C Chlamydial Diseases

180 *Chlamydia trachomatis* (Trachoma and Urogenital Infections)

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SHORT VIEW SUMMARY

Definition

- *Chlamydia trachomatis* is an obligate intracellular bacterium that mainly infects ocular, genitourinary, and rectal epithelium.
- The ocular disease trachoma is the leading infectious cause of blindness worldwide.
- Chlamydial genital infections are the most common bacterial sexually transmitted infections in the world.
- Lymphogranuloma venereum (LGV), caused by distinct serovars of *C. trachomatis,* is a less common disease characterized by enlarged inguinal lymph nodes or severe proctocolitis.

Epidemiology

- Trachoma is common in poor, rural areas of developing countries, with an estimated 21.4 million active cases worldwide in 2011.
- Active infection in trachoma largely affects young children, often those younger than 1 year of age, whereas the scarring sequelae that produce blindness occur later in life.
- The sexually transmitted urogenital infections are common worldwide, with an estimated 131 million new cases in 2012.
- Chlamydial genital infections are most prevalent among adolescent women and men, in whom repeated infections are common.
- Chlamydial genital infections are often asymptomatic but can cause reproductive sequelae in women, including pelvic inflammatory disease (PID), ectopic pregnancy, and involuntary infertility.
- Transmission during vaginal delivery can lead to neonatal conjunctivitis and pneumonia.
- *C. trachomatis* infection is a notifiable disease that is reportable to the Centers for Disease Control and Prevention and all state health departments in the United States.

Pathophysiology

- Disease manifestations are largely mediated by the host response to the infection, which is initiated and sustained by actively infected nonimmune host epithelial cells.
- In a substantial minority of persons with trachoma and genital infections, the host inflammatory response leads to tissue scarring, resulting in end-organ dysfunction and sequelae.

Microbiology

- *C. trachomatis* is a gram-negative bacterium, but Gram stain is not used for identification.
- The organism is cultivated in cell culture because it does not divide and grow axenically.
- This obligate intracellular bacterium replicates within a cytoplasmic inclusion in a host cell.
- The two morphologic forms are the infectious but nondividing elementary body and the reticulate body, which is an intracellular, noninfectious, replicating form.

Clinical Manifestations

- Trachoma is a chronic keratoconjunctivitis that may progress over years in a minority of cases to conjunctival scarring and corneal opacification.
- Active trachoma is frequently asymptomatic but may present as chronic conjunctivitis with redness, discomfort, photophobia, and mucopurulent discharge.
- *C. trachomatis* urogenital infection in men manifests as nongonococcal urethritis with scant, nonpurulent urethral discharge and dysuria.
- *C. trachomatis* urogenital infection in women is mostly asymptomatic, but may present as mild and nonspecific symptoms, such as vaginal discharge and bleeding, mild abdominal pain, or dysuria.
- Classic LGV is a chronic, systemic illness that progresses from a genital lesion to prominent inguinal lymphadenopathy.
- LGV proctocolitis presents with mucopurulent anal discharge and rectal pain, ulceration, and bleeding.

Diagnosis

- Diagnosis of trachoma in endemic areas is typically by physical examination of the eye according to criteria established by the World Health Organization.
- Diagnosis of genital infections requires microbiologic testing, ideally by nucleic acid amplification tests of first-catch urine in men and vaginal swabs in women.

Therapy (See Table 180-3)

- Therapy of trachoma is generally provided by repeated mass treatment of hyperendemic communities with single-dose azithromycin.
- Recommended treatment of uncomplicated urogenital infection is either azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice daily for 7 days. Treatment of sex partners is crucial to prevent repeated infection.
- Treatment of complicated infections requires longer durations of therapy: for salpingitis, 14 days; for epididymitis, 10 days; and for mild proctitis, 7 days.
- Classic LGV, and severe proctocolitis caused by LGV serovars, are treated with doxycycline for 21 days.

Prevention

- Trachoma can be prevented by face washing, access to clean water, and improvements in sanitation.
- In the United States and other developed countries, prevention of sexually transmitted genital infections and complications is largely focused on screening and treating nonpregnant, sexually active women under 25 years of age on an annual basis. Uptake of screening in the United States is relatively low.
- Screening of all pregnant women is recommended.
- Screening and treatment of women older than 25 years of age is recommended if risk factors are identified, such as new or multiple sexual partners.
- One-time screening and treatment in women decreases risk of symptomatic PID in the following year. Data are currently lacking as to whether screening programs reduce population prevalence of chlamydial genital infection.
- Screening of young men in high-risk settings (sexually transmitted infection and adolescent clinics, correctional facilities) should be considered if resources allow.
- No vaccine is currently available for either trachoma or chlamydial genital infections. Induction of sterilizing immunity appears unlikely, and future vaccine efficacy evaluation may be based on demonstrating reduced risk of inflammatory sequelae in the eye and symptomatic PID in women.

Chlamydia trachomatis imposes a tremendous burden on human health because it is one of the most common causes of bacterial infection. Worldwide in 2011, an estimated 21 million people had active trachoma, which is an ocular *C. trachomatis* infection that is the leading cause of infectious blindness.[1] The World Health Organization (WHO) estimated that in 2016, 1.9 million individuals were blind or had severe visual impairment as a long-term consequence of trachoma.[2] This disease has disappeared from the developed world coincident with improved sanitation and access to clean water. However, an estimated 190 million people live in endemic areas, mainly in poor, rural regions of 41 low-income countries, and require interventions to prevent blinding disease.[2]

C. trachomatis genital tract infection is the most common bacterial sexually transmitted infection (STI) in the world, with an estimated 131 million new cases in 2012.[3,4] Women disproportionately suffer the major complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility, and can transmit the bacterium to their newborns, who develop conjunctivitis and pneumonia. In 2016, 1.59 million new cases of *C. trachomatis* infection were reported to the Centers for Disease Control and Prevention (CDC),[5] making it the most commonly reported infectious disease in the United States.[6] Not all *C. trachomatis* infections are diagnosed, however, and the actual incidence is higher. For example, in 2008 the annual incidence of *C. trachomatis* genital infections in the United States was estimated to be 2.86 million cases, which was more than twice the number that were reported.[7] In a population-based assessment, chlamydial prevalence in the United States in the 14- to 39-year-old age group was estimated to be 2.0% for women and 1.4% for men for 2007 to 2012, with stable overall prevalence during this period.[8] Women have had consistently higher rates, which may be due to increased screening and diagnosis compared to men. Higher chlamydial prevalence is reported when screening targeted populations, who tend to be higher-risk groups. For example, in women 15 to 24 years old, chlamydial prevalence ranging from 6.6% to 15.6% has been reported, with the highest prevalence in STI clinics and juvenile detention centers.[5] In the United States, reported case rates continue to rise, largely as a result of increased screening and improved diagnostic tests (Fig. 180.1A), but population prevalence appears stable, with notable disparities by age and race/ethnicity (Fig. 180.1B). Data from 2016 demonstrate that the disease is most common in adolescents and young adults ages 15 to 24 years, with higher rates in women and among African Americans compared with Hispanics/Latinos and non-Hispanic whites.[5] *C. trachomatis* infections thus continue to be a major public health problem.

Human *C. trachomatis* infections are associated with ocular, genitourinary, and respiratory disease syndromes, and although clinically distinct, they share common characteristics. These include the propensity to cause (1) infections that are often long-lasting in the absence of treatment; (2) repeated infections after natural clearance or antibiotic treatment of an initial infection; (3) infections that are often asymptomatic or minimally symptomatic; and (4) infections that produce inflammatory and scarring sequelae in the absence of treatment, often with few or no symptoms until end-organ dysfunction is manifest.[9]

CHLAMYDIAL BIOLOGY

Chlamydiae are obligate intracellular bacteria that replicate within eukaryotic cells.[10] *C. trachomatis,* like all other *Chlamydia* spp., has one of the smallest bacterial genomes. Its 1.04-Mb genome encodes approximately 900 genes, which is less than a fifth of the genes in *Escherichia coli.*[11] The large majority of these genes (668) are shared among all

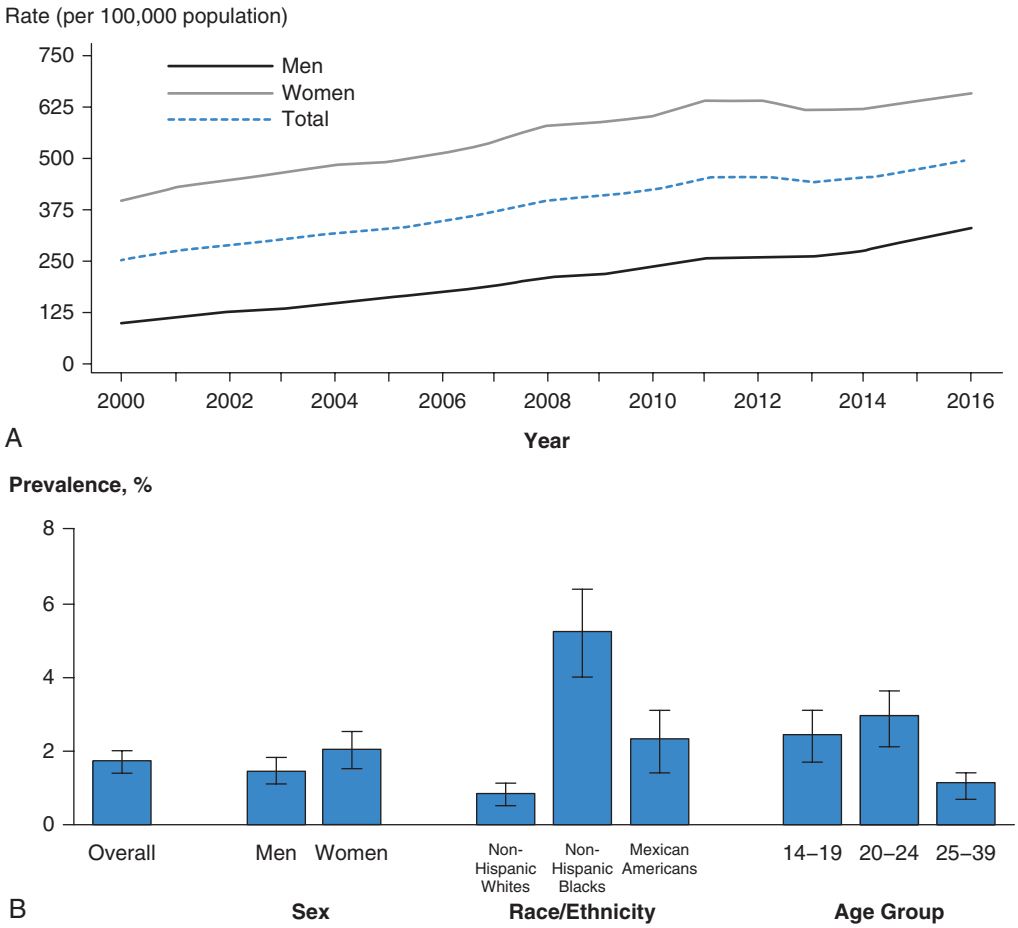

FIG. 180.1 Prevalence of *Chlamydia trachomatis*. (A) Reported case rates of *Chlamydia trachomatis* in the United States. (B) Population prevalences of *Chlamydia trachomatis* in the United States, 2007–2012. Note: Since 2000, all 50 states and the District of Columbia require the reporting of *Chlamydia* cases. *(From Centers for Disease Control and Prevention.* Sexually Transmitted Disease Surveillance, 2016. *Atlanta: US Department of Health and Human Services; 2017; and data from Torrone E, Papp J, Weinstock H; Centers for Disease Control and Prevention. Prevalence of* Chlamydia trachomatis *genital infection among persons aged 14–39 years—United States, 2007–2012.* MMWR Morb Mortal Wkly Rep. *2014;63:834–838.)*

Chlamydia, representing a core set of genes necessary for the intracellular chlamydial lifestyle.[12] The small genome size is due to reductive evolution in which *C. trachomatis* has lost enzymes and metabolic pathways for nutrients that are readily available from the host cell.[13] As a consequence, however, *Chlamydia* is dependent on its host cell to complete its developmental cycle. Other common obligate intracellular bacteria include *Rickettsia,* which also has a small genome, and *Mycobacterium leprae,* which is undergoing active genome reduction.

Chlamydia is unusual among bacteria in having two developmental forms, which are morphologically different and functionally specialized.[10] The reticulate body (RB) is the vegetative form that grows and divides by binary fission.[14] Chlamydiae lack many enzymes and pathways for biosynthesis of amino acids and nucleotides[15] and thus are auxotrophic for many essential metabolites. The RB compensates by having numerous membrane transport systems to acquire nutrients—including amino acids, oligopeptides, nucleotides, ATP, carbohydrates, and metal ions—from the host cell.[15,16] The RB thus resembles a typical bacterium, but it occupies an intracellular niche in which it can obtain many nutrients from its host cell.

The spore-like elementary body (EB) is a unique chlamydial adaptation that is the transmission vehicle to infect another host cell.[10,17] It is environmentally stable, with a rigid envelope of cross-linked membrane proteins,[18–20] and DNA condensed into chromatin by two *Chlamydia*-specific histone-like proteins, Hc1 and Hc2.[21–23] The most abundant of its membrane proteins is the major outer membrane protein (MOMP), which is also the immunodominant antigen.[18] Extracellular EBs have limited metabolic and biosynthetic activity.[24,25] However, EBs contain the machinery for central metabolism and glucose catabolism,[15] and large stores of ATP,[26] which prime it for a burst of metabolic activity upon entry into a host cell.

For many years, it was not clear if chlamydiae have peptidoglycan, which is a major structural component of the bacterial cell wall.[27] It has now been shown that *C. trachomatis* RBs form a narrow peptidoglycan ring at the septum during chlamydial cell division.[28] However, EBs lack peptidoglycan, and the classical mesh-like exoskeleton of peptidoglycan found in most other bacteria is not present in pathogenic *Chlamydia* spp.[28]

All *Chlamydia* spp. share an unusual biphasic developmental cycle in which there is conversion between RBs and EBs inside an infected host cell (Fig. 180.2).[10,14,29] An EB binds and enters a susceptible epithelial cell, within a membrane-bound vacuole called the chlamydial inclusion, and converts into an RB within 2 to 8 hours. RBs divide repeatedly by binary fission and then begin converting into EBs at about 24 hours after entry. The delayed and asynchronous timing of RB-to-EB conversion has been proposed to be controlled by RB size, which progressively decreases by sixfold as the RB population in an infected cell expands via successive rounds of replication.[14] By 48 hours, several hundred to 1000 EBs are released by lysis of the host cell, or by extrusion of the inclusion from an intact host cell.[30]

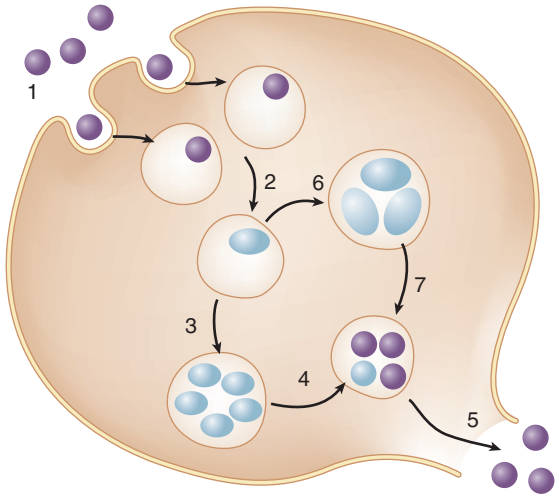

FIG. 180.2 Developmental cycle of *Chlamydia trachomatis* in cell culture. *1,* Infectious elementary bodies (EBs) attach to and are taken up by epithelial cells. *2,* Inclusions fuse and EBs differentiate into reticulate bodies (RBs). *3,* RBs divide by binary fission. *4,* Delayed and asynchronous conversion of RBs into EBs. *5,* EBs release, often with cell death, to infect other cells. *6,* Altered growth state under stressful conditions (e.g., interferon-γ exposure) leading to enlarged aberrant RBs but no EBs. *7,* With removal of stress, reactivation back to normal developmental cycle and production of EBs.

EB attachment to an epithelial host cell occurs through a two-step process.[31] The first step is a low-affinity interaction between an EB surface protein OmcB,[32] and heparan sulfate proteoglycan associated with the host cell surface.[33] The second step is an irreversible, high-affinity interaction between chlamydial ligands, including MOMP and the Pmp family of polymorphic membrane proteins,[34] and specific host receptors.[31] *C. trachomatis* has been reported to bind a number of host receptors, which may contribute to strain-specific differences in tissue tropism, but could also represent a strategy to increase the chance of binding to a host cell.[17]

This bacterium plays an active role in promoting its own entry into a host cell, which allows it to infect nonphagocytic cells.[35–37] Upon contact, the EB induces actin cytoskeletal rearrangements in the host cell by secreting preformed protein effectors via a polarized, type III secretion (T3S) mechanism.[38,39] For example, a chlamydial effector called Tarp (translocated actin-recruiting phosphoprotein) nucleates actin polymerization directly, while also activating the host actin-nucleating machinery,[40–42] and another effector (CT694) binds the actin-binding protein AHNAK.[43] In addition, binding of the EB to receptor tyrosine kinases, such as epidermal growth factor receptor, fibroblast growth factor receptor, and platelet-derived growth factor receptor, activates the MEK-ERK and phosphoinositide 3-kinase signaling pathways, promoting chlamydial entry and host cell survival.[31,44,45]

The inclusion provides a protected intracellular niche within which chlamydiae grow and replicate. Maintenance of this intracellular compartment depends on inhibition of phagolysosomal fusion and selective recruitment of vesicles carrying nutrients such as lipids and iron.[31] *Chlamydia* inserts inclusion membrane proteins into the inclusion membrane, where they interact with host regulators of vesicular trafficking and fusion, including Rab guanosine triphosphatases and SNARE proteins.[36,46,47] There may initially be multiple small inclusions in a host cell, from infection by more than one EB, but they coalesce into a single inclusion through fusion mediated by an inclusion membrane protein called IncA (see Fig. 180.2).[48]

The inclusion has interactions with a number of organelles in the host cell that help promote the intracellular infection.[31] Early in the infection, the inclusion migrates along microtubules from the cell periphery to the microtubule organizing center in the perinuclear region of the host cell.[49] Chlamydiae acquire host lipids from the Golgi apparatus via exocytic vesicles, from the endoplasmic reticulum by recruiting the host lipid biosynthetic machinery to the inclusion, and via lipid-rich organelles called lipid droplets.[31,50] These host lipids are necessary for the enlarging inclusion membrane and may contribute to the chlamydial cell membrane.[50–52] The inclusion also recruits recycling endosomes and mitochondria to facilitate acquisition of iron and ATP, respectively.[31,53]

Chlamydial genes are expressed as three major temporal groups that correspond to three main stages of the developmental cycle.[54–56] Early genes are first transcribed during EB-to-RB conversion and have a likely role in establishing the chlamydial inclusion. The largest temporal group consists of midcycle genes, which include most housekeeping genes as well as virulence genes that are expressed during RB replication. Data indicate that midcycle genes and a subset of early genes are activated by higher levels of negative DNA supercoiling in midcycle.[57,58] This novel use of its DNA topology to regulate developmental gene expression makes *Chlamydia* dependent on enzymes such as DNA gyrase that modulate supercoiling levels.[59] It also provides a *Chlamydia*-specific target for the fluoroquinolones, including ofloxacin and levofloxacin, which are DNA gyrase inhibitors used to treat chlamydial infections. Late genes, which are the third and last temporal group, have roles in RB-to-EB conversion and EB function. They are negatively regulated by the transcription factor EUO, providing a mechanism to prevent their premature expression.[60,61]

Chlamydia actively promotes the survival of its host cell so that it can complete its developmental cycle.[31,62] At the time of entry, binding of the EB to receptor tyrosine kinases and ephrin receptors activates prosurvival pathways involving MEK-ERK and phosphoinositide 3-kinase.[31] In addition, chlamydiae block intrinsic apoptosis by downregulating and sequestering proapoptotic factors, and by upregulating and stabilizing antiapoptotic factors.[62] *Chlamydia* also inhibits extrinsic apoptosis by blocking caspase activation.[63]

Chlamydiae grown in cell culture can enter an altered growth state of viable but noncultivable bacteria that is called in vitro persistence.[64,65] Infectious progeny cannot be recovered because there is no RB-to-EB conversion. Persistence can be induced by exposing *Chlamydia*-infected cells to interferon (IFN)-γ, which induces host indoleamine 2,3,-dioxygenase to deplete tryptophan.[66] It can also be induced by penicillin treatment, iron or nutrient starvation, herpesvirus coinfection, or host cell differentiation.[65] In vitro persistence is reversible because removal of the inducing agent causes reactivation back to the normal developmental cycle and production of EBs.[67] The relevance of in vitro persistence to clinical infection and disease is not clear.[65]

Chlamydia causes centrosomal and DNA defects that may explain its association with cervical cancer.[68] *Chlamydia* alters centrosomal function by inducing the formation of extra centrosomes, centrosome clustering, and mitotic spindle defects, each of which may lead to chromosome segregation errors.[69–71] *Chlamydia* causes DNA damage in an infected cell by inducing DNA double-strand breaks and impairing the DNA damage response.[72] In addition, *Chlamydia* can transform infected cells in cell culture and cause cervical dysplasia in mice.[73]

ANTIGENIC AND GENETIC DIVERSITY

C. trachomatis has been historically classified into 15 serovars on the basis of antigenic cross-reactivity in the microimmunofluorescence test of Wang and Grayston.[74] The serovars can be grouped into two biovariants that are associated with different clinical presentations. The trachoma biovar consists of serovars A, B, Ba, and C, which cause trachoma, and serovars D through K, which cause urogenital disease and inclusion conjunctivitis. The lymphogranuloma venereum (LGV) biovar includes serovars L1, L2, and L3, which cause LGV and proctocolitis in men who have sex with men (MSM). Occasionally, serovars B and Ba have been isolated from the genital tract, but A and C have not. Alternatively, serovars can be divided into two subgroups on the basis of cross-reactivity patterns: the B complex consists of serovars B, Ba, D, E, L1, and L2, and the C complex is composed of serovars A, C, H, I, J, K, and L3. Serovars F and G bridge the two complexes, although they are more closely related to the B complex.[74] Additional serovars have been proposed (Da, Ia, and L2a),[75] as well as serovars such as L2b that have caused a recent outbreak of LGV proctitis.[76] When full-length sequencing of *ompA* is used for strain identification, additional stable variants have been described.[77]

Species-, subspecies-, and serovar-specific antibodies recognize epitopes located in four variable sequence regions of MOMP.[78] Serovar-specific epitopes are mostly found in variable sequence regions 1 and 2, whereas the more broadly shared epitopes cluster in variable region 4.[78] However, some serovars contain more than one serovar-specific epitope, and serovar-specific epitopes are found in variable region 4 as well.[79] The immunodominant genus-reactive epitope is in lipopolysaccharide (LPS), which is closely related to LPS of other gram-negative bacteria, particularly the deep rough (Re) mutants of enterobacteria.[80] Chlamydial LPS is technically a lipooligosaccharide composed of a 3-deoxy-D-manno-octulosonic acid trisaccharide in a 2,4 linkage and a *Chlamydia*-specific 2,8 linkage.[81] Additional genus-reactive epitopes have been identified in MOMP,[82] the 60-kDa cysteine-rich protein (OmcB),[82] and the 60-kDa heat shock protein..[83]

With the advent of affordable whole-genome sequencing, chlamydial strains are increasingly being typed by genetic differences on a genomic scale.[84,85] Genomic comparison has confirmed that *C. trachomatis* can be divided into three lineages, with the urogenital and ocular clades more closely related to each other than to the LGV clade.[85] There is strong conservation of gene content and order between *C. trachomatis* strains, with only 4860 single nucleotide polymorphisms, out of just over 1 million bases, between the trachoma and LGV biovars.[86] This overall genetic similarity has led to a search for specific genes that contribute to differences in tropism and clinical presentation. A hot spot of genetic diversity called the plasticity zone is located at the replication terminus of the chlamydial genome, which is a site of large-scale rearrangements in many bacterial genomes.[12,87] Between *Chlamydia* spp., there is considerable variation in the size of the plasticity zone, from 18 kb to 81 kb, and the presence or absence of genes encoding virulence factors, such as membrane attack complex/perforin,[88] phospholipase D,[89] cytotoxin,[90] and tryptophan synthetase.[91,92] Notably, only *C. trachomatis* serovars that cause urogenital disease, and not trachoma strains, encode a functional tryptophan synthase.[91,92] Urogenital strains may have retained this enzyme to convert indole, produced by vaginal microbiota, into tryptophan as a defense mechanism to counter IFN-γ–mediated tryptophan depletion.[93,94] Another difference is that the enzymatic portion of the cytotoxin gene is only present in urogenital, but not ocular or LGV, strains.[95] A family of autotransporters, called the polymorphic membrane proteins, has a high concentration of single nucleotide polymorphisms, which appear to correlate with tissue tropism.[96,97] Genetic differences have also been identified between urogenital and rectal clinical isolates of *C. trachomatis*.[98] This identification of strain-specific differences will be made easier by new methods for whole-genome sequencing of chlamydial clinical isolates without cultivation.[99,100]

Recombination between *C. trachomatis* strains is another source of genetic diversity.[86,101] It occurs in nature, most commonly between strains that infect the same tissue site, providing evidence for mixed infections with more than one *C. trachomatis* strain.[102] In addition recombination has been documented between urogenital and ocular strains, and even between trachoma and LGV biovars.[102] As a dramatic example, a hypervirulent *C. trachomatis* LGV strain isolated from a patient with severe hemorrhagic proctitis was found to be a recombinant of an invasive L2 strain and a noninvasive urogenital D strain.[103] Nevertheless, the high degree of genomic conservation among *C. trachomatis* strains suggests that most recombination events are not fixed as gene gain or loss. Recombination events have been fixed at a higher rate near the MOMP gene (*ompA*), most likely because of selective pressure from the host immune system. *ompA* has transferred between phylogenetically unrelated strains on multiple occasions, leading to serovar switching and chimerism.[86,102] These findings explain why MOMP is not a good phylogenetic marker[104] and why MOMP strain typing does not correlate well with clinical manifestations.[105–107]

Experimental methods to genetically manipulate chlamydiae have recently become available.[108,109] These methods include transformation of chlamydiae,[110–112] generation of mutants by chemical mutagenesis,[113] and, in a few instances, targeted gene disruption and replacement.[114,115] Chlamydiae appear to be naturally competent because recombinant progeny can be readily obtained by coinfecting the host cell with two chlamydial strains.[116] The phenotypes of mutants can be studied with linkage analysis, conditional mutants, and complementation.[117,118] These approaches have been aided by the use of whole-genome sequencing to identify genetic alterations that can then be related to phenotypes in cell culture and infection models.[119] These methodologic breakthroughs provide the potential for forward and reverse genetic approaches to study chlamydial gene function. However, their widespread use has been limited by the low efficiency of EB transformation and the time (in months) to produce and isolate mutants. The downsized *C. trachomatis* genome is expected to contain a high proportion of essential genes that cannot be deleted.

PATHOGENESIS

The target cells of the trachoma biovar of *C. trachomatis* are the squamocolumnar epithelial cells of the endocervix and upper genital tract in women, the epididymis and perhaps the prostate in men, and the conjunctiva, urethra, rectum, and to a lesser extent the pharynx in both women and men.[120–123] In infants, the columnar epithelial cells of the respiratory tract are also commonly infected.[120] *C. trachomatis* strains exhibit strong tissue tropism during natural infection, thereby producing very different clinical presentations; genital serovars preferentially infect genital tract epithelial cells, ocular serovars favor conjunctival epithelial cells, and LGV serovars infect macrophages and spread to lymph nodes.[124]

Regardless of the site, the initial response to infection appears to be primarily a polymorphonuclear leukocyte response.[125–127] Infected epithelial cells produce proinflammatory cytokines, such as the chemokine interleukin (IL)-8, that direct the initial innate and acquired responses to chlamydial infection and drive inflammatory responses that both produce disease and resolve the infection.[128–131] LPS may be the predominant chlamydial antigen capable of inducing proinflammatory cytokines.[132] In immortalized human cells, IL-8 induction depends on host cell signaling,[133] in part via the MEK-ERK pathway.[134] Independently, IL-8 is also induced via the pattern response receptor nucleotide-binding oligomerization domain 1, which is a peptidoglycan sensor.[135] *C. trachomatis* pathogenesis is often studied with the related species *Chlamydia muridarum,* which offers a convenient mouse model of human genital infection.[136,137] *C. muridarum* infection of mouse oviduct epithelial cell lines induces a variety of cytokines, including IL-1α, IL-6, tumor necrosis factor-α, and cytokines that augment IFN-γ production, including type I interferons IFN-α/β and IL-12p70.[131,138] In this mouse model of chlamydial genital infection, Toll-like receptor 2 (TLR2) is the principal pattern response receptor responsible for induction of acute-phase mediators and chronic inflammatory pathology.[139,140] Chlamydiae can also be detected by the intracellular nucleotide sensors cyclic GMP-AMP synthase (cGAS) and STING (stimulator of interferon genes), which induce expression of type I interferons.[141,142] Mice deficient in receptors for type I interferons (α and β) have reduced duration and shedding of organisms during infection, less oviduct pathology, and enhanced CD4 recruitment to cervical tissue compared with wild-type mice, suggesting that induction of type I interferons exacerbates infection by inhibiting the specific CD4 response to chlamydiae.[143]

The initial neutrophilic infiltration is followed by tissue infiltration with lymphocytes, macrophages, plasma cells, and eosinophils.[120] In ocular and genital infections, plasma cells are generally present in large numbers,[144,145] whereas in infant pneumonia, eosinophils and neutrophils predominate.[126] In ocular and genital disease with the trachoma biovar, lymphoid follicles (aggregates of lymphocytes and macrophages in the submucosa) form as the acute inflammation begins to subside. There is thinning or loss of epithelium overlying the follicles, and they may become necrotic as the disease progresses.[120] In the conjunctiva, these follicles are clinically apparent as raised avascular lesions. Epithelial proliferation leads to formation of papillae and papillary hypertrophy. As the infection then begins to resolve, fibrosis and scarring occur.

Initial infection of the eye in humans[120] and of the eye[145] and genital tract in monkeys[146] resolve with little or no residual tissue damage. However, in the human eye[147] and the monkey genital tract,[148] as few as two to three repeated infections produce an accelerated and more intense inflammatory response with scarring and tissue damage; multiple rechallenges amplify the effect in the primate eye.[149] The potentially important role of reinfection in chlamydial disease was first recognized during human trachoma vaccine trials in which volunteers were immunized and then subsequently challenged with live organisms.[150] In these and other studies in humans and monkeys, limited serovar-specific protection against infection could be induced. However, when infection did occur after immunization in humans, it was more severe than in unvaccinated people and the increased severity was not serovar specific.

Studies have raised questions about the relative contributions of conjunctival infection by *C. trachomatis* and other bacterial pathogens to trachoma development.[151] It was previously thought that a chlamydial component, the chlamydial 60-kDa heat shock protein (cHSP60), was driving disease expression by hypersensitivity mechanisms. Although serologic data show an association between antibody response to cHSP60 and risk of PID[152] and scarring trachoma,[153] these responses are likely due to complicated or severe disease and are not reflective of a causal link. In early follicular and intense trachoma, the inflammatory response and early disease expression are driven and sustained by *Chlamydia*-infected nonimmune epithelial cells. However, trachomatous scarring in adults shows a greater association with other bacteria than with *C. trachomatis* infection.[151] It has therefore been proposed that *C. trachomatis* plays an initiating role but that chronic conjunctival inflammation and tissue damage may be due to the host immune response to other bacterial pathogens after the *C. trachomatis* infection.

Chlamydial infection induces production of IFN-γ,[154] which inhibits chlamydial replication[155] and in animal models shortens the duration of infection.[156] In cell culture, it induces a dose-related persistent infection as described in "Chlamydial Biology" earlier. However, subsequent removal of IFN-γ allows the organisms to be rescued and to resume replication and conversion into infectious EBs. Long-lasting infections are known to occur relatively frequently in the absence of treatment in women[157,158] and in men.[159] The in vivo bacterial and inclusion morphology of these long-lasting infections in people is unknown. However, it is possible that cyclic changes in inhibitory cytokines, chlamydial replication, and antigen production and the resulting continued inflammatory signaling from nonimmune epithelial cells could explain the chronic inflammation and scarring often associated with chlamydial infections.[160]

The LGV biovar of *C. trachomatis* is distinctive in being able to cause a systemic infection. This organism gains entrance through breaks in the skin or infects epithelial cells of the mucous membranes of the genital tract or rectum. It has tropism for the lymphatic system and is carried by lymphatic drainage to the regional lymph nodes, where it multiplies inside mononuclear phagocytes.[120] Bacteremic spread may also occur, and the central nervous system may be infected. The characteristic histopathology is that of granuloma formation with development of small abscesses that may become necrotic or coalesce into suppurative foci.[161,162]

Almost all strains of *C. trachomatis* carry a conserved 7.5-kb plasmid that has a role in pathogenesis.[163] It encodes eight proteins, including Pgp3, which is an effector that is secreted into the host cytosol, and an immunodominant antigen that is being used as a serologic marker.[164,165] Another plasmid gene, *pgp4,* regulates transcription of chromosomal genes involved in virulence and glycogen synthesis.[166] Rare *C. trachomatis* clinical isolates lacking this plasmid have been described.[167] Studies with chlamydial strains experimentally cured of the plasmid show that the plasmid modulates chlamydial exit from host cells, infectivity, and virulence.[163,168,169] For example, a plasmid-cured *C. muridarum* strain was able to infect the lower genital tract but did not produce upper tract disease in the mouse oviduct because it failed to activate TLR2-dependent cytokine production.[170] These results suggest that the plasmid controls expression of the chlamydial TLR2 ligand, which has not yet been identified. Plasmid exchange between different *C. trachomatis* strains only occurs infrequently.[102]

There is evidence that chlamydial infections may involve a mixed population of genetic variants. These variants may differ in pathogenicity, such as their ability to cause lower versus upper genital tract disease.[171] Experimental work with animal models indicates that these variants can compete or cooperate, and that mixed infections may differ from monoinfection with a single variant.[171]

IMMUNITY

Natural infection with *C. trachomatis* appears to confer little protection against reinfection, and this limited protection is short-lived.[172] Multiple or long-lasting infections are an essential factor in the pathogenesis of trachoma.[173] Rates of repeated infection are also high in young, sexually active individuals with genital tract infections: 29% of men and women attending an STI clinic during a 3½ -year period,[174] as well as 38.4% of adolescent women observed prospectively for up to 2 years, had repeated infections.[175] However, other data suggest that genital tract infections confer at least partial immunity against reinfection. In women with endocervical infection, the presence of secretory immunoglobulin (Ig) A correlated inversely with the numbers of organisms shed.[176] Men experiencing their first episode of nongonococcal urethritis (NGU) are more likely to have *C. trachomatis* recovered from their urethra than men with a prior history of NGU.[177] A study of commercial sex workers demonstrated reduced chlamydial detection with increasing duration of sex work.[178] In another study of female sex workers in Nairobi, protective immunity against incident infection was observed and correlated with cHSP60-specific IFN-γ production in peripheral blood mononuclear cells.[179] In the trachoma vaccine trials mentioned previously, partial serovar-specific immunity could be elicited, but protection lasted for only 1 or 2 years.[173] Repeated genital infections in adolescent women are common after effective treatment,[77] and it has even been suggested

that treatment may inhibit the development of protective immunity.[180] In support of this intriguing hypothesis, women who spontaneously resolved an uncomplicated chlamydial infection before they could be treated were less likely to have reinfection at 1 to 12 months than women whose infections were ended by treatment.[181]

In mouse models of *C. muridarum* infection, CD4 lymphocytes of the Th1 type that traffic to the genital mucosa are crucial for restriction of intracellular growth and resolution of infection.[182] Antibodies directed at epitopes on MOMP are neutralizing in cell culture and may play a role in reducing acquisition of infection.[183] In the mouse genital infection model, antibodies against MOMP or LPS partially protect against reinfections but not primary infections, indicating that the protective effect is dependent on CD4-mediated adaptive immunity acquired at the initial infection.[184] Antibodies may also influence the severity of upper genital tract pathology in the mouse.[185] IgA antibodies are not absolutely required for protective immunity in this model.[186] Both antibody and cell-mediated mechanisms are important in protective immunity in the guinea pig model.[187] It is possible that antigen presentation in natural mucosal infection may be relatively ineffective in producing strong protective immunity, because dendritic cells pulsed in vitro with inactivated chlamydiae are capable of conferring protective immunity in the mouse model.[188] The current consensus is that $CD4^+$ T cells and B cells are most critical in mediating recall immunity to *C. trachomatis* infection and $CD8^+$ T cells are less important.[189] The latter may exert antichlamydial activity by production of IFN-γ rather than by cytotoxic activity. In *C. muridarum* genital tract infections in the mouse, two independent subsets of *Chlamydia*-specific CD4 T cells exhibit different effector functions, one dependent on inducible nitric oxide synthase (iNOS) and the other requiring expression of *Plac8.* If *Plac8*-deficient mice are treated with an iNOS inhibitor, the mice are unable to resolve genital tract infections.[190]

The natural history of human infection is not well defined, but we know that many infections resolve without treatment, while many are long-lasting. In one of the few studies of its kind, Molano and colleagues[158] found that 46% of untreated women had persistent infection with identical strains at 1 year. These infections were likely to represent long-lasting infection rather than reinfection because most of these women were older than 30 years of age, and 83% reported a single lifetime sexual partner. It is not clear how *C. trachomatis* is able to avoid immune clearance for prolonged intervals or why an effective response is so slow to develop.[191] One hypothesis is that *C. trachomatis* may utilize nutrients produced by the genitourinary microbiota to evade inhibitory effects of IFN-γ, which restricts chlamydial replication by inducing tryptophan starvation. *C. trachomatis* is auxotrophic for tryptophan, but urogenital serovars can use indole to synthesize tryptophan. The microbiomes of the vagina in women and the urethra in men sometimes contain *Prevotella* species and other bacteria that produce indole.[192,193] In vitro studies indicate that vaginal secretions, and bacterial supernatants containing indole, mitigate the inhibitory effect of IFN-γ on chlamydial growth.[93,94] Additional chlamydial genes not involved in the tryptophan biosynthetic pathway appear to contribute to IFN-γ resistance.[194]

Vaccine development efforts over the past 20 years have been directed at defining relevant epitopes for use as components in a synthetic or genetically engineered vaccine.[195,196] This subunit approach is important because of the combination of protective and deleterious effects produced by infection or vaccination with the whole organism.[129] Studies of DNA vaccines utilizing the *ompA* gene of *C. muridarum* showed reduced organism burden and mortality in a mouse pneumonia model,[197] but similar studies have not demonstrated an influence on the course of experimental genital infection in mice.[198] Vaccines composed of native conformations of trimeric MOMP of *C. muridarum* compounded with human vaccine adjuvants have been shown to induce partial protection in genital tract challenge in mice.[199] Chlamydial protease–like activity factor (CPAF) is a possible vaccine candidate because it is an immunodominant antigen, and mice immunized with recombinant CPAF, together with IL-12 or CpG, are protected against genital challenge.[200] Promising recent data have established that strains of *C. trachomatis* and *C. muridarum* lacking the 7.5-kb plasmid are attenuated for pathology in the primate eye[201] and in the mouse oviduct[202] even in the absence of sterilizing immunity. Such plasmidless strains hold promise as live-attenuated vaccines against both trachoma and complications of genital infection, including PID.[201,203]

Efforts are also underway to improve vaccine response by developing mucosal vaccines. For example, a vaccine composed of ultraviolet light–inactivated *C. trachomatis* conjugated to synthetic adjuvant nanoparticles induced a robust systemic memory T-cell response and long-lived protection from genital infection in mice immunized via the intrauterine or intranasal route.[204]

LABORATORY DIAGNOSIS

Among *C. trachomatis* infections, only trachoma can be diagnosed on clinical grounds alone, given the proper epidemiologic setting in a high-prevalence area.[205] Other chlamydial infections are often associated with specific clinical syndromes but require laboratory confirmation for definitive diagnosis.[206,207] Detection of *C. trachomatis* indicates the presence of an infection because the organism is intrinsically invasive as an intracellular pathogen and there is no extracellular colonization state. The mainstay of modern diagnostic testing of *C. trachomatis* is the nucleic acid amplification test (NAAT). Other laboratory methods for detection of chlamydial antigens and nucleic acids have been largely superseded as routine diagnostic tests. Microbiologic identification by isolation of *C. trachomatis* in cell culture remains important but in a specialized role.

Nucleic Acid Amplification Tests

NAATs are the preferred diagnostic and screening test for *C. trachomatis* genital infection in the United States because they are sensitive and can be used for noninvasive testing without the need for a pelvic examination or a urethral swab.[208–210] The optimal specimens are a vaginal swab in women,[211,212] which can be obtained by either a clinician or the patient,[213] and first-catch urine in men.[214] Some NAATs have been cleared by the US Food and Drug Administration (FDA) for use on liquid-based cytology specimens collected for Papanicolaou smears.[215] NAATs target and amplify conserved nucleic acid sequences that are present in almost all clinical strains of *C. trachomatis,* including the urogenital, LGV, and ocular serovars.[210] However, NAATs are only approved by the FDA for diagnosis of *C. trachomatis* urogenital infections and have not been cleared to detect the organism in extragenital sites, such as the rectum, oropharynx, and eye.[209] Some laboratories have performed in-house validations for NAAT testing of extragenital specimens in order to conform to Clinical Laboratory Improvement Amendments (CLIA) regulations. Some NAATs can also detect *Neisseria gonorrhoeae,* providing a convenient means to test for chlamydial infection and gonorrhea in the same clinical specimen.[210]

NAATs are considerably more sensitive and nearly as specific as culture, which was the historical gold standard for identifying *C. trachomatis.*[206,207] The high sensitivity is due to the amplification step, which allows detection of chlamydial nucleic acids from a single infected host cell. NAATs are 15% to 20% more sensitive than culture performed in an experienced laboratory and may be up to 40% to 50% more sensitive than culture or enzyme immunoassay tests in other settings (Table 180.1). NAATs can be used to detect *C. trachomatis* in traditional urogenital specimens, including endocervical swabs in women and urethral swabs in men, which require an invasive procedure for collection. However, a major advantage is that NAATs have a comparable high sensitivity for noninvasive specimens, such as a vaginal swab or first-catch urine.[211,212,214] Thus noninvasive testing of chlamydial infections is now possible, allowing innovations such as self-collected vaginal swabs that make widespread screening and prevention programs feasible.[213] NAATs have also been shown to be sensitive and specific for detection of *C. trachomatis* infection of the eyes in patients with trachoma[216] and in the rectum and oropharynx in MSM.[217,218]

Although NAATs are sensitive, a number of disadvantages have been discussed.[219] They are expensive and thus may not be affordable by health departments for comprehensive screening. In addition, they may be technically demanding for some routine laboratory settings, resulting in reproducibility issues and false-positive and false-negative rates that are higher than the reported rates. Another disadvantage is that the current FDA-approved NAATs do not distinguish between LGV and

TABLE 180.1 Comparative Performances of Selected Diagnostic Tests in the Detection of *Chlamydia trachomatis*

TEST	SENSITIVITY RELATIVE TO EXPANDED GOLD STANDARD (%)[a]	SPECIFICITY (%)	DETECTABILITY LEVEL (ELEMENTARY BODIES)
Enzyme immunoassay	40–60	99.5[b]	1000–10,000
Nonamplified genetic probe	40–65	99.0	1000–10,000
Direct fluorescent antibody	50–80	99.8	50–1000
Cell culture	50–90	99.9	10–100
Nucleic acid amplification tests	Cervix: 81–100 Vagina: 91–98 Male urine: 90–96	99.7	1–10

[a]Defined using a combination of different test methodologies, including culture, direct fluorescent antibody, and polymerase chain reaction (PCR) or ligase chain reaction (LCR) directed against a target sequence distinct from that used in the routine PCR or LCR assays.
[b]Specificity using confirmatory assays.

non-LGV strains, which is important because the duration of treatment is longer for LGV infections. Nonapproved NAATs that can distinguish between LGV and non-LGV strains have been described but are not widely available.[220] Another issue is that NAATs detect chlamydial DNA or RNA rather than live organisms, and positive NAATs are not uncommon 3 weeks after completion of antibiotic therapy.[221] Thus the NAAT should not be used as a test-of-cure assay, except in pregnant women in whom it is justified to document cure at 3 to 4 weeks after completion of therapy in efforts to prevent infection in the infant.[209]

Commercially available NAATs differ in the method used to amplify chlamydial nucleic acids from a clinical sample.[210] Although the tests also amplify different DNA or RNA sequences, these targets are not intrinsic to the amplification method and can be altered if necessary. Transcription-mediated amplification is sensitive because it amplifies *C. trachomatis*–specific 23S ribosomal RNA, which is present in high copy number. The DNA strand displacement assay amplifies sequences on the chlamydial plasmid. It has excellent sensitivity because the chlamydial plasmid is present in all clinical isolates, with rare exceptions, and there are approximately four to eight copies of the plasmid in each organism.[222] Polymerase chain reaction (PCR) is another commonly used amplification method that amplifies different sequences on the chlamydial plasmid or chromosome. Ligase chain reaction was a chlamydial NAAT method that was used for many of the initial validation studies, but it is no longer available commercially.[223]

In 2006, a new variant strain of *C. trachomatis* that was not detectable with a PCR-based NAAT was discovered in Sweden.[224] This strain has a 377-bp deletion in the region of the chlamydial plasmid that was amplified by two different PCR-based NAATs (AMPLICOR [CT/NG] Test [Roche Diagnostics, Indianapolis, IN] and *m*2000 RealTi*m*e [Abbott Laboratories, Abbott Park, IL]), resulting in false-negative tests. Its discovery was prompted by an unexpected decrease in chlamydial prevalence in Sweden due to underreporting from counties that used these two NAATs compared with other counties that used a strand displacement NAAT assay that targeted a different region on the plasmid.[225] Once recognized, the strain was found to be highly prevalent in counties where it was not being detected, demonstrating that missed diagnosis and treatment of infected individuals can confer a diagnostic selective advantage that promotes the spread of a particular strain. It also provides an important lesson about the limitation of detecting a pathogen by only targeting a single small region of DNA or RNA. In addition, the plasmid may not be an ideal NAAT target because other deletion and recombination events within the plasmid have been reported.[102] Improved versions of the Roche and Abbott PCR-based NAATs targeting two regions of the *C. trachomatis* genome are now available. However, there are still other FDA-approved *Chlamydia* NAATs that only target a single sequence on the *C. trachomatis* plasmid or chromosome.[210] The Swedish new variant strain of *C. trachomatis* has been largely restricted to the Nordic countries, where its prevalence has decreased with the use of NAATs that detect both wild-type and variant strains.[226] So far, this clonal strain has not been detected in the United States.[227]

Antigen Detection and Nucleic Acid Hybridization

Before the introduction of NAATs, a number of molecular tests were developed to detect chlamydial antigens or nucleic acids in clinical samples without the need to culture the organism.[207] The three main nonculture tests are direct fluorescent antibody (DFA) staining and enzyme-linked immunoassay (EIA), which both detect chlamydial antigen, and nucleic acid hybridization, which uses a DNA probe to detect chlamydial ribosomal RNA without an amplification step. These tests have sensitivities of 60% to 80% and specificities of 99% at best, which provide acceptable positive and negative predictive values, except in low-prevalence populations (<5% infected) where the false-positive rate is high. However, this level of sensitivity can only be achieved with invasive specimens, such as a cervical swab in women or a urethral swab in men. In general, the sensitivity is higher in patients who are symptomatic and shedding large numbers of chlamydiae compared with asymptomatic patients shedding fewer organisms.[228,229] The *C. trachomatis* nonculture tests have been largely replaced because of the superior performance of NAATs even when performed on noninvasive specimens.

Isolation in Cell Culture

Culture has been supplanted by NAATs as the routine method for detecting *C. trachomatis* in clinical samples because it has lower sensitivity and is more difficult to perform. Even under optimal conditions, the sensitivity of culture is estimated at between 70% and 80% on the basis of the comparison to the more sensitive NAAT and may be as low as 40% to 50% outside experienced laboratories.[206,207] Many of the relative disadvantages of culture stem from the need to grow the organism in cell culture, which requires expertise, proper handling and storage of specimens, and a long turnaround time.[230] *C. trachomatis* is most commonly cultivated in HeLa or McCoy cells, but the bacterium grows well in a variety of cell lines.[231] The most sensitive and specific way to identify intracytoplasmic inclusions is by direct immunofluorescent staining with antichlamydial monoclonal antibodies, which is usually performed after 36 to 72 hours of incubation. The chlamydial inclusion can also be detected by staining with Giemsa stain.[230] *C. trachomatis* differs from other *Chlamydia* spp. in containing glycogen, which can be stained with iodine.[206]

The isolation rate of cell culture depends on the number of viable organisms in the clinical specimen.[228] For example, the highest concentrations of chlamydiae, and thus the highest isolation rates, are with ocular infections such as active trachoma and neonatal or adult inclusion conjunctivitis. In urogenital infections, many more organisms are recovered from the endocervix compared with the male urethra, and even fewer from the female urethra.[232] Infections associated with symptoms and signs, as well as infections in younger individuals, generally have higher isolation rates.[233]

Chlamydial culture is still used as a specialized diagnostic method and for research purposes.[230] One important advantage is that it allows recovery of the clinical isolate, which molecular methods such as NAATs

do not. Culture can be important for public health issues, including antibiotic susceptibility testing and identification of outbreak or mutant strains. The high specificity of culture, which approaches 100%, makes it the recommended test for medicolegal evaluation of child abuse, although the NAAT can be used for *C. trachomatis* detection from the urine or vagina in girls.[209] In sexual assault in adults and adolescents, NAATs are recommended because they are more sensitive and widely available.[209]

Cytologic Diagnosis

Typical intracytoplasmic inclusions and free chlamydiae can be identified in Giemsa-stained cell scrapings from the eye. Stained conjunctival scrapings are positive in 90% of infants with neonatal conjunctivitis and 50% of adults with inclusion conjunctivitis. It is less sensitive for active trachoma, in which only 10% to 30% of conjunctival scrapings are positive.[234] Cytology has also been used to evaluate endocervical scrapings, including those obtained for Papanicolaou smears, but the sensitivity and specificity are low.[235]

Rapid Tests

There has been great interest in the development of rapid tests for *C. trachomatis* infection, to shorten the time between sample collection and test results. Currently, the best rapid test for *C. trachomatis* is the Xpert CT/NG (Cepheid, Sunnyvale, CA) rapid PCR test, which can produce results in approximately 90 minutes.[236] This test is a rapid NAAT for *C. trachomatis* and *N. gonorrhoeae,* and it has performance in both low- and high-prevalence populations similar to existing NAATs, which typically have a processing time of 1 to 2 days. The CT/NG GeneXpert has been approved by the FDA as a "moderate complexity" test, which means that it can be performed in many more clinic and hospital laboratories than other NAATs, which are classified as "high complexity" tests. It has been approved for use on endocervical and vaginal swabs and for urine.

The ideal rapid *Chlamydia* test is a point-of-care (POC) test that is performed while the patient is being evaluated, producing immediate, yet sensitive and specific, results. POC *Chlamydia* tests have been developed that use a rapid immunoassay to detect chlamydial antigens in a patient specimen. These tests are similar in principle to a rapid group A streptococcus or pregnancy test and have the potential to be simple to perform and to interpret. However, current non-NAAT *C. trachomatis* POC tests have low sensitivities compared with NAATs, and reported performances have been highly variable from study to study.[237–239] These *Chlamydia* POC tests are commercially available in some countries but have not been approved for use in the United States. A portable NAAT that can produce results in 1 hour has been developed and evaluated as a POC test in a hospital emergency department setting.[240] It utilizes a microfluidic cartridge to capture and process chlamydial nucleic acids, and a mobile phone to operate the cartridge and to collect the optical signal with its built-in camera.

Serology

Serologic tests are not recommended for diagnosis of most common oculogenital *C. trachomatis* infections.[206] Anti-*Chlamydia* IgM is infrequent in adults with genital tract infection, whereas anti-*Chlamydia* IgG is long lived and cannot distinguish past from current infection. Although a rise in convalescent titers can be used to diagnose an acute infection, it is not a practical option given the wide availability of *Chlamydia* NAATs. Serology can be used to support a diagnosis of LGV infection because commercial NAATs do not distinguish between LGV and non-LGV serovars of *C. trachomatis,* but this role is limited because reliable serologic testing for LGV is not widely available.[210]

The complement fixation test has been used to diagnose LGV, but it is technically demanding and performed in few clinical laboratories.[206] It measures complement-fixing antibodies against the genus-specific LPS antigen, which is present in *C. trachomatis* as well as *Chlamydia pneumoniae* and *Chlamydia psittaci.* Thus it lacks specificity because it can also be positive in patients with recently acquired *C. pneumoniae* infections and some patients with oculogenital infections, although titers tend to be higher with LGV because it is a more invasive disease. A confirmed result requires a fourfold or greater rise in titer between acute and convalescent sera, but patients with LGV often present 3 or 4 weeks into their illness when their antibody titers are stable. Complement fixation serology has no diagnostic utility for *C. trachomatis* urethritis, cervicitis, or conjunctivitis because most patients with these infections have low or nonexistent titers.

The microimmunofluorescence (MIF) test is the historical gold standard for detection of chlamydial antibodies because of its sensitivity, and species and serovar specificity. It is not widely used because it is primarily available in research laboratories,[206] although less well-characterized immunofluorescence serologies are commercially available. In its most common format, elementary bodies from each of the 15 *C. trachomatis* serovars are used as antigens to detect antibodies against chlamydial cell wall components.[74] This test reliably differentiates among *C. trachomatis, C. pneumoniae,* and *C. psittaci,* and occasionally, but not reliably, among *C. trachomatis* serovars. In an experienced laboratory it is more sensitive than the complement fixation test. Many adult patients with symptomatic eye or genital infection have a fourfold rise in antibody titers between acute and convalescent sera. Titers are especially high in women with PID or perihepatitis, in whom its diagnostic sensitivity may be highest. However, among *C. trachomatis* infections, the MIF test is most useful for diagnosing neonatal chlamydial pneumonia because almost all affected infants test positive, with an IgM titer of 1:32 or greater.[241] Anti-*Chlamydia* IgM is also present in approximately 30% of infants with neonatal inclusion conjunctivitis.[241]

The limited availability of the MIF test and the interlaboratory variability in its interpretation emphasize the need for more clinically practical serologic tests. As an example of an assay in development, an enzyme-linked immunosorbent assay test using whole EBs as antigens has been reported to be more sensitive than a commercially available MOMP peptide-based assay and able to distinguish between *C. trachomatis* and *C. pneumoniae* infection.[242] This and other assays may prove useful in defining seroprevalence and duration of antibody positivity after infection in advance of future vaccine trials. In addition, reproducible assays will be necessary to document preexisting antibodies and the response after vaccination.

CLINICAL MANIFESTATIONS

C. trachomatis infections can be divided into four clinical categories: (1) trachoma, (2) oculogenital diseases in adults, (3) perinatal infections, and (4) LGV.

Trachoma

Trachoma is a chronic keratoconjunctivitis caused by repeated infections with serovars A, B, Ba, and C of *C. trachomatis.*[205,243] The disease has been recognized as a cause of blindness since antiquity, with therapy for trachoma and its complications described in China in the 27th century BC and in Egypt in the 19th century BC.[205] The countries with the highest prevalence are in sub-Saharan Africa, but about half of all active trachoma cases are located in just five countries: Ethiopia, India, Nigeria, Sudan, and Guinea.[244,245] In high-prevalence areas, active trachoma is present in more than 50% of children younger than 10 years of age.[245] The economic burden from blindness and lost productivity has been estimated to be $5.3 billion annually.[246]

Active *C. trachomatis* infection mostly occurs in children, but a small proportion of individuals go on to have complications of impaired vision and blindness in adulthood.[205] In areas where trachoma is endemic, the first infection usually occurs in the first 2 years of life, and active infection and inflammation persist for several months. Although initial infections may resolve spontaneously, they are frequently followed by reinfection or by superimposed bacterial conjunctivitis. This active stage of trachoma manifests as a chronic follicular conjunctivitis with papillary hypertrophy and inflammatory infiltration. Individuals are frequently asymptomatic, but can have mild symptoms of chronic conjunctivitis with redness, discomfort, photophobia, and mucopurulent discharge.[205] Many individuals will clear the infection and inflammation without sight-threatening consequences. However, in a significant minority there is scarring of the tarsal conjunctiva lining the eyelid. Over years, this distortion of the eyelid causes the eyelashes to turn in and abrade the cornea (trichiasis), resulting in ulceration, scarring, corneal opacification, and loss of vision as an adult, typically at 30 to 40 years of age. Trachoma

is a clinical diagnosis, and laboratory detection of *C. trachomatis* is not used for diagnosis.[1] The WHO simplified grading scheme for evaluating trachoma by direct examination of the conjunctiva is presented in Table 180.2. The assessment can be easily performed in the field, and the only equipment necessary is a pair of binocular loupes, which are simple magnifying glasses.

In endemic areas, children with ocular infection are the primary reservoir.[205] Transmission occurs by hand-to-eye contact between children and their caregivers, by contact with fomites contaminated with ocular or nasal secretions, and via the feet of flies. Flies feed on the exudate from children with active conjunctivitis and act as a mechanical vector. Hygienic factors important for control of disease include face washing, access to clean water, improvements in sanitation, and reduction of household fly density. Although global estimates of trachoma prevalence have limitations, a downward trend has been noted in both active trachoma and trachoma-related blindness since 1981.[245]

Treatment

Individuals can be successfully treated with systemic antibiotics, but in high-incidence areas they will be easily reinfected, and treatment of the entire community is necessary to have an impact.[205,243] WHO recommends mass drug administration in communities in which more than 10% of children have follicular trachoma. More than 85 million people worldwide received single-dose azithromycin for trachoma in 2016.[2] Trials of mass treatment with azithromycin at the village level indicate that both infection and clinical disease are markedly decreased at 6 and 12 months, but multiple rounds of treatment over 3 to 5 years are necessary in hyperendemic areas.[247] Of the regimens recommended by WHO, single-dose oral azithromycin is the preferred treatment.[205] Topical application of tetracycline ointment to the eyes is an alternative, but adherence is difficult because of the 6-week course. In addition, topical treatment does not clear the bacterium from extraocular sites, such as the nasopharynx and rectum, that are also infected in children with trachoma.[205] Surgery for trichiasis is performed to correct in-turned eyelashes and has some value in preventing progression to corneal opacity and blindness, but recurrence of trichiasis after surgery is common, occurring in about 20% of persons at 1 year and in up to 60% at 3 years.[205] An added benefit of trachoma treatment programs is an associated reduction in childhood mortality, diarrhea, and respiratory infections.[243]

WHO has initiated a program to eliminate blinding trachoma by 2020.[205] The objective is not necessarily to eliminate the trachoma serovars of *C. trachomatis,* but to eliminate or at least markedly reduce clinically active disease. The key elements of the S-A-F-E strategy are *S*urgery for deformed eyelids, *A*ntibiotics administered as periodic mass treatment of villages with single-dose azithromycin, *F*ace washing and hygiene, and *E*nvironmental improvements to control flies, using approaches such as building latrines outside villages. In some areas, trachoma appears to be disappearing coincident with economic gains and without the introduction of specific control programs, illustrating the strong historic link between poor socioeconomic conditions and endemic trachoma.

TABLE 180.2 World Health Organization Simplified Grading Scheme for Trachoma

Trachomatous inflammation—follicular (TF): ≥5 follicles in the upper tarsal conjunctiva (follicles must be at least 0.5 mm in diameter).
Trachomatous inflammation—intense (TI): Pronounced inflammatory thickening of the tarsal conjunctiva, which obscures half of the normal deep tarsal vessels.
Trachomatous conjunctival scarring (TS): The presence of easily visible scars in the tarsal conjunctiva.
Trachomatous trichiasis (TT): At least one eyelash rubs on the eyeball. Evidence of recent removal of in-turned lashes was also graded as trichiasis.
Corneal opacity (CO): Easily visible corneal opacity present over the pupil, which was so dense that at least part of the pupil margin was blurred when seen through the opacity.

Modified from Thylefors B, Dawson CR, Jones BR, et al. A simple system of the assessment of trachoma and its complications. Bull World Health Organ. *1987;65:477–483.*

Oculogenital Disease in Adults

C. trachomatis serovars D through K, and occasionally B and Ba, produce a wide variety of oculogenital infections (Fig. 180.3).

Inclusion Conjunctivitis

C. trachomatis ocular infection is called inclusion conjunctivitis because of the pathognomonic presence of the prominent chlamydial inclusion inside infected epithelial cells. In adults, chlamydial eye infection manifests as an acute follicular conjunctivitis, often with a foreign body sensation in the eye. Symptoms are usually unilateral. The clinical picture in the first 2 weeks is dominated by hyperemia and a mucoid discharge that becomes purulent.[248] This is followed by lymphoid follicle formation (frequently with corneal lesions and epithelial keratitis[249,250]) and infiltration of the upper margin of the cornea by small blood vessels (pannus), which is indistinguishable from early trachoma. Preauricular lymphadenopathy and otitis media may be present.[249] The condition may persist for many months if untreated but usually resolves without complications.[251] Scarring similar to that seen in mild trachoma may occur in occasional cases. A common scenario for chlamydial ocular infection is conjunctivitis for weeks to months that has not responded to topical antibiotics. The differential diagnosis is primarily conjunctivitis caused by adenovirus or other viruses.[250]

Slightly more than half of adults with inclusion conjunctivitis have a concurrent *C. trachomatis* genital tract infection, although they may not have genital symptoms.[251,252] In such individuals, the presumed mode of transmission is autoinoculation of the eye with infected genital secretions or, in some cases, direct inoculation from an infected partner. Eye-to-eye spread between individuals by transfer of infected secretions without sexual contact may also occur.[252] In adolescents and adults, the peak frequency of *C. trachomatis* as a cause of keratoconjunctivitis is in 16- to 20-year-olds, accounting for 9% of cases and most likely reflecting high sexual activity and risk of chlamydial genital infection.[251] However, for perspective it is important to remember that chlamydial ocular infection is an uncommon complication that is seen in less than 1% of individuals with proven genital tract infection.[251,252]

A definitive diagnosis can be made by demonstration of the organism by culture if it is available. Of the nonculture tests, DFA has been approved for detection of chlamydiae in conjunctival swabs. NAATs can also be used, but are not FDA approved. The condition responds promptly to the administration of appropriate systemic antibiotics such as azithromycin or doxycycline with decreased discharge, hyperemia, and keratitis symptoms within 48 hours.[248] The patient and partners should be evaluated and treated for genital tract infection.

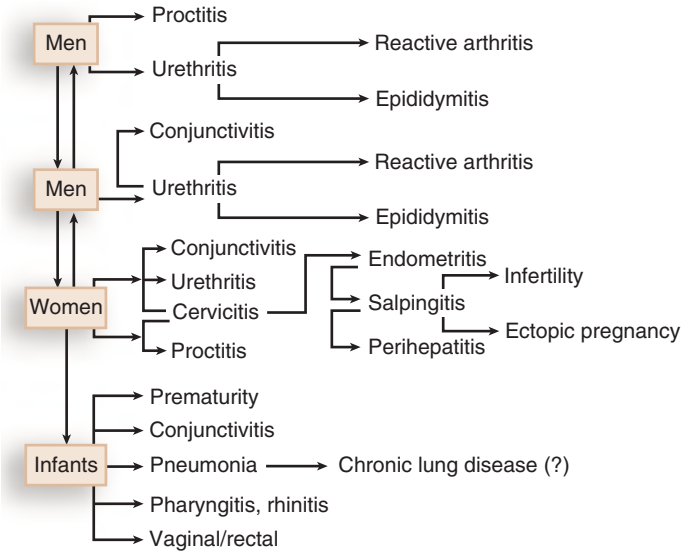

FIG. 180.3 Clinical manifestations and sequelae of *Chlamydia trachomatis* urogenital infections and patterns of transmission.

Urogenital Infections

C. trachomatis urogenital infections are extremely prevalent, accounting for 60% of infections reported to the CDC each year.[253] *C. trachomatis* is the most common cause of bacterial STI in the United States and the world.[4,6] Direct medical costs of chlamydial genital infections in the United States are estimated to be more than $500 million each year (in 2010 dollars).[254]

The risk of acquisition of *C. trachomatis* from a single episode of sexual intercourse with an infected partner has been estimated to be substantially less than that of *N. gonorrhoeae.*[255] By extrapolating data from partner notification programs, and from couples with discordant cell culture–proven infection, the transmission probability within a heterosexual partnership has been estimated to be 0.39 from men to women and 0.32 from women to men.[256] Another heterosexual partnership study that used a NAAT to establish infection found that 72.9% of male partners of positive women, and 68.6% of female partners of positive men, were themselves infected.[257] A modeling study used similar concordance data from an earlier partnership study[258] to estimate transmission probabilities of 0.40 to 0.68.[259] However, these estimates are based on the average frequency of intercourse within a partnership and do not represent the transmission probability of a single encounter. The transmission probability from a single act of unprotected coitus has been estimated to be 0.13 from a stochastic model[260] that used data from a longitudinal study of young women[77] and 0.10 from a partnership study.[259]

In contrast to gonococcal infections, *C. trachomatis* genital infections have characteristics of a prevalent infection rather than an incident infection. Recent exposure to a new partner was much more strongly associated with gonorrhea than with chlamydial infection.[261] In adolescents, chlamydial infection was strongly associated with frequency of intercourse, both in those reporting one lifetime partner and in those reporting more than one partner.[262] These differences may be explained by the less overt presentation of chlamydial infection. Most individuals infected with *N. gonorrhoeae* develop symptoms and seek care quickly, but many men and most women with *C. trachomatis* are either asymptomatic or minimally symptomatic and are only diagnosed as a result of screening or because a contact is symptomatic.[261] Women were also less likely to be infected with *C. trachomatis* if their male partners were identified through a screening program rather than a symptomatic infection.[263]

C. trachomatis is recovered more often from women who acquire gonorrhea than from similarly exposed women who do not have gonorrhea.[264] In individuals with gonorrhea, the recurrence of *C. trachomatis* infection with the same serovar is significantly greater than can be explained by variables related to likely exposure.[265] Furthermore, individuals infected with both *C. trachomatis* and *N. gonorrhoeae* shed larger numbers of *C. trachomatis* than those infected with *C. trachomatis* alone.[228] These data, based on cell culture diagnosis, suggest that acquisition of a gonococcal infection increases the likelihood of acquiring or detecting a chlamydial infection.

Urogenital Infections in Men

Urethritis

C. trachomatis is well known for being a major cause of symptomatic NGU.[266] There are differences in the clinical presentation of chlamydial and gonococcal urethritis, but a reliable distinction cannot be made on clinical grounds without testing.[267] The incubation period for symptomatic chlamydial urethritis is longer, taking 7 to 14 days compared with 4 days for gonococcal urethritis. Both infections present with dysuria, but the urethral discharge with chlamydial urethritis is usually white, gray, or sometimes clear, in contrast to the more purulent discharge observed with gonococcal urethritis.[268] The discharge of NGU may be so slight as to be demonstrable only after penile "stripping" or "milking" and only in the morning. Some patients may deny the presence of discharge but may note stained underwear in the morning. The preferred rapid diagnostic test for urethritis is a Gram stain of the urethral discharge. Previously, the definition of NGU for treatment purposes was 5 or more white blood cells per oil immersion field (1000× magnification), without organisms that looked like *N. gonorrhoeae.* The most recent 2015 STI treatment guidelines have reduced this threshold to 2 or more white blood cells per oil immersion field,[209] based on the finding that *C. trachomatis* is detected by a NAAT with appreciable frequency at this lower cutoff.[269]

In the United States, the incidence of NGU exceeds that of gonococcal urethritis by more than 2:1 in most STI clinics, with a higher ratio in many private practice settings.[270] *C. trachomatis* is a major cause of symptomatic NGU, outnumbering cases caused by *Ureaplasma urealyticum, Trichomonas vaginalis, Mycoplasma genitalium,* or herpes simplex virus.[271,272] The proportion of NGU due to *C. trachomatis* was 23%,[273] 43%,[274] and 42%[262] in three recent treatment trials. In addition, *C. trachomatis* can be recovered from approximately 20% of men with gonococcal urethritis.[275] Risk factors for chlamydial urethritis in men include age younger than 20 years, black race, and heterosexual orientation.[268]

There is limited information about the natural history of untreated urethral infection.[191] Several small studies suggest that untreated chlamydial infection of the urethra may persist in men for up to 6 months.[159,276,277] Asymptomatic infections are common in men, as shown by a study in which only one of eight infected men who were observed without treatment for a minimum of 21 days developed symptomatic urethritis.[278] The primary complications of chlamydial urethritis in men are (1) epididymitis, (2) sexually reactive arthritis, and (3) transmission to women.

Epididymitis and Prostatitis

C. trachomatis[279] and *N. gonorrhoeae* are the most frequent causes of epididymitis in men younger than 35 years, whereas Enterobacteriaceae (primarily *E. coli*) are the usual pathogens in men older than 35 years.[280] In younger men, urethritis is usually also present but may be asymptomatic and only noted on examination. The absence of urethritis does not exclude chlamydial infection or gonorrhea as the cause of epididymitis. Most men with *E. coli* epididymitis have other risk factors for urinary tract infection, including recent catheterization, urologic surgery, or rectal insertive intercourse. Chlamydial epididymitis is often associated with oligospermia during the acute phase,[281] but there are no data indicating that future fertility is impaired. In addition, epididymitis is usually unilateral, and attempts to correlate chlamydial infections with male factor infertility have been unsuccessful.[282] A presumptive diagnosis of epididymitis in the setting of NGU can be confirmed as chlamydial in etiology by a NAAT on first-void urine sample (Table 180.3).

Typically, acute epididymitis presents with unilateral testicular pain and tenderness, hydrocele, and palpable swelling of the epididymis.[209,280] Patients also have dysuria, fever, and, in some cases, shaking chills. Many patients can be managed in the outpatient setting, but others require hospitalization for parenteral antibiotics, scrotal elevation, analgesia, and observation. An alternative diagnosis of testicular torsion should always be considered in a young man with acute onset of severe unilateral scrotal pain and should be ruled out with ultrasound.

The role of *Chlamydia* in prostatic infection remains controversial. From available data, this bacterium does not appear to play a role in acute prostatitis, which is mainly caused by *E. coli,* other gram-negative rods, or enterococci. Its role in chronic nonbacterial prostatitis remains more controversial. Although some investigators have recovered *C. trachomatis* from prostatic expressate or biopsies, convincing evidence that *Chlamydia* plays an etiologic role in chronic nonbacterial prostatitis has yet to be developed and antibiotic therapy is not recommended.[283]

Sexually Reactive Arthritis

Reactive arthritis is defined as an immune-mediated inflammatory response in the joints due to a primary infection at a distant mucosal site.[284] Approximately 10% to 15% of adults report rheumatologic symptoms following a bacterial enteric infection caused by *Salmonella, Shigella, Campylobacter, Yersinia* spp., or *E. coli* O157; two-thirds of these cases are confirmed as reactive arthritis by a rheumatologist.[285] Reactive arthritis can also be triggered by chlamydial and other STIs, but its frequency is not well defined because only the more severe cases are identified. A commonly cited estimate is that 0.8% of men with NGU develop sexually reactive arthritis. This estimate is based on hospitalized arthritis cases ($n = 182$) from 1942 to 1956 normalized to

TABLE 180.3 Clinical Characteristics of Common *Chlamydia trachomatis* Infections

INFECTION	SYMPTOMS AND SIGNS	PRESUMPTIVE DIAGNOSIS	DEFINITIVE DIAGNOSIS	TREATMENT
Men				
Nongonococcal urethritis	Urethral discharge, dysuria	Urethral leukocytosis; no gonococci seen	Urine or urethral NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Epididymitis	Unilateral epididymal tenderness, swelling; pain; fever; presence of NGU	Urine or urethral NAAT	Urethral leukocytosis; pyuria on urinalysis	*STI likely:* Ceftriaxone, 250 mg IM, plus doxycycline, 100 mg PO bid, for 10 days *History of insertive anal intercourse:* Ceftriaxone, 250 mg IM, plus levofloxacin, 500 mg once daily, for 10 days
Women				
Cervicitis	Frequently asymptomatic or mild symptoms of vaginal discharge and bleeding, mild abdominal pain, dysuria; a small minority have mucopurulent cervical discharge; ectopy, easily induced bleeding	≥20 PMN/OIF on cervical Gram stain	Urine, vaginal, or cervical NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Urethritis	Dysuria, frequency; no hematuria	Pyuria on UA; negative urine Gram stain and culture	Urine, cervical, or urethral NAAT	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
PID	Lower abdominal pain, adnexal pain, cervical motion tenderness	Evidence of mucopurulent cervicitis	Urine or cervical NAAT	*Inpatient:* Cefoxitin 2 g IV q6h or cefotetan 2 g IV q12h, plus doxycycline 100 mg PO bid; transition after clinical improvement to doxycycline alone to complete 14-day course *Outpatient:* Ceftriaxone, 250 mg IM (single dose), plus doxycycline, 100 mg PO bid, for 14 days, with or without metronidazole, 500 mg PO bid, for 14 days
Men and Women				
Conjunctivitis	Ocular pain, redness, discharge; simultaneous genital infection	Gram stain of conjunctival swab negative for bacterial pathogens; PMNs on smear	DFA or NAAT on conjunctival swab	Azithromycin, 1 g PO (single dose) *or* Doxycycline, 100 mg PO bid, for 7 days
Proctitis (non-LGV)	Rectal pain, discharge and bleeding; history of receptive anal intercourse	≥1 PMN/OIF on rectal Gram stain; no gonococci seen	Urine or urethral NAAT; rectal culture or NAAT	Doxycycline, 100 mg PO bid, for 7 days
LGV	Painful, tender inguinal lymphadenopathy; women more likely to have backache and pelvic pain	"Groove sign"	Urine, urethral, lymph node, or rectal NAAT; rectal or lymph node culture; LGV-specific testing if available	Doxycycline, 100 mg PO bid, for 21 days
LGV proctitis and proctocolitis	Rectal pain, discharge, and bleeding in MSM; absence of inguinal lymphadenopathy	≥1 PMN/OIF on rectal Gram stain; no gonococci seen	Urine, urethral, or rectal NAAT; rectal culture; LGV-specific testing if available	Doxycycline, 100 mg PO bid, for 21 days
Newborns				
Conjunctivitis	Ocular pain, redness, discharge; simultaneous genital infection	Gram stain of conjunctival swab negative for bacterial pathogens; PMNs on smear	DFA or NAAT of conjunctival swab; vagina, rectum, pharynx also often positive	Erythromycin base, 50 mg/kg/d PO, divided into four doses for 14 days; evaluate and treat parents as well
Pneumonia	Staccato cough, tachypnea, hyperinflation	Diffuse interstitial infiltrate, eosinophilia	Nasopharyngeal NAATs or culture; MIF serology (IgM)	Erythromycin base, 50 mg/kg/d PO, divided into four doses for 14 days; evaluate and treat parents as well
Children				
Trachoma	Frequently asymptomatic or mild eye symptoms of redness, discomfort, photophobia, mucopurulent discharge; a small minority have progression to trichiasis, corneal opacification, and blindness	Residence in endemic area; direct examination of conjunctiva	DFA or NAAT of conjunctival swab (not usually performed)	Community-wide mass treatment with azithromycin, 1 g PO (single dose)

DFA, Direct fluorescent antibody; *IgM,* immunoglobulin M; *LGV,* lymphogranuloma venereum; *MIF,* microimmunofluorescence; *MSM,* men who have sex with men; *NAAT,* nucleic acid amplification test; *NGU,* nongonococcal urethritis; *OIF,* oil immersion field; *PID,* pelvic inflammatory disease; *PMN,* polymorphonuclear neutrophil; *STI,* sexually transmitted infection; *UA,* urinalysis.

22,010 outpatient cases of urethritis of any etiology at the same institution over this time period.[286] Better data come from a study in an STI clinic in which 271 consecutive patients at risk and treated for chlamydial infection were carefully screened for rheumatologic symptoms; 4.1% had features of reactive arthritis, which was relatively mild and mostly short-lived.[287] Retrospective data from a sexual health clinic in Sydney, Australia, found that reactive arthritis diagnoses declined from 1992 through 2012 despite increasing rates of *Chlamydia* detection.[288]

The full complex of sexually reactive arthritis consists of arthritis, conjunctivitis, urethritis, and skin lesions. However, limited data suggest that NGU-associated cases of reactive arthritis predominantly present with enthesitis and oligoarthritis,[287] similar to patients with reactive

arthritis following enteric infection.[285] In men with untreated reactive arthritis who have urethritis, *C. trachomatis* can be recovered from the urethra in up to 69% at the onset of the acute arthritis.[289] Reactive arthritis patients also have elevated synovial and serum antibody levels to *C. trachomatis*.[289] In older studies, approximately 80% of reactive arthritis patients had the histocompatibility marker human leukocyte antigen (HLA)-B27,[290] but this percentage is much lower in more recent population-based studies.[285,287] In addition to reactive arthritis, there appears to be an association between chlamydial infection, HLA-B27, and undifferentiated oligoarthritis.[291]

There is some evidence that *C. trachomatis* may be present in the joints of reactive arthritis patients in a form that either cannot be cultured or is difficult to culture.[292] Synovial lymphocytes from reactive arthritis patients show higher proliferative responses in vitro to chlamydial antigens than do peripheral blood lymphocytes from the same patients or synovial fluid lymphocytes from control patients.[284] Early reports of *C. trachomatis* recovery by culture from the synovial membranes of at least some patients with reactive arthritis have not been replicated even with improvements in isolation technique.[284] However, organisms with morphology consistent with *Chlamydia* have been identified in the synovium of reactive arthritis patients by electron microscopy, immunocytochemical staining,[284,289] and molecular hybridization and amplification techniques,[284] suggesting that *Chlamydia* may persist in some form in the synovial membranes of patients with reactive arthritis. Chlamydial DNA was found in synovial biopsies together with messenger RNA detected by reverse-transcriptase PCR, suggesting that the detectable forms and DNA represent viable, persistent organisms.[293] A double-blind study comparing lymecycline (tetracycline-L-methylene lysine) and placebo in patients with reactive arthritis suggested that 3 months of treatment was efficacious in arthritis associated with chlamydial infection but not in reactive arthritis associated with other causes.[292] Furthermore, when patients with reactive arthritis were treated for genitourinary infections with antichlamydial agents or broad-spectrum antibiotics, the incidence of arthritic relapses was significantly reduced compared with patients left untreated or treated with penicillin.[294] However, several other human treatment studies have not shown antibiotics to be of benefit.[295] A more recent prospective, placebo-controlled trial compared a 6-month treatment course of either doxycycline or azithromycin plus rifampin versus placebo in patients with chronic *Chlamydia*-associated reactive arthritis and suggested improvement in clinical end points.[296] The PCR assays used in this study to establish the association with chlamydiae are specialized research assays and not standard commercially available amplification assays used for screening purposes.

Urogenital Infections in Women

Most women with *C. trachomatis* genital infection are asymptomatic, and yet most of the serious consequences occur in women. Risk factors vary in different population groups, but in general the risk of *C. trachomatis* infection is in excess of 30% for the female sexual partner of a man with either gonococcal urethritis or NGU.[270] In the United States, higher prevalence rates in sexually active individuals have been associated with younger age (15 to 24 years old), African-American ethnicity, unmarried status, new or multiple sexual partners, and oral contraceptive use.[270,297] Higher rates are also seen in the southeastern part of the country. Young age is the single factor most strongly associated with increased risk of chlamydial infection among sexually active females. For example, chlamydial prevalence in sexually active individuals is nearly three times greater among 14- to 24-year-olds compared with 25- to 39-year-olds.[298] In addition, young age is associated with an increased risk of repeated infection[299] and with an associated increased risk of PID, ectopic pregnancy, and infertility.[300] Oral contraceptives may increase susceptibility or enhance detection because of increased cervical ectopy, resulting in more exposure of susceptible endocervical cells. Alternatively, oral contraceptive use may be a surrogate marker for increased sexual activity.[297] In some studies,[301] but not others,[261] a recent change of sexual partners and increased numbers of partners have also been associated with increased prevalence of chlamydial genital infection.

The natural history of endocervical infection with *C. trachomatis* in women is not fully known.[191] In most animal models, including primates, an immune response is mounted after infection or reinfection, and the organism can no longer be detected.[302] It is likely that a similar response occurs in a substantial proportion of infected women, but other data suggest that chlamydiae can cause chronic asymptomatic infections in the female genital tract. For example, 68 of 85 (80%) infected but asymptomatic adolescent women were still infected when retested 2 months or more after their initial evaluation.[303] Two epidemiologic studies indicate that untreated genital infection persists for 1 year in 45% to 50% of women.[157,158] Further evidence of a prolonged infection comes from reports of young women who repeatedly tested positive for up to 5 years with a genetically identical strain of *C. trachomatis*.[304,305]

The epidemiology and natural history of anorectal infections in women is another area of uncertainty. In two large studies, the prevalence of anorectal chlamydial infection among women giving a history of anorectal intercourse was 14.6%[306] and 16%,[307] respectively; all women were asymptomatic. A recent Canadian study carried out at two different clinic sites (Calgary and Edmonton) assessed genital and anorectal chlamydial infection by sampling female STI clinic attendees who received a pelvic examination as routine clinic procedure (Calgary) or were screened for either symptoms, contact with STI, anorectal sex, sex work, or sexual assault (Edmonton).[308] The prevalence of rectal infection was 11.7% at Calgary and 13.5% at Edmonton. A substantial proportion of chlamydial infections were detected only at the rectal site (44.6% at Calgary, 17.8% at Edmonton). Reported anorectal sex among women with rectal-only infection was only 15.9% at Calgary and 22.6% at Edmonton. The reason(s) for the disparity between anorectal infection and anorectal intercourse could relate to underreporting of anorectal sex, autoinoculation from the genital site, difference in spontaneous clearance rates at the genital and rectal sites, or false-positive NAATs at the rectal site.[308] Alternatively, Rank and Yeruva[309,310] hypothesized an asymptomatic reservoir in the lower gastrointestinal tract established via the fecal-oral route, which is a well-documented and common characteristic of chlamydial infections in birds and mammals. More research is needed to confirm the frequency and mechanism of rectal carriage in women, to determine whether routine screening at the anorectal site is warranted to increase case finding, and to evaluate the response of anorectal infection to the currently recommended treatment regimens.

Cervicitis and Urethritis

The cervix is the most common site of *C. trachomatis* infection in women. Indeed, mucopurulent cervicitis caused by *C. trachomatis* has been called the female counterpart of male NGU.[125] However, only a minority of patients have the classic presentation of mucopurulent cervicitis with discharge of mucus and pus from the cervical os and easily induced endocervical bleeding by gentle passage of a cotton swab through the os.[125,311] Instead, approximately 70% of women with endocervical infection are asymptomatic or have mild symptoms, such as vaginal discharge and bleeding, mild abdominal pain, or dysuria.[270] Vaginal discharge is likely due to endocervical rather than vaginal infection because *C. trachomatis* cannot infect the squamous epithelium of the adult vagina. However, vaginitis can be present in girls because the vagina is lined with transitional cell epithelium before puberty. Dysuria may reflect concurrent urethral infection. On examination, the cervix may appear normal or may exhibit edema, erythema, and hypertrophy. Diagnostic testing with a NAAT is necessary to distinguish between *C. trachomatis* and *N. gonorrhoeae* as the two common causes of cervicitis.

Acute urethral syndrome is defined as dysuria and urinary frequency with fewer than 10^5 organisms per milliliter of urine.[312] In one study of 59 women with this syndrome, 42 also had pyuria and 11 of the 42 were infected with *C. trachomatis,* as were 3 of 66 women without symptoms and 1 of 35 women with cystitis related to *E. coli.* Most of the remainder of the women with pyuria and acute urethral syndrome had low urine concentrations of *E. coli* or *Staphylococcus saprophyticus* demonstrated by culture of urine obtained by suprapubic aspiration.[312] Young, sexually active women with this clinical syndrome should be evaluated for possible *C. trachomatis* infection, and they respond to appropriate antibiotics such as doxycycline. *C. trachomatis* has also been isolated from the Bartholin glands in women with bartholinitis.

C. trachomatis has been associated with cervical cancer in seroepidemiologic studies and proposed as a cofactor for human papillomavirus (HPV) in the development of this common cancer in women. In a meta-analysis of 19 retrospective studies and 3 prospective studies, *C. trachomatis* infection in women was significantly associated with increased risk of cervical cancer (odds ratio [OR], 2.21; 95% confidence interval [CI], 1.88–2.61).[68] The risk was present with *C. trachomatis* as an independent factor (OR, 1.76; 95% CI, 1.03–3.01), and increased when there was also HPV infection (OR, 4.03; 95% CI, 3.15–5.16).[68] Case-control studies have found an association between cervical dysplasia or neoplasia and *C. trachomatis* infection.[313–317] This association has been noted for both cervical squamous cell carcinoma and cervical adenocarcinoma,[68] and is more pronounced for specific strains of *C. trachomatis*.[318,319] Coinfection with *C. trachomatis* was associated with more prolonged shedding of HPV in a longitudinal study of adolescents.[320] Biologic support for a cofactor role comes from studies indicating that *C. trachomatis* infection may cause chromosomal and genetic instability and induce cellular transformation, as described earlier in the "Chlamydial Biology" section. Chronic inflammation induced by *Chlamydia* could also potentially contribute to cancer development.

Also of concern is the possible association between chlamydial cervicitis and acquisition of human immunodeficiency virus (HIV) infection by women.[321] In a case-control study of female prostitutes in Zaire, the adjusted OR for HIV seroconversion with *C. trachomatis* infection was 3.6, with a 95% CI of 1.4 to 9.1.[321] Other studies support the idea that *C. trachomatis* and other sexually transmitted agents that produce genital mucosal inflammation cause increased shedding of HIV in genital secretions.[322]

Pelvic Inflammatory Disease

PID is an upper genital tract disease that encompasses some combination of endometritis, salpingitis, and peritonitis. For example, in a study of women with suspected PID, 70% had histologic evidence of plasma cell endometritis, 67% had laparoscopically verified salpingitis, and 67% had peritonitis.[323] Endometritis is present in 40% of women with mucopurulent cervicitis, suggesting early spread of infection from the cervix to the endometrium.[144] In clinical practice, PID is rarely confirmed pathologically or by direct visual inspection of the fallopian tubes by laparoscopy. Instead the diagnosis is usually made on a clinical basis in a sexually active woman with lower abdominal pain and cervical, uterine, and adnexal tenderness on pelvic examination.[324] Approximately 20% of women with acute PID have *C. trachomatis* detected in urogenital samples, although the percentage ranges from 5% to 51%, depending on the population studied and the techniques used.[270] A more recent estimate of acute PID cases attributable to *C. trachomatis* was derived by examining multiple data sources from the United Kingdom; the overall rate was estimated at 20%, but was 35% in younger women (ages 16–24).[325]

The proportion of women with endocervical *C. trachomatis* infections who develop acute symptomatic PID is difficult to determine accurately. Estimates vary widely, which may be due to differences in the patient populations, with clinic-based studies having more cases of recently acquired incident infections. For example, 18 of 109 (16.5%) infected but asymptomatic adolescent women who were observed for 2 months or more became symptomatic, but only 2 (1.8%) developed clinical PID.[303] In contrast, 6 of 20 (30%) women infected with both *C. trachomatis* and *N. gonorrhoeae,* but only treated for gonorrhea, developed acute salpingitis during a 7-day follow-up interval.[326] More consistent estimates can be obtained from randomized controlled trials that screen asymptomatic women who are followed longitudinally to detect clinical PID. For example, the Prevention of Pelvic Infection (POPI) trial of *Chlamydia* screening and treatment in London suggested that the rate of clinical PID observed in the year after a positive *Chlamydia* screening test is about 10%.[327] More recently, data from the POPI trial[327] and two other randomized controlled trials[328,329] were reanalyzed using Markov modeling.[325] The estimated risk of PID following chlamydial infection was 14.8% (95% CI, 4.8–24.8%) for symptomatic PID and 17.1% (95% CI, 5.6–28.9%) if asymptomatic PID was also included.[330] The timing of progression to PID after acquisition of a chlamydial infection is not established. Although it may occur early, modeling of data from the POPI trial suggests that clinical PID attributed to *Chlamydia* may occur at a constant rate over the course of a year after infection.[331] These findings support a role for screening and treatment to prevent PID even after acquisition of *C. trachomatis* infection.

The spectrum of PID associated with *C. trachomatis* infection ranges from acute, severe disease resembling gonococcal salpingitis, with associated perihepatitis and ascites, to asymptomatic or "silent" PID.[332] Undiagnosed PID appears to be more common than diagnosed acute disease,[330] while completely asymptomatic disease may only account for about 11% of cases.[330] Compared with women with gonococcal or nongonococcal, nonchlamydial salpingitis, women with chlamydial salpingitis are more likely to experience a chronic, subacute course with a longer duration of abdominal pain before seeking medical care. Yet they have as much or more tubal inflammation at laparoscopy.[333] In a prospective study, one-time screening of asymptomatic women for chlamydial infection, followed by treatment if infected, reduced the incidence of PID in a health maintenance organization setting.[328]

Infertility and Ectopic Pregnancy

The long-term consequences of both acute PID and silent, subclinical disease are tubal infertility, ectopic pregnancy, and chronic pelvic pain syndrome.[330,334] In developed countries, infertility affects approximately one in six couples and occlusion of the fallopian tubes is a factor in 10% to 30% of these cases.[324] The general mechanisms responsible for tubal occlusion are not understood, but *Chlamydia* is believed to induce chronic inflammation, scarring, and eventual blockage of the fallopian tubes from repeated or prolonged infection. In a nonhuman primate model, repeated endocervical infections followed by direct tubal inoculation of *C. trachomatis* produced peritubular adhesions and plasma cell endometritis.[335] Consistent with the greater inflammation seen with chlamydial disease,[333] women with nongonococcal salpingitis are more likely to have an adverse reproductive outcome than women with gonococcal salpingitis.[336]

The relationship between PID and infertility was examined in a prospective study of women with laparoscopy-verified salpingitis.[337] Over a mean period of 94 months, 16% of the patients and 2.7% of the control subjects failed to conceive. Ten percent of patients and none of the control subjects had confirmed tubal infertility. Tubal infertility increased with severity of infection from 0.6% after a case of mild PID to 21.4% after severe PID. It also increased with the number of episodes of PID, from 8% after one episode to 19% after two and 40% after three. The ectopic pregnancy rate was 9.1% among patients versus 1.4% among control subjects.[337]

Tubal infertility and ectopic pregnancy have a strong association with serologic evidence of prior chlamydial infection, even though most women with these reproductive health issues do not have a known history of an STI or PID.[270] *Chlamydia* has been recovered from fallopian tube biopsies in women undergoing microtuboplasty for surgical correction of damaged tubes.[338,339] In one study, 22% of women experiencing ectopic pregnancy had histologic evidence of plasma cell salpingitis and antichlamydial antibodies.[340] Repeated *Chlamydia* diagnosis is associated with ectopic pregnancy risk[341]; however, further studies are necessary to define the relative role of prolonged versus repeated infection in increasing the risk of ectopic pregnancy. Recent analyses have led to contemporary estimates that *C. trachomatis* infections are complicated by PID in 17% of cases, by tubal factor infertility in 0.5%, and by *Chlamydia*-attributable ectopic pregnancy in 0.2%.[334]

Pregnancy Complications

Some data indicate that *C. trachomatis* infections affect pregnancy outcome. In one study, women experiencing recurrent spontaneous abortions had high titers of antichlamydial IgG but negative endocervical cultures for *C. trachomatis,*[342] suggesting an association between prior or chronic *C. trachomatis* infection and spontaneous abortion. Some women with recent *C. trachomatis* infection had infants with lower birth weight compared with women lacking *Chlamydia*-specific IgM.[343,344] An association between chlamydial infection and prematurity and premature rupture of membranes has been reported.[345,346] In a large study, treatment of *C. trachomatis* infection was significantly associated with decreased premature rupture of membranes, and there was a trend

toward increased perinatal survival.[347] Pregnancy outcomes have also been compared in 244 treated women who were cured of a *C. trachomatis* infection and 79 treated women who had a treatment failure or recurrent infection.[348] Successful treatment significantly decreased the frequency of premature delivery (OR, 0.16; 95% CI, 0.06–0.47), premature rupture of membranes (OR, 0.31; 95% CI, 0.14–0.69), and small-for-gestational-age infants (OR, 0.45; 95% CI, 0.23–0.88).

These studies suggest that identification and treatment of *C. trachomatis* infection in pregnancy is likely to improve pregnancy outcome. A major difficulty in these studies is the potential interaction of other infections that may influence pregnancy outcomes, including genital infections caused by *Mycoplasma, Ureaplasma,* herpesviruses such as cytomegalovirus, and *Trichomonas,* as well as bacterial vaginosis, urinary tract infections, and vaginal colonization with gram-negative rods. Studies need to evaluate and analyze all of these factors comprehensively, making such studies both large and complex.[349]

Extragenital Infections

Proctitis (Rectal Infections)

C. trachomatis genital serovars D through K can cause proctitis, but this rectal infection is not as severe as LGV proctitis.[350] It results from direct inoculation of the rectum in either men or women through receptive anal intercourse but can also be caused by secondary spread of secretions from the cervix in women. The primary manifestations are anal pruritus and pain and a mucous rectal discharge that may become mucopurulent. However, many patients are asymptomatic. The infection remains superficial, is limited to the rectum, and closely resembles gonococcal proctitis. Leukocytes are usually present on rectal Gram stain even if the infection is asymptomatic.[351,352] Proctitis with concomitant infection by LGV and non-LGV strains of *C. trachomatis* has been reported in MSM.[353] Non-LGV serovars of *C. trachomatis* can also cause asymptomatic rectal carriage in infants[354] and adults.[355] Proctitis caused by LGV serovars of *C. trachomatis* is discussed in "Lymphogranuloma Venereum" later in this chapter.

Oropharyngeal Infections

Oropharyngeal infections have been documented by cell culture in heterosexual men (3.7%) and women (3.2%),[121] and more recently in MSM, by NAATs (2.9%).[122] Oral sex is a risk factor, and virtually all of the infections are asymptomatic. However, transmission to genital sites likely occurs,[356,357] and treatment is recommended if detected. Routine screening for oropharyngeal infection is not recommended, although screening is suggested for MSM who engage in oral sex.[209]

Other Infections

Pneumonia caused by *C. trachomatis* is primarily a disease of infants, although there have been isolated reports of *C. trachomatis* pneumonia in immunocompromised patients.[358] There are rare reports of pulmonary infection in laboratory workers exposed to high concentrations of LGV serovars.[359] The route of transmission is believed to have been by inhalation of aerosolized organisms, and the individuals responded promptly to treatment with tetracycline or erythromycin. In retrospect, an older report of serologic association of *C. trachomatis* with community-acquired pneumonia[360] was likely due to cross-reacting antibody with then-unrecognized *C. pneumoniae*. Case reports and case-control studies have reported serologic association of *C. trachomatis* with meningoencephalitis,[361] myocarditis,[362] and endocarditis.[363]

Treatment of Genital and Ocular Infections in Adults

A number of antibiotics have excellent activity against *Chlamydia* in cell culture, including the tetracyclines, macrolides and related compounds, rifamycins (including rifampin), and some of the fluoroquinolones.[364] The fluoroquinolones block bacterial DNA replication and generate double-strand DNA breaks by targeting DNA gyrase and topoisomerase IV, which are enzymes that regulate DNA supercoiling levels. *C. trachomatis* DNA gyrase and topoisomerase IV have been confirmed to be susceptible to ciprofloxacin,[59] and mutations in these enzymes confer fluoroquinolone resistance.[365] Because DNA supercoiling levels control the temporal expression of chlamydial genes, the fluoroquinolones are also likely to have a *Chlamydia*-specific activity in blocking progression of the chlamydial developmental cycle.[56,59] Chlamydiae are susceptible to ampicillin and penicillin because they contain peptidoglycan, which has been shown to be present in RBs during chlamydial cell division, but not in EBs.[28] Cephalosporins lack activity due to poor affinity for chlamydial penicillin-binding proteins.[366] Aminoglycosides are not effective because they do not penetrate the eukaryotic host cell.

Considerable clinical data are available on the treatment of uncomplicated urogenital tract infections in both men and women.[367] For many years, standard therapy for uncomplicated genital tract infection has been doxycycline, 100 mg orally twice daily for 7 days. However, azithromycin given as a single 1-g dose is as effective as a 7-day course of doxycycline[368] and is a recommended regimen in the 2015 CDC guidelines for the treatment of STIs.[209] Single-dose therapy is attractive because it minimizes treatment failure from lack of medication adherence, but so far azithromycin is the only approved single-dose option for treating *C. trachomatis* infection. Rifalazil, which is highly active against *C. trachomatis* and has a long half-life, had shown promise as a single-dose treatment for chlamydial NGU.[369] However, a randomized trial did not establish its noninferiority with azithromycin in treatment of uncomplicated chlamydial genital infections in women,[370] and it is no longer in development because of severe adverse effects.

Questions have been raised about the efficacy of azithromycin for treating chlamydial genital infections. Early repeated chlamydial infection has been reported in up to 5% to 13% of adolescents treated with azithromycin, which could represent reinfection, but treatment failure could not be excluded in some cases.[371,372] Although a meta-analysis found an azithromycin cure rate of 97%, it was based on trials that used cell culture for diagnosis.[373] In a recent randomized controlled trial of NGU in men that used NAATs as a more sensitive test, there was an unexpectedly low chlamydial cure rate of 77.4% for azithromycin versus 94.8% for doxycycline.[274] In contrast, another NAAT-based study showed a cure rate for azithromycin of 86% versus 90% for doxycycline,[273] and a third study found an azithromycin cure rate of 94%.[369] The authors of these three trials harmonized their data and calculated an overall azithromycin treatment failure rate of 6.2% to 12.8% for symptomatic urethral chlamydial infection in men, although acquisition of new infection could not be completely excluded.[374] In women with uncomplicated infection, two observational studies using NAATs documented use effectiveness of azithromycin therapy of 92%.[77,375] A recent randomized trial, with observed treatment, in a youth correctional setting found that largely asymptomatic men and women with uncomplicated urogenital chlamydial infection had cure rates of 97% for azithromycin and 100% for doxycycline, although the noninferiority of azithromycin was not established.[376] The high cure rates in this institutionalized group suggest that lower cure rates in the wider population could be due to acquisition of new infection, although it is also possible that asymptomatic infections have a higher cure rate than symptomatic ones. In addition, a meta-analysis of randomized controlled trials suggested a degree of increased efficacy for doxycycline, particularly among symptomatic urethral infection in men.[377]

Despite these efficacy questions, azithromycin continues to be recommended for treatment of uncomplicated chlamydial infections in the 2015 Sexually Transmitted Diseases Treatment Guidelines.[209] This recommendation takes into consideration the major advantage of azithromycin as a single-dose regimen, which enhances adherence and makes directly observed therapy feasible. Azithromycin is now available as a generic drug, and its cost to our STI clinic in Indianapolis of about 60 cents per 1-g dose is about the same as a 7-day course of doxycycline (J. Arno, personal communication). The FDA released a warning on March 12, 2013 that azithromycin can cause potentially life-threatening arrhythmias. Patients with known QT interval abnormalities, or who take drugs to treat arrhythmias, should receive doxycycline instead.

For *C. trachomatis*, ofloxacin, 300 mg twice daily for 7 days, and levofloxacin, 500 mg once daily for 7 days, are included as alternative agents in the 2015 CDC treatment guidelines.[209] However, neither should be used in adolescents younger than 18 years or in pregnant women.[209] Delayed-release doxycycline (Doryx) 200 mg daily for 7 days has been approved by the FDA for treatment of uncomplicated chlamydial genital infection in men and women.[209] This once-daily preparation could

improve treatment adherence and was shown to be noninferior to the standard twice-daily regimen in both men and women.[378]

To date, antimicrobial resistance among wild-type chlamydiae has not been demonstrated to be a clinically important problem.[379] Reports of tetracycline resistance in *C. trachomatis* clinical isolates have not been substantiated.[380] However, resistance can be induced in the laboratory to fluoroquinolones, rifampin, and other drugs.[365,381,382] There have been isolated reports of patients with persistently symptomatic genital infection with repeatedly positive *C. trachomatis* NAATs despite antibiotic treatment, suggestive of treatment failure.[383] Currently there is no active surveillance program in the United States to detect emergence of resistance in *C. trachomatis.*

Guidelines from the CDC in 2015 recommend azithromycin, 1 g orally, as the treatment for chlamydial infections in pregnancy.[209] Amoxicillin, 500 mg orally three times a day, and erythromycin preparations are listed as alternatives.[384–386] Doxycycline, ofloxacin, and levofloxacin are contraindicated in pregnancy. Clindamycin is only partially effective in eradicating *C. trachomatis* in men with NGU,[387] but it appears to be as efficacious as erythromycin in pregnant[388] and nonpregnant[389] women with *C. trachomatis* infection.

Test of cure after treatment is not necessary in most patients because of high cure rates with first-line antibiotics. It is reserved for specific circumstances, including pregnancy, persistent symptoms, and suboptimal antibiotics (erythromycin or amoxicillin). However, retesting 3 months after therapy is recommended because of the high risk of reinfection in women and men (see "Prevention Strategies" later).[209]

Presumptive treatment of *C. trachomatis* for both patient and sexual partners is warranted for clinical conditions with a high likelihood of a chlamydial infection, such as NGU in heterosexual men, epididymitis in men younger than 35 years, PID, and gonococcal infection in either men or women.[209] Presumptive treatment of mucopurulent cervicitis is more controversial, but current guidelines accept it as a reasonable approach in women at high risk for chlamydial infection (≤25 years old, with new or multiple sex partners, partners who themselves have concurrent partners, or partners with known STI). Otherwise, diagnostic testing with NAATs and follow-up for treatment is the recommended approach.[209]

Proctitis in homosexual men and the acute urethral syndrome in women may be managed either by presumptive therapy or by therapy based on test results. Empirical therapy of both proctitis and epididymitis should include treatment for gonorrhea—for example, ceftriaxone, 250 mg IM in a single dose followed by 7 days of doxycycline, 100 mg orally twice daily.[209] Azithromycin should probably be avoided for treatment of symptomatic rectal infections due to reports of treatment failure.[390] Doxycycline treatment should be extended to 21 days when an LGV serovar is known or suspected to be the causative agent in a patient with more severe symptoms.[209,391] Treatment of asymptomatic rectal infection in MSM is with either single-dose azithromycin or 7 days of doxycycline according to the 2015 CDC guidelines,[209] but doxycycline is preferred in the 2015 European guidelines.[392] A survey of observational studies suggests that azithromycin may be less effective for treatment of rectal chlamydial infection in MSM,[393] and a randomized trial comparing azithromycin with doxycycline is underway in Australia.[394]

The management of PID, even when gonorrhea is present, should always include therapy directed against *C. trachomatis,* as well as *N. gonorrhoeae* and anaerobic bacteria. Randomized trials have shown that parenteral and oral regimens have similar clinical efficacy for mild-to-moderate PID, although doxycycline is given orally if possible because intravenous infusion is painful.[209] Recommended parenteral regimens include cefoxitin or cefotetan along with a 14-day course of doxycycline or, alternatively, clindamycin plus gentamicin, or ampicillin-sulbactam plus doxycycline.[209] Outpatient regimens include initial, single-dose, intramuscular therapy with a second- or third-generation cephalosporin plus 14 days of doxycycline, with or without metronidazole, 500 mg twice daily for 14 days. Due to the emergence of quinolone-resistant *N. gonorrhoeae,* regimens that include a quinolone are no longer recommended for PID treatment.

Few comparative data exist on the treatment of adult inclusion conjunctivitis, but doxycycline is effective when given for a 2- or 3-week period of time.[395] Erythromycin is an alternative.

Perinatal Infections

Neonatal Inclusion Conjunctivitis

C. trachomatis was recognized as a cause of ophthalmia neonatorum when the introduction of perinatal ocular prophylaxis for gonorrhea did not eliminate all cases of neonatal conjunctivitis. However, even before the modern antibiotic era, cytoplasmic inclusions, which are identical to those seen in patients with trachoma, had been observed in conjunctival scrapings from neonates with conjunctivitis and subsequently in cells from the cervix of mothers and the urethra of fathers of infected infants.[396] Infant infection is usually acquired during passage through an infected birth canal. Exceptions include occasional infants who appear to have acquired infection perinatally despite birth by cesarean section, or postnatally from an infected caregiver by hand-to-eye contact.[397] Between 30% and 50% of infants born to *C. trachomatis*–infected women develop neonatal conjunctivitis.[398] The usual incubation period is 5 to 12 days from birth, although onset may be as late as 6 weeks of age.[399] Typically, a watery ocular discharge appears, which becomes progressively more purulent. The eyelids swell, and the conjunctivae become erythematous and thickened. At birth, the conjunctiva lacks a lymphoid layer, so follicles do not develop initially but may become apparent after 3 to 6 weeks.

The progression of the disease is similar to inclusion conjunctivitis in adults, with spontaneous resolution occurring in most untreated infants after 3 to 12 months.[161] However, mild or subclinical infection may persist for several years,[400] and late sequelae such as scars and corneal lesions occur in a small proportion of cases.[401] A mucopurulent rhinitis and vulvovaginitis in female infants are often associated with the conjunctivitis. The primary differential diagnosis in a newborn is gonococcal ophthalmia, which is uncommon in children who receive ocular prophylaxis at birth, but can still occur.[402] Topical erythromycin ointment, which is the standard prophylaxis for gonococcal ophthalmia in neonates, does not prevent perinatal transmission of *C. trachomatis* infection.[209]

Infant Pneumonia

Between 11% and 20% of infants born by vaginal delivery to mothers with *C. trachomatis* genital infection develop chlamydial pneumonia.[403] Infected infants usually become symptomatic before 8 weeks of age with nasal obstruction or discharge, tachypnea, and cough, or a combination of these.[404] Typically, infants have been symptomatic for 3 or more weeks when they present for care between 4 and 11 weeks of age. Most are afebrile and only moderately ill,[405] but half have a history of conjunctivitis and a majority have middle ear abnormalities.[404] Paroxysms of staccato coughing that interfere with sleeping and eating are sometimes present. Auscultation may reveal scattered crackles, but breath sounds are usually good and wheezing is usually absent. Chest radiographs show bilateral interstitial infiltrates with hyperinflation (Fig. 180.4).[404] Peripheral eosinophilia, arterial hypoxemia, and elevated serum immunoglobulins are characteristic.[404–406] *C. trachomatis* can usually be recovered from nasopharyngeal swab specimens, and antichlamydial IgM titers are elevated.[403]

Untreated, the course is protracted, often lasting weeks to months.[405] Especially in very young infants, the initial respiratory manifestations of *C. trachomatis* infection may be more severe and include prolonged spells of apnea or respiratory failure.[407,408] Although published reports emphasize more serious disease, it is likely that most patients with *C. trachomatis* infant pneumonia are treated as outpatients, often without laboratory confirmation of diagnosis. Long-term respiratory sequelae may be significant in more severe cases, as shown by a higher frequency of obstructive airway disease and physician-diagnosed asthma in children who were diagnosed with chlamydial pneumonia in the first 6 months of life.[409,410]

Diagnosis of Neonatal Inclusion Conjunctivitis and Infant Pneumonia

Sensitive and specific methods for diagnosis of ophthalmia neonatorum and infant pneumonia include cell culture and nonculture tests, such as DFA, EIA, and NAAT. DFA is the only nonculture test cleared by the FDA for conjunctival and nasopharyngeal samples.[209] NAATs have not been cleared by the FDA for conjunctival and nasopharyngeal

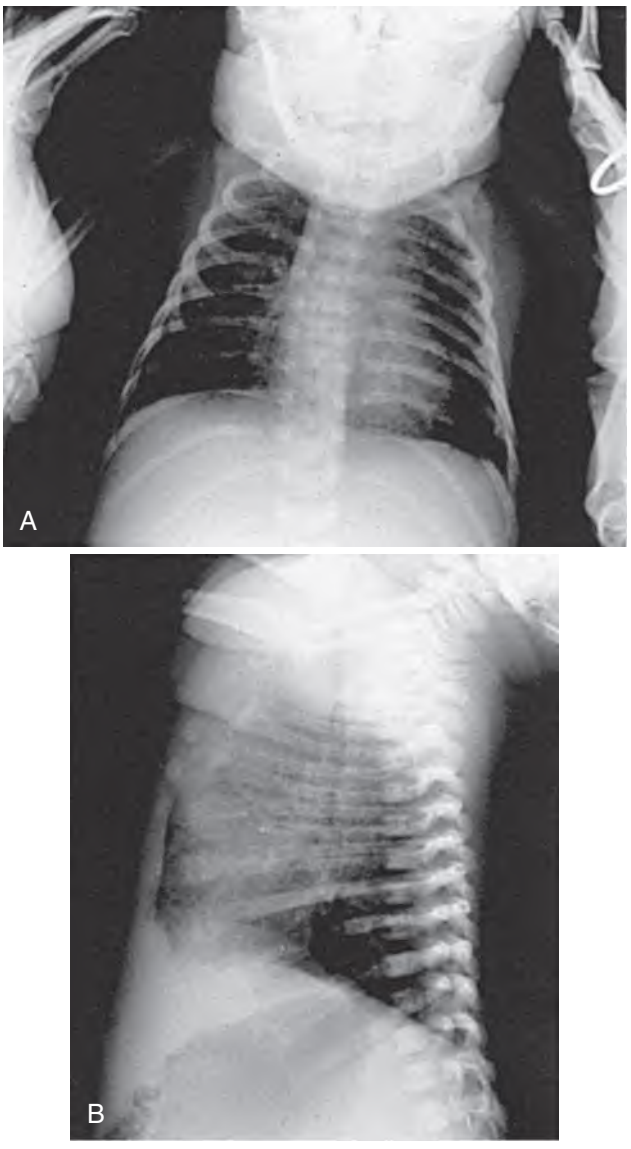

FIG. 180.4 *Chlamydia trachomatis* infant pneumonia. (A and B) Chest radiographs of a 2-month-old infant with *Chlamydia trachomatis* pneumonia demonstrate typical patchy interstitial infiltrates and flattened diaphragms. *(Courtesy Dr. John Gaebler, Indianapolis.)*

samples, but they can be used for testing these sites if the laboratory has performed in-house validations to conform to CLIA regulations.

Prevention and Treatment of Infant Infections

Oral erythromycin is the treatment for *C. trachomatis* conjunctivitis or pneumonia in infants. The recommended dose is 50 mg/kg of body weight per day, divided into four doses, for 14 days, and the efficacy of therapy is approximately 80%, although a second course may be required.[398] Topical treatment of inclusion conjunctivitis is not recommended because of difficulty in application and failure to eliminate concurrent nasopharyngeal carriage, which can result in recurrent conjunctivitis, pneumonia, or both.[411] Mothers of infants with *C. trachomatis* should be evaluated and treated, if necessary, as should their sexual partners.[209]

Prenatal screening and treatment of *C. trachomatis* infection is the best strategy for preventing neonatal chlamydial infections because it is approximately 90% effective in preventing transmission during birth.[412,413] However, in populations at high risk for reinfection, particularly adolescents, reacquisition of infection after the first trimester is frequent,[414] and repeated prenatal screening is warranted. Current recommendations are to obtain a test of cure at 3 to 4 weeks after treatment, preferably by a NAAT. Such a test of cure is not routinely done for chlamydial genital infections but is recommended for pregnant women to ensure therapeutic cure in light of the serious sequelae of neonatal infection. Women younger than 25 years of age should be retested during the third trimester. Thus a woman who is positive in the first trimester should receive a test of cure, a follow-up test at 3 months, and a retest during her third trimester.[209] The incidence of neonatal chlamydial infections has greatly decreased in the United States because of widespread screening and treatment of pregnant women, but *C. trachomatis* remains a common cause of neonatal conjunctivitis in countries that do not perform prenatal screening.[398]

Chlamydial Infections in Prepubertal Children

Perinatally acquired *C. trachomatis* infection may persist in the nasopharynx, urogenital tract, or rectum for 2 to 3 years.[209,400] Consequently, differentiating infection acquired at birth from infection related to sexual abuse may be particularly difficult in children younger than 3 years of age. However, chlamydial infection among prepubertal children 3 years and older should be considered indicative of sexual abuse. NAATs can be used for vaginal and urine specimens from girls, but the CDC does not recommend NAATs for boys due to insufficient data.[209] In the setting of possible sexual abuse, data are insufficient to support use of NAATs at extragenital sites. The CDC continues to recommend *Chlamydia* culture for detection of urogenital *C. trachomatis* in boys and at extragenital sites in both boys and girls.[209]

Lymphogranuloma Venereum

LGV is an STI caused by serovars L1, L2, and L3 of *C. trachomatis*.[415] These serovars produce a more invasive disease than the genital infections caused by serovars D to K.[416] In endemic areas in Africa, India, Southeast Asia, South America, and the Caribbean, LGV commonly presents in its classic or bubonic form as a chronic, systemic illness with a genital lesion, and prominent regional lymphadenopathy. LGV can also cause a severe proctitis and proctocolitis, sometimes with prominent systemic symptoms, that occurs as clusters of cases outside endemic areas, primarily in MSM.

Classic LGV

There are three distinct stages in classic LGV.[416] The first stage is formation of a primary lesion, usually on the genital mucosa or adjacent skin. *C. trachomatis* cannot infect squamous epithelial cells, and when the primary lesion occurs on the external genitalia or in the vagina, the organism probably gains entry through minute lacerations or abrasions.[417] The primary lesion is usually a small papule or herpetiform ulcer that produces few or no symptoms and is generally not noticed. It appears between 3 and 30 days after acquisition of infection[161] and heals rapidly without leaving a scar. Depending on the site of inoculation, the initial infection can present as symptomatic urethritis, cervicitis, or proctitis.

Days to weeks after the primary lesion has resolved, there is a secondary stage with lymphadenopathy and systemic symptoms.[416] Lymphadenopathy is unilateral in two-thirds of patients and involves lymph nodes that drain the area of the primary lesion.[161,417] Thus in men, inguinal lymphadenopathy is most common because the primary lesion is usually on the penis or in the urethra, which drain to the inguinal nodes. In contrast, inguinal lymphadenopathy is the main manifestation in only 20% to 30% of women, occurring when the primary infection is in the vulva or lower vagina, which drain into the inguinal and femoral nodes. In contrast, inguinal lymphadenopathy is not prominent with an upper vaginal or cervical infection because these sites drain to the iliac and obturator nodes in the pelvis. The internal iliac nodes are involved when there is a rectal infection. Initially, the lymph nodes are discrete and tender with overlying erythema, but because of extensive periadenitis, the inflammatory process spreads from the lymph nodes into the surrounding tissue, forming an inflammatory mass. Abscesses within the mass coalesce, forming a bubo that may rupture spontaneously with development of loculated abscesses, fistulas, or sinus tracts.[418] Mild leukocytosis, with an increase in monocytes and eosinophils, is frequent.[417]

The secondary stage is marked by systemic manifestations, including fever, headache, and myalgias.[416] Women often have backache and pelvic pain from enlarged, inflamed pelvic lymph nodes. Meningitis may occur, and in some cases the organism has been recovered from blood or cerebrospinal fluid.[417] Rupture of the fluctuant inflammatory mass (bubo) relieves pain and fever,[417] although sinus tracts may continue to drain thick, yellowish pus for several weeks or months before fully resolving.[419] Excised inguinal nodes often have a characteristic inflammatory response, with central stellate coalescing abscesses that contain neutrophils and necrotic debris, surrounded by a zone of palisaded epithelioid cells, macrophages, and occasional multinucleated giant cells, and an outer layer of lymphocytes and plasma cells. With time, the nodal architecture is effaced and replaced by progressive fibrosis. This histopathologic presentation is suggestive of the diagnosis of LGV or cat-scratch disease but is not unique to these entities.

Healing leaves some scarring in the inguinal region but does not result in significant sequelae in most cases. Relapse occurs in approximately 20% of untreated cases.[417] Only approximately one-third of buboes become fluctuant and rupture. The others harden and form inguinal masses, which gradually involute over time.[419] The separation of enlarged femoral and inguinal nodes by the inguinal ligament produces the "groove sign," which is characteristic of LGV.[417,420] Inguinal or femoral lymphadenopathy may be misdiagnosed as inguinal hernia, whereas internal iliac node involvement may raise a question of appendicitis.

If untreated, some patients progress to a chronic tertiary stage of the infection.[416] Complications include elephantiasis of the male or female genitalia from lymphatic obstruction and esthiomene (Gr., "eating away"), which refers to hypertrophic chronic granulomatous enlargement with ulceration of the external genitalia (vulva or scrotum and penis).[419]

The differential diagnosis of inguinal lymphadenopathy in the age group likely to have LGV includes genital herpes, syphilis, chancroid, and, occasionally, lymphoma.[418] LGV manifesting as inguinal lymphadenopathy can be distinguished from genital herpes by the presence of multiple painful ulcers at the site of the primary herpes infection, in contrast to the painless primary lesion of LGV, and by matting of the lymph nodes in LGV. Also, lymphadenopathy is frequently bilateral in herpes but not in LGV. A diagnosis of syphilis is suggested by a primary lesion with indurated margins (chancre) and bilateral and nontender inguinal lymphadenopathy. Large ulcers that are multiple and extremely tender in association with lymphadenopathy suggest chancroid. The pseudobuboes that occur in granuloma inguinale are nodules in the skin and subcutaneous tissue, with lymph node involvement being the result of secondary infection.[161] However, the clinical presentations of sexually transmitted agents causing inguinal lymphadenopathy clearly overlap, and appropriate laboratory tests are usually required to distinguish among them. When an ulcer is present, a darkfield examination would be helpful to identify *Treponema pallidum,* but it is not usually available, and serologic tests for syphilis should be obtained.[161,418]

LGV Proctitis and Proctocolitis

LGV proctitis or proctocolitis is a severe rectal infection, caused by *C. trachomatis* LGV serovars, that primarily occurs in MSM.[421,422] This infection is called *proctitis* if it is restricted to the distal 15 cm of the rectum, or *proctocolitis* if there is more extensive involvement into the colon.[207] Infection results from direct inoculation of the rectum via receptive anal intercourse. In women, there can also be lymphatic spread to the rectum from the posterior vaginal wall or cervix, or spread of infected secretions from the cervix.[417,423] The disease can extend into the colon, and a granulomatous inflammatory process is present in the bowel wall, with both noncaseating granulomas and crypt abscesses. As the disease progresses, muscle layers are replaced by fibrous tissue, which contracts to form rectal strictures. Sinus tract formation can lead to rectovaginal fistulas in women.[419,423] Involvement of the distal rectal mucosa can lead to perirectal abscesses and anal fissures. Lymphatic drainage from the rectum leads to lymphadenopathy of the internal iliac nodes, which may cause lower abdominal and back pain.[419] Lymphatic obstruction can lead to outgrowths of lymphatic tissue resembling hemorrhoids.

Since 2003, there has been an increase in reports of chlamydial proctitis and proctocolitis caused by LGV strains.[76,415] This outbreak has occurred as clusters of cases in large cities in Europe, North America, and Australia, outside regions where LGV is endemic. Almost all the cases have been in MSM. The majority of these patients have been infected with HIV, and they have had high rates of other STIs. However, the patients were typically not immunosuppressed, and LGV proctitis does not appear to be an opportunistic infection. Rectal ulcers commonly present in LGV proctitis may facilitate transmission and acquisition of HIV and other infections.

Patients with LGV proctitis and proctocolitis often have mucopurulent anal discharge, as well as rectal pain, ulceration, and bleeding.[76] Tenesmus and constipation are reported less consistently. A minority of patients have systemic symptoms, such as fever, malaise, and weight loss.[76] LGV proctitis may also be asymptomatic, as shown by a UK screening study that found an absence of rectal symptoms in 15 of 55 (27%) MSM with LGV detected by rectal swab PCR.[424] One unusual feature of the current outbreak of LGV proctitis is that most patients have not had genital lesions or inguinal lymphadenopathy typical of classic LGV.[76,415] These cases have been caused by a clonal strain of *C. trachomatis* serovar L, designated L2b.[102] By retrospective testing, this serovar was already present in San Francisco in the early 1980s, leading to questions about how new the outbreak is.[422,425] Endoscopic findings include mucopurulent exudate with hyperemic and friable mucosa, easy bleeding, multiple ulcers, erosions, and granulomatous tissue, but are not specific for LGV proctitis.[76] The differential diagnosis of severe infectious proctitis in a high-risk population includes gonorrhea, genital herpes, and syphilis.[350,415] Proctocolitis caused by LGV strains has clinical and histologic similarities to other inflammatory bowel diseases, such as Crohn disease, which can lead to misdiagnosis and inappropriate therapy.[350,351]

Diagnosis of Lymphogranuloma Venereum

The diagnosis of LGV has been hampered by the lack of a good, widely available test.[209,415,426] Standard NAATs will identify *C. trachomatis* but not the serovar; additional PCR-based testing is required to identify LGV serovars but is not available outside specialized laboratories.[209,426] Specimens that can be tested include a swab of the primary genital ulcer, if present; lymph node aspirate or biopsy; rectal swab or biopsy; urine; and urethral swab. NAATs are not FDA approved for rectal specimens, but some laboratories can perform the test because they have done validation studies.[209] In 30% of cases, *C. trachomatis* can be recovered by culture from bubo aspirates, genital tissue, or rectal tissue, but *Chlamydia* culture is no longer routinely performed in clinical microbiology laboratories.[417] A complement fixation titer greater than 1:64 supports a diagnosis of LGV in the appropriate clinical setting but is not widely available.[209] Histopathologic changes in LGV are not specific. A skin test (Frei test) has been used in the past but is no longer available.[417,427] With this lack of widely available LGV-specific diagnostic tests, it is recommended that patients with a clinical presentation of LGV or LGV proctitis be treated presumptively.

Treatment of Lymphogranuloma Venereum

For classic LGV, the CDC recommends 21 days of doxycycline, 100 mg twice daily, for treatment of LGV, with erythromycin as an alternative.[209] Data are lacking, but if doxycycline cannot be used because of allergy or intolerance, azithromycin, 1 g orally once weekly for 3 weeks, is probably effective, and levofloxacin for extended intervals might be effective as well.[209,391] In addition, fluctuant buboes should be aspirated to prevent rupture and sinus tract formation.[417] Antibiotic therapy results in rapid abatement of constitutional symptoms but has only a limited effect on bubo resolution.[417] Effects on late complications such as strictures are variable.[161,417] Sexual partners should be examined and treated. Pregnant women with LGV should be treated with erythromycin because doxycycline should be avoided.[209]

With the overall lack of access to specific testing for LGV serovars, all cases of severe chlamydial proctitis and proctocolitis should be assumed to be due to LGV strains and treated with the 21-day regimen of doxycycline.[209] However, this regimen from the CDC is based on the experience with classic LGV, and concern about the deep-seated nature of LGV buboes, and is not based on comparative studies of treatment duration for LGV proctitis.[391] The optimal duration of treatment for asymptomatic or minimally symptomatic LGV proctitis is not known.

A recent observational study of MSM from the United Kingdom showed that most asymptomatic or mildly symptomatic rectal infections due to an LGV serovar resolved microbiologically with 7 days of doxycycline.[428] However, clinical trials are needed to confirm that a shorter 7-day doxycycline course would suffice.

PREVENTION STRATEGIES

Primary prevention strategies are centered on (1) inducing behavioral changes that reduce the risk of acquisition of chlamydial infections, as well as other STIs; and (2) identification and treatment of people with genital infection before they can transmit the infection or develop complications. Behaviors that reduce risk of infection include delaying age of first intercourse, decreasing numbers of partners, and promoting use of condoms. Vaginal microbicides such as vaginal sponges containing the spermicide nonoxynol-9 were previously thought to be of value, but a meta-analysis of multiple trials concluded that nonoxynol-9 was ineffective in preventing cervical gonorrhea, chlamydial infection, or HIV infection.[429] Other microbicides that may be more effective and less toxic than nonoxynol-9 are under development, but none is yet available.

Many developed countries have initiated programs with the stated goal of reducing transmission and sequelae of *C. trachomatis* by screening high-risk populations for asymptomatic infections and by aggressive partner notification and treatment programs. These efforts are based on the previous success of such measures with gonococcal infections and on a few controlled clinical trials that sought to determine the effect of screening on development of symptomatic PID.[430] Most clear-cut is a randomized trial that used selective screening criteria in a health maintenance organization and demonstrated a 60% reduction in PID among screened women during 12 months of follow-up.[328] Largely on the basis of these data, the US Preventive Services Task Force recommendations for screening women have been published and are summarized in Table 180.4.[431,432] However, adoption of such opportunistic screening in the United States has been relatively low,[433] and the effects on population prevalence are less clear. A small longitudinal study of repeated infections in adolescent women showed that screening and treatment as frequently as every 3 months did not reduce point prevalence over time in a high-risk population.[77] On a larger scale, a recent controlled trial of registry-based screening did not demonstrate a reduction in prevalence of *C. trachomatis* infection.[434] At present there is insufficient evidence to recommend routine *C. trachomatis* screening in men.

Patients with *C. trachomatis* urogenital infection should be retested 3 months after treatment because of the high frequency of repeated chlamydial infections within the first several months after treatment.[209,372,435] This retesting is to identify any new chlamydial infection, which is usually from an untreated sex partner or a new partner, and is not to test for cure of the initial infection. However, despite these recommendations, a recent study found that only 22.3% of men and 38% of nonpregnant women were retested.[436] Interventions under evaluation to improve these rates include home-obtained vaginal swabs or urine collection using mailed kits.[437,438]

TABLE 180.4 Screening for Chlamydial Infection, US Preventive Services Task Force, 2014

Screen all sexually active women ≤24 years old
Screen older, high-risk women[a]
Screen pregnant women ≤24 years old
Screen older, high-risk pregnant women[a]
Insufficient evidence to recommend male screening at present

[a]High risk for chlamydial infection as defined in various populations can include women ≤24 years old; new or multiple sexual partners, a sex partner with concurrent partners, or a sex partner with a sexually transmitted infection; previous or concurrent sexually transmitted infection; inconsistent condom use among persons who are not in mutually monogamous relationships; and exchanging sex for money or drugs.

Noninvasive testing of urine and self-collected vaginal swabs with NAATs has facilitated screening efforts in sexually active young men and women. Target groups include nontraditional settings such as high schools, military intake centers, juvenile detention centers, and other nonclinic sites in the community.[439–441] The benefits of screening women have been assessed primarily in ecologic studies in which there have been declining case rates of infection and PID in the screened population during the first several years of screening implementation. However, some programs have noted an unexpected increase in chlamydial case rates and repeated infections as screening continued,[180] while the PID rate has continued to decline.[442] The "arrested immunity" hypothesis attempts to account for these observations by proposing that early identification and treatment of *C. trachomatis* infections interferes with the development of protective immune responses, leaving the individual more susceptible to reinfection,[443] while also interfering with deleterious immune responses that lead to PID.[442]

Notification and treatment of sexual partners are also effective in reducing infection and can be cost-effective despite the resources required.[444,445] The effectiveness of sex partner management has been shown to be improved by expedited partner-delivered therapy.[446] In this innovative approach, the sex partner does not have direct medical contact or evaluation, but instead receives empirical single-dose antibiotic therapy through the index case, often with solicitation of mailed-in urine or vaginal swabs for NAAT testing. Collectively, these prevention strategies have the goal of reducing the high prevalence of *C. trachomatis* genital infections.

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The complete reference list is available online at Expert Consult.

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181 Psittacosis (Due to *Chlamydia psittaci*)

David Schlossberg

SHORT VIEW SUMMARY

Definition
- Psittacosis is a systemic infection that frequently causes pneumonia.

Epidemiology
- It is acquired by inhalation after exposure to infected birds, or less commonly from domesticated animals. Occasional patients provide no history of relevant exposure.

Microbiology
- The etiologic agent is *Chlamydia psittaci* of the family Chlamydiaceae.

Diagnosis
- Diagnosis is made serologically in most cases; when available, polymerase chain reaction may be positive. Because diagnostic methods are imperfect, treatment should not await definitive diagnosis.

Therapy
- Doxycycline or tetracycline is the treatment of choice. Macrolides and fluoroquinolones have good in vitro activity, but there is limited clinical experience.

Prevention
- Treat imported birds and birds that have suspected infection.

Psittacosis is a systemic infection that frequently causes pneumonia. Its relationship to bird exposure has been known for more than 100 years. In 1879, Ritter[1] studied an outbreak in Switzerland and called it pneumotyphus. Morange[2] applied the term *psittacosis* (from the Greek word for parrot) in 1892 after studying cases associated with sick parrots. In 1930, the organism was identified in several laboratories, by Bedson in the United Kingdom, Kromwede in the United States, and Levinthal in Germany.[3]

The name *psittacosis* has persisted, even though the term *ornithosis* more accurately depicts the potential for all birds to spread this infection. In fact, even mammals, including humans, are rare sources of psittacosis.

The causative agent of psittacosis is *Chlamydia psittaci.* A proposed reclassification grouped *C. psittaci* with *Chlamydia pneumoniae, Chlamydia pecorum, Chlamydia abortus, Chlamydia caviae,* and *Chlamydia felis* in a new genus, *Chlamydophila*, but use of the latter has declined.[4]

EPIDEMIOLOGY

Chlamydia psittaci is common in birds (often as asymptomatic carriers) and domestic animals. Infection is therefore a hazard to pet owners, pet shop employees, poultry farmers (turkey-associated psittacosis has the highest attack rate in psittacosis epidemics), workers in abattoirs and processing plants (psittacosis is the most common abattoir-associated pneumonia), and veterinarians. However, anyone in contact with an infected bird or animal is at risk. Human cases occur both sporadically and in outbreaks.[5,6]

Most patients with psittacosis have had some contact with a bird, usually as a pet. In fact, the importation of exotic birds (sometimes illegally) has been correlated with an increase in human psittacosis in the United States, Sweden, England, and Wales. Often, the bird was recently acquired or was ill. Bird contact may achieve surprising levels of intimacy. Patients have acquired psittacosis by kissing their parrot or by performing mouth-to-mouth resuscitation on a dying bird. Other patients have had more trivial or transient exposure, such as visits to public bird parks, transporting pigeons by car, passing through a room in which infected birds were sitting, sharing a stage with a parrot, or guarding crates of pigeons at a railroad depot. Still, some patients (25%) have had no avian exposure,[7] and other animal sources may rarely be implicated, as in a recent outbreak in a veterinary school following exposure to equine fetal membranes.[8]

Birds transmit the infection to their nestlings, which in turn shed the organism during periods of both illness and good health. In bird populations studied, there is a baseline prevalence of 5% to 8% of *C. psittaci* carriage. This may increase to 100% when birds are subjected to the stress of shipping, crowding, and breeding.[3,7]

It is likely that all birds are susceptible. More than 130 avian species have been documented as hosts of *C. psittaci*.[3] These include members of the parrot family (macaws, cockatoos, parakeets, budgerigars), finches (canaries, bullfinches, goldfinches, sparrows), poultry (hens, ducks, geese, turkeys), pigeons, pheasants, egrets, seagulls, and puffins.

Infection may appear in birds years after exposure. Infected birds may be asymptomatic or obviously sick. In the latter case, birds may exhibit shivering, depression, anorexia, emaciation, dyspnea, and diarrhea, frequently with closed eyes and ruffled feathers. Spontaneous relapse and remittance of the illness may occur, although it is during periods of illness that infected birds excrete the largest numbers of organisms. Discharge from their beaks, eyes, feces, and urine is infective; their feathers and the dust around their cage become contaminated.

The infection is generally spread by the respiratory route, by direct contact or aerosolization of infective discharges or dust. Rarely, the bird may spread the infection by a bite. If untreated, 10% of infected birds become chronic asymptomatic carriers.[7]

Strains from turkeys and psittacine birds are the most virulent for humans. Although most human exposure comes from avian strains of *C. psittaci,* disease has occurred in ranchers after exposure to infected tissues from parturient cows, goats, and sheep. Endocarditis has been attributed to avian and nonavian strains, and cats have spread feline pneumonitis to humans and other mammals. The growing practice of pet-associated therapy in nursing homes has produced a new epidemiologic risk for psittacosis.[9]

Human-to-human[10,11] and nosocomial[12] transmissions are rare, and it is therefore thought unnecessary to isolate patients in the hospital or to give antibiotic prophylaxis to contacts. However, cases acquired from humans tend to be more severe than avian-acquired disease. Environmental sanitation is important because the organism is resistant to drying and can remain viable for months at room temperature.[3,7]

CLINICAL MANIFESTATIONS AND DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS

The disease begins after an incubation period of 5 to 15 days. Onset may be insidious or abrupt, and the clinical manifestations tend to be nonspecific. Several syndromes may result. The infection may be

subclinical, or it may resemble a nonspecific viral illness with fever and malaise or a mononucleosis-like syndrome with fever, pharyngitis, hepatosplenomegaly, and adenopathy. A typhoidal form manifests as fever, bradycardia, malaise, and splenomegaly. However, the presentation most suggestive of the cause is atypical pneumonia, with nonproductive cough, fever, headache, and chest film abnormalities more dramatic than would be suggested by the physical findings. The illness ranges in severity from an inapparent or mild disease to a fatal systemic illness with prominent respiratory symptoms.

Because many patients have an illness with nonspecific findings, the list of initial diagnoses for which patients have been referred to hospitals is extensive. This list reflects the various organ systems that may be involved in *C. psittaci* infection and includes the diagnoses of meningitis, tonsillitis, pneumonia, pulmonary embolism, myocardial infarction, gastroenteritis, hepatitis, peritonitis, pancreatic carcinoma, urinary tract infection, endocarditis, vasculitis, septicemia, malaria, brucellosis, fever of unknown origin, and polymyositis.[9,13]

The list of considerations in the differential diagnosis is extensive, and the diagnostic possibilities depend on the presentation. A typhoidal picture suggests the mononucleosis syndrome, typhoid fever, brucellosis, tularemia, influenza, malaria, or subacute bacterial endocarditis. Respiratory signs and symptoms plus headache and myalgias should orient the clinician to causes of atypical pneumonia, such as viral pneumonia, Q fever, legionellosis, and infection with mycoplasma and *C. pneumoniae.* Helpful clues to a diagnosis of psittacosis, when present, are relative bradycardia, rash, hemoptysis, epistaxis, and splenomegaly.

The most common sign is fever, occurring in 50% to 100% of patients. Cough has been reported in 50% to 100%, but often it appears late in the illness and is not present initially. Headache, myalgias, and chills are reported in 30% to 70% of patients. The nonspecificity of these signs and symptoms may be puzzling until cough supervenes. Even then, the long list of other signs and symptoms that occur in fewer than half the patients may be particularly confusing due to the lack of specificity: diaphoresis, photophobia, tinnitus, ataxia, deafness, anorexia, nausea and vomiting, abdominal pain, diarrhea, constipation, sore throat, dyspnea, hemoptysis, epistaxis, arthralgia, and rash. Chest soreness is reported, but true pleuritic pain is rare.[5,7,13,14]

The signs most frequently reported are fever, pharyngeal erythema, rales or other abnormalities on chest auscultation, and hepatomegaly. These occur in more than half of cases. Fewer than 50% of patients show the signs of somnolence, confusion, tachycardia, relative bradycardia, pleural rub, splenomegaly (this occurs toward the end of the first week and is helpful diagnostically), adenopathy, palatal petechiae, herpes labialis, Horder spots (see later), and muscle tenderness.[5,7,13]

Specific end-organ involvement reflects the systemic nature of psittacosis. The organ most commonly involved in humans is the lung. This is manifested clinically by cough, dyspnea, and a variety of nonspecific auscultatory findings on physical examination. On occasion, the pneumonitis may progress to acute respiratory distress syndrome. Cardiac manifestations include pericarditis (rarely with effusion and tamponade), myocarditis, idiopathic dilated cardiomyopathy,[15] and "culture-negative" endocarditis. *Chlamydia psittaci* endocarditis is associated with preexisting heart disease and may cause valvular destruction. Arterial embolism to major vessels occurs rarely. The source of these emboli and the mechanism are unknown; some are attributed to endocarditis or mural thrombi.[16]

Hepatitis may develop, sometimes with jaundice. Anemia may result from hemolysis (both Coombs test positivity and cold agglutinins are reported) and from a reactive hemophagocytosis, in which case pancytopenia may be present. Disseminated intravascular coagulation (DIC) also complicates psittacosis.[17,18] Reactive arthritis occurs 1 to 4 weeks after the initial illness; although most of the described cases are polyarticular, monarticular arthritis has also been described.

Neurologic abnormalities include cranial nerve palsy (including sensorineural hearing loss), cerebellar involvement, transverse myelitis, confusion, meningitis, encephalitis, transient focal neurologic signs, and seizures. Results of cerebrospinal fluid examination on lumbar puncture are usually normal; a small number of white cells (predominantly lymphocytes) may be seen, and the protein level on occasion is greatly elevated.[19–24]

Dermatologic phenomena include Horder spots, which are a pink, blanching, maculopapular eruption on the face or trunk resembling the rose spots of typhoid fever. Also described are erythema multiforme, erythema marginatum, erythema nodosum, and urticaria, as well as acrocyanosis, subungual splinter hemorrhages, and superficial venous thromboses. Acute glomerulonephritis, acute tubulointerstitial nephritis, and acute tubular necrosis have been reported. Psittacosis has severe consequences in pregnancy and often causes DIC, hepatic dysfunction, and placentitis, with fetal compromise.[25,26] Additional clinical complications of psittacosis include phlebitis, pancreatitis, and thyroiditis. Bacteremia has been demonstrated in a patient with a sarcoid-like illness.

Recent observations have suggested that *C. psittaci* is associated with ocular adnexal lymphomas involving orbital soft tissue, lacrimal glands, and conjunctiva. *Chlamydia psittaci* has been detected in lymphoma biopsies by polymerase chain reaction (PCR) assay, and tumors in some patients have regressed after treatment with doxycycline. However, the prevalence of *C. psittaci* in these lymphomas varies, particularly geographically,[27–29] and some tumors with no evidence of *C. psittaci* have also responded to doxycycline therapy, suggesting that *C. psittaci* detection methods are inadequate or that other doxycycline-responsive organisms may cause this malignancy, or that the presumed lymphoma may not be a true malignancy but a hyperproliferative response to an infecting agent.[30,31]

In addition to ocular adnexal lymphomas, *C. psittaci* has recently been associated with nongastrointestinal mucosa–associated lymphoid tissue lymphomas and autoimmune precursor lesions (Hashimoto thyroiditis and Sjögren syndrome).[32]

There is no documented protection after infection, and second infections have been seen in spite of elevated levels of complement-fixing (CF) antibodies.[3] Treated birds can also be reinfected.

LABORATORY FINDINGS

The total white blood cell count is usually normal or slightly elevated. Two-thirds of patients have a leftward shift. Eosinophilia has been seen in convalescence. Results on liver function testing are mildly abnormal in 50% of cases and may suggest cholestasis. Culture of the organism is possible from blood in the first 4 days of illness and from sputum in the first 2 weeks. However, although the organism can be isolated in cell culture and by animal inoculation, these methods are dangerous, and serologic diagnosis is preferred (see "Diagnosis" later).

Appearance on chest radiography is abnormal in approximately 75% of patients (range, 50%–90%) and is usually more abnormal than auscultation would predict. The most frequent finding is consolidation in a single lower lobe, seen in 90% of the abnormal chest radiographs. However, a variety of patterns have been reported, including a homogeneous ground-glass appearance, a patchy reticular pattern radiating from the hila, segmental or lobar consolidation with or without atelectasis, a miliary pattern, unilateral or bilateral hilar enlargement, and the halo sign. These chest radiograph findings may take as long as 20 weeks to resolve, with resolution occurring by 6 weeks on average. Pleural effusions are seen in up to 50% of cases but are usually small and asymptomatic.[5] As noted, hilar enlargement may be present but never as the sole manifestation of disease.

PATHOLOGIC FINDINGS

Birds show involvement predominantly in the liver, spleen, and pericardium, but in humans the lung is most frequently and characteristically involved. The trachea and bronchi become inflamed, with widespread mucous plugging. The inflammation spreads from respiratory bronchioles to the alveoli in a lobular pattern. Alveolar and then interstitial exudates accumulate; these are composed of mononuclear cells with a few polymorphonuclear leukocytes, red blood cells, epithelial cells, and fibrin. There is hyperplasia, proliferation, and desquamation of alveolar lining cells, which contain basophilic intracytoplasmic inclusions. Hilar lymph nodes swell, and the lungs become rubbery and solid. The classic sequence of congestion, edema, and red and then gray hepatization is seen.

The brain is congested and edematous, with diffuse arachnoiditis. Meningeal exudate contains macrophages with intracytoplasmic inclusions. The heart shows monocytic infiltration, edema, fatty degeneration,

and subendocardial hemorrhage. The pathologic findings in acute glomerulonephritis include hyaline glomerular occlusion, with subepithelial electron-dense deposits on electron microscopy. The liver may show nonspecific hepatitis or granulomas. Infected placental tissue shows intervillositis with trophoblastic cytoplasmic inclusions.[25] In emboli, polymorphonuclear leukocytes, platelets, and fibrin are seen but not organisms or chlamydial antigen.[18]

DIAGNOSIS

Culture from sputum, pleural fluid, and clotted blood is possible but dangerous because of the risk to technicians of laboratory-acquired infection, and direct identification in tissue specimens is not standardized, so diagnosis depends on serology. The Centers for Disease Control and Prevention considers a *confirmed* case as one with a compatible clinical illness plus either laboratory confirmation by culture from respiratory secretions, or fourfold or greater rise in CF or microimmunofluorescence (MIF) antibody (immunoglobulin G) in specimens drawn at least 2 weeks apart.[32a] A *probable* case is one associated with a compatible illness plus either an antibody (immunoglobulin M) titer of at least 1:32 in a single specimen or detection of *C. psittaci* DNA in a respiratory specimen by PCR. There are both false-positive and false-negative PCR results. Also, the complement fixation test is only genus-specific and does not distinguish *C. psittaci* from *Chlamydia trachomatis* or *C. pneumoniae,* both of which are common pathogens. MIF testing is species-specific but is not generally available, and cross-reactions still occur. Thus serologic testing remains imperfect. In addition, antibiotic therapy can delay or diminish the antibody response. PCR assay can detect *C. psittaci* in avian and human tissues[33–36] and has been used for real-time diagnosis in both birds and humans.[33,37] A recent study in the Netherlands utilized PCR successfully to diagnose psittacosis in patients with community-acquired pneumonia.[38] However, PCR assay is not routinely available and may yield false-negative results. A recent report described rapid identification of *C. psittaci* inclusion bodies in mucosal lesions stained by the Diff-Quik cytologic stain.[39] Thus, since none of the diagnostic methods combines the features of sensitivity, specificity, speed, and availability, therapy for psittacosis should be initiated on the basis of clinical suspicion, usually in the setting of a relevant exposure history.

THERAPY

The treatment of choice is doxycycline, 100 mg PO twice daily, or tetracycline hydrochloride, 500 mg PO four times daily for 10 to 21 days. Some observers recommend the longer course to prevent relapse, but this is controversial. Minocycline is probably equivalent to doxycycline, but clinical data are limited. Macrolides have good in vitro activity, and azithromycin has been effective in birds; thus some have recommended azithromycin as alternative therapy in children and pregnant patients. However, macrolides may be less efficacious in severe cases and might not protect the fetus when administered during pregnancy. Fluoroquinolones, particularly levofloxacin and moxifloxacin, demonstrate good in vitro activity, but clinical experience with these agents is also lacking.[40]

Most patients respond within 24 hours subjectively. Without treatment, the fatality rate is approximately 20%; with treatment, it drops to 1%. The best therapy for endocarditis is valve replacement and prolonged antimicrobial therapy with doxycycline, possibly with macrolides or fluoroquinolones.[7,16] A theoretical advantage of fluoroquinolone chlamydiacidal activity in intravascular infection awaits further clinical experience.

PREVENTION

Infected birds should be treated with tetracycline, chlortetracycline, or doxycycline for at least 45 consecutive days. The US Department of Agriculture (USDA) requires that imported birds be quarantined for 30 days to prevent introduction of Newcastle disease. During this period, birds are treated with chlortetracycline. The USDA recommends that importers continue treatment for an additional 15 days, but this is not always done, and, if treated for fewer than 45 days, some infected birds will continue to shed the organism.[7]

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The complete reference list is available online at Expert Consult.

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182 Chlamydia pneumoniae

Margaret R. Hammerschlag, Stephan A. Kohlhoff, and Charlotte A. Gaydos

SHORT VIEW SUMMARY

Microbiology and Epidemiology
- Obligate intracellular bacterium, must be grown in tissue culture.
- Capable of causing persistent infection, often subclinical.
- Worldwide distribution, infects many animals as well as humans.
- Primarily a respiratory pathogen in humans, causing community-acquired pneumonia.
- Can cause epidemics in enclosed populations: military bases, schools, nursing homes.

Diagnosis
- *Chlamydia pneumoniae* causes pneumonia; clinically it cannot be differentiated from other causes of atypical pneumonia, especially *Mycoplasma pneumoniae*.
- The most accurate method of diagnosis is identification of the organism in respiratory samples by culture or nucleic acid amplification test (NAAT).
- Serology is of limited value and requires paired sera, and many patients who are positive by culture or NAAT will be seronegative.

Therapy
- *Chlamydia pneumoniae* is susceptible to macrolides, quinolones, and tetracyclines. Data on efficacy, including optimal dose and duration of therapy, are limited.
- Ten- to 14-day courses of erythromycin, clarithromycin, doxycycline, levofloxacin, or moxifloxacin or 5 days of azithromycin are clinically effective and result in approximately 80% microbiologic eradication.

Chlamydiae are obligate intracellular bacterial pathogens whose entry into mucosal epithelial cells is necessary for intracellular survival and subsequent growth. Chlamydiae cause a variety of diseases in animal species at virtually all phylogenic levels, from amphibians and reptiles to birds and mammals. In 1999, Everett and coworkers[1] reported a taxonomic analysis involving the 16S and 23S ribosomal RNA (rRNA) genes and found that the order Chlamydiales contained at least four distinct groups at the family level. Moreover, within the family Chlamydiaceae there were two distinct lineages, which suggested splitting the genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*. This classification was not universally accepted by the *Chlamydia* scientific community, and recently it was agreed that the family Chlamydiaceae contains a single genus, *Chlamydia*.[2] This position has been supported by additional data on chlamydial genome sequences. The genus *Chlamydia* contains nine recognized species: *Chlamydia trachomatis, Chlamydia psittaci* (agent of psittacosis; many species of birds), *Chlamydia pneumoniae, Chlamydia pecorum* (ruminants and koalas), *Chlamydia muridarum* (formerly the agent of mouse pneumonitis), *Chlamydia suis* (an important pathogen of swine), *Chlamydia abortus* (causes abortion in cattle and sheep; rarely causes abortion in humans), *Chlamydia caviae* (formerly *C. psittaci,* the guinea pig inclusion conjunctivitis strain), and *Chlamydia felis* (causes epidemic keratoconjunctivitis in cats).[1–4] *Chlamydia trachomatis* and *C. pneumoniae* are the most significant human pathogens, and *C. psittaci* is an important zoonosis.

Recently, several chlamydiae-like organisms that are endosymbionts of free-living amebas have been identified.[3,5] These organisms, which include *Parachlamydia acanthamoebae, Simkania negevensis,* and *Neochlamydia hartmannellae,* have been termed *environmental chlamydiae.* Analyses of nearly full-length 16S rRNA gene sequences of these isolates showed that they clustered with other members of the order Chlamydiales but in a lineage separate from those of *Chlamydia* (16S rRNA sequence similarities >88%). This bacteria-protozoa interaction might have been a driving force for the development of effective mechanisms by bacteria to survive phagocytosis by unicellular eukaryotes, which in turn may have been a first step in the evolution of intracellular bacterial pathogens of higher organisms.[5]

Although *C. pneumoniae* is a human respiratory pathogen, the organism has also been isolated from nonhuman species, including horses, cats, koalas, bandicoots, and amphibians.[4,6,7] Molecular data suggest that the animal strains are ancestral to human strains and that the organism may have crossed from animals to humans as the result of a relatively recent zoonotic event.[4] Whole-genome sequencing has found that human isolates are highly conserved, whereas a *C. pneumoniae* strain from a koala was 12 kb larger, with several interesting differences, including a plasmid that has not been described in human isolates.[4]

HISTORY

The first isolates of *C. pneumoniae* were serendipitously obtained during trachoma studies in the 1960s. After the recovery of a similar isolate from the respiratory tract of a college student with pneumonia in Seattle, Grayston and colleagues[8] applied the designation TWAR after their first two isolates, TW-183 and AR-39. Only one serotype of *C. pneumoniae* has been identified so far. Studies have found a high degree of genetic relatedness (>98%) among human *C. pneumoniae* isolates tested.[4,9]

MICROBIOLOGY

Chlamydiae have a gram-negative envelope without detectable peptidoglycan; however, recent genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode for proteins that form a nearly complete pathway for synthesis of peptidoglycan, including penicillin-binding proteins.[10] Chlamydiae also share a group-specific lipopolysaccharide antigen and use host ATP for the synthesis of chlamydial protein.[10] Although chlamydiae are auxotrophic for three of four nucleoside triphosphates, they do encode functional glucose-catabolizing enzymes, which can be used for generating ATP.[10] As with peptidoglycan synthesis, for some reason these genes are turned off, which may be related to their adaptation to the intracellular environment. All chlamydiae also encode an abundant protein called the major outer membrane protein (or outer membrane protein A) that is surface exposed in *C. trachomatis* and *C. psittaci* but apparently not in *C. pneumoniae*.[10] The major outer membrane protein is the major determinant of the serologic classification of *C. trachomatis* and *C. psittaci* isolates. Chlamydiae are susceptible to antibiotics that interfere with DNA and protein synthesis, including tetracyclines, macrolides, and quinolones. *Chlamydia pneumoniae* lacks a tryptophan recovery or biosynthesis pathway and is resistant to sulfonamides and trimethoprim.[4]

Chlamydiae have a unique developmental cycle with morphologically distinct infectious and reproductive forms: the *elementary body* (EB) and the *reticulate body* (RB; Fig. 182.1). After infection, the infectious EBs, which are 200 to 400 nm in diameter, attach to the host cell by a

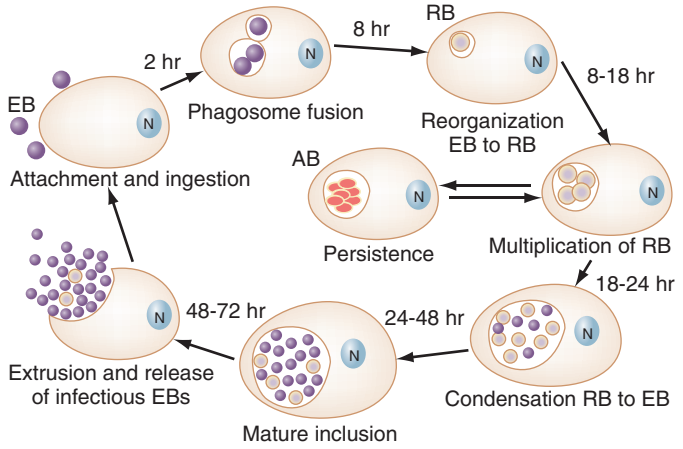

FIG. 182.1 Life cycle of chlamydiae in epithelial cells. *AB,* Aberrant body; *EB,* elementary body; *N,* nucleus; *RB,* reticulate body. *(Modified from Hammerschlag MR, Kohlhoff SA, Darville T:* Chlamydia pneumoniae *and* Chlamydia trachomatis. *In: Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.)*

process of electrostatic binding and are taken into the cell by endocytosis that does not depend on the microtubule system. EBs are sporelike; they are metabolically inactive but stable in the extracellular environment. Within the host cell, the EB remains within a membrane-lined phagosome, with inhibition of phagosomal-lysosomal fusion. The inclusion membrane is devoid of host cell markers, but lipid markers traffic to the inclusion, which suggests a functional interaction with the Golgi apparatus. Chlamydiae appear to circumvent the host endocytic pathway, inhabiting a nonacidic vacuole that is dissociated from late endosomes and lysosomes. EBs then differentiate into RBs that undergo binary fission. After approximately 36 hours, the RBs differentiate back into EBs. Despite the accumulation of 500 to 1000 infectious EBs in the inclusion, host cell function is minimally disrupted. At about 48 hours, release may occur via cytolysis or a process of exocytosis or extrusion of the whole inclusion, leaving the host cell intact. This strategy is very successful and enables the organism to cause essentially silent chronic infection.

A number of in vitro studies have challenged this biphasic paradigm. Chlamydiae may enter a persistent state in vitro after treatment with certain cytokines, such as interferon-γ (IFN-γ); treatment with antibiotics, specifically penicillin; restriction of certain nutrients, including iron, glucose, and amino acids; infection in monocytes; and heat shock.[4,11] While in the persistent state, metabolic activity is reduced and the organism is often refractory to antibiotic treatment. These different systems produce similar growth characteristics, including loss of infectivity and development of small inclusions that contain fewer EBs and RBs, and ultrastructural findings, specifically morphologically abnormal RBs, which suggests that they are somehow altered during their otherwise normal development. These abnormal RBs are often called aberrant bodies (ABs). Restriction of certain nutrients has also been shown to induce persistence in chlamydiae. Ultrastructural analysis of IFN-γ–treated *C. pneumoniae* also reveals atypical inclusions that contain large reticulate-like ABs with no evidence of redifferentiation into EBs.

Another model of persistent *C. pneumoniae* infection is long-term continuous infection. In contrast to the previously described models, continuous cultures become spontaneously persistent when both chlamydiae and host cells multiply freely in the absence of stress. *Chlamydia pneumoniae* infection was maintained in human epithelial type 2 (HEp-2) and A549 cells for more than 4 years without centrifugation, addition of cycloheximide, or IFN-γ treatment.[12] Infection levels in these infected cells were high (70%–80%). Ultrastructural studies revealed three types of inclusions in these cells. Approximately 90% were typical large inclusions that ranged approximately from 5 to 12 μm in diameter. The second type (altered inclusions) contained both normal EBs and RBs, but in considerably lower numbers than typical inclusions, and pleomorphic ABs, which were up to four to five times the size of normal RBs (2.5 μm in diameter); their cytoplasm was homogeneous. The third type of inclusion was small aberrant inclusions, on average 4 μm in diameter, containing about 60 ABs that were similar in size to normal RBs but appeared electron dense and no longer retained a smooth spherical shape. These dense ABs retained the characteristic chlamydial outer membrane structure, with very little periplasmic space, and the membranes were more tightly bound to the chlamydial body, similar to normal RBs. No EBs were observed in these inclusions. These findings show that the developmental cycle of *C. pneumoniae* can combine the typical development forms with the persistent phase in tissue culture.

Another possible mechanism of chlamydial persistence could be through a direct effect on the host cell, possibly through an effect on apoptosis, which is an important regulator of cell growth and tissue development. Apoptosis is a genetically programmed, tightly controlled process, unlike necrosis, which involves nonspecific inflammation and tissue damage and intracellular enzymes, condensation of nucleus, and cytoplasm and fragmentation. Many microbial pathogens, including chlamydiae, have been found to modulate cellular apoptosis to survive and multiply. *Chlamydia* spp. have been shown to both induce and inhibit host cell apoptosis, depending on the stage of the chlamydial developmental cycle.[13] Chlamydiae protect infected cells against apoptosis as a result of external stimuli during early stages of infection and may induce apoptosis of the host cell during later stages of the life cycle. Thus chlamydiae may protect infected cells against cytotoxic mechanisms of the immune system, and the apoptosis observed at the end of the infection cycle may contribute to the inflammatory response because apoptotic cells secrete proinflammatory cytokines and facilitate the release of the organism from the infected cells. Studies with IFN-γ–treated cultures have reported that cells infected with *C. trachomatis* and *C. pneumoniae* resist apoptosis as the result of external ligands, via inhibition of caspase activation. Data from studies with the long-term continuously infected cell model showed marked differences in the effect of *C. pneumoniae* on apoptosis in acute and chronically infected A549 cells.[13] Acute *C. pneumoniae* infection *induced* apoptotic changes in A549 cells within the first 24 to 48 hours after infection. Induction of apoptosis in acute infection may facilitate release of *C. pneumoniae* from the host cell. Chronic *C. pneumoniae* infection *inhibited* apoptotic changes within the first 24 hours and up to 7 days. These results suggest that inhibition of apoptosis may help to protect the organism when it is in the intracellular, persistent state.

LABORATORY TESTING FOR *CHLAMYDIA PNEUMONIAE*

Although numerous methods can be used to detect *C. pneumoniae* in clinical samples, in practice detection is very difficult, primarily because of the lack of standardized well-validated methods. Determination of whether *C. pneumoniae* infection is an acute primary infection or reinfection, a chronic persistent stage, or a past infection is also very difficult. Cell culture, immunohistochemistry (IHC), and nucleic acid amplification tests (NAATs) detect living bacteria, antigen, and nucleic acid, respectively. These techniques are primarily used in research settings or require experienced specialized laboratories. In clinical settings, routine diagnosis of *C. pneumoniae* infection has been based on results of serologic testing to identify anti–*C. pneumoniae* immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) antibodies. This approach is problematic for a number of reasons subsequently outlined in detail.

Two new commercially available multiplex nucleic acid amplification assays for the detection of *C. pneumoniae* in conjunction with other respiratory pathogens were cleared by the US Food and Drug Administration (FDA) in 2012 and 2017 (discussed later under "Single and Multiplex Nucleic Acid Amplification Tests").[14,15]

Cell Culture

Chlamydia pneumoniae, as an obligate intracellular parasite, can be isolated by means of cell culture, but the organism is fastidious and slow growing. *Chlamydia pneumoniae* will grow, although not as readily, in cell lines that are usually susceptible to *C. trachomatis,* such as McCoy and HeLa cells. Growth has been observed to be somewhat easier in HL (human line) and HEp-2 cells.[16,17]

Chlamydia pneumoniae has been isolated from the respiratory tract (nasopharyngeal and throat cultures, bronchoalveolar lavage fluids) and tissue biopsies, including lung and adenoids. The organism can also be isolated from sputum, but sputum can be toxic to cell culture and often is contaminated by overgrowing fungi or bacteria. If nasopharyngeal or pharyngeal swab specimens are collected, use of aluminum- or plastic-shafted Dacron-tipped swabs is mandatory because calcium alginate on cotton-tipped swabs and those with wooden shafts may inhibit the growth of the organism in tissue culture and may be toxic to cells. Specimens for culture must be stored in a suitable transport medium optimized for chlamydiae. A suitable medium is sucrose-phosphate-glutamate buffer with antibiotics and fetal calf serum, but ready-to-use media are also commercially available.

Specimens that can be processed within 24 hours should be kept refrigerated at 4°C and shipped on wet ice. Samples that cannot be processed within 24 hours should be held at 4°C before freezing at –70°C because more rapid freezing decreases the titer of viable organisms. Specimens need to be treated with sterile glass beads or sonication to disrupt cells and then centrifuged onto the cell monolayers to facilitate absorption. Cell cultures are incubated at 37°C with 5% carbon dioxide for at least 72 hours per passage. Culture confirmation is assessed by staining inclusion bodies, using a *Chlamydia* genus–specific fluorescent antibody and epifluorescence microscopy (Fig. 182.2). More than one subculture may be necessary for isolation; thus culture is not a straightforward attempt to diagnose the microorganism in a timely fashion. Because the organism has been difficult to grow and because of the lack of any other commercially available diagnostic assay, most original associations with respiratory diseases have been via use of serology with the microimmunofluorescence (MIF) test. Patients who have IgG autoantibodies against IgM may cross react with anti–*C. pneumoniae* IgM antibody.[18]

Antigen Detection

Chlamydia pneumoniae has also been detected in tissue sections or cells with monoclonal antibodies labeled with a peroxidase (IHC) or fluorescent (immunofluorescent) marker. Antigen detection testing, in general, allows preservation of tissue morphology. On the other hand, interpretation of the staining pattern to distinguish the organism from background or nonspecific staining is subjective and influenced by a number of technical issues.[19] In complex biologic samples, IHC gives rise to cross-reactions between antitarget antibodies and nontarget proteins that produce nonspecific signals (e.g., immunoreactivity for *C. pneumoniae* was frequently present in atheroma and nonatheroma sections of vessel walls). The sites with positive results with *C. pneumoniae* IHC assays precisely matched the sites with autofluorescent ceroid deposits.[20] The interpretation of IHC staining must be performed with the utmost caution.

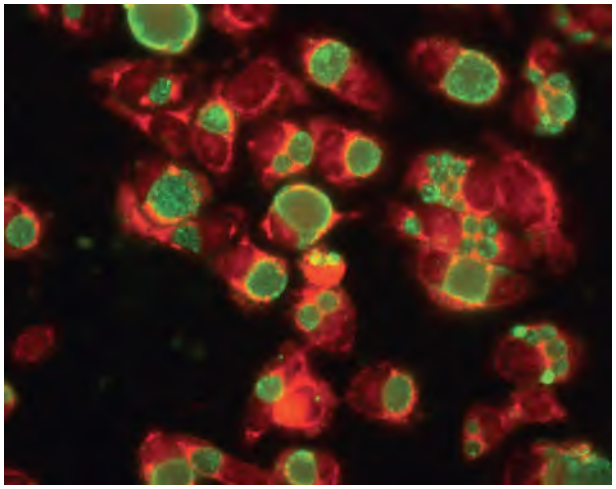

FIG. 182.2 Direct immunofluorescence staining of cell culture 72 hours after infection. The apple-green *C. pneumoniae* inclusion bodies are seen in red counterstained HEp-2 cells (magnification, ×600). *(Courtesy P. Apfalter.)*

Single and Multiplex Nucleic Acid Amplification Tests

Multiple in-house NAATs (such as polymerase chain reaction [PCR]) methodologies have been published, but the older literature has been confounded by lack of standardization and validation. In 2000, a Centers for Disease Control and Prevention (CDC) workshop suggested a few assays that were considered to be "validated" enough to be used for research.[19] Some of these included early developed and validated ones.[21–25] These have been improved, and others have been developed since then.[26–29] The advantages of these NAAT or PCR assays are their sensitivity, decreased possibility of contamination, and ability to quantify DNA. Nearly a decade later, however, many of these tests turned out to be highly prone to false-positive results.[30,31] Until recently, not a single NAAT for the detection of *C. pneumoniae* was commercially available or listed in the in vitro diagnostic database of the FDA (Devices @ FDA; http://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm). Numerous in-house PCR-based tests still are performed, but these assays range from those that are well validated to those that are not validated at all. Although NAATs offer the promise of exquisite sensitivity, theoretically allowing detection of a single organism in a clinical sample, both false-negative and false-positive results can and do occur because of a large number of technical issues that were summarized recently.[32] Currently, real-time PCR technology for the detection of *C. pneumoniae* should be used.[33,34] Real-time PCR offers significant advantages over conventional PCR in its rapidity, the ease with which it can be automated, the potential decreased risk of carryover contamination, and the potential provision of a quantitative result.

Until recently, there have been no commercially available NAAT assays. Abbott Laboratories (Abbott Park, IL) developed a research-use-only PCR assay that was used in a multicenter study comparing PCR results by using in-house PCRs from five different laboratories. The assay performed very well but it was never taken to a clinical trial.[35] Becton Dickinson (Franklin Lakes, NJ) performed a clinical trial for a strand displacement assay, but it was not cleared by the FDA. BioFire Technologies (formerly Idaho Technologies; Salt Lake City, UT) developed a FilmArray assay for the detection of 17 viruses, which is FDA cleared.[36,37] The FilmArray system now includes assays on the same platform for some of the atypical agents of pneumonia, including *C. pneumoniae, Mycoplasma pneumoniae,* and *Bordetella pertussis.* This assay received FDA clearance in July 2012. The BioFire FilmArray system combines nucleic acid extraction, nested PCR, detection, and data analysis in a single-use pouch.[14]

The BioFire FilmArray automated system is simple to perform, and results are ready in 1 hour. This single-test platform enables the detection of numerous viral and bacterial respiratory pathogens in a single test. The pouch is loaded into the FilmArray instrument, and the remainder of the test is completely automated. The system is very robust, detecting a low concentration of pathogen in the presence of a high concentration of a second pathogen. The respiratory panel detects adenoviruses, bocaviruses, coronaviruses, influenza A and B, influenza A subtypes (novel H1-2009, H1, H3), metapneumovirus, parainfluenza viruses types 1 to 4, respiratory syncytial virus, and rhinoviruses, as well as *B. pertussis, C. pneumoniae,* and *M. pneumoniae.*[14,36] However, data on the clinical performance of this assay for detection of *C. pneumoniae* from clinical specimens from patients with community-acquired pneumonia (CAP) are very limited. Most of the data on sensitivity and specificity were derived from contrived specimens.

The GenMark Respiratory Pathogen (RP) Panel (GenMark Diagnostics, Carlsbad, CA), another new multiplex panel for molecular amplification identification of respiratory viruses and bacteria, including *C. pneumoniae* and *M. pneumoniae,* was recently cleared by the FDA. Results were reported from a large multicenter evaluation that compared this assay to the BioFire FilmArray. However, *C. pneumoniae* was detected in only 6 of 2908 (0.3%) patients tested, and the data on performance parameters were derived from contrived specimens.[15,38] The GenMark RP Panel is a qualitative nucleic acid multiplex test for use on GenMark's new ePlex instrument. The ePlex RP Panel is a sample-to-answer multiplex assay that runs on a single-use cartridge that automates all aspects of nucleic acid testing, including extraction, amplification, and detection.[39] The ePlex RP Panel detects a similar range of viral and bacterial targets

as the Biofire FilmArray assay: viral targets include adenovirus, coronavirus (subtypes 229E, HKU1, NL63, OC43), human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A subtypes (H1, H1-2009, H3), influenza B, parainfluenza viruses types 1 to 4, and respiratory syncytial virus types A and B.[40] From specimen receipt to result, time to identification is 1 hour 45 minutes with a hands-on time per sample of 1 minute 35 seconds. Overall agreement between the ePlex RP Panel and BioFire FilmArray in this clinical trial was greater than 95% for all targets. Having new commercially available assays that can detect copathogens, as well as single organisms, will improve and better define the role of *C. pneumoniae* in diseases processes. However, we need more data on the performance of both assays for detection of *C. pneumoniae* in clinical specimens.

Specimens for research PCR testing have previously included nasopharyngeal swabs; secretions from the respiratory tract, including sputum and bronchoalveolar lavage fluid; tissue; and peripheral blood mononuclear cells (PBMCs). Swabs should be sent in tubes without transport medium. Sputum, bronchoalveolar lavage fluid, and tissue should also be collected in a sterile device without transport medium. Of note, currently FDA-cleared multiplex NAATs are only approved for use with nasopharyngeal swab specimens.

Serologic Testing

Several types of serologic assays are currently commercially available for the detection of antibodies to *C. pneumoniae.* However, none is currently approved by the FDA for this indication. The test used most frequently and recommended by the CDC remains the MIF assay.[19] Tests based on an enzyme-linked immunosorbent assay (ELISA) format are particularly easy to perform and do not need sophisticated laboratory equipment, which makes them the preferentially offered diagnostic chlamydial tool for laboratories. *Chlamydia pneumoniae,* however, is an intracellular pathogen, and the poor correlation between direct detection (e.g., with culture or NAAT) and serologic results is not surprising. Besides specificity issues, it is not at all clear which classes and titers of antibodies might represent acute first infection or reinfection, or chronic, persistent, or past *C. pneumoniae* infection.[19] This is true for complement fixation tests (measurement of antibodies against chlamydial lipopolysaccharide, therefore not specific for *C. pneumoniae*), ELISA-based tests (purified *C. pneumoniae* EBs or recombinant antigens detected; specificity unclear), and also the gold standard MIF test (formalinized *C. pneumoniae* EBs fixed onto glass slides). A serologic test can only be as specific as the antigen used. Cross-reactivity between *C. pneumoniae* and other *Chlamydia* species has been shown with the MIF test. Factors such as strain type, purity, and concentration of antigen used, and the assay procedure itself, might contribute to the fact that the MIF is less specific for *C. pneumoniae* than was thought 20 years ago. Data also show significant problems with subjective interpretation and intralaboratory and interlaboratory reproducibility.[41] The problems in context with *C. pneumoniae* serology have been discussed in detail in two review articles.[42,43] For an example of the complexity of this issue, consider that two multicenter pneumonia treatment studies in children showed that although 7% to 13% of the patients in the study had positive culture results and 7% to 18% met the serologic criteria with the MIF test for acute infection, they were not the same patients. Only 1% to 3% of the patients with positive culture results met the serologic criteria, and approximately 70% with positive culture results for *C. pneumoniae* were seronegative.[44]

Benitez and coworkers[34] reported similar data in adults from an investigation of a *C. pneumoniae* outbreak in a prison. MIF serology (IgG and IgM) had a positive predictive value of only 30% compared with real-time PCR. Another problem with serologic diagnosis of *C. pneumoniae* infection is that the MIF method used to detect serum antibodies is not standardized; studies have shown substantial interlaboratory variation in the performance of these tests.[41] In summary, serology seems not only to be insufficient for diagnosis of *C. pneumoniae* respiratory tract infection but also to be an inadequate methodology to study associations between *C. pneumoniae* and other diseases. *C. pneumoniae* serology is most problematic in terms of defining specificity, reproducibility, and titer in a given clinical picture or disease, even if prospectively defined.

It is important to know that new environmental *Chlamydia* spp. are being steadily described. Ample evidence exists for a huge diversity and wide distribution of chlamydiae in nature, and humans are exposed to that diversity of species. As an example, the recovery of a novel environmental *Chlamydia* strain from activated sludge with cocultivation with an *Acanthamoeba* sp. was reported; it was shown to also invade mammalian cells.[45] These new environmental chlamydiae (i.e., *Simkania, Waddlia,* and *Parachlamydia*) may interfere with serologic testing for traditional Chlamydiaceae (*Chlamydia*).[46,47]

EPIDEMIOLOGY

The mode of transmission of *C. pneumoniae* remains uncertain but probably is through infected respiratory secretions. Acquisition of infection via droplet aerosol was described during a laboratory accident.[48] *Chlamydia pneumoniae* can remain viable on Formica countertops for 30 hours and can survive small particle aerosolization.[49] Spread within families and enclosed populations, including military recruits, prisons, and nursing homes, has been described.[34,50–52] A review of the role of *C. pneumoniae* in respiratory illness in US Army training centers from January 2013 through December 2016 found that the organism was responsible for approximately 10% of illness, following rhinovirus and *M. pneumoniae* (J. Gaydos, personal communication).

Several serologic surveys have documented rising chlamydial antibody prevalence rates, beginning in school-age children and reaching 30% to 45% by adolescence.[8] Seroprevalence antibody, as determined with the MIF method, can exceed 80% in some adult populations.[53,54] The proportion of CAP in children and adults associated with *C. pneumoniae* infection has ranged from 0% to more than 44%, varying with geographic location, the age group examined, and the diagnostic methods used (Table 182.1).[55] The proportion of CAP attributable to *C. pneumoniae* appears to be significantly lower in studies published after 2000. Whether this is secondary to the methods used (most of the more recent studies have used real-time PCR) or possible cycling, as is seen with *M. pneumoniae,* is unknown. Four studies published after 2010, from diverse geographic areas (Europe, Africa, and Thailand), that used real-time PCR found prevalences of *C. pneumoniae* infection ranging from 0% to 3.8%.[56–59] This was compared with 11.4% in a Chinese study that used MIF serology; 30% of the patients only had a single serum sample (see Table 182.1).[60] In Germany from 2011 to 2012, CAP as assessed by molecular methods was more often attributable to *M. pneumoniae* (12.3%) and *C. psittaci* (2.1%) than *C. pneumoniae* (1.4%).[61] Early studies that relied on serology suggested that infection in children younger than 5 years was rare; however, subsequent studies with culture or PCR assay have found the prevalence rate of infection in children beyond early infancy to be similar to that found in adults.[55] Approximately 50% or more of children with culture-documented *C. pneumoniae* respiratory infection (pneumonia and asthma) show seronegativity with the MIF.[55] Prolonged respiratory infection, documented with culture, that lasts from several weeks to several years after acute infection has been reported.[62]

Coinfections with other organisms, specifically *Streptococcus pneumoniae* and *M. pneumoniae,* may occur frequently.[55,63] Clinically, these patients cannot be differentiated from those infected with a single organism. In these cases, *C. pneumoniae* may not be the primary cause of the pneumonia but might disrupt the normal clearance mechanisms and enable other pathogens to invade. This may have been the case in an outbreak of pneumonia and fatal pneumococcal meningitis among US Army trainees.[52] Six of 12 trainees with pneumonia were infected with *C. pneumoniae,* which suggested a simultaneous outbreak of both infections. Asymptomatic respiratory infection may occur in 2% to 5% of adults and children.[53,63] The role asymptomatic carriage plays in the epidemiology of *C. pneumoniae* is not known. Acute respiratory infection with *C. pneumoniae* does not appear to vary by season, but no systematic surveillance for *C. pneumoniae* infection exists in the United States.

Prior to the availability of research and commercial multiplex NAAT assays for viruses and atypical pathogens, few studies had focused on the role of *C. pneumoniae* as a pathogen in CAP with multiple viral etiologies and as a copathogen. A recent study reported the presence of 14 respiratory viruses and atypical bacteria (*M. pneumoniae, C. pneumoniae*), via multiple PCR assays in patients under 18 years old

TABLE 182.1 Summary of Selected Studies of Respiratory Infection From *Chlamydia pneumoniae* in Adults and Children Published Since 2001

LOCATION	AGE (NO. TESTED)	DIAGNOSTIC METHODS	NO. POSITIVE	COMMENTS
United Kingdom	>16 yr (316)	Serology,[a] gene amplification	55 (17%)	All positive with serology
Netherlands	1–88 yr (159)	EIA, Pst1-based PCR	5 (3.1%)	All positive with serology alone
Netherlands	≥18 yr (107)	Conventional test or PCR[b]	0	Test not described
Taiwan	17–99 yr (168)	MIF	12 (7.1%)	—
Japan	17–99 yr (232)	MIF, PCR, culture	15 (6.5%)	Proportion of results positive with each test not stated
Germany	≥18 yr (546)	MIF, PCR	5 (0.9%)	All 5 positive with 2 different PCR assays, negative with MIF
Thailand	1 mo–15 yr (333)	MIF	149 (44.7%)	14 of 149 positive with single titers
United States	6 wk–18 yr (154)	MIF, EIA	14 (9%)	Proportion positive with MIF and EIA not stated
Switzerland	Ages not stated (1583)	RT-PCR	2 (0.013%)	
Kenya	>2 mo (2158)	RT-PCR	81 (3.8%)	
China	≥18 yr (507)	MIF	60 (11.4%)	Paired sera were obtained from 320 (63%) patients
Thailand	All ages, range not specified (3417)	RT-PCR, MIF	92 (2.7)	Proportion diagnosed by PCR or serology not specified
Sweden	18–93 yr (184)	PCR, EIA	0	

[a]PCR or serology method not described.
[b]Study enrolled both patients and age-matched control subjects.
EIA, Enzyme immunoassay; *MIF,* microimmunofluorescence; *PCR,* polymerase chain reaction, *Pst1,* a restriction enzyme isolated from *Providencia stuartii*; *RT-PCR,* real-time PCR.
Modified from references 55–60.

hospitalized due to CAP.[64] Atypical pathogens were detected in 40% (58/146), viral etiologies in 36% (52/146), and coinfections in 19% (27/146). The most common etiologic agent was *M. pneumoniae* (n = 47), followed by *C. pneumoniae* (n = 11). The most frequent respiratory viruses detected were respiratory syncytial virus subtype A (n = 35), influenza virus (n = 21), and parainfluenza virus (n = 10). Virus-bacterium and bacterium-bacterium coinfections were found in 27 cases. *Mycoplasma pneumoniae* and *C. pneumoniae* were detected as frequently as respiratory viruses (36%) in this population. However, the prevalence of *C. pneumoniae* was significantly higher in this pediatric population compared to what has recently been reported in similar populations in the United States. The prevalence of *C. pneumoniae* was only 0.3% in the multicenter study evaluating the GenMark RP Panel; 58% (1222/2098) were ≤21 years of age and 842 (40%) were <5 years of age.[15]

Until more prospective studies are conducted with commercially available multiplexed NAAT assays for viruses and atypical bacteria, which also include diagnoses of typical bacteria such as *S. pneumoniae,* the role of *C. pneumoniae* in CAP remains less clear than when only serology was used for diagnosis of *C. pneumoniae.*[65]

CLINICAL MANIFESTATIONS

Most respiratory infections from *C. pneumoniae* are probably mild or asymptomatic. Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *M. pneumoniae.*[8] Subsequent studies have found that pneumonia associated with *C. pneumoniae* has been clinically indistinguishable from other pneumonias.[55,66] The spectrum of disease in a recent outbreak in which molecular diagnosis with multiplex PCR was used is illustrative. *Chlamydia pneumoniae* was found in 73% of samples from patients with pneumonia but also in 36% of samples from patients with upper respiratory disease without pneumonia, all of whom recovered without therapy.[38] *Chlamydia pneumoniae* has been associated with severe illness and even death, although the role of preexisting chronic conditions as contributing factors in many of these patients is difficult to assess. *Chlamydia pneumoniae* can be a serious pathogen even in the absence of underlying disease; it was isolated from the respiratory tract and the pleural fluid of a previously healthy adolescent boy with severe pneumonia complicated by respiratory failure and pleural effusions.[67]

The role of host factors remains to be determined. Although *C. pneumoniae* has been detected in bronchoalveolar lavage fluid from 10% of a group of patients with acquired immunodeficiency syndrome and pneumonia, its clinical role in these patients is uncertain because most were coinfected with other well-recognized pathogens such as *Pneumocystis jirovecii* and *Mycobacterium tuberculosis.*[68] Gaydos and colleagues[23] identified *C. pneumoniae* infection with PCR assay in 11% of a group of immunocompromised adults with human immunodeficiency infection, malignant neoplasms, and other immune disorders, including systemic lupus erythematosus, sarcoidosis, and common variable immunodeficiency. *Chlamydia pneumoniae* was responsible for 6 of 31 episodes (19%) of acute chest syndrome in children with sickle cell disease in New York.[70] *Chlamydia pneumoniae* infection in these patients appeared to be associated with more severe hypoxia than was infection with *M. pneumoniae.*

The relationship of *C. pneumoniae* and upper respiratory infections, including pharyngitis, sinusitis, and otitis media, is less clear.

THERAPY

Data on treatment of *C. pneumoniae* respiratory infection are limited. *Chlamydia pneumoniae* is susceptible to antibiotics that affect DNA and protein synthesis, including macrolides; azalides, specifically azithromycin; tetracyclines; and quinolones (Table 182.2). However, in vitro activity may not always predict in vivo efficacy. Most published pneumonia treatment studies, including all recent studies, have used serology alone for diagnosis of *C. pneumoniae* infection, which is at best a clinical end point. Results of several multicenter treatment studies that used culture showed 70% to 86% efficacy of treatment with erythromycin, clarithromycin, azithromycin, levofloxacin, and moxifloxacin in eradicating *C. pneumoniae* from the nasopharynx of children and adults with CAP.[44] Most patients had clinical improvement despite persistence of the organism. Persistence did not appear to be the result of the development of antibiotic resistance because the minimal inhibitory concentrations of the isolates obtained after treatment did not change. Antibiotic resistance is unusual in chlamydiae.[44,71] Investigators were unable to select for macrolide resistance after passage of *C. pneumoniae* in subinhibitory concentrations of azithromycin.[72] In contrast, resistance to quinolones has been selected in vitro after passage of *C. pneumoniae* in subinhibitory concentrations of moxifloxacin.[73] These isolates were found to have a point mutation in the *gyrA* gene. Studies with long-term continuously infected cells suggest that *C. pneumoniae* may be refractory to antibiotics when in the persistent state.[74]

On the basis of these limited data, the following regimens can be used for respiratory infection from *C. pneumoniae* in adults: doxycycline, 100 mg orally twice daily for 14 to 21 days; tetracycline, 250 mg orally

TABLE 182.2 Comparative in vitro Activities of Currently Available Antimicrobials Against *Chlamydia pneumoniae*

ANTIMICROBIAL AGENT	MIC RANGE (μg/mL)
Doxycycline	0.015–0.5
Tigecycline	0.125–0.25
Erythromycin	0.015–0.25
Azithromycin	0.05–0.25
Clarithromycin	0.004–0.03
Ciprofloxacin	1–4
Levofloxacin	0.25–1
Moxifloxacin	0.125–1
Rifampin	0.0075–0.03
Trimethoprim	≥128
Sulfamethoxazole	≥500

MIC, Minimal inhibitory concentration.
Modified from Hammerschlag MR, Kohlhoff SA. Treatment of chlamydial infections. Expert Opin Pharmacother. *2012;13:542–552.*

four times daily for 14 to 21 days; azithromycin, 500 mg orally once daily followed by 250 mg/day for 4 days; clarithromycin, 500 mg orally twice daily for 10 days; levofloxacin, 500 mg intravenously or orally once daily for 7 to 14 days; or moxifloxacin, 400 mg orally once daily for 10 days. For children, the following regimens can be used: erythromycin suspension, 50 mg/kg/day for 10 to 14 days; clarithromycin suspension, 15 mg/kg/day for 10 days; or azithromycin suspension, 10 mg/kg once on the first day, followed by 5 mg/kg once daily for 4 days. Some patients may need re-treatment.

CHLAMYDIA PNEUMONIAE AND CHRONIC DISEASE IN HUMANS

One of the distinguishing characteristics of chlamydiae is their ability to cause persistent, often subclinical, infections. From a clinical standpoint, chlamydiae may be the persistent infection par excellence, capable of persisting in the host for months to years, often without causing obvious illness. From a microbiologic standpoint, persistence also refers to long-term intracellular infection that can be detected with antigen, microscopy, or nucleic acid–based amplification methods. Chronic persistent infection with *C. pneumoniae* has been implicated in the pathogenesis of several chronic diseases initially not thought to be infectious, including asthma, arthritis, and atherosclerosis. However, studies of the association of *C. pneumoniae* and these disorders have been hampered with difficulty in diagnosis of chronic persistent infection with the organism, which, in turn, makes determination of the efficacy of interventions difficult, especially with antibiotics.

Chlamydia pneumoniae and Asthma

Infection with *C. pneumoniae* has been linked to asthma by a large number of epidemiologic and clinical studies. The controversy about definition of infection and diagnostic tests contributes to the difficulty in interpretation and comparison of studies. The field is further complicated by differences in study populations in regard to asthma phenotype and the presence of acute symptoms. Table 182.3 summarizes selected studies that have examined the association of *C. pneumoniae* infection and asthma. The wide range of positivity illustrates the sometimes contradictory findings regarding an association between *C. pneumoniae* and asthma, some of which may be explained by differences in populations and diagnostic methods.

In 1991, Hahn and colleagues[75] reported an association between serologic evidence of acute *C. pneumoniae* infection and adult-onset asthma and asthmatic bronchitis in the United States. Studies that have shown an association or lack of association between the presence of antibodies (IgG, IgM, and IgA) and higher antibody titers (IgG) against *C. pneumoniae* with asthma have been reported since then in a variety of populations.[76] Studies that used direct detection methods (culture or PCR) were more consistent in establishing a role of *C. pneumoniae* in exacerbations of asthma (see Table 182.2). In patients with stable asthma symptoms, evidence for infection with *C. pneumoniae* of up to 22% with PCR, alone or in combination with *M. pneumoniae,* may suggest chronic infection.[76] The clinical implications of *C. pneumoniae* infection in patients with asthma who have no acute symptoms are not clear; the obvious concern is that the persistent presence of the pathogen may lead to ongoing inflammation and thus contribute to severity and progression of asthma.[77]

Currently, minimal data exist to examine the immunologic basis for the association between *C. pneumoniae* and asthma pathology. Persistent infection with *C. pneumoniae,* which has been shown in patients with asthma, might be the result of an insufficient Th1 response in these patients, which is critical for clearance of the intracellular bacterium.[66,78] In analogy to the correlation of abnormal host immune response to *C. trachomatis* infection and tissue sequelae, a similar relationship may conceivably exist between respiratory infection with *C. pneumoniae* and asthma pathology.[79] Abnormal cellular immune responses to respiratory infections with *C. pneumoniae* in patients with asthma may in part be related to genetic variation in immune mediator genes.[80] Genetic variation of Toll-like receptor 2 is under investigation as a major factor in the development of asthma and may be related to susceptibility to *C. pneumoniae.* In Toll-like receptor $2^{-/-}$ mice, decreased IFN-γ and adaptive cell responses led to poor control of respiratory infection with *C. muridarum* and prolonged inflammation.[81] Differences in *C. pneumoniae* IgG antibody responses were seen in children with asthma, depending on variant mannose-binding lectin alleles.[82] An association between wheezing and anti–*C. pneumoniae* IgE in children infected with *C. pneumoniae* was shown, which suggests a Th2 response to the bacterium in patients with asthma.[83] The role of stress and host genetics in delayed or suboptimal Th1 response to chlamydial infection and development of complications in certain individuals, and the role of specific *C. pneumoniae* antigens in eliciting harmful immune responses in patients with asthma, are currently unclear.

Therapy

If infection with *C. pneumoniae* contributes to inflammation in patients with allergic asthma, diagnosis and treatment of these infections is important. Interactions may also exist between *C. pneumoniae* infection and asthma drugs. Treatment of asthma exacerbations frequently includes systemic steroids, which have been shown to enhance the in vitro infectivity of *C. pneumoniae*[84]; this was reflected in significant increases of inclusions but did not affect the in vitro activities of azithromycin, erythromycin, and doxycycline against *C. pneumoniae.*[84]

Several studies have addressed the question of whether antibiotic treatment of *C. pneumoniae* infection in patients with asthma leads to improvement in disease activity. Study design has been complicated by the fact that macrolides, quinolones, and tetracyclines all have immunomodulatory activity independent of their antimicrobial activity.[85,86] Any positive treatment outcomes may therefore be the result of antichlamydial or immunomodulatory effects, or a combination of the two. Several uncontrolled studies showed beneficial effects of antibiotics on patients with asthma with proven or presumed *C. pneumoniae* infection.[66,87] Subsequent placebo-controlled trials attempted to confirm the benefits suggested by these preliminary studies. A placebo-controlled 6-week trial of roxithromycin in patients with asthma who were seropositive for *C. pneumoniae* showed significantly higher morning peak expiratory flow in the treatment group at the end of treatment but not at subsequent time points.[88] In the absence of clear evidence that patients with asthma in this study had persistent *C. pneumoniae* infection, one could conclude that the treatment effect was the result of the antiinflammatory action of roxithromycin, which disappeared after stopping the drug. A double-blind, randomized, placebo-controlled study of telithromycin in patients with acute exacerbations of asthma found reduction of asthma symptoms among those treated with the active drug; however, the study could not adequately assess the effect of infection because only 1 of 278 enrolled patients was positive for *C. pneumoniae* with PCR assay of upper airway samples.[89] Similarly, in a randomized, controlled study of clarithromycin, the number of asthma patients who were PCR-positive for either *C. pneumoniae* or *M. pneumoniae* was only 12 and therefore the

TABLE 182.3 Summary of Clinical Studies of the Role of *C. pneumoniae* in Asthma

POPULATION (yr)	NO. WITH ASTHMA/CONTROL	CULTURE+ ASTHMA/ CONTROL (%)	PCR+ ASTHMA/ CONTROL (%)	SEROLOGY: MIF ASTHMA/ CONTROL	COMMENTS
United States, adults (1991)	365	—	—	Positive correlation between IgG titers (MIF) and wheezing	
Italy, adults (1994)	74/—	—	—	IgG seroconversion (10%/—)	Asthmatics with acute exacerbation
United States, children (1994)	118/41	11/4.9	—	No significant difference in IgG titers between groups; 58% of culture-positive asthmatics without IgG/IgM response	Asthmatics with acute exacerbation
Japan, adults (1998)	168/108	1.2/0	5.4/0.9	Higher prevalence rate of IgG and IgA in asthmatics (85%/68% and 48%/17%); mean IgG titers: 39/18	Asthmatics with acute exacerbation
Great Britain, adults (1998)	123/1518	—	—	No difference in prevalence rate of IgG titers (≥512 and ≥64–256) between groups (5.7%/5.7% and 15%/13%, respectively)	IgG ≥64–256 more common in subgroup of severe asthmatics (34.8%)
New Zealand, children and adults (2000)	96/102	—	—	No positive correlation between diagnosis of asthma and IgG titer at 11 yr and 21 yr of age	Asthma-enriched birth cohort; self-reported asthma
Italy, children (2000)	71/80	—	8/2.5	Serologic response consistent with acute infection: 13%/0%	Asthmatics with acute exacerbation
United States, adults (2001)	55/11	0/0	12.7[a]/0	Serologic response consistent with acute infection in 42% of PCR-positive asthmatics	Stable asthmatics
Great Britain, adults (2004)	74/74	—	22[b]/9[b]	—	Cases: stable atopic asthmatics; Controls: nonatopic spouses
Finland, adults (2005)	83/162	—	—	No difference in titers or conversion rates	Population-based cohort
Finland, adults (2006)	103/30	—	21[c]/37	—	Stable asthmatics

[a]Respiratory specimens obtained from lower airway (bronchoalveolar lavage, biopsy, or airway brushing).
[b]Positivity rate during 3-month (October to December) longitudinal study (at least one positive sample obtained on repeat sampling).
[c]Positivity rate in mild asthmatics: 20.8%; in moderate asthmatics: 22%.
Ig, Immunoglobulin; *MIF,* microimmunofluorescence; *PCR,* polymerase chain reaction.
Modified from Hammerschlag MR, Kohlhoff SA, Darville T. Chlamydia pneumoniae *and* Chlamydia trachomatis. *In: Fratamico PM, Smith JL, Brogden KA, eds.* Post-infectious Sequelae and Long-term Consequences of Infectious Diseases. *Washington, DC: American Society for Microbiology; 2008.*

study was underpowered to test the effect of clarithromycin on asthma outcome variables.[90] A randomized controlled trial of minocycline in patients with allergic asthma showed improved asthma symptoms and reduced total serum IgE, a beneficial effect that did not appear to be the result of a respiratory infection with *C. pneumoniae;* seropositivity for *C. pneumoniae* was not significantly different between patients and control subjects, and no patient had positive nasopharyngeal cultures for *C. pneumoniae.*[91]

Comparing studies of antibiotic treatment of patients with asthma is complicated by the use of different criteria of *C. pneumoniae* infection status (culture, PCR, serology, or a combination of these tests), use of nonstandard methods, and the unclear definition of chronic infection. Most studies have been underpowered to show effects of infections status. In conclusion, although diagnosis and treatment of *C. pneumoniae* infections in patients with asthma with signs and symptoms of an airway infection are recommended, the benefit of using antibiotics with activity against atypical bacteria in patients with asthma without laboratory evidence of infection remains controversial.

Chlamydia pneumoniae and Other Chronic Diseases

Persistent *C. pneumoniae* infection has also been implicated in the pathogenesis of several chronic diseases initially not thought to be infectious, including atherosclerosis, multiple sclerosis (MS), temporal arteritis, stroke, Alzheimer disease, lung cancer, and macular degeneration.[76] Studies in mice have shown that *C. pneumoniae* disseminates to the spleen and other organs after respiratory infection via macrophages.[92] However, this effect has not been conclusively shown to occur in humans. In addition, studies of the association of *C. pneumoniae* and these disorders have been hampered by difficulty in diagnosis of chronic persistent infection with the organism; no validated serologic or other surrogate markers exist for chronic *C. pneumoniae* infection.[19] The high prevalence of chlamydial infections and transient immunity after infection makes differentiation of persistent infection from reinfection or even past infection difficult. This, in turn, makes determination of the efficacy of any therapeutic intervention difficult.

Chlamydia pneumoniae and Atherosclerosis

Conventional risk factors, including cigarette smoking, hypertension, and high serum lipid levels, do not fully explain the incidence, prevalence, and distribution of coronary artery disease (CAD). Inflammation of the vessel wall plays an essential role in the initiation and progression of atherosclerosis, erosion, fissure, and eventual rupture of the atheromatous plaques.[93] Various markers of systemic inflammation, including C-reactive protein, have been found to predict future cardiovascular events, including nonfatal and fatal myocardial infarction and stroke. Although inflammation is present, the exact cause is still not known. Infectious agents, including cytomegalovirus, human herpesviruses, enteroviruses, *Helicobacter pylori,* bacteria involved with periodontal disease, and *C. pneumoniae,* have also been investigated as possible causes for this inflammation.

The first report that suggested a possible association between *C. pneumoniae* infection and CAD came from a case-control study from Finland published in 1988, showing that patients with proven CAD were significantly more likely to have antibodies to *C. pneumoniae* than control subjects selected at random.[94] This report was quickly followed by additional seroepidemiologic studies and studies that identified *C. pneumoniae* in atheroma with various methods, including culture, immunohistochemical staining (IHS), and PCR.[42,43] Animal studies have shown that *C. pneumoniae* can either induce or enhance the development

of atherosclerosis in mice.[95] In vitro studies have shown that *C. pneumoniae* can infect and replicate within monocytes, macrophages, and vascular endothelial and smooth muscle cells and that all are important components of atherosclerotic plaque.[93,96] In vitro infection also results in oxidation of cellular low-density lipoprotein; the production of proinflammatory cytokines involved in atherogenesis, including tumor necrosis factor-α, interleukin-6 and interleukin-1β, and interferon-α; and the transendothelial migration of neutrophils and monocytes.[93,96] *Chlamydia pneumoniae* can induce human macrophage foam cell formation in vitro, a key event in early atheroma development.[97] However, this may not be specific because the key component appears to be the chlamydial lipopolysaccharide, which is conserved in all chlamydial species, including *C. trachomatis*.

However, no single serologic, PCR, or IHS assay has been used consistently across all studies, and these assays are not standardized. In 2002, Boman and Hammerschlag[42] reviewed 14 seroepidemiologic studies published from 1992 to 2000 and found a great deal of heterogeneity among these studies in terms of the serologic tests used and the criteria for seropositivity. In some studies, an IgG or IgA titer of 1:64 or more was used as an indicator of chronic infection; in others, the same criteria were used as indicators of *past* infection. Nine of these studies used the MIF assay; all were in-house tests. The antigen used was only specified in four of the MIF assays. The remaining studies used a variety of other methods, including genus-specific enzyme immunoassays and whole-cell immunofluorescence. As stated previously, MIF assays are not standardized and are subject to significant operator variation.[41] Background seropositivity rates in the general population often exceed 70%, which can also make demonstration of an association between the presence of *C. pneumoniae* antibodies and CAD difficult. Earlier case-control studies that showed an association were generally small and based on single serum samples, which does not take into account that antibody titers fluctuate over time. A meta-analysis of 12 studies only found combined odds ratios of 1.15 and 1.13 for IgG and IgA antibodies, respectively.[98]

Boman and Hammerschlag[42] also analyzed 43 studies, published from 1992 through 2000, that examined 2679 samples of atheromatous tissue for the presence of *C. pneumoniae* with culture, electron microscopy, PCR, and IHS. The overall rates of detection of *C. pneumoniae* ranged from 0% to 100%, with 49.7% being positive by at least one method. However, when specimens were analyzed with more than one method, the prevalence rate of specimens positive by at least two methods (usually IHS and PCR) was only 15.14%. As with the serologic studies, major variation was found in the methods, including the antibodies and techniques for IHS and PCR. IHS has also been found to have problems with interpretation and reproducibility. Studies from Hoymans and colleagues[20] reported that ceroid, an insoluble lipid present in plaque, could cause nonspecific reactions with IHS.

The extent of interlaboratory variation with performance of PCR was shown by Apfalter and colleagues,[30] who sent a panel of 15 homogenized clinical atheroma specimens (carotid and coronary) and control specimens to nine laboratories in Europe and the United States for detection with PCR. The positivity rate in the clinical specimens ranged from 0% to 60%, and three laboratories identified *C. pneumoniae* in negative control specimens. The concordance between the assays was only 25% for one specimen. Subsequently, Apfalter and colleagues[32] showed that contamination was practically impossible to avoid with nested PCR assays. Ieven and Hoymans[43] published an analysis of studies reported through 2003, many of which used real-time PCR and were found to be predominantly negative. Real-time PCR is much less subject to contamination from amplicon carryover. Ieven and Hoymans[43] also noted that in studies where serology was done in addition to PCR, no correlation was found between presence of *C. pneumoniae* in the atheroma tissue and presence of anti–*C. pneumoniae* antibodies in individual patients.

With extrapolation from the observation that *C. pneumoniae* can disseminate systemically in mice after intranasal inoculation,[92] the presence of *C. pneumoniae* in PBMCs has been suggested to act as a surrogate marker for infection with *C. pneumoniae* in individuals with cardiovascular and other diseases.[42,43] More than 20 studies that examined the presence of *C. pneumoniae* DNA in PBMCs have been published, and as seen with studies of vascular tissue, the reported prevalence rate of *C. pneumoniae* DNA in PBMCs has also varied significantly, from 0% to 59% of patients with CAD and 0% to 46% of healthy blood donors.[99] Kohlhepp and others[100] examined PBMCs from more than 300 blood donors, either younger than 20 years or older than 60 years. The samples were divided and sent to two different laboratories, where they were tested for *C. pneumoniae* DNA with real-time, touchdown, and nested PCR assays. Only two samples from the younger–than–20-year-old group were positive in one of the laboratories but negative in the second. None of the samples for the more–than–60-year-old group was positive in either laboratory. This study showed that two different laboratories, with different extraction methods and real-time PCR targets, did not detect *C. pneumoniae* DNA in both cohorts of patients, but evidence of interlaboratory discrepancy was found with two specimens. More recently, West and coworkers[101] examined PBMCs from 86 patients with angiogram-documented CAD and 91 age and gender control subjects for the presence of *C. pneumoniae* DNA by using two different real-time PCR assays that used different genetic targets. No *C. pneumoniae* DNA was detected in any of the specimens, including serial specimens from a subset of patients followed for 8 months. The background prevalence of anti–*C. pneumoniae* IgG of greater than or equal to 1:16 was 74% in case and control subjects.

Results of the initial seroepidemiologic and organism detection studies led to several preliminary studies that investigated the efficacy of antibiotic treatment directed at *C. pneumoniae* for the prevention of secondary cardiac events. The results of these preliminary studies suggested an effect but the studies were underpowered and raised questions about the antibiotic regimens used and methods of identification of patients with *C. pneumoniae* infection. The major assumption of many of the seroepidemiologic studies of the association of *C. pneumoniae* and atherosclerosis and other chronic conditions is that the presence of anti–*C. pneumoniae* antibody implies the presence of the organism somewhere in the body. However, earlier studies of patients with respiratory infection often found a poor correlation between serology and isolation of the organism from the respiratory tract.[55]

Gupta and others[102] randomized 60 men with prior myocardial infarction and who were seropositive with MIF (IgG ≥8) to receive either azithromycin 500 mg/day for 3 or 6 days or placebo. They found that the patients who received azithromycin showed a decrease in MIF IgG titers and had a lower risk of a secondary adverse cardiac event than the patients who received placebo. The antibiotic regimen used by Gupta and colleagues[102] was never studied for treatment of *C. pneumoniae* infections. A meta-analysis of 11 randomized trials, which enrolled a total of 19,217 patients, was published in 2005.[103] Seven of these trials used azithromycin; length of treatment ranged from 500 mg/day for 3 to 6 days to 500 to 600 mg/wk for 6 weeks to 1 year. Three studies used roxithromycin for 30 days to 6 weeks; one used clarithromycin, 500 mg/day for 85 days; and one used gatifloxacin 400 mg/day for 10 days/month for 2 years. The duration of follow-up ranged from 3 months to 2 years. The results of two of six of the earlier small studies (≤150 patients in each arm) favored antibiotic treatment, but all of the remaining five large studies favored placebo for all end points, including total mortality; subsequent myocardial events, including infarction; and unstable angina. Also, no relationship was found between outcome and *C. pneumoniae* serologic status. A similar analysis with similar results was published by Baker and Couch in 2007.[104]

A number of possible reasons have been proposed for the failure to show a positive effect of antibiotic treatment, including populations studied, trial design, and duration of treatment. Given lack of a reliable marker for endovascular *C. pneumoniae* infection and the largely negative results in recent organism detection studies, additional studies are unlikely to show any benefit of long-term antibiotic treatment in reducing mortality or cardiovascular events in patients with CAD.

Chlamydia pneumoniae and Multiple Sclerosis

During the past 60 years, 20 different bacteria and viruses have been proposed to be associated with MS. The results were often inconsistent. The possible association of *C. pneumoniae* and MS was first described in

a case study from researchers at Vanderbilt University Medical Center (VUMC); this study was then followed by a series of patients from VUMC in which the researchers claimed that they identified the organism with culture and PCR.[105] The hypothesis of how *C. pneumoniae* might cause MS was not clear. The results of subsequent studies from a number of other groups were conflicting, some finding *C. pneumoniae* DNA in approximately 30% to more than 80% of cerebrospinal fluid (CSF) specimens from patients with MS and in approximately 20% of CSF specimens from patients with other neurologic diseases, and others finding none in CSF and brain tissue with culture and PCR.[106,107]

In an effort to deal with the issue of laboratory-to-laboratory differences in methods used to detect *C. pneumoniae* in studies of MS, prospectively collected CSF specimens from patients with MS and other neurologic diseases were sent to laboratories at VUMC, Johns Hopkins University (JHU), and the University of Umea (UU) in Sweden and subsequently also to the CDC.[108] Thirty specimens from patients with MS and 22 control specimens were tested; none was positive with PCR at JHU, UU, and the CDC, but 73% of the CSF specimens from patients with MS and 23% of the control specimens were positive with PCR at VUMC. Reasons for these discrepant results were discussed and included poor sensitivities of the assays used by JHU, UU, and the CDC or specificity problems with the assays used by VUMC. The primer sets used by VUMC in the multicenter study were analyzed at the CDC and were found to have high sequence similarity to human DNA, as determined with a BLAST (basic local alignment search tool), suggesting that they were not specific for *C. pneumoniae*.[109]

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The complete reference list is available online at Expert Consult.

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D *Mycoplasma* Diseases

183 *Mycoplasma pneumoniae* and Atypical Pneumonia

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SHORT VIEW SUMMARY

Definition
- Atypical pneumonia caused by *Mycoplasma pneumoniae* is a syndrome that in contrast to lobar pneumonia is:
  - Slower in onset
  - Generally milder
  - Not associated with lobar consolidation
  - Not associated with resolution by crisis
- Many other viral and bacterial agents can also produce an atypical pneumonia syndrome.

Etiologic Agent
- *M. pneumoniae*
  - Bacterium lacking cell wall
  - Adhesins and adhesion organelle important in pathogenesis
  - Fastidious
  - May be cultivable from sputum after symptoms of illness resolve

Extrapulmonary Sites and Manifestations of Infection
- Skin (maculopapular or vesicular rashes, Stevens-Johnson syndrome)
- Cardiac (pericarditis, myocarditis)
- Central nervous system (encephalitis or meningitis, myelitis, radiculopathy)
- Musculoskeletal (myalgia/arthralgia, rhabdomyolysis, arthritis)
- Raynaud phenomenon
- Renal (glomerulonephritis, nephrotic syndrome)
- Hematologic

Diagnosis
- Clinical suspicion by syndrome
- Presence of cold agglutinins in the presence of the syndrome and absence of another explanation
- Detection of *M. pneumoniae*–specific genetic components
- Detection of rising titers of antibodies to *M. pneumoniae*
- Detection of *M. pneumoniae*–specific antigens

Treatment
- Macrolides (e.g., azithromycin, 500 mg day 1, 250 mg day 2 to 5)
  - Macrolide resistance is increasing and may limit usefulness
- Tetracyclines (e.g., doxycycline, 100 mg twice daily for 7–14 days)
- Fluoroquinolones (e.g., moxifloxacin, 400 mg daily for 7–14 days)

The concept of atypical pneumonia antedated the start of the antibiotic era. At least as early as World War I it was recognized that "…in the larger number of cases observed in the [military] camps the pneumonia was of an atypical nature. The onset tended to be slower than that of the lobar pneumonia of civil life; the course more prolonged. Crisis was relatively rare; physical signs were slow of development and of patchy distribution and scattered in several lobes."[1]

With the introduction of sulfonamides in the 1930s and penicillins in the 1940s, it was recognized that some cases of pneumonia did not respond to these antibiotics and that many of these could not be attributed by Gram stain or culture to a known bacterial cause. The term "primary atypical pneumonia" was given to these cases; the prefix "primary" indicated that no causative agent could be determined.

Since then, as diagnostic microbiology and virology advanced, it was recognized that, in addition to *Mycoplasma pneumoniae*, multiple etiologic agents can produce the atypical pneumonia syndrome, including influenza virus, adenovirus, respiratory syncytial virus, cytomegalovirus, *Chlamydia* spp., *Legionella* spp., *Pneumocystis jirovecii*, and metapneumovirus. Additional agents will surely be recognized in the course of time, and the prefix "primary" is now mostly of historical interest. Atypical pneumonia is best regarded as a syndrome to be contrasted with the classic symptom complex of "typical" or lobar pneumonia, as exemplified by pneumococcal pneumonia.[2]

HISTORY

In 1938 Reimann described seven patients with similar clinical characteristics that he termed *atypical pneumonia*.[3] During World War II the syndrome assumed special importance because the majority of pneumonias encountered in the military were atypical.[4]

In 1944 *M. pneumoniae* was first identified as a transmissible cause of atypical pneumonia by Monroe Eaton and coworkers[5,6] and became known as the Eaton agent. In these original experiments sputum and homogenized lung tissue from autopsied patients with primary atypical pneumonia were inoculated into cotton rats, where they produced pneumonitis. The agent was then serially passed and titered in cotton rats and in hamsters, where it also produced pneumonitis. The agent produced no lesions when inoculated and passed in chick embryos. However, when transferred from chick embryo into cotton rats or hamsters, it produced pneumonia. The agent could be passed through a Millipore filter, was neutralized by sera from patients convalescing from atypical pneumonia, and could not be grown on standard bacteriologic media. Thus it was originally thought to be a virus. Demonstration that the agent could be inactivated by certain antibiotics cast doubt on this hypothesis, and the organism was subsequently grown on artificial media and shown to share properties with the group of infectious agents known as the pleuropneumonia-like organisms, or PPLOs.[7]

In 1943 Maxwell Finland and coworkers identified "cold agglutinins," isohemagglutinins that were active at 4°C, in the blood of some patients with atypical pneumonia, and in subsequent work they showed that the cold agglutinins were present in cases associated with the Eaton agent but not in cases associated with influenza virus.[8,9]

The relation of the Eaton agent to the atypical pneumonia syndrome was established by the observations that human serum from some patients recovering from atypical pneumonia neutralized the agent.[10,11] The causal link with atypical pneumonia was strengthened with the demonstration that neutralizing activity was present in convalescent serum from volunteers who were infected by inoculation of ultrafiltrates from those with naturally occurring disease.[12] Subsequently, the agent was grown in tissue culture[13] and cell-free media,[14] and its morphology was described.[15] The Koch postulates were completely fulfilled when the cultivated organism produced the disease in volunteers.[16] Although the similarity of the Eaton agent with organisms that caused pneumonia

in cattle led to its initial description as a pleuropneumonia-like organism, they were rapidly identified as mycoplasmas and named *Mycoplasma pneumoniae*.[6,17]

MICROBIOLOGY

The genus *Mycoplasma* falls within the class Mollicutes, prokaryote microorganisms that lack cell walls. *M. pneumoniae* is one of several members of this genus that cause disease in humans. It is among the smallest of free-living organisms that cause disease in man (≈10 × 200 nm) and has a relatively small genome of 816,394 base pairs of DNA encoding 679 genes.[18] Because it lacks a cell wall, *M. pneumoniae* is insensitive to penicillin and other β-lactam antibiotics and does not take up Gram stain.

M. pneumoniae can be grown in cell-free artificial media but has complex growth requirements and grows slowly. On horse serum and yeast extract–enriched agar medium, it requires 6 to 7 days to form granular or characteristically "fried egg"–appearing colonies. The fried egg appearance results because the centers of the colonies are dense and embedded in the agar medium, whereas the less dense periphery is spread on the agar surface. At maturity colonies may range in size from 50 to 100 microns in diameter. When stained with Dienes stain, colonies contain densely stained small granules. *M. pneumoniae* ferments glucose and other sugars, including xylose, mannose, maltose, dextrin, and starch. It produces a hemolysin that will lyse human, guinea pig, or horse erythrocytes within 24 to 48 hours in artificial media.[19]

Lacking a cell wall, *M. pneumoniae* is bounded by a trilaminar cell membrane that is rich in sterols. It divides by binary fission and is pleomorphic when grown on an inert surface.

M. pneumoniae adheres to respiratory epithelial cells and to red blood cells via sialic acid receptors.[20,21] Adherence is mediated by a complex set of adhesion proteins, including P1, P30, proteins B and C, P116, and HMW1-3. These form an organelle at one end of the rodlike structure of the organism.[22,23] The adhesion organelle also allows the organism to have gliding motility.[24,25] Types 1 and 2 *M. pneumoniae* strains differ because of major variations of the P1 adhesion protein.[26] Distinguishing among these types is important for surveillance, epidemiologic, and clinical purposes.[24,27]

M. pneumoniae also elaborates a cytotoxin that has been called the community-acquired respiratory distress syndrome (CARDS) toxin. This is a cell-associated adenosine diphosphate ribososylating and vacuolating cytotoxin that is present in the inflamed airways of infected experimental animals and elicits antibodies that appear as the infection wanes.[28]

M. pneumoniae forms biofilms.[29] These are volcano-like structures composed of polysaccharide, protein, and lipid. The organisms become encased in the biofilm. It has been shown that types 1 and 2 *M. pneumoniae* strains form very different biofilms and that these confer differential resistance to antibiotics and may alter the ability of antibodies, complement, and white blood cells to penetrate to and attack the organisms.[30]

As macrolide resistance has become more prevalent, case reports have suggested that resistant isolates might be more virulent than sensitive ones[31,32]; however, in one family, a wide spectrum of severities were found.[33] Also, in a single case report severe disease was seen in a 14-year-old adolescent with infectious mononucleosis. At present, any association between macrolide resistance and disease severity must be judged speculative.

EPIDEMIOLOGY

M. pneumoniae are distributed worldwide with minor, if any, effects of climate on the incidence of disease. Long-term studies have shown a pleomorphic annual cycle: In some years there are seasonal peaks in fall and winter, whereas in other years there is little evidence of seasonality.[34–38]

Infection has been described at all ages; however, it is primarily a disease of childhood and adolescence, with the peak incidence of infection between 5 and 15 years of age. It has been reported that children younger than 3 years develop primarily upper respiratory tract infection, whereas those 5 to 20 years of age tend to develop bronchitis and pneumonia.[39] In older infected adults pneumonia predominates.[40] Because asymptomatic infection is common, descriptions of the disease's epidemiology must be interpreted in light of whether the data was collected from cases of symptomatic disease or from systematic survey of defined populations.

Seroepidemiologic studies in the United States showed an incidence of mycoplasmal pneumonia of 5 per 1000 per year among those 10 years of age and 1 per 1000 per year among those 25 to 50 years of age. Annual incidence fell as age increased further. There were no major sex differences.[34] It was estimated that, overall, at least one case of mycoplasmal pneumonia occurs annually for each 1000 persons, or more than 2 million cases annually. The total incidence of mycoplasmal infection at all sites in the respiratory tract may be 10 to 20 times higher.[3]

A prospective study of all adults from two counties in Ohio, who required admission for community-acquired pneumonia during 1991, demonstrated similar rates of hospitalization for *M. pneumoniae* and *Streptococcus pneumoniae* for those between 15 to 34, 34 to 65, and 66 to 79 years of age, with a continued increase in the incidence of disease from each pathogen in those older than 80 years, but in this oldest group there was a divergence, with the rate of *S. pneumoniae* disease rising above that resulting from *M. pneumoniae*. *M. pneumoniae* was not seasonal in this study, whereas *S. pneumoniae* had a clearly defined seasonality.[41]

M. pneumoniae is spread from person to person. Spread of infection appears to be by droplet infection. The infection is transmitted by coughing and spread of droplets, and patients may remain infectious for prolonged periods after many of the symptoms other than cough have disappeared. The organism and disease associated with it can relapse or be transmitted even after treatment of patients with effective antibiotics.[42]

When assessed within families, the rate of spread is slow, but, because of prolonged carriage, it is extensive. In one study, spread occurred in 23 of 36 families, and among the 23, 84% of children and 41% of the adults became infected.[43] In another study the total infection rate was 58% (81% in children).[44]

In closed populations, such as military recruit camps and boarding schools, *M. pneumoniae* disease can be epidemic.[1,45,46] Outbreaks have been described in schools,[47] hospital workers,[48] and even the confined spaces of nuclear submarines.[49] *M. pneumoniae* also can cause nosocomial infections in long-term care (LTC) facilities. A report from the Centers for Disease Control and Prevention (CDC) described an outbreak that occurred in an LTC facility in Nebraska.[50] The respiratory illness, characterized by cough and fever, occurred in 55 residents of the LTC and resulted in 12 hospitalizations and 7 deaths. *M. pneumoniae* DNA was detected by polymerase chain reaction (PCR) in 40% of the specimens collected. The outbreak was terminated by strict observance of good infection control practices. This report points out that serious *M. pneumoniae* infections are not confined to children and young adults, can cause disease in the elderly, and should be considered in clusters of nosocomial pneumonia.

Reports of two outbreak scenarios illustrate how transmission occurs and the disease spreads. One occurred among 26 of 55 members of a college fraternity who attended a pledging banquet.[51] Symptoms occurred in the first of the infected fraternity members 1 week after the banquet, and the epidemic reached its peak 6 days later. In this instance multiple members of the fraternity were simultaneously exposed to a high concentration of infectious *M. pneumoniae* and developed disease synchronously. In a second outbreak employees of a large hospital in New York City developed illness over a 6-month period (July–December), but a single source for the outbreak could not be identified.[52]

Because infection is often asymptomatic or mild in children, and shedding persists long after illness, even after treatment children are often the reservoir from which spread can occur.[53,54]

IMMUNOLOGY AND RESISTANCE

Innate Immunity

Initial host-parasite interactions with *M. pneumoniae* are likely to involve the innate immune system; however, the details are just beginning to be elucidated. Cathelin-related antimicrobial peptide, a molecule that protects the host from other infectious agents, was shown to inhibit the growth of *M. pneumoniae*, and its presence was increased in the cells of *M. pneumoniae*–infected mice.[55] Mice lacking the gene for heat

shock factor 1, a transcription-controlling molecule that is active in the response to a wide variety of stresses, had a higher concentration of *M. pneumoniae* in their lungs than did those with the gene.[56] Mice that lacked the gene for human SPLUNC1 (short palate, lung, nasal, and epithelial clone 1) protein had lower concentrations of *M. pneumoniae* than did those in which it had been inserted.[57]

Infection of cell cultures of A549 lung cancer cells with *M. pneumoniae* has been shown to increase the level of proinflammatory cytokines (interleukin [IL]-8, IL-1) in the cultures.[58]

In a guinea pig model peritoneal and alveolar macrophages ingested and killed opsonized *M. pneumoniae* at a rate slightly slower than other bacteria; however, the authors, Erb and Bredt,[59] concluded that the delay was not clinically significant. Of note, the cell-adherent but unopsonized organisms were relatively resistant to killing mediated by complement.

Adaptive Immunity

M. pneumoniae induces a rich immune response. The humoral response includes protective immunoglobulin G (IgG) and IgA antibodies. Mice born to immune mothers were protected from infectious challenge, whereas those born to nonimmune mothers were not. The protection was shown to be due to IgG antibody in maternal colostrum.[60] Similar protective effects were shown in a hamster model in which passive transfer of serum conferred humoral but not cellular immunity.[61] In human studies the level of IgA antibody in nasal secretions correlated with protection after experimental challenge.[62] Protection is probably mediated by antibody against *M. pneumoniae* polysaccharide rather than against its proteins, and the antibody may act by blocking the binding of the pathogen to epithelial surfaces.[63–65]

One of the striking aspects of the immune response to *M. pneumoniae* is the production of isohemagglutinins directed against the I antigen expressed on the surface of adult erythrocytes.[66] As discussed above, the presence of "cold agglutinins" was described in 1942 by Finland and coworkers.[67] They showed that such antibodies were present in 50% to 70% of patients with Eaton agent pneumonia and that the agglutination, produced by holding erythrocytes at 4°C, was reversible by warming them to 37°C.[8,68]

Cold Agglutinins

Cold agglutinins are IgM antibodies and are often present at the start of or in the first week of clinical illness. They may persist for several months after illness. Several theories have been suggested to account for their appearance. One is that the I antigen, present on erythrocytes, is also present in respiratory epithelium and is part of the receptor through which the organism attaches to the cell. Thus the antibody is actually directed at the binding complex or a part of it.[69] Another theory suggests that the antibody is actually directed against a polysaccharide component of *M. pneumoniae*. This is based on the observation that immunization of rabbits with either *M. pneumoniae*, *Streptococcus* MG (a group F α-hemolytic streptococcus), or *Listeria monocytogenes* produced cold agglutinins that were inactivated by incubation with *M. pneumoniae* membrane-associated lipopolysaccharide.[70,71]

Cold agglutinins have no known role in the development of pneumonitis, but they may occasionally be directly pathogenic, particularly when the antibody titers are high, producing hemolysis, capillary obstruction with the Raynaud phenomenon, renal failure, and, rarely, gangrene (Fig. 183.1B and C).[72–74]

Complement-fixing *M. pneumoniae*–specific antibodies also appear early in the course of illness and high levels persist for several months after infection. Seroprevalence surveys suggest that these antibodies persist at a low level for much more prolonged periods.[75–77]

Resistance and Susceptibility to Infection

There are few or no data relating specific conditions to the infectious inoculum of *M. pneumoniae*. Knowledge about which factors affect severity and duration of *M. pneumoniae* infection rests primarily on case reports or small series of patients with severe disease. Because severe disease is also seen in those without obvious predisposition, such associations must be considered tentative.[78–80]

FIG. 183.1 Skin conditions associated with *Mycoplasma pneumoniae* infection. (A) Stevens-Johnson syndrome in a child with *M. pneumoniae* infection. (B) Raynaud phenomenon in a young woman with *M. pneumoniae* infection and high titers of cold agglutinins. (C) Necrosis of distal extremities in a patient with sickle cell disease who contracted *M. pneumoniae* infection accompanied by very high cold agglutinin titers.

Although the immune systems may play a role in pathogenesis of clinical disease, the humoral response to infection provides immunity to reinfection for some period of time. Reinfections with *M. pneumoniae* are generally milder than initial infections,[81–83] and both antibody (human and murine) and colostrum (murine) were protective against *M. pneumoniae* in murine models.[60,84] Intensity of antibody response was inversely correlated with severity of *M. pulmonis* infections in rats.[85] Additional studies in mice showed that in comparison with immunocompetent mice, those with X-linked immunodeficiency failed to show severe lung lesions despite *Mycoplasma pulmonis* in their lungs. Mice with severe combined immunodeficiency also had milder lung lesions but allowed the mycoplasma infection to spread to the joints, where they produced severe pathology.[86]

Several reports have suggested that children with sickle cell SS or SC hemoglobinopathy are predisposed to severe disease,[87,88] but severe disease has not been reported in association with sickle trait (hemoglobin SA). *M. pneumoniae* has been found to be responsible for 16% to 20% of cases of sickle cell anemia–associated "chest syndrome."[89,90] Children with Down syndrome have also been reported to be disposed to severe disease.[91,92] Neither adults with chronic obstructive lung disease nor cigarette smokers are predisposed to *M. pneumoniae* infection.[3,93,94]

M. pneumoniae is not generally considered an opportunistic pathogen in immunocompromised patients; however, a few reports suggest that cytotoxic chemotherapy and neutropenia may be associated with severe and prolonged disease.[95,96] A 7-year-old boy with hypogammaglobulinemia was reported to recover completely from mycoplasmal pneumonia but had continued carriage over a 2-year period despite additional courses of antibiotics.[97]

Many studies suggest that genetic factors, as yet undefined, may play a role. In the mouse there are marked strain differences in disease severity, and male sex is associated with severe disease.[98,99] Reviews of severe mycoplasmal pneumonia in humans have also shown a predominance of males.[100] One case report documents markedly different disease severity in identical twins, demonstrating that severity is not solely conditioned by genetic factors.[101]

Suprainfection during the course of mycoplasmal pneumonia is unusual; however, there are suggestions that when there is concomitant pulmonary inflammation or coinfection the clinical severity is worsened.[102] Thus, in a study of Kenyan refugee camps, persons coinfected with "atypical pathogens" and respiratory viruses were significantly more likely to have severe respiratory illness.[103] Acute exposure of mice to nitrogen dioxide worsened the pathologic findings when the exposed mice were subsequently infected with *Mycoplasma pulmonis*.[104]

PATHOLOGY

M. pneumoniae infections are common in children and young adults but are very uncommonly fatal. Postmortem examination in one fulminant case showed extensive exudates in the lungs, with abscess formation, pericarditis, and disseminated intravascular coagulation with involvement of skin, lungs, and adrenals. A review of 11 other autopsied cases showed similar findings.[105]

There are several mechanisms by which *M. pneumoniae* can cause disease. First, attachment of the organism to sialic acid receptors on respiratory epithelium, via its attachment organelle, may directly damage the respiratory epithelium and its ciliary activity.[20,23] Second, *M. pneumoniae* elaborates a cytotoxin, the CARDS toxin, that may also directly damage the respiratory tract.[28] Third, the organism may alter antigens on the surface of cells to which it is attached to the extent that autoantibody formation is elicited. This is presumed to be the mechanism by which the anti-I cold hemagglutinin antibody is formed.[66] Finally, *M. pneumoniae* and/or its CARDS cytotoxin may elicit the influx of inflammatory cells, which then produce proinflammatory cytokines that may result in damage to the host.[106,107]

DISEASE SYNDROMES

Respiratory Illnesses

Most *M. pneumoniae* infections are symptomatic, and most of these occur in children and adolescents. Although disease in children younger than 5 years does occur, it predominantly manifests itself as coryza, but *M. pneumoniae* may also be responsible for as much as 5% of episodes of bronchiolitis. In older children and adolescents the most common syndrome is a combination of tracheobronchitis and upper respiratory tract infection, such as coryza, pharyngitis, and otitis media.[39]

Atypical pneumonia is the predominant syndrome seen in young adults and adults (Fig. 183.2). The incubation period was 9 to 13 days in volunteer experiments, but estimates from clinical studies suggest that it may be as long as 3 weeks. After the incubation period, low-grade (<102°F) fever, malaise, headache, myalgia, and cough develop over a few days. The gradual onset of atypical pneumonia is in contrast to the sudden onset of classic lobar pneumonia, with its high fever, shaking chill, and pleurisy, as well as to classic influenza, with its high fever and rapid progression of symptoms from the nose to the throat and chest.

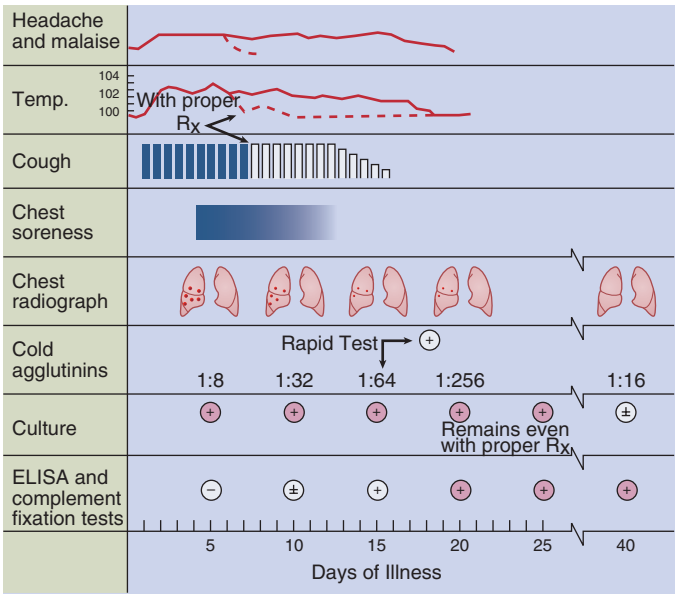

FIG. 183.2 Major clinical and laboratory manifestations of mycoplasmal pneumonia. *ELISA,* Enzyme-linked immunosorbent assay; *Rx,* treatment; *Temp.,* temperature. *(Modified from Baum SG. Mycoplasmal infections. In: Wyngaarden JB, Smith LH Jr, eds.* Cecil Textbook of Medicine. *17th ed. Philadelphia: Saunders; 1985:1506.)*

Coughing or tracheitis may result in substernal soreness, but pleuritic chest pain is rare. Gastrointestinal symptoms are unusual.

Physical examination of a patient with mycoplasmal pneumonia shows relatively few findings for observed degree of malaise. Cough is persistent, frequent, and debilitating. Nonexudative pharyngitis may be seen, but cervical lymphadenopathy is unusual. Examination of the chest by palpation and percussion is generally normal. Auscultation may indicate patches of rales at one or more sites, but they are often absent or heard only inconsistently. Wheezes and ronchi are not characteristic. The remainder of the examination is usually unrevealing unless there is a rash or extrarespiratory infection, as discussed in the following section.

Otitis and bullous myringitis were commonly seen in experimentally induced disease in adults[16] and in case series,[108] but other studies suggest that in clinical practice *M. pneumoniae* is only an occasional cause of otitis and that bullous myringitis is due to the same spectrum of pathogens as are other ear infections.[109] No useful diagnostic information is provided by the absence of bullous myringitis and its presence is of uncertain sensitivity and specificity.

Routine laboratory data are generally normal. There may be a mild leucocytosis in as many as 25% of cases, but the total white blood cell count rarely exceeds 15,000 per mL. Expectorated sputum is generally scanty and not viscous. Gram stain of sputum, if of sufficient quality for interpretation, shows a mixture of polymorphonuclear and mononuclear cells. Chest radiographs or other imaging may show one or more patches of alveolar fluid or bronchopneumonia, and some consolidation may be evident in approximately a quarter of the cases. Severe cases may show a bronchopneumonia extending from the hilum and involving the entire lung. Pleural effusions, generally small and transudative, may be seen in 5% to 20% of cases.[108] Typically, imaging shows more disease than was anticipated from the physical signs. Lobar consolidation is unusual but has been reported.[110]

The natural history of such a case is one of extended illness, with fever and cough lasting well into the third week of illness, and then followed by gradual recovery. There is no "crisis" as there is in untreated pneumococcal pneumonia. Appropriate antibiotics shorten the duration of illness but do not reliably eradicate the organism from the respiratory tract, and convalescent cases can serve as sources of infection to others.

That *M. pneumoniae* infection might precipitate exacerbations of childhood asthma was suggested in 1970.[111] Although such a role seems

well established, it is not unique to this organism.[112,113] Whether *M. pneumoniae* has any role as an initiator of childhood asthma remains unclear and controversial.[114] In infants without asthma, *M. pneumoniae* may be an etiologic agent for acute bronchiolitis.[115]

Other Illnesses

Although respiratory illness is the most common syndrome in *M. pneumoniae* infections, other organs of the host can be involved, and extrapulmonary symptoms may be present or predominant. The extrapulmonary sites that are most commonly involved are the skin and mucous membranes, central nervous system (CNS), heart, hematopoietic system, kidneys, and musculoskeletal systems.

DERMATOLOGIC SYNDROMES

Skin disorders are common in *M. pneumoniae* infection, occurring in up to 25% of cases.[116] Signs may range from relatively transient exanthems[43] to severe Stevens-Johnson syndrome (see Fig. 183.1A).[117] Among 29 patients with *M. pneumoniae* infection, cutaneous lesions were macular in 4 patients, maculopapular in 14, vesicular in 14, bullous in 6, urticarial in 2, and petechial in 1 patient. The rash involved the trunk and extremities in most of the patients. It generally began with the onset of fever and lasted for a week or more in most. Twenty-one of the patients had oral lesions, 14 had ulcerative stomatitis, and 7 had tonsillitis or pharyngitis. Eleven patients had conjunctivitis, 8 of them deemed severe.[118]

In a review of reports of dermatologic complications of *M. pneumoniae* infection, exanthematous rashes were noted to be reported in 8% to 33% of patients, urticaria in 7%, and Stevens-Johnson syndrome in 1% to 5%.[119] Because respiratory symptoms do not always predominate, *M. pneumoniae* infection should be considered in the differential diagnosis of patients who present with Stevens-Johnson syndrome. In a recent series from the Mayo Clinic, *M. pneumoniae* infection was present in 6 (22%) of such patients.[120]

Bullous erythema multiforme has also been reported in association with *M. pneumoniae* infections. The bullous lesions seen in this condition are commonly caused by herpes simplex virus, and an early review concluded that bullous erythema multiforme was not caused by *M. pneumonia*,[121] but a more recent review identified 33 such cases in association with *M. pneumoniae* infection and concluded that this infection could also be a cause.[119]

Raynaud Phenomenon

The Raynaud phenomenon is a vasospastic disorder characterized by blanching of the fingers and/or toes after exposure to cold temperatures, followed by extreme cyanosis, and then flushing when the extremities are rewarmed. During this process the digits go from white to blue to red in color. The Raynaud phenomenon occurs more commonly in women than in men, and it is often seen in patients with rheumatologic disorders.

The Raynaud phenomenon has been reported very rarely in association with *M. pneumoniae* infection (see Fig. 183.1B). A 3-year-old child developed the Raynaud phenomenon in association with respiratory symptoms, a patchy pulmonary infiltrate, elevated titers of cold hemagglutinins, and an elevated titer of complement-fixing antibody to *M. pneumoniae*.[73] In another series of cases, "cold hands" but none of the other features of the Raynaud phenomenon, were observed in 1 of 40 patients with proven *M. pneumoniae* infection seen during an outbreak in Scotland.[66]

CARDIAC SYNDROMES

M. pneumoniae infection can affect the heart. Among 560 patients with serologically confirmed *M. pneumoniae* infection seen in hospitals in Helsinki, Finland, 25 (4.5%) had evidence of carditis. Patients with carditis ranged in age from 1 to 68 (mean, 38) years. Thirteen were female and 12 were male. Six had pericarditis (2 without and 4 with pericardial effusion). Electrocardiographs (ECGs) were abnormal in 19 patients. In 5 patients there were ST-T wave changes only, and in 4 patients there were ST-T wave changes with atrioventricular block. Two patients had atrial fibrillation, 1 had atrial flutter with variable conduction, and 1 had supraventricular tachycardia. Two patients had ventricular ectopic beats, 1 patient had ventricular fibrillation, and 2 had ventricular bigeminy. Nineteen patients had myopericarditis: 2 with ECG changes only; 5 with ECG changes and cardiac enlargement; 8 with ECG changes and a transient murmur; 4 with ECG changes, a transient murmur, and enlargement of the heart. Fourteen of 23 patients developed long-term sequelae of carditis (2 were not followed). Four of the patients had persistent heart failure, and 9 had persistent rhythm disturbance. One individual, who was 25 years of age at his initial presentation with *M. pneumoniae* and carditis, had acute myocardial infarctions 3 and 5 months later.[122]

In another series from Pennsylvania, 13 (9.2%) cases of pericarditis and myopericarditis occurred among 141 patients with proven *M. pneumoniae* infection. Five patients had myopericarditis, and 8 had pericarditis alone. Six were deemed critically ill, and 1 was moderately ill. Three of the 5 patients with myopericarditis developed heart failure, and 1 developed a hemopericardium. All recovered from the infection and were discharged from the hospital, but 2 died during the follow-up period.[123]

Individual case reports confirm the presence of *M. pneumoniae* in cardiac sites. In two cases *M. pneumoniae* was cultured from pericardial fluid or tissue.[124] A marrow transplant recipient developed pericarditis, and the organism was demonstrated by electron microscopy and PCR.[125]

NEUROLOGIC SYNDROMES

Neurologic complications are a well-described complication of *M. pneumoniae* infection, and CNS symptoms occur in up to 7% of patients hospitalized with this infection. A cluster of encephalitis cases among schoolchildren led to the discovery of a much more widespread outbreak of *M. pneumoniae* respiratory infections in the community.[47]

Manifestations include encephalitis, aseptic meningitis, transverse myelitis, stroke, and radiculopathy.[126–129] In one series of 38 cases of atypical pneumonia in which neurologic complications occurred, 24 patients had meningoencephalitis, 5 patients had transverse myelitis, 4 had stroke, 4 patients had ascending paralysis, and 1 patient had cranial nerve palsy.[130] Three recently published articles illustrate the severe neurologic complications of *M. pneumoniae* infection that can occur in children. The first described 7 children who developed severe Guillain-Barré syndrome (GBS) in association with IgM antibody to *M. pneumoniae*.[131] Five of the children required mechanical ventilation, 1 died, and only 2 recovered completely. A second series from France reported on 9 children ranging in age from 4 to 14 years of age with neurologic problems complicating *M. pneumoniae* infection, including meningoencephalitis in 6 children, ophthalmoplegia in 2 children, myositis in 1 child, and GBS in 1 child.[132] The third series involved 42 children who developed neurologic diseases of 365 children with proven *M. pneumoniae* infection seen at the Hospital for Sick Children in Toronto, Canada over a 16-year period from 1996–2013. Of these children with neurologic disease, 22 had encephalitis, 5 had acute disseminated encephalomyelitis, 5 had transverse myelitis, and 7 had other conditions, including 2 with GBS.[133] The authors of this series noted two patterns of onset of neurologic disease. First were the children who developed with little (<7 days) or no prodromal respiratory disease, and the second group had respiratory symptoms (usually dry cough) for 7 days or more. The former were predominantly PCR positive for *M. pneumoniae* in cerebrospinal fluid (CSF) but not in the respiratory tract, whereas the latter were predominantly PCR positive in the respiratory tract but not in CSF. The authors concluded that the first group had direct invasion of the CNS by the infection, whereas that mechanism in the latter CNS involvement was indirect and most likely immunologically mediated.

Brainstem involvement and dysfunction may be seen.[134] Bickerstaff encephalitis and Miller Fisher syndrome, two syndromes associated with ataxia and ophthalmoplegia, have been reported to occur.[135,136] Both syndromes are also associated with increased CSF protein concentrations without pleocytosis and with increased concentrations of anti-CQ1b antibody. Both may respond to corticosteroids, plasmapheresis, or immunoglobulin therapy.

MUSCULOSKELETAL, RENAL, AND HEMATOLOGIC SYNDROMES

Myalgia and arthralgia are common symptoms in *M. pneumoniae* infections, occurring in up to 45% of patients.[137,138] Rhabdomyolysis has been reported.[139–141] *M. pneumoniae* osteomyelitis is very rare but

has been reported in immunocompromised individuals.[142,143] A case report of periprosthetic joint infection caused by *M. pneumoniae* has been published.[144]

M. pneumoniae infection has been associated with acute glomerulonephritis and the nephrotic syndrome.[145–149]

Hemolytic anemia is an uncommon, but reported, complication of *M. pneumoniae* infection.[118,137,150,151] Other hematologic complications include thrombocytopenia, pancytopenia, and disseminated intravascular coagulation. These complications are generally seen in the most fulminant cases[105,138,152–154] and are associated with the presence of cold agglutinins (see "Immunology and Resistance").

DIAGNOSIS

As a common cause of illness in children and young adults, *M. pneumoniae* should be regularly considered as a possible etiology in any upper respiratory infection in a child or young adult, as well as in older adults who have not responded to β-lactam antibiotics. In adults of any age with community-acquired pneumonia of mild-to-moderate severity, the present of persistent intractable cough is an important clinical clue that should suggest the possibility of mycoplasmal infection, as well as adult pertussis, especially in older adults. In the immunocompromised patient *M. pneumoniae* may cause severe pneumonia that is typically not lobar or consolidating in its radiologic appearance.

Multiple laboratory tests are available for diagnosis of *M. pneumoniae* infection. It appears that the best of these tests are those based on the detection of *Mycoplasma*-specific nucleic acids, although no single test is so good as to make the others obsolete.

Cold Agglutinins

The use of cold agglutinins as a diagnostic test is mainly of historical interest. A 1944 survey of the prevalence of cold agglutinins in various conditions in adults indicated that titers greater than 1:40 could be found in 137 of 200 patients with the atypical pneumonia syndrome (sensitivity, 68.5%) but only in 5 of 200 patients with acute bacterial pneumonia (specificity, 97.5%) and 9 of 658 patients with other conditions (specificity, 98.6%).[67] Similar results (sensitivity, 70%; specificity 97%) were found in a 1990 survey of 130 children with pneumonia by using a rapid cold agglutinin test with a diagnostic cutoff at a titer of greater than 1:32.[155] The description of a bedside test for cold agglutinins and evidence of a strong correlation with a titer of 1:64 by a more quantitative test[156] promoted widespread use; however, it is not clear if the performance characteristics of the bedside test, which may be performed by inexperienced physicians, match those of the laboratory test (Fig. 183.3).

FIG. 183.3 A positive bedside test for cold agglutinins. *(Modified from Callahan CW. Pneumonia and bacterial pulmonary infections. In: Panitch H, ed.* Pediatric Pulmonology. *Philadelphia: Mosby; 2005.)*

A test with a sensitivity of 70% and a specificity of 98% can provide useful diagnostic information. Applied to a person with pneumonia in whom the pretest probability of *M. pneumoniae* infection is low, for instance, 20%, a positive test would yield a posttest probability of 90%. Unfortunately, a negative test provides relatively little information. Applied to a person with pneumonia in whom the pretest probability is 80%, a negative test would only reduce the posttest probability to 55%.

Culture

Most clinical microbiology laboratories do not perform routine *Mycoplasma* cultures on respiratory secretions. Recovery and speciation of *M. pneumoniae* by routine methods takes too long to be useful in management; however, a rapid (24 hours to a presumptive positive) culture system has been developed.[157] Unfortunately, even when recovered, an isolate of *M. pneumoniae* cannot be regarded as diagnostic of illness etiology. As previously noted, asymptomatic carriage may be prolonged after infection, and many from whom the organism is recovered lack evidence of current infection.[158]

Detection of *Mycoplasma pneumoniae*–Specific Antibodies

A fourfold rise in the titer of antibodies to an uncultured organism is generally deemed indicative of concomitant infection with that organism. Because determination of a rising titer requires two separate samples of blood, spaced in time, saved, and then tested simultaneously in the same assay, the method is onerous and does not return results soon enough to be useful for decisions regarding management. To avoid delay, methods have been explored to identify single high titers of IgM antibody, which would presumably reflect current initial infection, and rising titers of IgG, which would be present on either initial or reinfection. Multiple commercial tests are available based on enzyme-linked immunosorbent assay, complement fixation, and particle agglutination.[159–161] A comparison of 12 such tests in 31 patients with positive and 96 with negative PCR tests for *M. pneumoniae* showed widely different sensitivities (35%–77%) and specificities (49%–100%) when using a diagnostic criterion of a single titer of IgM greater than or equal to 1:64. For the combined criterion of either seroconversion or a threefold rise in titer of IgG, the sensitivities ranged from 26% to 68%.[161] Using the specific PCR as the gold standard, another study demonstrated a sensitivity of 62.2% for a commercial IgM assay. Of note, many discordant results for PCR and IgM tests were observed, and IgM^+/PCR^- patients had frequent coinfections documented, including adenoviral and pneumococcal infections.[162]

Detection of *Mycoplasma pneumoniae*–Specific Antigens

Antigen capture immunoassays have been developed for the detection of *M. pneumoniae* in respiratory secretions. The sensitivity of one early test was 91.3% and the specificity was 100% when compared with seropositivity as the gold standard.[163] More recently, a commercial rapid antigen kit in Japan for the detection of the *Mycoplasma pneumoniae* ribosomal protein L7/L12 was found to have an approximately 60% sensitivity using PCR as the standard.[164] Although antigen detection remains an area of research interest, it has largely been supplanted by PCR-based diagnostic methods.

Detection of *Mycoplasma pneumoniae*–Specific Nucleic Acids

The detection of bacterial or viral nucleic acids for diagnostic purposes has been an active area of transitional research in the past 2 decades, and detection of *M. pneumoniae* DNA is now the diagnostic method of choice where available. PCR was applied to throat swabs from sputum to permit the detection of *M. pneumoniae* DNA encoding the *P1* adhesin gene as early as 1989 and to the detection of bacterial 16S RNA by 1991.[165,166] Early evaluation suggested that, compared with culture, sensitivity was 73% to 92%[167–169] and specificity was 96% to 100%, although at least one study subsequently showed lower sensitivity (57%).[170] Another study evaluated PCR and serologic testing in clinically diagnosed cases in outbreak settings and noted a 48% sensitivity of PCR if testing was performed during the first 3 weeks of illness.[171] PCR has been found

to be superior to IgM testing in acute infection.[172] In a comparison of 12 commercially available serologic tests with PCR, none of the serologic tests appeared to have superior performance.[161]

Differences in sensitivity between individual reports are likely due in part to differences in case definitions and timing of the tests over the course of illness, as well as in differences in the concentration of organisms or nucleic acids in clinical specimens. Sputum samples are more likely to yield positives than are nasopharyngeal aspirates or throat swabs.[173] Occasional detection of *M. pneumoniae* nucleic acids in clinical specimens from asymptomatic individuals suggests that a positive result should be deemed to have the same significance as would a positive culture. It does not prove that *M. pneumoniae* is the cause of the symptoms that generated the test. Currently, two US Food and Drug Administration–cleared or –approved multiplex PCR panels include testing for *M. pneumoniae*.

TREATMENT

Many *M. pneumoniae* infections in children produce few or no symptoms and resolve spontaneously without treatment. The more severe infections are brought to medical attention and, if recognized, can be treated with antibiotics; however, the carriage of *M. pneumoniae* is not always eradicated. In a classic study Smith and coworkers[42] treated 35 Eaton agent–infected volunteers with either tetracycline or erythromycin. They demonstrated that treatment shortened the duration of symptoms, with improvement in symptoms generally within 24 hours. They noted that 8 of 25 tetracycline-treated and 5 of 10 erythromycin-treated continued to shed *M. pneumoniae* after completion of therapy.[42]

M. pneumoniae lacks a cell wall. Hence it is insensitive to the penicillins and other β-lactam antibiotics. The organism has generally been susceptible to fluoroquinolones, both older and newer macrolide antibiotics, and tetracyclines. Azithromycin has been favored due to its lower minimum inhibitory concentration (MIC) in *M. pneumoniae* and fewer contraindications in children than quinolones and tetracyclines. However, macrolide-resistant *M. pneumoniae* strains have become increasingly prevalent, particularly in Asia, and resistance is primarily due to point mutations in the peptidyl-transferase loop of the 23S rRNA, most commonly the A2063G mutation.[174,175] A 2010 report from China documented a 69% prevalence of resistance and treatment failure when the MIC was 2 μg/mL or greater,[176] A survey of Chinese isolates from 2008–12 found a rate of macrolide resistance of 90.6%,[177] and a nationwide survey of pediatric isolates in Japan over the same 5-year period found macrolide-resistance mutations in 70.3% of isolates.[178] Rates of resistance in the United States appear lower. In a study of 91 *M. pneumoniae* specimens collected from six locations in the United States from 2012–14, 13.2% of isolates were found on genotypic and phenotypic analyses to be macrolide resistant.[179] Tetracycline antibiotics remain effective against macrolide-resistant strains.[180] In a retrospective review of outcomes in hospitalized patients with *M. pneumoniae* pneumonia in Japan from 2010–13, during which macrolide-resistance rates were high, fewer patients begun on tetracyclines or fluoroquinolones required switches to other agents than those begun on macrolides.[181] Although a newer fluoroquinolone antibiotic has been approved for use in the pediatric population in Japan, it is not clear which agents might be acceptable for use in the pediatric populations of Europe and the United States if these macrolide-resistant organisms become more prevalent as a cause of symptomatic disease.[182]

The optimum duration of therapy is uncertain, although reports of success with 1, 3, and 5 days of azithromycin therapy have been reported.[183,184] Some authors suggest that shorter durations of treatment may lead to relapse.[185] Selection of the optimum antibiotic in a particular case involves considerations of local prevalence of macrolide resistance, patient age, potential drug interactions, risk of photosensitivity, presence of liver or renal disease, and pregnancy. For otherwise healthy adults in areas with a low prevalence of macrolide resistance, treatment options include a second-generation macrolide (e.g., azithromycin, 500 mg orally on day 1 and 250 mg daily on days 2–5), a tetracycline (e.g., doxycycline, 100 mg orally twice daily for 7–14 days, or a fluoroquinolone (e.g., moxifloxacin, 400 mg orally once daily for 7–14 days).

PREVENTION

Infection Control

M. pneumoniae infection is believed to spread by droplet transmission, and CDC guidelines for hospital infection control recommend droplet precautions for the duration of illness. As shedding continues to occur for prolonged periods, convalescent cases are a reservoir from which new infections arise in small groups such as families or larger ones such as military recruits.[43,82]

In an observational, nonrandomized study of an outbreak in a facility for the developmentally disabled, the investigators assessed effect of "standard" epidemic control measures (i.e., active surveillance, use of standard and droplet precautions for cases, cohorting of residents and staff, and promoting hand washing) and standard measures plus preventive use of azithromycin. The adjusted secondary attack rate in the absence of any measures was 17.6%; for standard measures alone, it was 7.5%; and for standard measures plus azithromycin, it was 0.9%. The observational nature of this study prevents the drawing of any definitive conclusion, but the results are consistent with the efficacy of both standard control measures and preventive antibiotic therapy.[186]

Vaccination

The occurrence of epidemic *M. pneumoniae* in military camps and the importance of mycoplasmas as veterinary pathogens have maintained interest in the creation of vaccines for human and animal diseases. Several live-attenuated or inactivated vaccines are in veterinary use in cattle, goats, sheep, and poultry; however, a review in 2009 concluded that evidence for efficacy was weak, and they "provide only transient or partial immunity and often induce unpleasant side effects."[187]

Human vaccine development has not yet yielded a vaccine suitable for general use. Formalin-inactivated vaccines were developed for human use in the 1960s and 1970s and have been tested on relatively large groups of people. In a meta-analysis of six such studies, four of them involving 66,458 military recruits and two of them involving 810 pediatric patients, there was an overall efficacy for preventing pneumonia of 41% in the military studies but only 14% among the children. This difference, although large, was not statistically significant. Overall efficacy, considering all six studies, was 30%.[188]

Live-attenuated *M. pneumoniae* vaccines have been developed and tested using a hamster model, and these vaccines were also shown to have low efficacy: 50% for one strain and 10% for a second.[189–191]

Cell-free protein-based vaccines have not been superior to the whole-cell preparations. Immunization of guinea pigs with the adhesion protein, which binds the organism to respiratory epithelium, was not protective. In immunized animals severe pneumonia developed with infectious challenge, despite the fact that the animals had enhanced levels of *M. pneumoniae*–specific IgG, IgA, and adherence-neutralizing antibody.[192]

DNA vaccination of mice against *M. pulmonis* was described as protective in 1997.[193] DNA vaccine candidates are currently being evaluated for the prevention of porcine enzootic pneumonia caused by *M. hyopneumoniae*.[194,195]

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The complete reference list is available online at Expert Consult.

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