groups of patients for the presence of *C. pneumoniae* by cell culture and PCR and tested serial serum specimens from them by MIF. We found excellent correlation between positive serology by MIF and detection of the organism. MIF serology was always the most sensitive measure of infection. The organism was demonstrated by isolation and/or PCR in 62 to 75% of persons positive by serologic tests but not in patients who did not have serologic evidence of infection (27, 41, 125, 127).

Whereas fourfold rises in antibody titer in the IgM or IgG serum fraction are clearly related to current *C. pneumoniae* infection, a high titer of antibody in a single specimen may suggest current infection or persistence after a recent or past infection. We have found a small number of persons who, after a TWAR infection, have had IgM antibody persisting for many months. In a study of persons more than 65 years old, we found that 8% had a persistent IgG antibody titer of >512. Nevertheless, a high titer of antibody in individual specimens suggests that treatment for *C. pneumoniae* infection may be a reasonable course to follow. We studied middle-aged adults with pneumonia and bronchitis whose only evidence of TWAR infection was a high titer of IgG antibody. The infection was confirmed years later by a positive PCR test on their throat specimens, which had been stored frozen (125). A similar sensitivity of the MIF test has been found by others (26, 27, 68, 115, 142).

The MIF test is technically demanding, and some laboratories have been unable to obtain species-specific results. The test was originally developed for *C. trachomatis* and was used to immunotype the now 18 serovars of *C. trachomatis*. A test of a laboratory's ability to perform the MIF procedure properly is whether it can distinguish, by serologic testing alone, eight individual genital serovars or three serogroups (B, C, and intermediate) of *C. trachomatis*. Differentiating the three *Chlamydia* species is an easier task than is differentiating serovars and is a goal that should be attained by laboratories employing the MIF test for *C. pneumoniae* serology.

In contrast to the MIF test, the chlamydial CF test, which detects antibodies against chlamydial LPS, is widely available but is unable to distinguish between antibodies to the three chlamydial species. This test has been widely used for the diagnosis of psittacosis. Since TWAR infection is much more common, many persons diagnosed as having psittacosis on the basis of the CF test, particularly in the absence of either exposure to a new or a sick pet bird or occupational exposure, in reality had a TWAR infection.

Antigen Detection

C. pneumoniae species-specific MAbs have been useful in detection of the organism in cell culture (46, 72). However, they are insensitive for demonstration of the organism in direct smears from clinical specimens. The sensitivity of antigen detection from throat swab smears is only about 50% of isolation (27, 46). Antigen detection by ELISA, using genus-specific antibody that is commonly used for *C. trachomatis*, has also shown poor sensitivity.

DNA Probes

Several *C. pneumoniae*-specific primers have been used in PCR detection of organisms. The targets amplified include a *C. pneumoniae*-specific sequence of unknown coding function (17), rRNA gene sequences (37), the MOMP gene (10, 57, 62, 110), and the 60-kDa cysteine-rich outer membrane protein gene (138). PCR or PCR enzyme immunoassay has been used successfully for detection of the organisms in pharyngeal swab specimens (17, 41), nasopharyngeal swab specimens (38), bronchoalveolar lavage (34), and sputum (130). In these studies, PCR appeared to be more sensitive than isolation for detection of TWAR organisms. PCR has also been successfully applied to the detection of *C. pneumoniae* organisms in fresh or formalin-fixed, paraffin-embedded human and animal tissues (80, 84). This tool has been used to study the association of *C. pneumoniae* with various clinical manifestations. In conjunction with other diagnostic methods, these studies have provided evidence that *C. pneumoniae* causes community-acquired pneumonia (41, 130), bronchitis (41), and pneumonia in immunocompromised patients (35). Lastly, PCR has been used to demonstrate the presence of *C. pneumoniae* in coronary atheroma and atherectomy specimens (18, 80).

PATHOGENESIS

In *C. trachomatis* infection, immunopathology causes scarring of the conjunctivae (47) and fallopian tubes (103) as a consequence of reinfection. The delayed hypersensitivity antigen has been implicated in the pathogenesis of blindness from trachoma (98, 122) and tubal infertility (13) and ectopic pregnancy (14) from salpingitis. The 60-kDa chlamydial HSP is a delayed hypersensitivity antigen (98) which provokes a chronic inflammatory reaction. For *C. trachomatis*, antibodies against the 60-kDa HSP are frequently found in women with tubal factor infertility and ectopic pregnancy associated with this organism. Whether the *C. pneumoniae* GroEL gene product might play a similar role in immunopathology of chronic *C. pneumoniae* infections is unknown.

In contrast to *C. trachomatis* infection, there is evidence that recent past infection with *C. pneumoniae* may confer protection from severe disease on reinfection. In an outbreak of *C. pneumoniae* affecting male military trainees in Finland, persons with an MIF serologic pattern indicating reinfection were less likely to have pneumonia and more likely to have a febrile upper respiratory infection than patients with evidence of primary infection (26). Similar results were found in a prospective study of middle-aged adults with symptoms of upper respiratory tract infection in Seattle (125).

C. trachomatis is known to be a causative agent of reactive arthritis (Reiter's syndrome) (88). Recent studies have shown an association between *C. pneumoniae* and reactive arthritis (11). In these studies, peripheral blood and synovial lymphocytes from patients with reactive arthritis were shown to react specifically with TWAR organisms in the lymphocyte transformation assay. The antigens involved in the pathogenesis of Reiter's syndrome have not been analyzed.

ANIMAL MODELS

Three animal models of *C. pneumoniae* infection, mouse, rabbit, and monkey, have been evaluated. The most susceptible animals are mice, which are susceptible to inoculation with TWAR organisms by intranasal (65, 72, 144, 145), intravenous (72, 145), subcutaneous (72, 145), and intracerebral (72) routes. Lung infection induced by intranasal inoculation runs a prolonged course. Organisms are recoverable from lungs for 42 days, and the lung pathology persists for over 60 days (144).

The lung pathology in mice is characterized by patchy interstitial pneumonitis, with polymorphonuclear leukocyte infiltration in the early stage and with mononuclear cell infiltration in the late stage (144). Ultrastructural examination revealed *C. pneumoniae* inclusions in ciliated bronchial epithelial cells and interstitial macrophages (143). This is similar to pneumonitis induced in mice with *C. trachomatis*, except infection with *C. trachomatis* is cleared in 14 days. A striking pathologic feature is the accumulation of lymphoid cells in the perivascular and peribronchial areas, which appear on day 11 and persist through day 60 following the primary infection. For *C. trachomatis*, formation of lymphoid cell foci is observed only in chronic or repeated ocular and salpingeal reinfection in both humans (129) and monkeys (102–104). Human lung pathology in *C. pneumoniae* infection has not been well described, and whether a pathology similar to that in mice develops in humans is unknown.

C. pneumoniae is shown to spread systemically in mice following intranasal inoculation (145). Isolation of TWAR from spleen and peritoneal macrophages is as frequent as that from lungs in the intranasally inoculated mice. This finding that *C. pneumoniae* infection in mice is a systemic disease suggests that it may be a systemic infection in humans.

C. pneumoniae has been shown to be of low virulence in baboons (*Papio cynocephalus anubus*) and rhesus (*Macaca cyclopid*) and cynomolgus monkeys. Nasopharyngeal, oropharyngeal, or intratracheal inoculation of baby baboons (6) and cynomolgus monkeys (58) with TWAR resulted in no disease attributable to the experimental infection, although the organisms were recoverable from the nasopharynx. Unlike that of *C. trachomatis*, inoculation of TWAR into conjunctiva results in only mild inflammation (72).

New Zealand White rabbits are susceptible to intranasal and intratracheal inoculation with TWAR (94). Respiratory disease in the rabbit model is characterized by moderate multifocal interstitial pneumonia with bronchiolitis and vasculitis. Single inoculation results in a self-resolving pneumonitis, 3 weeks in duration, composed of heterophils initially and then changing to predominately mononuclear cells. With repeated inoculations, scattered microgranulomas consisting of a central core of macrophages surrounded by activated lymphocytes develop and persist through day 42 of infection. Organisms could be cultured from the upper respiratory tract during the early stages of the disease but never from lung tissue. However, lung tissue was intermittently positive by PCR through day 42. Chlamydial DNA was also detected by PCR in spleen tissue and peripheral blood mononuclear cells, indicating systemic disease similar to that in the mouse model.

FUTURE DIRECTIONS

The early seroepidemiologic studies provided a framework for understanding the epidemiology of *C. pneumoniae* infection. It seems unlikely that there will be any major changes discovered in the epidemiology as described in this article. On the other hand, the role of *C. pneumoniae* in chronic diseases is not well understood. Clearly, the organism remains viable in the body long after an acute infection has subsided. Probably the most important current question about *C. pneumoniae* pathogenesis is, What is the pathologic significance of the organism in atheroma, lung, and other tissues in the body?

Because the MIF test is technically demanding and isolation of the organism from the throat is disappointing, a rapid reliable laboratory test of infection for the clinical laboratory is a major need in the field.

The lability of the specific antigens of *C. pneumoniae* has so far prevented their specific identification. Since neutralizing antibodies can be produced, it will be important to identify, isolate, and produce the specific antigen for both laboratory tests and a possible vaccine.

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