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Chronic Chlamydial Diseases: From Atherosclerosis to Urogenital Infections

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Abstract Chlamydiae cause a wide range of diseases in human and animal hosts. *Chlamydia pneumoniae* and *Chlamydia trachomatis* are important human pathogens with worldwide distribution that produce significant morbidity. Acute infections with *C. pneumoniae* cause respiratory tract infections, while chronic infection has been linked to chronic bronchitis, asthma and atherosclerosis. Ocular serovars of *C. trachomatis* induce trachoma, the leading cause of infectious blindness worldwide. *C. trachomatis* is the most common bacterial cause of sexually transmitted diseases (STDs) worldwide. Acute infections with genital serovars of *C. trachomatis* remain clinically silent in most women, but can progress to upper genital tract infection leading to pelvic inflammatory disease (PID), infertility and ectopic pregnancy. *Chlamydia*-induced, reactive arthritis can develop as a late-term condition after genital *C. trachomatis* or respiratory *C. pneumoniae* infection. In this review, we address recent information on pathogenesis, immune response, diagnosis and treatment of chronic diseases induced by *C. trachomatis* and *C. pneumoniae*.

Keywords Atherosclerosis · Arthritis · *Chlamydia pneumoniae* · *Chlamydia trachomatis* · Pelvic inflammatory disease · Trachoma

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Introduction

The chlamydiae, Gram-negative, obligate intracellular bacteria, cause a broad range of diseases, affecting a wide range of economically important non-human animals and humans. All chlamydiae share a biphasic developmental cycle. The primary developmental forms of chlamydiae are elementary bodies (EB) and reticulate bodies (RB). EB, the extracellular form of the bacteria, are small (0.2 μm) and infectious, targeting host mucosal epithelial cells. After attachment and entry into the host cell, EB develop within a membrane-bound endocytic vacuole called an inclusion. Inside the inclusion, EB differentiate into larger (0.8 μm), metabolically active but noninfectious RB, which undergo multiple rounds of division. RB redifferentiate into infectious EB, and mature EB complete the developmental cycle, exiting the host cell via lysis or extrusion of the inclusion [1]. Stressors of developing chlamydiae, including the host immune response, nutrient deprivation, antibiotic exposure or co-infection with viruses or parasites, can result in a form called aberrant bodies (AB). AB develop when RB replicative division and maturation of RB to EB is interrupted, resulting in abnormally large chlamydiae. This divergence from the typical developmental cycle constitutes a viable but noninfectious form of chlamydiae, and is termed persistence or the chlamydial stress response, a reversible condition that can eventually allow continued production of infectious EB [1, 2, 3•, 4].

In humans, chlamydiae usually cause eye, urogenital or respiratory infections. *Chlamydia trachomatis* is the most common bacterial sexually transmitted disease (STD) and the leading cause of infectious blindness worldwide. *Chlamydia pneumoniae*, an agent of respiratory infection, is nearly ubiquitous in humans, with seropositivity rates of 70–80 % in older populations, suggesting most people experience infection during their lifetime [5]. *Chlamydia psittaci,* a common pathogen of birds, has the best known zoonotic potential of the

human pathogenic chlamydiae and causes relatively rare respiratory infections associated with severe clinical manifestations, while several other animal pathogenic chlamydial species, including *Chlamydia abortus*, *Chlamydia felis*, and *Chlamydia suis*, are known, or suspected, to cause infrequent human infections with various clinical presentations [6, 7]. Chronic chlamydial infections in animals, particularly ruminants and pigs, are sub-clinical and ubiquitous in nature [8, 9]. Although their pathogenic significance is debateable, recent data suggest clinical impact when these infections coincide with various epidemiological risk factors. Chronic chlamydial human disease has well-recognized medical significance, encompassing the most detrimental outcomes of chlamydial disease, and is the topic of this review.

By definition, chronic diseases are long-lasting conditions that can be controlled, but not cured. The term chronic, however, is usually applied when the disease lasts more than three months, regardless of the eventual outcome [10]. Chronic disease is the leading cause of death and disability in the United States [11]. In Europe, chronic conditions account for 86 % of all deaths and 77 % of the disease burden [12]. Chronic diseases also significantly increase health care costs related to long-term medical care. Exposure to infectious agents has been implicated as a risk factor for development of chronic diseases, and strong association has been recently shown for five pathogens: human immunodeficiency virus (HIV), hepatitis C virus, *Helicobacter pylori*, and *Chlamydia (C.) pneumoniae* [12]. Association of chlamydiae other than *C. pneumoniae* with chronic disease is also well supported.

In this article, we review the role of chlamydiae in chronic disease conditions including atherosclerosis, trachoma, urogenital infections and arthritis. Chlamydiae have been implicated in neurological chronic diseases, such as multiple sclerosis and Alzheimer's disease, and in neurobehavioral diseases such as autism and schizophrenia [13], but because supporting evidence is contradictory, these diseases will not be discussed here. Additionally, chlamydial infection has been associated with cervical, ovarian, and prostate cancers [14, 15], but again, evidence supporting the association is inconsistent and this topic will not be discussed at length. Because of length limitations, we focus on the intensively investigated chlamydial species *C. trachomatis* and *C. pneumoniae* in humans. We will: 1) address the clinical significance and public health impact of chronic chlamydial diseases; 2) summarize pathogenesis emphasizing host-pathogen interactions, including host immune response and bacterial factors associated with disease; 3) discuss diagnostic methods and therapeutics in the light of chronicity; and 4) indicate future research directions.

Chlamydia trachomatis: Trachoma and Blindness

Trachoma, caused by ocular strains of *C. trachomatis*, causes visual impairment of about 2.2 million people, of whom 1.2 million are irreversibly blind [16]. Globally, it is estimated that more than 50 countries are endemic for blinding trachoma, mainly in Africa and the Middle East, but also in Asia, Latin America and the western Pacific [17]. However, estimates for global trachoma vary considerably due to limited reliable survey data from endemic regions [18]. Trachoma is associated with poor hygiene status and extreme poverty [17]. It is a family-based disease clustering in certain communities and specific households within these communities. The disease is spread by direct contact with ocular and nasal discharges, contact with fomites, or contact with eye-seeking flies, which are vectors for the disease. Trachoma is thus a disease specific to poor rural regions in less developed countries and is part of the Neglected Tropical Diseases Program [16].

Ocular serovars (A, B, Ba, and C) of *C. trachomatis* have highly specific tropism for mucosal epithelia of ocular conjunctiva. Infection of the conjunctival epithelium leads to conjunctivitis and triggers an immune response characterized by a marked inflammatory cell infiltrate and release of pro-inflammatory cytokines in the conjunctiva [18, 19•]. One episode of infection results in self-limiting chlamydial conjunctivitis, an acute phase referred to as active trachoma. The World Health Organization (WHO) estimates 40 million people worldwide have active trachoma [16]. Infection is usually acquired in infancy in hyperendemic regions and active trachoma is mostly seen in children, progressing to eyelid scarring and blindness in adulthood. Active trachoma is frequently found in the absence of detectable *C. trachomatis* infection, and is clinically represented by papillary and/or follicular inflammation of the tarsal conjunctiva. Repeated and/or persistent infections trigger sustained inflammation and scarring of the upper tarsal conjunctiva. Scarring and fibrosis, in turn, distort the upper eyelid and facilitate inturning of the eyelid (entropion) and eyelashes (trichiasis), causing irritation of the corneal surface and irreversible blindness. The scarring and trichiasis that lead to corneal opacity and sustained pathological tissue reaction to inflammation constitute the second phase of trachoma.

The World Health Organization Simplified Trachoma Grading System divides active trachoma into two often coexisting phenotypes: Trachoma Inflammation Follicular (TF) and Trachoma Inflammation Intense (TI) [18]. Chronicity and progression to inflammatory eye lesions are classified in the WHO system as Trachomatous Scarring (TS), reflected by tarsal conjunctiva scarring, and Trachomatous Trichiasis (TT), including at least one eyelash rubbing on the eyeball. The most severe disease sequela is blinding Corneal Opacity (CO) [18]. The immune response to *C. trachomatis* provides only partial protection, is serovar-specific and does not

prevent reinfection [19•]. Tissue damage and scarring result from chronic, pathological immune reactions. Related immunity and immunopathogenesis have been studied in mouse and guinea pig animal models, and data more comparable to human trachoma have been obtained from non-human primate studies. Scarring complications result from complex interactions between infectious burden, local immune response and host genetic polymorphisms related to immune function.

Repeated reinfections are implicated in the development of chronic scarring disease [19•]. The role of persistent, non-replicating chlamydial forms in ocular infections is unclear and controversial within the chlamydial field [20]. Tissue damage and fibrosis in *Chlamydia*-related diseases are thought to result from cell-mediated immunity responses against chlamydial antigens, either by delayed type hypersensitivity or molecular mimicry [21]. In trachoma, infected conjunctival epithelial cells secrete pro-inflammatory cytokines, chemokines, and growth factors, which recruit inflammatory immune cells. Inflammatory cells such as neutrophils and macrophages disrupt normal tissue architecture by releasing mediators such as toxic reactive oxygen and nitrogen species and matrix metalloproteinases (MMP). MMP9 can degrade collagen IV, leading to basement membrane disruption, and pro-fibrinogenic factors are thought to stimulate activated fibroblasts to produce collagen, causing scarring.

Key factors influencing trachoma development and progression are the presence of different strains circulating within communities, pathogen burden of infected individuals and polymorphisms in specific host genes. Host polymorphism in immune response genes is hypothesized to play a significant role in trachoma disease progression [22•]. Single nucleotide polymorphisms in the Interleukin-10 (IL-10) gene, the tumor necrosis factor (TNF) locus and MMP-9 have been implicated in trachoma pathogenesis [22•]. Recently, specific combinations of polymorphisms in Human Leucocyte Antigen C (HLA-C) ligands and their inhibitory Killer-cell Immunoglobulin-like Receptors (KIRs) were associated with increased risk of conjunctival scarring in trachoma patients [23]. Besides host polymorphisms, genetic variation of *C. trachomatis* impacts disease severity and tissue tropism [22•]. Major outer membrane protein (MOMP) serovar predicts chlamydial disease biovars (A-C: endemic trachoma), but does not reflect disease severity differences. Polymorphisms in chlamydial genes such as Tarp, Inc, CT229, pmp and cytotoxin appear to influence disease severity and tissue tropism; however, clear links and mechanisms are unknown. Distinction between ocular and genital strains can also be made based on mutations in the tryptophan synthase genes [24].

Documented *C. trachomatis* infection correlates poorly with clinical sequelae [25], complicating diagnosis of trachoma. Nucleic acid amplification tests (NAATs) are sensitive and specific, but results do not correlate with clinical grading. In vivo confocal microscopy has been recently used to visualize progression of inflammatory and scarring changes [26]. Commercial NAATs do not detect *Chlamydiaceae* species other than *C. trachomatis*. Single and mixed infections with *C. trachomatis*, *C. psittaci, C. suis, C. pecorum* and *C. pneumoniae* were detected in conjunctival samples of trachoma patients by ArrayTube microarray [27]. This finding and the potential zoonotic origin of these *Chlamydiaceae* species other than *C. trachomatis* might have implications for immunopathology and disease outcome in trachoma patients, therapeutic treatment and future vaccine development.

The WHO launched an initiative with the ambitious goal of eliminating blinding trachoma globally by 2020. The SAFE strategy includes: surgery for trichiasis, antibiotics for active trachoma, facial cleanliness, and environmental improvement [16]. Current WHO recommendations constitute mass treatment with a single dose of azithromycin. The risk of adverse events and possible antibiotic resistance development due to azithromycin treatment merit consideration, but ancillary benefits such as reduced infectious disease and decreased childhood mortality outweigh these concerns [25]. Success of trichiasis surgery is impeded by high recurrence rate (5-40 %), poor surgical technique, limited accessibility to surgery and lack of acceptance of surgery among the local population [28]. Limiting exchange of ocular secretions can be achieved by facial cleanliness and improvement of hygiene conditions (environmental improvement) to decrease transmission [28].

Chlamydia trachomatis: Genital Infections

C. trachomatis is the most common bacterial cause of STD worldwide. In the United States, 1.4 million chlamydial infections were reported by the Centers for Disease Control and Prevention (CDC) in 2011 [29], and the WHO estimates that more than 90 million persons are infected worldwide [30]. The greatest burden is in sexually active women aged 14 to 19 years, with a prevalence of approximately 6.8 % in the United States [31]. The major age groups for chlamydial STD are women aged 18 to 20 and men aged 20 to 24 years. Moreover, many patients are asymptomatic (70–90 % of women, 30–50 % of men), thus most cases likely remain undiagnosed/unreported [29]. Risk factors include young adulthood, multiple sex partners, intermittent condom use, cervical ectopy, history of other STD such as HIV, low education status, low socioeconomic class, and anal receptive intercourse [29]. High-risk types of human papillomavirus (HPV) are principle causative agents in cervical cancer, but *C. trachomatis* infection is a co-factor in development of cervical neoplasia [32]. Women with untreated chlamydial diseases have increased risk of HIV infection [33]. Additionally, HIV-infected women with reduced CD4+ T cell counts have increased risk for developing *C. trachomatis* pelvic inflammatory disease (PID) [34•]. Forty-six percent of men

and women infected with *Neisseria gonorrhoeae* also have co-infection with *C. trachomatis* [29].

Nineteen serovars based on MOMP seroreactivity predict chlamydial disease biovars: A–C (endemic trachoma), D–K (genital diseases) and L1–L3 (lymphogranuloma venereum). *C. trachomatis* genital serovars infect superficial mucosal epithelia of the urethra in men or endocervix in women, initiating disease. In women, *C. trachomatis* cervical infections are mostly asymptomatic and can either resolve spontaneously or progress for weeks to months, causing complications [33]. Approximately 25 % of women with chlamydial cervicitis have concomitant urethritis [33]. Cervical infection can ascend into the endometrium and fallopian tubes and develop into chronic infection and PID. PID is characterized by infection and inflammation of the upper genital tract, frequently involving the endometrium, fallopian tubes, and pelvic peritoneum (endometritis, salpingitis or tubo-ovarian abscess and peritonitis). PID is caused by common sexually transmitted infections, such as *Neisseria gonorrhoeae*, *Mycoplasma genitalium, C. trachomatis* (in 30 % of clinical cases), and by anaerobic vaginal microbes causing bacterial vaginosis [35]. Presumptive diagnosis of PID is made clinically (women of reproductive age with pelvic or abdominal pain), while definitive diagnosis is made by laparoscopy [35]. If untreated, 8–10 % of *C. trachomatis*-infected women develop PID [35]. Tubal damage is mediated by innate immune responses and adaptive T-cell responses. Long-term sequelae of PID include tubal infertility, ectopic pregnancy, and chronic pelvic pain caused by tubal damage and scarring from inflammation. The duration of an infection or repeated infections affect the pathogenesis of PID, but the relative importance if each has not been elucidated. Repeated *C. trachomatis* infections are common, indicating limited natural immunity.

Women with active infection can transmit *C. trachomatis* to their infant during delivery, leading to conjunctivitis and pneumonia in the newborn. *C. trachomatis* infection in pregnant women has been also linked to chorioamnionitis, placentitis, premature rupture of membranes, and preterm birth [36], however, existing evidence is weak [32]. In men, *C. trachomatis* causes non-gonococcal urethritis, epididymitis, prostatitis, and proctitis [37]. Urethritis is the most frequent STD syndrome in men, and *C. trachomatis* is the causative agent in 15–40 % of cases. The more invasive strains causing lymphogranuloma venereum (LGV) are named L1, L2, L2a and L3. LGV serovars infect and replicate within macrophages, spread systemically through lymph nodes, and cause necrosis and abscesses in inguinal and femoral lymph nodes. LGV proctitis can vary from clinically silent to severe. Traditionally, LGV is found most often in Africa, India, Southeast Asia, and the Caribbean, and almost exclusively in men who have sex with men (MSM). Since 2004, increasing incidence has been found in North America, Europe, and Australia and occurs mostly in MSM with proctitis [29].

C. trachomatis infection of the female genital tract is recognized by Toll- like receptor (TLR)-2 and TLR-4 and nucleotide-binding oligomerization domain (NOD)1, leading to induction of interferon (IFN) gamma. Cell-mediated immunity is important in clearance of *C. trachomatis* infection. However, the immune response against *C. trachomatis* infection does not provide long-lasting protection and may contribute to pathology. Th1 T helper cell response helps resolve the infection, but also leads to secretion of pro-inflammatory factors such as TNF alpha, IL-1alpha and IL-6. Induction of IL-10 might down regulate the chlamydial-specific T-cell responses, leading to chronic inflammation and tissue damage in persistent infections [38]. Matrix metalloproteinase 9 (MMP9) expression by fallopian tube cells infected with *C. trachomatis* is associated with scarring [34•]. Clearance of *C. trachomatis* infection might be delayed by pathogen immune evasion strategies, such as enhanced survival inside and outside host cells, reduced inflammatory and adaptive immune responses and ability to persist within host cells as AB [39]. Adding complexity, sex hormones modulate female genital tract immune responses [40, 41]. Women are more susceptible to chlamydial infection under the influence of estradiol, and estradiol enhances disease sequelae [40]. T-cell-driven INF gamma and Th17 responses are critical for clearing infection and play a role in protection from disease [38, 42]. Initiation of autoimmunity by molecular mimicry has been suggested in the pathogenesis of PID [43]. The most important factor in molecular mimicry might be chlamydial heat shock protein 60 (Hsp60). Chlamydial Hsp60 is considered the key antigen in immunopathogenesis of tubal infertility, stimulating humoral and cell mediated immune responses in women with PID/tubal infertility [43]. Increased antibody response to chlamydial Hsp60 in women is strongly associated with PID, ectopic pregnancy, and tubal infertility [44].

Variability in the MOMP gene (ompA) is unrelated to disease severity and MOMP serovars fail to correlate with virulence [45]. However, recent data indicate that variation in the pmp genes may contribute to disease severity [22•]. Pmps B, D, and H are strongly immunogenic and elicit pro-inflammatory cytokine responses [45]. Genital, but not ocular, strains of *C. trachomatis* possess functional tryptophan synthase (trpBA) to convert indole, secreted by local vaginal flora, into tryptophan. A perturbed vaginal microbiome (bacterial vaginosis) might provide a source of indole, enabling genital *C. trachomatis* serovars to circumvent tryptophan limiting bacteriostatic/bactericidal effects of IFN gamma [46] and establish persistent infection [4]. Persistence versus clearance is likely driven by IFN gamma responses. High levels of IFN gamma eradicate chlamydiae, but low levels result in the persistent state [42]. Inflammation of infected tissues promotes local oxygen consumption, resulting in hypoxia [47]. A recent study indicates hypoxia reactivates IFN gamma-induced persistent *C. trachomatis*, causing increased

bacterial growth and progeny, while dampening the host inflammatory response [48].

The *C. trachomatis* genome is highly conserved, and diversity seems to have evolved through genetic recombination [49] and might result in hypervirulent strains [22•]. Deletion events also occur and have been described in the cryptic plasmid of *C. trachomatis*. Chlamydial plasmids are present in *C. trachomatis*, but are non-conjugative and nonintegrative; they do not encode antibiotic resistance nor show signs of genetic flexibility. Therefore, they are targeted for NAAT diagnosis of *C. trachomatis* infection [50]. However, emergence of the new Swedish variant (nvCT), a mutated strain from serovar E strains carrying a 377-base-pair deletion within its plasmid, was reported in 2006. Several commercial NAATs targeted this region, leading to diagnostic failure [51]. Retrospective studies suggest nvCT in the Swedish population arose after 2000, perhaps by importation of the variant or by spontaneous mutation [49]. Genetic predisposition and host immune response are also important in pathogenesis of long-term complications of genital *C. trachomatis* infection [32]. Specific HLA DQ alleles and polymorphisms in the promoter of IL-10 and TNF alpha are associated with high risk of tubal infertility [22•], whereas polymorphisms in TLR-2 are associated with protection against tubal disease following *C. trachomatis* infection [36].

Diagnosis of *C. trachomatis* infection is recommended by NAAT from urine, vaginal, or endocervical swabs. NAAT from non-genital samples, such as rectal swabs, is performed but not US Food and Drug Administration (FDA)-approved and is essential to diagnose LGV proctitis [33]. Self-collected vaginal or urine swabs are most commonly used in screening programs. The Centers for Disease Control and Prevention (CDC) recommends annual screening for *C. trachomatis* in sexually active women aged 25 years and younger [31]. Upon diagnosis, primary uncomplicated urogenital infections can be effectively treated with antibiotics. The CDC recommends either single-dose azithromycin or a 7-day course of doxycycline in adolescent and adult men and women, and amoxicillin in pregnant women [52]. Early treatment of *C. trachomatis*-infected individuals shortens infection duration and prevents sexual transmission and complications including PID, while treatment of pregnant women prevents transmission to the infant during delivery [52]. However, most chlamydial infections are asymptomatic and undiagnosed/untreated. Treatment failure occurs [42], perhaps due to re-infection, persistent infection or, less likely, acquired antibiotic resistance. Recurrent *C. trachomatis* infections result from re-infection from an untreated partner or infection from a new partner [52]. Moreover, prevalence of anorectal chlamydia is high; almost all women with anorectal chlamydia had concurrent urogenital chlamydia [53]. This finding has implications for the diagnosis of *C. trachomatis* infections (both sites should be tested) and for the therapy (anorectal chlamydial infections are more difficult to treat). Moreover, anorectal infection might lead to recurrent vaginal infection by autoinoculation [54]. Treatment of LGV infection requires prolonged therapy (21 days) and the CDC recommends doxycycline [52].

Screening programs aim to identify and treat asymptomatic cases of cervicitis before *C. trachomatis* infection can progress to PID [35]. Trials indicate that *Chlamydia* screening and treatment reduce risk of PID among young women [55]. Screening has contributed to the decline of PID; however, the magnitude of benefit might have been overestimated in initial trials. Asymptomatic chlamydial infections may have been present for months when detected by screening, and thus might have already progressed to chronic inflammation in the upper genital tract. The role of chlamydial screening in reducing complications has not been confirmed and benefit on an individual level is difficult to assess [55]. Future focus should include screening young, sexually active women; determining optimal frequency of screening and benefit of screening for repeat infections; and, of major importance, treatment of partners of infected women [56]. In addition to preventing adverse sequelae of *C. trachomatis* infection, reducing the incidence of new infections through interruption of transmission might be equally important [55]. Despite screening and control programs, reported *C. trachomatis* cases have not exhibited sustained declines. This may be explained by the arrested immunity hypothesis: early treatment interrupts acquisition of protective immunity, increasing risk of reinfection [57, 58]. A recent clinical study provides data supporting this concept, indicating spontaneous resolution of chlamydial infection in the absence of antibiotic treatment may reduce risk of subsequent reinfection [59]. Development of a safe and effective *C. trachomatis* vaccine to prevent acquisition and transmission of infection, and prevent development of inflammatory sequelae, remains the ultimate goal [42, 57].

Chlamydia pneumoniae: Respiratory Infections, Coronary Artery Disease and Atherosclerosis

Chlamydia (C.) pneumoniae causes a wide range of acute and chronic respiratory diseases and has been associated with atherosclerosis and cardiovascular disease. The chlamydial organism Taiwan acute respiratory agent (TWAR) was classified in 1989 as a new species, *C. pneumoniae* [60]. Human *C. pneumoniae* strains are highly conserved, in contrast to animal strains, which are thought to be ancestral [61]. Although *C. pneumoniae* is an acute human respiratory pathogen, much research has focused on its role in chronic infections. *C. pneumoniae* is essentially ubiquitous, with seropositivity of 50 % by the age of 20 years and 70–80 % by 60–70 years [62]. Most acute infections are asymptomatic or manifest as mild, self-limiting upper respiratory tract infections. However, progression to severe upper respiratory tract infections (pharyngitis, sinusitis, and otitis) and lower respiratory

tract infections, such as acute and chronic bronchitis, asthma, and pneumonia, occurs [63, 64]. *C. pneumoniae* is believed to account for around 10 % of community-acquired pneumonia (CAP), 5 % of pharyngitis, bronchitis and sinusitis [65], and can exacerbate chronic bronchitis and asthma.

CAP can be of bacterial and/or viral etiology [66]. Bacterial CAP is usually caused by *Streptococcus pneumoniae*; other common pathogens involved are *Staphylococcus aureus*, *Haemophilus influenzae*, *Enterobacteriaceae*, *Legionella pneumoniae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. A prospective study of German CAP patients identified *C. pneumoniae* in respiratory samples in only 0.9 % cases by PCR [67]. In contrast, another study identified *C. pneumoniae* as the second most common pathogen in CAP patients in North and South America, with a global incidence of 7 % [68]. The incubation period is around 21 days, with transmission by respiratory secretions [64].

Clinically, the causative pathogen of CAP is often unidentified, and commercially available FDA-approved NAATs for *C. pneumoniae* are not available [69]. Microimmunofluorescence testing (MIF) is the reference standard for serodiagnosis of *C. pneumoniae* infection. However, MIF cannot differentiate acute versus chronic infection, nor discriminate between past and persistent infections [70]. Common treatment for CAP is empiric antibiotic therapy with a beta-lactam in combination with macrolides, respiratory fluoroquinolones or tetracyclines [71]. Specific treatment of *C. pneumoniae* -induced acute respiratory infection is successful with macrolides, tetracyclines, or respiratory fluoroquinolones [66]. However, the benefit of treatment is difficult to assess, as the majority of the studies have relied on serology for the diagnosis of *C. pneumoniae*-induced respiratory infections [52]. Serology often does not correlate with pathogen detection by culture or PCR, especially in children, where more than 70 % of culture positive children with CAP are seronegative [69].

In respiratory *C. pneumoniae* infections, the innate immune response is likely mediated by Toll-like receptor (TLR) -2 and TLR4-induced early cytokine and chemokine production via MyD88 [72•]. NOD and NOD-like receptors might play a role in prolonged activation of host cells. Bronchial epithelial cells are the first line of defense during *C. pneumoniae* infection, but after initial infection, the pathogen resides in macrophages and neutrophils. *C. pneumoniae* infection is also associated with asthma, and in particular, asthma exacerbation. *C. pneumoniae*-specific IgE is prevalent in asthma patients, and occurrence of these species-specific antibodies is associated with disease severity [73]. The most widely accepted association of *C. pneumoniae* respiratory infection with exacerbation of asthma is documented by multiple epidemiologic and clinical studies, some of which investigated whether antibiotic treatment in asthma patients improves outcome. However, macrolides, quinolones, ketolides and tetracyclines all have immunomodulatory activity independent of their antimicrobial activity, which might influence outcome. To date, the benefit of antibiotics in asthma patients without evidence of acute infection remains controversial [52].

Atherosclerosis, the leading cause of death in industrialized countries, causes coronary artery disease (CAD) and cerebrovascular disease, and is defined as a chronic inflammatory disease of multifactorial etiology. It is characterized by endothelial cell injury, proliferation of vascular cells and ensuing accumulation of inflammatory cells that secrete pro-inflammatory molecules such as IL-1, IL-6 and TNF alpha, inciting an oxidative stress response [74]. Besides well-known cardiovascular risk factors (hypertension, hyperlipidemia, diabetes, smoking and obesity), several infectious agents such as cytomegalovirus, influenza A virus, hepatitis C virus, HIV, *Helicobacter pylori*, periodontal pathogens, and *C. pneumoniae* have been implicated in pathogenesis of atherosclerosis [75, 76]. However, *C. pneumoniae* is the only one of these pathogens that has been repeatedly detected in atherosclerotic lesions and cultivated from atheromatous plaques. Association of *C. pneumoniae* with atherosclerosis and coronary heart disease is based on both seroepidemiological studies and direct detection of the microorganism in the atheroma by PCR, immunohistochemistry, in situ hybridization, electron microscopy and culture. Animal models have also demonstrated that progression of atherosclerotic lesions is accelerated by *C. pneumoniae* infection [75, 77•]. In human patients, after initial respiratory infection, the pathogen can disseminate from the lungs through peripheral blood mononuclear cells, replicating and persisting in vascular tissue cells—further suggesting a role in the pathobiology of atherosclerosis.

Chlamydial lipopolysaccharide (LPS) and chlamydial Hsp60 are important *C. pneumoniae* virulence factors. Molecular mimicry between bacterial and self antigens such as heat shock proteins might contribute to pathogenesis [75]. In response to infection with *C. pneumoniae*, acute phase proteins enhance progression and destabilization of atherosclerotic plaques [76]. *C. pneumoniae* can induce proliferation of vascular smooth muscle cells (VSMCs) and stimulate production of numerous pro-inflammatory mediators and adhesion molecules in VSMCs and endothelial cells [76, 78, 79]. *C. pneumoniae* infection can also induce reactive oxygen species (ROS) in macrophages, platelets, endothelial cells and VSMCs, contributing to atherosclerotic lesion development [74]. Progression of atherosclerotic lesions is viewed as host response to noxious stimuli, and *C. pneumoniae* infection might represent one among many such possible stimuli. Furthermore, it is unclear if *C. pneumoniae* is involved in induction of atherosclerotic lesions, or if it is a mere colonizer and bystander of already injured vascular tissue. Nevertheless, *C. pneumoniae* may be the only pathogen for which both a

direct and an indirect contribution to atherosclerosis have been demonstrated. In evidence of this, antigen and/or DNA/RNA of *C. pneumoniae* has been detected in human plaques, but never found in normal vascular tissue; viable organisms have been isolated from plaques; *C. pneumoniae*-related increased pro-inflammatory response has been shown; and atherosclerotic lesions in mouse and rabbit animal models were accelerated by *C. pneumoniae* infection.

The association between infectious agents, in particular *C. pneumoniae*, and CAD led to clinical trials assessing antibiotic treatment [75]. Cumulatively, these trials demonstrated that anti-chlamydial antibiotic treatment was ineffective in coronary heart disease patients, leading to the assumption that *C. pneumoniae* is not involved in the pathogenesis of atherosclerosis [77•]. However, several factors were not considered during these trials: treatment was given to patients suffering "end stage disease" with advanced atherosclerotic lesions unlikely to be influenced by antibiotic treatment; other related pathogens such as bacteria and viruses involved in the atherosclerotic process were not considered; a single antibiotic was used although a combination might have been more effective; and, finally, potential persistent forms within atherosclerotic lesions was not considered, though it is known from in vitro studies that persistent chlamydial organisms are refractory to antibiotic treatment [77•].

***Chlamydia*-Induced Arthritis**

The spondyloarthritides (SpA) are rheumatoid factor-negative, HLA-B27-associated arthritic diseases characterized by four clinical features: 1) inflammatory back pain, 2) asymmetric peripheral oligoarthritis, usually of the lower limbs, 3) enthesitis, and 4) extra-articular inflammatory symptoms, most commonly uveitis [80]. Five subgroups comprise the SpA, including ankylosing spondylitis, inflammatory bowel disease-associated SpA, psoriasis-associated SpA, reactive arthritis and undifferentiated SpA. Reactive arthritis (ReA) has similar clinical presentation to other SpA, but specifically develops subsequent to bacterial enteric, urogenital, or, less frequently, respiratory infection, typically within 1 to 3 weeks after the triggering infection [81]. In enteric-associated ReA, *Yersinia*, *Salmonella*, *Shigella*, and *Campylobacter* predominate, while *C. trachomatis* is the most common in urogenital-associated ReA, and the respiratory pathogen *C. pneumoniae* is also associated with the disease [82•]. *C. trachomatis* genital infections and *C. pneumoniae* infections account for about 50 % and 12 % of all ReA, respectively [82•, 83]. Septic arthritis, also initiated by infection, but not in the SpA group, is associated with actively infected synovium/synovial fluids, in contrast to ReA in which synovial cultures are typically negative [81]. However, in *Chlamydia*-induced ReA, bacteria/bacterial products can be found in affected joint(s) [83]. This indicates that the traditional description of ReA, having affected but non-infected joints, is inaccurate in the case of *Chlamydia*-induced ReA. The disease instead shares characteristics of both ReA, defined by autoimmune reaction-induced symptoms, and septic arthritis, defined by infection-induced inflammation [84•]. Here, we use the term *Chlamydia*-induced arthritis (CiA) in reference to ReA induced by *C. trachomatis* and/or *C. pneumoniae*.

In CiA, and other ReA, all associated clinical features are frequently not present and the clinical presentations can be quite variable. The College of Rheumatology general guidelines can be used as diagnostic criteria for ReA, indicating probable or definitive ReA depending on satisfaction of major and/or minor criteria based on clinical presentation, preceding symptomatic enteric or urogenital infection, and laboratory proof or evidence of triggering or persistent synovial infection [81]. Currently, however, no national or international guidelines exist for diagnosis or classification of ReA, and the incidence and prevalence of ReA and CiA remain unclear. The Office of Rare Diseases Research of the National Institutes of Health categorizes ReA as rare, affecting less than 200,000 individuals in the United States (USA) [85]. Notably, with up to 80 % of *Chlamydia* infections, and up to 90 % of *C. pneumoniae* infections specifically, expected to be asymptomatic [84•, 85], it is likely ReA and CiA are underdiagnosed. A high incidence of genital *Chlamydia* infections, estimated to be over 3 million annually in the USA and up to 115 million annually worldwide, and reports that approximately 4 % of those with acute genital chlamydial infection will develop ReA/CiA, have prompted some to calculate that at least 120,000 new cases of *C. trachomatis*-induced ReA are likely to occur annually in the USA [82•]. The USA and worldwide prevalence of CiA, if such calculations are accurate, might not be considered rare. Additionally, three recent case reports [86–88] of well-documented CiA following *C. trachomatis* LGV L2 proctitis, all in HIV-positive MSM, indicate that the ongoing increased incidence of *C. trachomatis* LGV L2 proctitis in Europe, Australia and North America [29] may be expected to correlate with increased CiA incidence. This has led some to suggest that screening for *C. trachomatis* LGV L2 in all HIV-positive MSM presenting with acute arthritis may be beneficial even in the absence of proctitis, because infection can be asymptomatic [86]. ReA is usually self-limiting, with spontaneous symptom resolution within 6 months in approximately 50 % to 70 % of cases; however, 30 % to 50 % of patients progress to chronic infection, often with remitting-relapsing disease course [82•]. Significant pain, loss of activity, and economic costs are reported for ReA, and are similar to other arthritic diseases, such as members of the SpA group and rheumatoid arthritis [89].

Host and bacterial factors contributing to CiA pathogenesis have not been clearly determined. HLA-B27 allele positivity has traditionally been associated with CiA/ReA and the other

SpA groups. Early studies reported that up to 80 % of ReA cases were HLA-B27 positive, while more recent studies indicate that 30 % to 50 % of all ReA cases are associated with the allele. Thus, HLA-B27 is currently thought to more accurately represent a risk factor for progression to chronic disease and/or increased disease severity in ReA, rather than a risk factor for ReA susceptibility [85]. In ReA other than CiA, the triggering organism is absent from affected joints, and pathogenesis is thought to occur largely based on autoimmune factors initiated by distal or resolved infection. However, viable *C. trachomatis* and/or *C. pneumoniae* have been demonstrated in affected joints by both electron microscopy and PCR [85]. This suggests that, although host autoimmunity might contribute to pathogenicity, the immune response to an ongoing joint infection may also affect disease pathogenesis.

Viable *Chlamydia* exists in the joint, apparently as a long-term infection, in a state usually referred to as persistence [85]. Persistent organisms are metabolically active, non-cultivable and exhibit altered patterns of gene expression. Persistence can be induced in vitro and in animal models by a variety of conditions, including amino acid or iron deprivation, antibiotic or IFN gamma exposure, co-infection with viruses or parasites, and heat shock [3•]. *Chlamydia* disseminates to joints from the initial site of infection via monocytes, and once in the joints, the chlamydiae infect macrophages and/or macrophage-like and fibroblast-like cells of the synovium [82•, 84•, 90]. Interestingly, the persistent state appears to occur, at least in part, due to *Chlamydia* infection of monocytes and macrophages, which do not support normal, productive infection [82•]. Cells of the synovium, along with T cells present in infected joint(s), mount a protective immune response to *Chlamydia* involving production of cytokines such as IFN gamma, TNF alpha, and various interleukins—a response that is important for clearance of *Chlamydia* infection, but also involved in inflammatory joint damage via cartilage and bone deterioration [90]. Chlamydial Hsp60 proteins play a major role in the host inflammatory response. In vitro, chlamydial Hsp60 genes show altered expression during persistent infection, and synovial samples from patients with chronic CiA reflect this [82•]. Diagnosis of continued bacterial presence indicates pathogen survival, and thus implies that the immune response is ineffective in clearing the infection. However, this immune response also causes inflammatory joint damage. It has been postulated that damage results from skewing of the T helper cell response from a Th1 dominant response to a Th2 dominant response, a process in which the persistent state of *Chlamydia* itself might play a role [84•]. A recent study showed that bacterial DNA and inflammatory cytokine/chemokine and Hsp60 transcripts can be found in the joint during the remitting phase of chronic CiA, suggesting damaging inflammation is ongoing even during symptom remission [91].

Host genetic variability, outside of HLA-B27 phenotype, is likely important in initial and continued immune response to infection, especially considering the low proportion of cases of acute *Chlamydia* infection resulting in CiA. Initially high levels of IFN gamma and TNF alpha in the synovium are associated with resistance to experimentally induced CiA [84•]; and it has subsequently been suggested that host-determined low initial cytokine levels at the time of acute *Chlamydia* infection might potentiate chronic disease progression, perhaps dependent to some degree on the persistent *Chlamydia* state [92]. Indirect evidence supports the concept that the *Chlamydia* persistent state may contribute to pathogenesis of CiA. A study evaluating 36 patients with confirmed CiA, all with synovial samples positive for *C. trachomatis* by PCR, exclusively identified ocular, not genital, strains of *C. trachomatis* [93]. This lead the authors to surmise *C. trachomatis* ocular strains might have a particular capacity for dissemination from the genital tract, and the small percentage of *C. trachomatis* cases progressing to CiA might reflect the small percentage of cases in which ocular strains are found in genital infection [93]. *C. trachomatis* ocular strains and *C. pneumoniae* human strains, the chlamydiae commonly demonstrated to be involved in CiA, are unable to synthesize tryptophan and thus have increased sensitivity to the host IFN gamma response and subsequent tryptophan deprivation, a characteristic that might facilitate persistence as a means of initiating and maintaining chronic infection [94, 95].

Treatment of CiA is not well studied, and therapy relies largely on managing the disease and treating symptoms. Non-steroidal anti-inflammatory drugs, corticosteroids and the disease modifying anti-rheumatic drug sulfasalazine have shown some efficacy for certain stages or severity of CiA [82•]. A meta-analysis of 12 antibiotic treatment studies showed that long-term antibiotic treatment does not significantly improve disease outcome [96], a finding that may support the concept that continued infection in CiA correlates with, but does not cause, ReA. However, a single trial examining combined 6-month use of a macrolide or tetracycline with rifampin, demonstrated that chronic CiA patients showed significant improvement and increased negative *Chlamydia* PCR diagnosis with combination antibiotic therapy compared to placebo, a finding the authors suggested might reflect that persistent chlamydiae are refractory to standard antibiotic treatment [85]. Anti-TNF alpha treatment, commonly used for rheumatoid arthritis, has shown efficacy for CiA [92]. However, given the importance of TNF alpha and other inflammatory cytokines in control of *Chlamydia* infection, more studies are needed to evaluate this therapy in CiA. In fact, many confounding factors encompass ReA and CiA, complicating pursuit of effective therapy. The relationship between continued joint infection, subsequent to initial *Chlamydia* infection, and CiA disease inception or progression has not yet been proven a causal relationship, and the importance of eradicating

underlying chlamydial infection for resolution of CiA is thus unknown. The basic biology underlying the persistent state of *Chlamydia* in joint infection is also poorly understood, and the in vivo diagnosis, as well as the consequence of persistent infection, remains to be elucidated [20].

Conclusions

Chronic chlamydial infections are implicated in trachoma, sexually transmitted genital diseases, atherosclerosis and arthritis. These infections are characterized by prolonged and chronic inflammation, scarring and fibrosis mediated by continuous stimulation of the host immune response. Multiple host and chlamydial factors contribute significantly to specific chlamydial chronic diseases. However, several characteristics of chlamydiae and chlamydial infection are especially relevant to the propensity of this organism to establish chronic disease in general: i) infection of multiple cell types and dissemination within the host, ii) the intracellular niche of the chlamydiae, iii) immune evasion allowing enhanced chlamydial survival by modulation of apoptosis, pathogen detection, and inflammatory and adaptive immune responses in the host [39, 97], and iv) frequent asymptomatic infection which can make detecting chlamydiae difficult.

Additionally, recent findings have broad implications for diagnosis and treatment of chlamydial infections. First, although the importance of chlamydial persistence in vivo remains unclear, persistent growth forms (AB) can be found in the human endocervix (by electron microscopy) where the local microenvironment at infection is similar to the in vitro model of IFN gamma persistence induction [98]. If the chlamydial persistent state is, in fact, present in vivo, both detection by culture (persistent chlamydiae are by definition non-cultivable) and treatment of chlamydial infection may be impacted. Persistent chlamydiae are resistant to killing by antibiotics in vitro; and a model of amoxicillin induced *C. muridarum* persistence in mice indicates persistent chlamydiae are resistant to killing by azithromycin in vivo as well [99]. Furthermore, clinically relevant concentrations of commonly used penicillins induce *C. trachomatis* persistence in vitro [100]. Second, tetracycline resistance in chlamydiae may be possible. Evidence of antibiotic resistance in human pathogenic chlamydiae is lacking, based on the observation that suspected tetracycline resistant strains lost the resistant phenotype during culture and failed to exhibit genomic evidence of resistance [101]. However, isolation of tetracycline resistant *C. suis* from pigs demonstrates an adaptive ability of chlamydiae to acquire antibiotic resistance under selective pressure [102], with implications for continued use of antibiotic treatment of chlamydial disease. Third, and finally, chlamydiae have long been known to colonize the gastrointestinal (GI) tracts of animal hosts, including poultry and sheep, without causing disease [54]. And although humans can become rectally infected with *C. trachomatis*, long-term intestinal infection in humans has not yet been confirmed [54]. Mouse model studies show chlamydiae infecting the GI tract can persist for up to 100 days with no pathology, and azithromycin treatment sufficient to cure genital infection did not similarly cure GI tract infection, despite drug levels in both anatomical sites [103•]. Thus, intestinal carriage of chlamydiae may not only allow auto- or re-inoculation of the genital tract, but may represent commensal association with the human host, providing a degree of incidental antibiotic resistance [54].

These findings indicate that future research in the areas of chlamydial persistence, mechanisms of protective immunity and immunopathology, and vaccine development is a priority. Given difficulties surrounding diagnosis and antibiotic treatment of chlamydial disease, development of an effective *Chlamydia* vaccine appears particularly advantageous in the context of chronic disease. Recent data from a non-human primate animal model demonstrated the efficacy of plasmid-deficient chlamydial strains as live attenuated vaccines against genital and ocular chlamydial infections [50], and vaccine development is underway [34•, 42]. Continued study of the intricate biology of the chlamydiae will facilitate advances in prevention, diagnosis and treatment of chronic chlamydial disease.

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Compliance with Ethics Guidelines

Conflict of Interest Cory Ann Leonard and Nicole Borel each declare that there are no conflicts of interest.

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