Chlamydia

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Glossary

Chlamydophila The genus consists of six species: *pneumoniae*, *felis*, *pecorum*, *abortus*, *caviae*, and *psittaci*.

Chlamydia muridarum A mouse-adapted *Chlamydia* species.

Chlamydophila pneumoniae The bacterium that causes community-acquired pneumoniae.

Chlamydia trachomatis The bacterium that is the most common cause of sexually transmitted disease and preventable blindness worldwide.

elementary body The infectious but nonmetabolically active form of *Chlamydia*.

inclusion The intracellular vacuole in which all *Chlamydia* and *Chlamydophila* species differentiate and replicate within.

lipid droplets Endoplasmic reticulum-derived lipid storage organelles that are a source of neutral lipids.

reticulate body The noninfectious but metabolically active form of *Chlamydia*.

sphingomyelin A Golgi-derived sphingolipid that usually consists of ceramide and phosphorylcholine

type III secretion system A secretion system present in Gram-negative bacteria that functions to deliver effectors directly from the bacteria into the host cytosol.

Abbreviations

CADD	*Chlamydia* protein associating with death domains
CPAF	Chlamydial protease-like or proteosome-like activity factor
cPLA2	cytosolic phospholipase 2
EAE	enzootic abortion of ewes
EB	elementary body
EEA1	early endosomal antigen
HGT	horizontal gene transfer
IDO	indolameanine 2,3-dioxygenase
Ig	Immununoglobulin
IL	Interleukin
Inc	Inclusion Membrane Proteins
INF-γ	gamma interferon
LCTs	large cytotoxins
LD	lipid droplets
LGV	lymphogranuloma venereum
MIF	microimmunofluorescence assays
MTOC	microtubule organizing center
MVBs	multivesicular bodies
OEA	ovine enzootic abortion
ORFs	open reading frames
p.i.	postinfection
PI3K	phosphatidylinositol-3-kinase
PZ	plasticity zone
RB	reticulate body
SNAREs	soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors
T3SS	type III secretion system
Tarp	translocated actin recruiting protein
TNF	tumor Necrosis Factor

Defining Statement

Chlamydiae are obligate intracellular bacteria that replicate within a nonacidified vacuole, termed the inclusion, which is actively modified by chlamydiae to promote their intracellular survival. To mediate their pathogenesis, chlamydiae secrete effector proteins into the host cytosol and inclusion membrane that directly target host signaling and trafficking pathways.

Introduction

Chlamydia and *Chlamydophila* species are Gram-negative obligate intracellular bacteria that are pathogenic for humans and a large variety of veterinary animal species. Chlamydiae replicate within a nonacidified vacuole, termed the inclusion, which is actively modified by chlamydiae to create a replication competent intracellular compartment. Due to the obligate nature of the organism as well as the inability to genetically manipulate the chlamydial genome, the molecular mechanisms that mediate the pathogenesis of chlamydiae have only recently begun to be elucidated. Over the last several years it has become clear that chlamydiae secrete both type III secretion system (T3SS)-dependent and -independent effectors into the host cytosol to directly target and exploit basic host cellular signaling and trafficking pathways.

Taxonomy

Chlamydiae belong to the Chlamydiaceae family (order Chlamydiales) that includes two genera: *Chlamydia* and *Chlamydophila*. Since 1999, three new families have been classified within the order Chlamydiales. These include the families Parachlamydiaeceae, Simkaniaeceae, and Waddliaceae, all of which contain *Chlamydia*-like or environmental *Chlamydia* species. The genus *Chlamydia* contains three species: *Chlamydia trachomatis*, *Chlamydia muridarum* (formerly *trachomatis* mouse pneumonitis agent or MoPn), and *Chlamydia suis*. The second genus *Chlamydophila* consists of six species: *C. pneumoniae*, *C. psittaci* (formerly *psittaci* avian), *C. pecorum*, *C. abortus*, *C. felis*, and *C. caviae* (formerly *psittaci* GPIC). Although the International Committee of Systematics of Prokaryotes supports the taxonomy that is described above, it is not used consistently in the literature. Readers may therefore come across a compromised taxonomy that many chlamydial researchers have adopted, which eliminates the genus *Chlamydophila* and places all nine species in the single genus *Chlamydia*.

Within the genus *C. trachomatis*, there are two biovariants (trachoma and lymphogranuloma venereum, LGV) that are distinguished based upon their anatomical distribution and severity of disease. The trachoma biovars are further classified serologically into at least 20 distinct serovars. Chlamydiae of serovars A–C primarily infect ocular epithelial surfaces and are associated with endemic blinding trachoma, a leading cause of preventable blindness worldwide. Chlamydiae of serovars D–K infect the urogenital epithelial mucosa and are the most frequent cause of bacteria-caused sexually transmitted disease, but they can also cause inclusion conjunctivitis in adults, presumably by autoinoculation from a genital site of infection. The LGV biovars (serovars L1, L2, and L3) are also sexually transmitted and infect urogenital epithelial mucosal surfaces. However, unlike the trachoma biovars, which remain localized to mucosal surfaces, the LGV serovars are more invasive. The LGV serovars penetrate the submucosa, resulting in spread to the lymphatic system.

The genus *Chlamydophila* is much more diverse than the genus *Chlamydia*. Although occasional zoonotic infections occur, with the exception of *C. pneumoniae*, members of this genus primarily infect nonhuman animals and birds. *C. abortus* infects ruminants; *C. caviae* infects guinea pigs; *C. felis* infects cats; *C. pecorum* infects mammals such as cattle, koalas, sheep, and swine; *C. psittaci* infects birds. *C. pneumoniae*, on the other hand, is primarily a human respiratory pathogen that is a leading cause of community-acquired pneumonia. Additionally, *C. pneumoniae* has been linked with several chronic conditions such as coronary heart disease and is thought to be an added risk factor for the development of atherosclerosis.

Developmental Cycle

Although the different *Chlamydia* and *Chlamydohila* species display extreme diversity in tissue tropism, disease expression, and *in vitro* growth properties, all *Chlamydia* and *Chlamydophila* are characterized by a unique biphasic developmental cycle that alternates between two morphological and functionally distinct cell types: the elementary body (EB) and the reticulate body (RB) (**Figure 1**). The EB is the smaller developmental form measuring 0.3–5 μm in diameter. It is metabolically inert and is the infectious form of the bacterium. The physical features that define the EB include a highly condensed nucleoid structure as well as an extensively disulfide cross-linked outer membrane. In the absence of measurable amounts of peptidoglycan, a cell wall component that provides structural support to most other bacterial species, the cross-linking between cysteine-rich outer membrane proteins imparts structural rigidity to the EB. The properties of the EB facilitate the extracellular survival of the organism at the initial infection or transmission stages and mediate internalization, and hence infection, of the organism into host eukaryotic cells.

In contrast to the EB, the RB is larger at approximately 1 μm in diameter; it is also metabolically active and noninfectious. In addition, the nucleoid is more diffuse within the bacteria, making the DNA accessible to the transcriptional and replication machineries. Furthermore, the outer membrane is less cross-linked, which makes the RB much more fragile than the EB and enables the replication of the organism. Whereas the EB functions in extracellular survival and internalization, the RB is the metabolic and replicative form and thus functions in intracellular survival and replication.

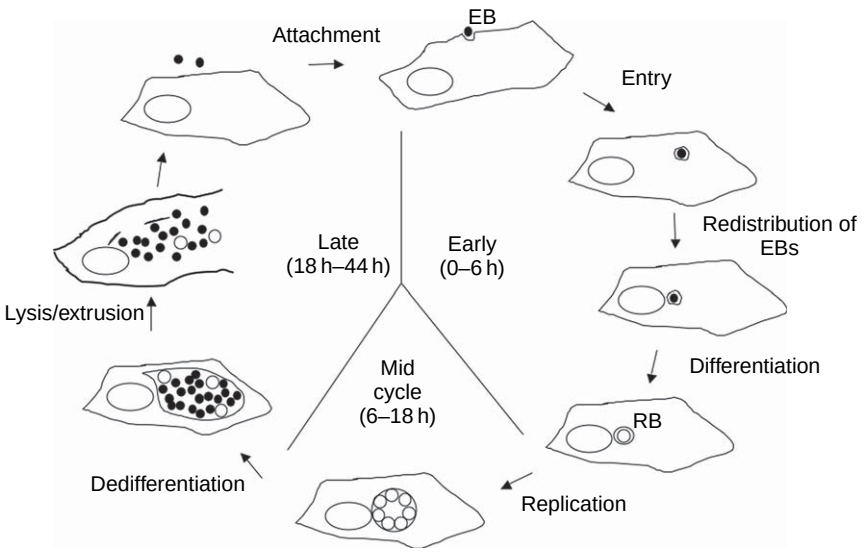

Figure 1 Chlamydial developmental cycle. The developmental cycle begins with the attachment and parasite-mediated endocytosis of the elementary body (EB). After internalization the EB is found within a membrane-bound vacuole, termed the inclusion. By 2 h p.i., the EB differentiates into the reticulate body (RB) and is trafficked to the peri-Golgi region of the host cell. Between 8 and 10 h p.i. the RBs begin to replicate and between 18 and 24 h p.i. some of the RBs begin to dedifferentiate back into infectious EBs. The cycle ends with either host cell lysis or extrusion of the inclusion from the infected cell, resulting in release of infectious EBs. All *Chlamydia* and *Chlamydophila* species undergo a similar developmental cycle. However, the time it takes to complete the cycle may vary from 36 to 96 h depending on the particular species.

The developmental cycle is initiated by the attachment and internalization of the EB into the host eukaryotic cell, resulting in the envelopment of the EB in a membrane-bound vacuole, which has been termed the inclusion (**Figure 1**). The bacteria remain confined within the inclusion during the entire developmental cycle. Within the first several hours postinfection (p.i.), the internalized EBs differentiate into RBs. Depending on the specific strain or species, the newly generated RBs replicate by binary fission starting between 10 and 18 h p.i. The developmental cycle is asynchronous. While some RBs continue to divide, others begin to differentiate back into EBs, resulting in a mixture of EBs and RBs. Since RBs are localized adjacent to the inclusion membrane, while EBs are localized to the lumen of the inclusion, it has been proposed that detachment from the inclusion membrane and inactivation of the T3SS apparatus may signal the dedifferentiation process. As the organisms replicate, the original inclusion membrane increases in size to accommodate the increasing numbers of organisms. By the end of the cycle, the inclusion occupies the majority of the host cytosol. The increase in size of the inclusion is likely due to incorporation of both *Chlamydia*- and host-derived proteins into the expanding inclusion membrane. The cycle ends with either activation of cysteine proteases and host cell lysis or extrusion of the inclusion out of the cell by an actin- and myosin-dependent mechanism. Release of newly generated infectious EBs that can infect neighboring cells ensures continuation of the chlamydial developmental cycle.

All *Chlamydia* and *Chlamydophila* species undergo a similar developmental cycle that differs primarily in the time it takes to complete the cycle, which can range from 36 h for the fast-growing *C. muridarum* strains to 96 h for the slower-growing *C. pneumoniae* strains. The *C. trachomatis* strains fall in the middle of this spectrum with cycle lengths of 44–72 h depending on the specific biovar or serovar in question. Completion of the intracellular developmental cycle is absolutely essential for intracellular survival and the production of infectious EBs.

Whole genome microarray analysis and RT-PCR analyses of specific subsets of chlamydial genes revealed that chlamydiae regulate the transcription of their genome in a temporal fashion. Three classes of gene transcription were identified that correlate with morphological aspects of the developmental cycle. Expression of 'early' genes is detected from 1 to 6 h p.i. 'Early' genes are likely to include genes involved in early metabolic processes, nutrient uptake, inclusion biogenesis, and EB to RB differentiation. Expression of 'mid cycle' genes is detected between 6 and 18 h p.i., and these genes are likely to be involved in replication and metabolic processes. Finally, expression of 'late' genes is detected 18 h and 24 h p.i. 'Late' genes include those involved in the dedifferentiation of RBs to EBs. Although the chlamydial genome encodes two alternative sigma factors, temporal regulation of chlamydial gene transcription is not regulated by these alternative sigma factors. Other types of regulation, such as response to stress, heat, nutrients, and host

inflammatory cytokines, are superimposed upon the global temporal regulation.

Intracellular Survival

As obligate intracellular bacteria, chlamydiae have evolved highly successful strategies to promote and maintain their intracellular lifestyle including specific mechanisms to (1) gain entry into host cells, (2) avoid or prevent degradation by degradative lysosomal enzymes, (3) obtain nutrients and biosynthetic precursors while sequestered within a protected niche, and (4) evade host immune detection and destruction. Importantly, chlamydiae accomplish these tasks while sequestered within the protected environment of the inclusion through the exploitation of host signaling and trafficking pathways. Because *Chlamydia* and *Chlamydophila* species display such differences in tissue tropism and *in vitro* growth properties, and because of differences arising from experimental protocols, observations made about one species or serovar may or may not apply to all *Chlamydia* or *Chlamydophila* species.

Entry

For most *Chlamydia* and *Chlamydophila* species, columnar epithelial cells, which are nonprofessional phagocytes, are the primary sites of infection. However, other cell types can be infected by some *Chlamydia* and *Chlamydophila* species. For example, the LGV biovars of *C. trachomatis* can replicate in monocytes/macrophages, while *C. pneumoniae* can replicate in many different cell types including epithelial, endothelial, and smooth muscle cells, and monocytes/macrophage. Chlamydiae enter nonprofessional phagocytes by a process that has been defined as 'parasite-mediated' endocytosis since it is 10–100 times more efficient than the uptake of inert particles such as latex beads or opsonized yeast. *In vitro*, multiple modes of entry have been documented including both clathrin- and non-clathrin-mediated endocytosis, microfilament-mediated phagocytosis, microfilament-independent pinocytosis as well as lipid raft-mediated endocytosis. Although different modes of entry have been established for *Chlamydia* species, a novel host cell actin-dependent entry mechanism has recently been reported. In this model, attachment of the EBs to the host cell surface is followed by the translocation of the T3SS effector, translocated actin recruiting protein (Tarp), into the host cell. Upon translocation, Tarp is rapidly phosphorylated by host cell kinases and concentrates on the cytosolic side of the eukaryotic plasma membrane at sites of EB attachment. Tarp is an actin-binding protein that facilitates localized actin recruitment and polymerization at sites of EB entry. Subsequent to Tarp phosphorylation, there is activation of host Rho GTPases, which are small ras-like GTPases that regulate actin dynamics. In *C. trachomatis*-infected cells, Rac1 is activated, while in *C. caviae*-infected cells, both Rac1 and Cdc42 are activated. The activation of Rho GTPases leads to the recruitment of several host proteins, including WAVE2, Abi-1, and Arp2/3, which are critical molecules in actin signaling and polymerization, to sites of EB attachment. Collectively, the activation and recruitment of these host signaling molecules, and others including another small GTPase, Arf6, leads to localized actin polymerization and cytoskeletal rearrangements that mediate the internalization of EBs. For other species, such as *C. pneumoniae*, the attachment of EBs induces the activation of other host signaling pathways including phosphatidylinositol-3-kinase (PI3K)-dependent and ERK1/2-dependent signaling pathways that are important for the entry of *C. pneumoniae* EBs.

Early Intracellular Events

During the first 2–3 h following internalization several critical events occur that are dependent on early *de novo* chlamydial gene expression (**Figure 2**). One of these critical events is EB to RB differentiation. Morphologically, differentiation is manifested by the reduction of the disulfide-linked outer membrane, the decondensation of the nucleoid, and an increase in size of the bacterium. The decondensation of the chlamydial chromatin plays an important role in silencing gene transcription in EBs. Two basic histone-like proteins Hc1 and Hc2 are thought to play critical roles in this process as overexpression of chlamydial Hc1 in *Escherichia coli* leads to condensation of the *E. coli* chromosome, gene silencing, and death. However, since Hc1 is expressed throughout the developmental cycle, degradation of Hc1 cannot be a mechanism that triggers DNA unwinding. Insights into this intriguing dilemma were revealed when researchers found that the death induced by overexpression of Hc1 in *E. coli* could be rescued by the overexpression of a second chlamydial gene product, CT804. CT804 is predicted to encode a kinase that shares homology to *E. coli IspE*, an enzyme that functions in a nonmevalonate pathway of isoprenoid biosynthesis. Currently, researchers believe that a metabolite of the pathway may act as a competitive inhibitor that blocks the DNA binding activity of Hc1, thereby allowing the DNA to unwind and enabling the initiation of the developmental transcriptional program.

Together with the physical changes in the organism, there are also critical interactions between the bacteria and the host that are established during this time period including the avoidance of lysosomal fusion, the intracellular redistribution of EBs, and the interception of host-derived secretory vesicles.

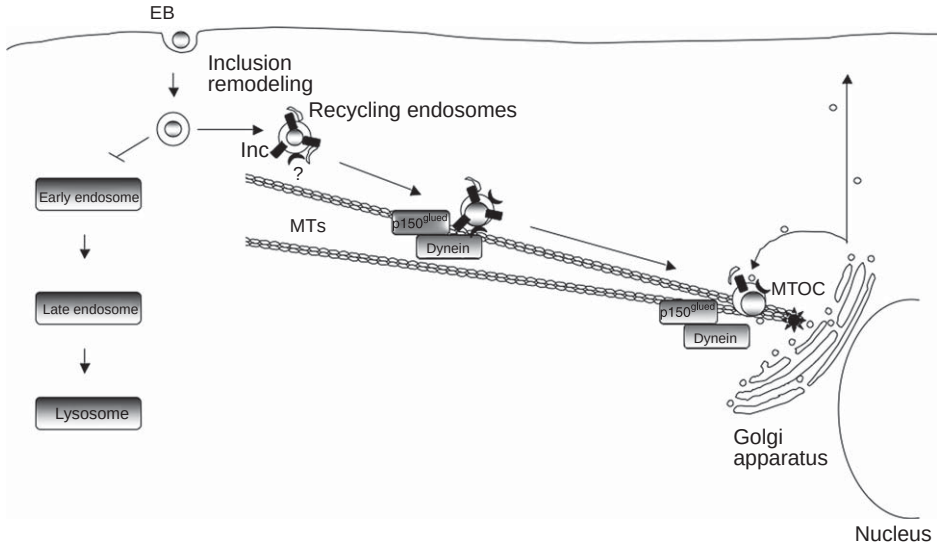

Figure 2 Early events during chlamydial infection. After entry into the host cell, the elementary body (EB) is contained within the inclusion that displays minimal interactions with the endosomal/lysosomal pathway. By 2 h p.i., the inclusion is actively remodeled in a process that is dependent on early chlamydial gene expression. Remodeling is most likely mediated through the secretion of chlamydial proteins into the inclusion membrane. As a result of this bacteria-mediated remodeling, the inclusion avoids fusion with the lysosomal pathway, and is instead directed to the peri-Golgi region or microtubule organizing center (MTOC) where it begins to fuse with a subset of Golgi-derived vesicles. Although the inclusion does not fuse with early endosomes, recycling endosomes containing transferrin are recruited to the inclusion. Redistribution of EBs is microtubule dependent and requires the microtubule motor, dynein. The chlamydial factors that mediate these early events have not been identified, but inclusion membrane proteins (Incs) or soluble proteins that localize to the cytosolic face of the inclusion are the most likely candidates.

Avoidance of lysosomal fusion

One of the obstacles that all intracellular bacteria encounter is the harsh degradative environment of host cell lysosomes, which a bacterial pathogen would normally encounter upon endocytosis and trafficking along the default endocytic/lysosomal pathway. Bacterial pathogens have evolved unique mechanisms to circumvent their destruction in lysosomes. Some alter their phagosomal maturation at an early prelysosomal stage such that they avoid fusion with lysosomes, while others like *Chlamydia* escape the default endocytic/lysosomal pathway and replicate in vacuoles that share properties with other host organelles, such as secretory organelles. Chlamydiae exit the classical endocytic/lysosomal pathway early during the developmental cycle as evidenced by absence of classical endocytic markers by processes dependent on early chlamydial protein expression (**Figure 2**). Avoidance of lysosomal fusion is thought to be a two-stage process. First, cell wall components present in the invading EB are thought to passively delay the trafficking of the nascent vacuole to the lysosome. The second stage is actively mediated by chlamydiae through an undefined mechanism that is dependent on early chlamydial protein expression.

Redistribution of EBs

By 2 h p.i, EB-containing vacuoles are transported from the periphery of the cell and aggregate at the peri-Golgi region or the region of the cell that corresponds to the microtubule organizing center (MTOC) by processes that are dependent on early chlamydial gene expression (**Figure 2**). During this initial infection period, at least 60 'early genes' are expressed. Several of these early expressed genes are localized to the inclusion membrane and thus are likely candidates to facilitate the redistribution of EBs.

Host factors are also required for the intracellular trafficking of EBs. Some chlamydiae exploit microtubule-dependent host trafficking pathways to mediate their intracellular redistribution. Consistent with the trafficking of EBs toward the minus-ends of microtubules, the redistribution of EBs has been shown to be dependent on dynein, a minus-ended microtubule-dependent motor protein. Most dynein-dependent trafficking pathways in the cell require the assistance of dynactin, a multiprotein complex that facilitates cargo binding to dynein. Interestingly, although the transport of EBs requires dynein and at least one component of dynactin, $p150^{glued}$, localizes to inclusions, trafficking does not require dynactin. These data suggest that EBs exploit a novel dynein-dependent but dynactin-independent transport pathway to facilitate their redistribution within the eukaryotic cell.

Chlamydia-mediated remodeling of the inclusion is absolutely essential for the intracellular survival of chlamydiae. In the absence of early chlamydial gene expression all of these early events fail to occur and eventually the chlamydiae will be trafficked to host lysosomes and destroyed.

Nutrient and Biosynthetic Precursor Acquisition

Chlamydiae are auxotrophic organisms that rely on their eukaryotic host for essential nutrients such as ribonucleotides, ATP, amino acids, and a variety of host-derived lipid species including glycerophospholipids, cholesterol, sphingomyelin, and neutral lipids. Chlamydiae acquire host-derived lipids by targeting both vesicular- and non-vesicular-mediated host trafficking pathways. Beginning by 2 h p.i., coincident with the redistribution of EBs to the peri-Golgi region, the inclusion becomes competent to fuse with a subset of Golgi-derived exocytic vesicles containing endogenously synthesized sphingomyelin and cholesterol. Subsequent to this discovery, it was revealed that chlamydiae also acquire sphingomyelin from the delivery of multivesicular bodies (MVBs) that originate from late endosomes to the inclusion. Chlamydiae also recruit lipid droplets (LD) to the inclusion. LDs are ER-derived lipid storage organelles that are a source of neutral lipids. Pharmacological inhibition of LD formation with triascin C alters inclusion morphology and greatly inhibits chlamydial development suggesting that LDs play an important role in chlamydial development. Several LD-associated chlamydial proteins (CT156/Lda1; CT163/Lda2; CT257; and CT473/Lda3) are translocated into the host cytosol and localize to structures adjacent to the inclusion membrane that partially colocalize with LDs. These data are consistent with Lda1/2/3 functioning to recruit or maintain the association of LDs with the inclusion membrane. Biochemical analysis of purified EBs revealed the presence of additional eukaryotic-derived lipids such as phosphatidylcholine and phosphatidylinositol. In contrast to sphingomyelin, host glycerophospholipids are delivered by a non-vesicular-mediated mechanism to the inclusion and are modified upon delivery to the inclusion. By a mechanism that is regulated by host cytosolic phospholipase 2 (cPLA2) activity and the Raf/MEK/ERK signal transduction pathway, both of which are induced during chlamydial infection, the straight chain fatty acid at the sn-2 position is removed and replaced by a *Chlamydia*-derived branch chain fatty acid.

Secreted Effectors/Virulence Factors

Intracellular pathogens exploit basic host cellular processes to mediate their entry and intracellular survival. Since chlamydiae remain sequestered within the inclusion, proteins that function to subvert host cellular processes need to directly access the host cytosol. Bioinformatics and heterologous expression systems have been used to demonstrate that chlamydiae express two categories of proteins that directly access the host cytosol: soluble secreted proteins that are translocated directly into the host cytosol and membrane-anchored proteins that localize to the inclusion membrane (**Figure 3**). Although many putative effectors have been identified, functional characterization of these effectors is difficult.

Type III Secretion System

Many Gram-negative bacterial pathogens, including *Chlamydia* and *Chlamydophila* species, share a unique virulence trait, a T3SS. T3SS is a specialized needle-like secretion system designed to translocate proteins from the bacteria directly into the host cell cytosol. T3SS effectors have been shown to modulate a large variety of host functions and facilitate many aspects of bacterial pathogenesis including entry, vacuolar biogenesis, intracellular trafficking, and inhibition or induction of host apoptotic pathways. Although electron micrographs obtained in the early 1970s depicted structures that resembled T3SS needle structures originating from the bacteria and extending through the inclusion membrane, genetic evidence that *Chlamydia* species encoded a T3SS was not obtained until 1997 when four genes with significant homology to known T3SS genes were identified in *C. caviae*. The following year genomic sequence analysis revealed that *C. trachomatis* encoded within their genome a complete, albeit abbreviated, T3SS.

T3SS consist of a conserved core secretory apparatus, T3SS-specific chaperones, mobile translocation proteins, and a highly diverse set of secreted effectors. Because the core secretory apparatus and associated accessory molecules have retained DNA sequence conservation, bioinformatics approaches have successfully identified genes homologous to these conserved components in all *Chlamydia* and *Chlamydophila* species. Based upon comparison to the DNA sequences of T3SS in other bacteria, chlamydiae do not appear encode the entire complement of T3SS genes, although they do encode enough of the components to produce a functioning T3SS. Chlamydiae likely encode T3SS structural components that have yet to be identified by conventional approaches due to divergence in DNA sequences. In support of this hypothesis, CdsF, which shares no sequence homology with other T3SS proteins, was recently identified as a possible functional homologue of the needle subunit protein SctF. Interestingly, there is no evidence that *Chlamydia* and *Chlamydophila* have acquired these genes through horizontal gene transfer (HGT), as is thought to be the case for T3SS in other facultative pathogenic bacteria. Although loosely organized into four genomic locations, genes encoding the T3SS are scattered throughout the genome. In addition, there are no differences in GC content of the T3SS-encoded genes as compared to the rest of the genome nor is there evidence of associated insertion or transposon-like elements.

In contrast to proteins that make up the secretion and translocation apparatus, effector proteins that are secreted

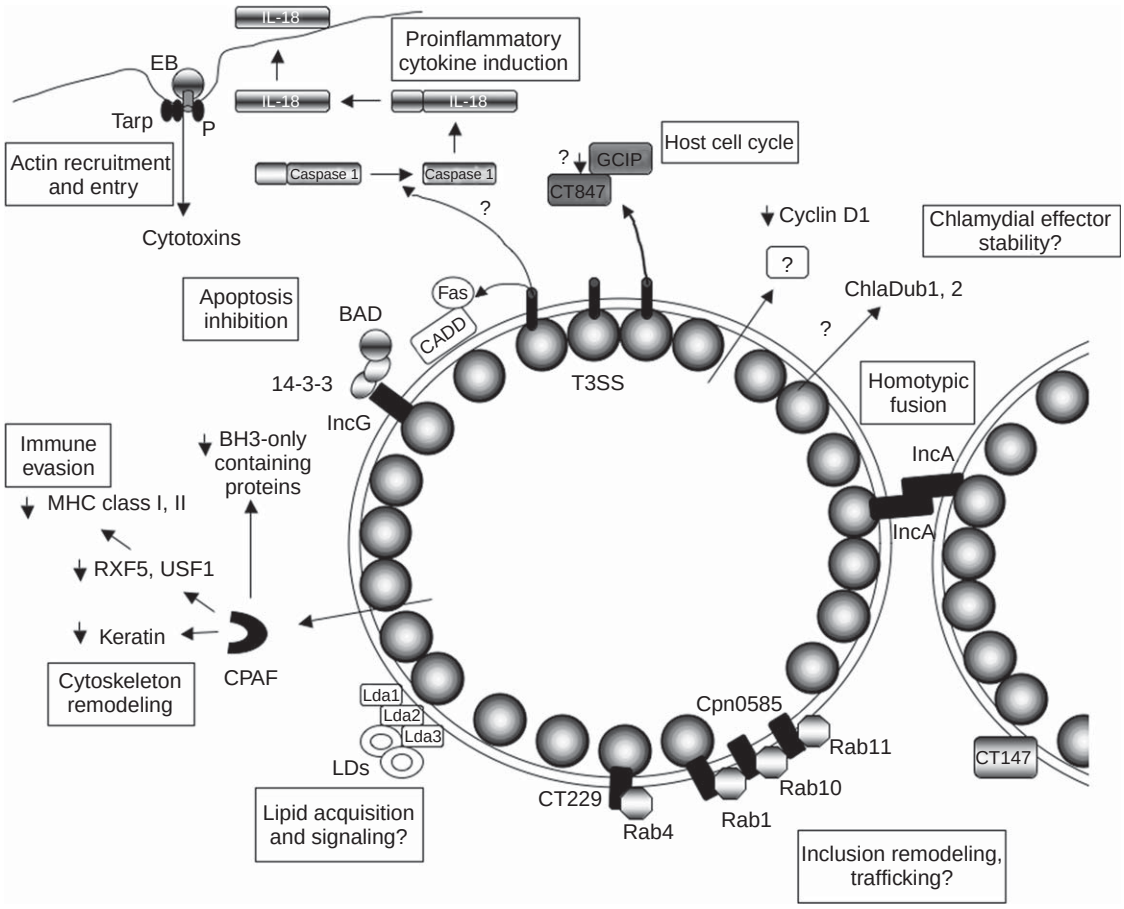

Figure 3 Secreted effectors. Chlamydiae secrete two classes of proteins across the inclusion membrane by both type III secretion system (T3SS)-dependent and -independent mechanisms. The first class of proteins consists of membrane proteins localized to the inclusion membrane, which include both inclusion membrane proteins (Incs) and non-Inc proteins such as CT147. The second class of proteins consists of soluble proteins secreted into the host cytosol and includes chlamydial protease-like or proteosome-like activity factor (CPAF), *Chlamydia* protein associating with death domains (CADD), and CT847. Effectors target multiple cellular pathways resulting in actin recruitment and rearrangement, inhibition of apoptosis and cell division, exploitation of lipid and vesicular-mediated trafficking pathways, induction of proinflammatory cytokines, and immune evasion. The predicted function of each effector is detailed in the main body of text.

into the host cytosol generally share no sequence homology as they normally execute functions specific to the bacterial pathogen and host cell type. Therefore, it has been more difficult to identify chlamydial effectors by DNA sequence homology alone. However, progress has been made in the identification and characterization of several important chlamydial type III effectors. By taking advantage of heterologous T3SS systems of *Yersinia*, *Salmonella*, and *Shigella*, several researchers have confirmed T3SS-dependent secretion of specific chlamydial proteins or have identified novel effectors among the numerous 'hypothetical open reading frames (ORFs)' present in the various chlamydial genomes. To date, known chlamydial T3SS effectors include several inclusion membrane proteins (Inc), Tarp, components of the T3SS apparatus (CopN, CopB, CopB2), CT847, Pkn5 (a serine/threonine protein kinase), *Chlamydia* protein associating with death domains (CADD), and at least 24 hypothetical ORFs. Putative functions for the best characterized of these effectors are described below.

Current data are consistent, with T3SS being an important virulence determinant that functions both early and late during *Chlamydia* development. First, genomic profiling has confirmed that all T3SS-associated genes are expressed in infected tissue culture cells. Second, several T3SS proteins have been localized to either the host cell cytosol or the inclusion membrane by immunofluorescence. Third, acylated hydrazones of salicylaldehydes, which are small molecule T3SS-specific inhibitors, have been shown to inhibit the secretion of several T3SS effectors in infected cells. Furthermore, the T3SS-specific inhibitors also inhibit the progression of the developmental cycle at different stages including partial inhibition of entry and inhibition of the dedifferentiation of RBs to EBs.

Although some of the components are not expressed until midcycle, it is believed that T3SS also functions early during development with infectious EBs containing a functional T3SS and preformed effectors. This model is supported by the translocation of the T3SS effector, Tarp, at the site of EB entry. Even with all of the recent data, it is still unclear whether the projections identified in the early EM studies are actually T3SS structures.

Effectors Localized to the Host Cell Cytosol

Chlamydiae translocate proteins into the host cell cytosol by both T3SS-dependent and -independent mechanisms. Chlamydial protease-like or proteosome-like activity factor (CPAF), a type II secreted protein, was the first chlamydial protein identified that localized to the host cytosol. CPAF is an important mediator of chlamydial survival that was identified biochemically due to its specific proteolytic activity in infected cells. In the host cytosol, CPAF functions to specifically degrade at least two host transcription factors, RXF-1 and USF-1, which are positive transcriptional regulators of gamma interferon (INF-γ)-induced MHC class I and class II and constitutively expressed MHC class I. CPAF-dependent degradation of RXF-1 and USF-1 results in decreased expression and presentation of MHC class I and II molecules on the cell surface of infected cells, and thus CPAF plays a critical role in immune evasion. CPAF has also been shown to cleave keratin, which may facilitate the unrestricted growth of the inclusion by breaking down the host cytoskeleton, and the pro-apoptotic BH3-only containing proteins, which are a subfamily of Bcl-2 family of pro-apoptotic proteins. BH3-only containing proteins localize to the mitochondria and promote the induction of apoptosis upon the receipt of various death signals from the cell. Apoptosis is a programmed cell death pathway that is vital to embryogenesis and homeostasis of the eukaryotic organism. Chlamydiae have been shown to inhibit the induction of mitochondrial-dependent apoptosis early in the developmental cycle, and either inhibit or induce apoptosis later during the developmental cycle. CPAF-mediated degradation of BH3-only containing proteins plays a major role in the *Chlamydia*-mediated inhibition of mitochondrial-dependent apoptosis. Inhibition of apoptosis early during the cell cycle helps to maintain the viability of the host cell, allowing chlamydiae to complete their developmental cycle and produce infectious EBs.

Chlamydiae also encode two other proteases, ChlaDub1 and ChlaDub2, which share homology with eukaryotic ubiquitin-like proteases and have been shown to possess deubiquinating and deneddylating activities. Eukaryotic ubiquitin-like proteins function to remove ubiquitin from proteins to prevent proteosomal degradation or to inhibit ubiquitin-mediated signaling pathways such as the NF-κB pathway. Since modification of proteins by ubiquitin is strictly a eukaryotic process, it has been postulated that ChlaDub1 and ChlaDub2 may function in the host cytosol to prevent destruction of secreted chlamydial effectors or alter host signaling pathways. However, the cytosolic localization of these two proteases has not yet been confirmed.

Several *Chlamydia* and *Chlamydophila* species encode several partial and complete ORFs that share significant homology to the catalytic domains of large cytotoxins (LCTs) produced by *Clostridium difficile*. LCTs inhibit the activity of Rho family GTPases, key regulators of the actin cytoskeleton. Some chlamydial strains are cytopathic to the host when infected at high multiplicities of infection in a phenomenon that has been termed 'multiplication independent immediate cytotoxicity'. Interestingly, there is a direct correlation between the ability to induce cytotoxicity and the number of cytotoxin-like genes that a strain encodes. More importantly, the immediate toxicity induced by chlamydiae is phenotypically indistinguishable from cells that have been exposed to LCTs. In both cases, there is a dramatic reorganization of the cytoskeleton that is manifested with the disassembly of actin stress fibers, which results in rounding up of the cells. Although the cytotoxins are expressed during later parts of the developmental cycle, it is thought that they are present in EBs as preformed effectors and function early during the developmental cycle. One possible model suggests that the cytotoxins act to inhibit host Rho GTPases at sites of chlamydial entry. Alternatively, the cytotoxins also share homologies to *E. coli* lymphostatin protein (LifA) and therefore may play a role in immunosupression.

CADD, a T3SS-secreted effector, was identified through a bioinformatics approach due to its homology with mammalian death domain receptors of the tumor necrosis factor (TNF) family. It has been shown to interact with several human death receptor domain family proteins including TNFR1, Fas, DR4, and DR5, and partially colocalizes with Fas adjacent to the inclusion membrane in infected cells. Although ectopic expression of CADD induces host cell apoptosis, neither endogenous expression of CADD or localization of Fas to the inclusion is sufficient to induce apoptosis in *Chlamydia*-infected cells. These conflicting results suggest that the ability to influence the host cell apoptotic pathways may be due to the specific localization of CADD, and therefore the exact role that endogenous CADD plays has not been elucidated.

Chlamydial infections induce strong host proinflammatory immune responses. The infected epithelial cell initiates the immune response through secretion of proinflammatory cytokines, which is thought to trigger further host immune responses and to recruit immune cells to sites of infections. One of the cytokines secreted by infected epithelial cells is interleukin (IL)-18. IL-18 is

secreted by infected cells in an IL-1α-independent but caspase-1-dependent manner. Although the mechanism by which chlamydiae activate caspase-1 has not been determined, recent data using T3SS inhibitors suggest that a T3SS effector mediates caspase-1 activation.

Effectors Localized to the Inclusion Membrane

A large number of chlamydial proteins localize to or are predicted to localize to the inclusion membrane. Proteins localizing to the inclusion membrane are classified as either Incs, a highly diverse family of *Chlamydia*-specific proteins that contain a signature hydrophobic motif, or non-Inc proteins that lack this hydrophobic motif.

Incs were originally identified in the pregenomic era through the isolation of proteins recognized by convalescent sera from guinea pigs infected with *C. caviae*. Upon completion of the genomic sequences from several *Chlamydia* and *Chlamydophila* species, based upon the presence of the signature hydrophobic motif, it was determined that *Chlamydia* and *Chlamydophila* species are predicted to encode 60–80 different Incs. Except for the conserved hydrophobic motif, Incs share no homologies with any other proteins in the databases nor do they share extensive homologies between themselves. In fact, *Chlamydia* and *Chlamydophila* species appear to express different complements of Incs. Furthermore, microinjection studies using domain-specific antibodies have demonstrated that many of the Incs directly access the host cytosol, making them likely candidates to exploit host cellular signaling and trafficking pathways through direct interaction with host proteins. Finally, Incs are expressed at all stages of the developmental cycle, suggesting that they may function at multiple times throughout infection. Biological roles for several inclusion-localized proteins have been proposed. For example, IncA is thought to promote homotypic vesicle fusion between *Chlamydia*-containing vesicles via either IncA–IncA interactions or the recruitment of host soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) to the inclusion. Consistent with a role in homotypic vesicle fusion, clinical *C. trachomatis* isolates that do not express IncA replicate in multiple nonfused inclusions. *C. trachomatis* IncG has been shown to recruit mammalian 14-3-3β to the inclusion. 14-3-3β is a phosphoserine binding protein that plays multiple roles in cell signaling and cell survival. The recruitment of 14-3-3β to the inclusion contributes to the ability of chlamydiae to inhibit cellular apoptosis by sequestering and inhibiting the pro-apoptotic protein BAD at the inclusion. Finally, two Incs have been shown to recruit Rab GTPases to the inclusion. Rab GTPases are small ras-like GTPases that play critical roles in vesicular-mediated trafficking pathways. *C. trachomatis* Inc CT229 recruits Rab4 to the inclusion, while *C. pneumoniae* Inc Cpn0585 interacts with multiple Rab GTPases, suggesting that chlamydiae may exploit Rab-dependent trafficking pathways to facilitate their intracellular trafficking or to modulate the identity of the inclusion.

CopN, a homologue of *Yersinia* YopN and component of the T3SS apparatus, CT147, and Cap1, a $CD8^+$ T cell epitope, are also localized to the inclusion membrane but lack the signature hydrophobic profile of Incs. CT147 is an early expressed inclusion localized protein that is homologous to the mammalian early endosomal antigen (EEA1). EEA1 contains a 'FYVE' domain that interacts with phosphatidylinositol (Ptd)-3-P, regulating tethering of early endosomes, and a separate domain that interacts with Rab5, regulating fusion of early endosomes. Interestingly, CT147 contains only the 'FYVE' domain that interacts with Ptd-3-P, suggesting that CT147 may function to tether early endosomes to the inclusion but since it lacks the Rab5 interacting domain that promotes fusion, fusion with early endosomes is prohibited. However, the specific function of CT147 remains to be determined.

Host Tropism

Surprisingly, comparative genomic analysis revealed that the human pathogen *C. trachomatis* and the mouse pathogen *C. muridarum* share near genomic synteny. Even more striking, *C. trachomatis* serovar A, an oculotropic serovar, and *C. trachomatis* serovar D, a genitotropic serovar, share 99.6% DNA sequence identity and yet display different tissue tropisms *in vivo*. Most of the strain-specific genes are localized to a single region of the chromosome, which has been termed the hypervariable plasticity zone (PZ). Several of the genes that are localized to the PZ encode tryptophan synthase and the cytotoxins, both of which are thought to play crucial roles in host and tissue tropism. First, genes encoding functional tryptophan synthase are present in the PZ of only *C. trachomatis* genital tract and not ocular species. The genes are completely absent from *C. muridarum*. None of the *C. trachomatis* strains are able to produce tryptophan, an essential amino acid, from most tryptophan metabolic precursors. However, the genital tract *C. trachomatis* strains that express tryptophan synthase are capable of generating tryptophan, from exogenously added indole, whereas strains deficient for this enzyme cannot. The important host proinflammatory molecule INF-γ acts in part to inhibit chlamydial growth by the induction of indolameanine 2,3-dioxygenase (IDO), which functions to degrade tryptophan. Therefore, genital tract *C. trachomatis* strains that can synthesize tryptophan from indole, perhaps derived from the vaginal flora, may use this as a mechanism to overcome INF-γ-induced IDO activity. As such, tryptophan synthase is considered a virulence factor that differentiates the ocular and the genital tract serovars of *C. trachomatis* as well as

differentiates the human genital tract *C. trachomatis* strains from murine *C. muridarum*.

Differences in susceptibility INF-γ also distinguish *C. trachomatis* strains from *C. muridarum*. Susceptibility to INF-γ correlates with the presence or absence of cytotoxins in each of the respective genomes. The growth of *C. trachomatis*, which lacks or contains truncated forms of the cytotoxins, is inhibited by INF-γ, whereas the growth of *C. muridarum*, which contains three complete copies of the cytotoxins, is not. Recent evidence suggests that differential susceptibility to INF-γ may be due to INF-γ-inducible GTPases such as the p47 GTPase, Iigp1, a mouse-specific immune regulator that inhibits the growth of intracellular pathogens. Iigp1 contains motifs similar to YopT targeting sequences. Since the chlamydial cytotoxins share similarities with YopT, the cytotoxins present in *C. muridarum* may target and inhibit Iigp1, thus providing a model for how *C. muridarum* may resist the inhibitory affects of INF-γ. Consistent with this hypothesis, knockdown of Iigp1 makes *C. trachomatis* strains, which do not express the cytotoxins, less susceptible to INF-γ-mediated growth inhibition. Based upon these observations, the prevailing model suggests that the diversity in the PZ arose to counteract species-specific host immune effectors such as INF-γ.

Clinical Disease, Diagnosis, and Treatment

Although chlamydiae cause a wide range of diseases in many different host species, most sequelae resulting from chlamydial infections is primarily due to the fibrosis and tissue damage that results from chlamydial-mediated induction of host proinflammatory cytokine responses that can occur during repeated or persistent infections.

C. trachomatis: Trachoma

C. trachomatis biovars A, Ba, Bb, and C infect ocular mucosal surfaces causing trachoma, the leading cause of preventable blindness worldwide. Although virtually absent from industrialized countries, trachoma is still endemic in tropical and subtropical areas with poor hygienic conditions, including North Africa, the Middle East, and Northern India. The disease is transmitted from human-to-human contact but may be potentiated by the transfer of human secretions by flies. Infection begins as conjunctivitis, which is followed by follicle formation. Scarring and distortion of the eyelids may be caused by subsequent follicle rupture, which in turn causes in-turning of the eyelids. In-turning of the eyelid causes the eyelashes to scrape the cornea, which causes physical damage to the cornea. It is the physical damage to the cornea that ultimately leads to blindness. Therefore, blindness does not result from an acute infection, but rather results after repeated or persistent infections.

Diagnosis of trachoma is made by isolation of the chlamydial organism from conjunctival scrapings or identification of inclusions in epithelial cells derived from conjunctival scrapings. Trachoma is still a disease associated with poverty and poor hygienic conditions. An eradication program known as the SAFE strategy has been in place in endemic countries to try to eliminate the disease. SAFE strategy includes *s*urgery to repair damaged eyelids, *a*ntibiotic treatment to eliminate the bacteria, *f*ace washing to improve personal hygiene, and *e*nvironmental improvements to reduce the fly population to reduce bacterial spread. The disease is currently treated with antibiotics such as doxycycline and azithromycin. The latter is more expensive but more convenient, as a single dose is all that is required.

C. trachomatis: Urogenital Tract Infections

Serovars D, E, F, G, H, I, J, and K of *C. trachomatis* infect the columnar epithelial cells of the urogenital tract and respiratory tract of newborns. Each year, close to 90 million new cases are reported, making *C. trachomatis* infections the leading bacterial cause of sexually transmitted disease. *C. trachomatis* causes a wide variety of disease manifestations in both men and women. These include adult and infant inclusion conjunctivitis and pneumonia, salpingitis, cervicitis, female urethral syndrome, postpartum endometris, male urethritis, epididymititis, and arthritis. Fibrosis and scarring are the hallmarks of chlamydial infections due to the elicitation of a strong proinflammatory host cell response to the invading bacteria. Scarring causes the devastating sequelae that develop upon repeated or persistent chlamydial infections. Due to the asymptomatic nature of most chlamydial infections, chlamydial infections often go undiagnosed and untreated. In women, untreated chlamydial infections can lead to pelvic inflammatory disease, ectoptic pregnancy, and infertility. In addition, chlamydial infections may lead to increased susceptibility to HIV infection. Laboratory diagnosis is made by isolation of the chlamydial organisms from cervical or urethral swabs or by visualizing infected cells with direct fluorescence antibody tests, enzyme-linked immunoassays for antigen, or nucleic acid detection. Nucleic acid amplification tests using either self-collected vaginal swabs or voided urine are much more sensitive than culture for detecting genital *Chlamydia* infections in women. Similar to ocular infections, genital tract infections are treated with tetracyclines or macrolides.

Lymphogranuloma Venereum

The LGV serovars (L1, L2, and L3) of *C. trachomatis* also infect mucosal surfaces of the urogenital tract. However, in contrast to infections by the trachoma, which remain

localized to the epithelium, the LGV serovars infiltrate the submucosa, resulting in infection of the lymphatic drainage system and a more systemic disease. Virtually eliminated in the United States, LGV infections are still prevalent in tropical areas of Asia, Africa, and Central America. In the initial stages of infection, asymptomatic primary lesions develop on the labia, vagina, cervix, or penis and may result in a mild proctitis. If the infection is not diagnosed and treated, lymphoadenopathy in the nodes draining the site of primarily infection (usually inguinal and femoral) may form. The nodes may rupture, resulting in chronic draining fistulas. At this stage, the disease can take two courses, the abscesses will either resolve or the disease will become systemic, which can result in fever, mylagia, and headache. Chronic inflammation and scarring may occur in some cases. Diagnosis is made upon isolation of the bacteria from either a primary lesion or aspirated pus, or by microimmunofluorescence assays (MIF).

C. pneumoniae

C. pneumonaie is a newly recognized species that is primarily a respiratory pathogen. Although the species was first isolated in 1965 from a child's conjunctiva during a trachoma vaccine trial study in Taiwan, it was not considered a respiratory pathogen until 1983, when it was isolated from the pharynx of a pharyngitis patient. The original isolate, TW-183, and the respiratory isolate, AR-39, were subsequently discovered to be the same organism, and thus have become known as the TWAR isolates. Infections by *C. pneumoniae* cause 10% of all community-acquired pneumonia infections and 5% of all bronchitis cases. Although most clinical manifestations are not easily distinguishable from other atypical pneumonias (nonproductive cough, headache, and malaise), there are several clinical signs that are more typical of infections caused by *C. pneumoniae*. These include a subacute onset and the presence of pharyngitis during the initial stages of infection. There may also be a biphasic pattern with resolution of the pharyngitis prior to the development of bronchitis. Finally, the presence of a prolonged cough and the absence of fever are also typical of *C. pneumoniae* infections. Most infections are mild or asymptomatic and do not require hospitalization. Despite antibiotic treatment, recovery is often slow with persistence of a cough for 2–6 weeks. All age groups are at risk; however, respiratory disease is most common among children 5–14 years old. The majority of adults are seropositive for *C. pneumoniae* antibody titers. These data suggest that by the time most people reach adulthood, they have been infected with *C. pneumoniae* at least one time during their life.

Laboratory diagnosis for respiratory infection is often unreliable due to the absence of well-standardized and commercially available diagnostic tests. The most common diagnostic test used involves serologic detection of *C. pneumoniae*-specific antibodies by MIF. *C. pneumoniae* infection is positively diagnosed with a single immunoglobulin (Ig)M titer greater than or equal to 1:16 or a fourfold increase in the IgG titer. In primary infections, *C. pneumoniae*-specific IgM and IgG titers do not appear until 2–3 weeks and 6–8 weeks after infection, respectively. Therefore, most physicians will prescribe antibiotic treatment that is effective against most bacteria-caused atypical pneumonias prior to a definitive diagnosis. Typically, antibiotic therapy consists of a macrolide such as azithromycin or clarithromycin. If a definitive diagnosis of *C. pneumoniae* is made, antibiotic therapy is usually switched to a 10–14 day course of doxycycline. *C. pneumoniae* infection may be linked to a variety of chronic inflammatory conditions such as asthma, atherosclerosis, myocardial infarctions, and multiple sclerosis.

C. psittaci

C. psittaci is a common avian pathogen and causes 'chlamydiosis'. In the past, infections of psittacine birds were termed 'psittacosis' and infections of wild or domestic fowl was called 'ornothosis'. Because the diseases caused are so similar, the term 'chlamydiosis' is used for infections in all birds. More than 130 species of birds are known to be infected with *C. psittaci*. Clinical signs vary between the different bird species. However, early signs may include dyspnoea, sinusitis, unilateral conjunctivitis, and diarrhea. During an acute infection, egg production may be reduced. In addition, weight loss, reduced body temperatures, dehydration, and loss of appetite may also be present. Morbidity and mortality rates may be as high as 50–80% and 30%, respectively. Presumed diagnosis may be made on physical examination and history. Definitive diagnosis of avian infections can be made by isolation of the organism and identification of inclusions in tissue culture cells or through the direct identification of the organism in infected tissue samples. Serological tests are not widely used for diagnosis. Chlortetracycline, delivered in the bird's food supply, is the antibiotic of choice. Oxytetracycline, doxycycline, and enrofloxacin are also used to treat infected populations. Quarantine and antibiotic treatment of imported birds are common control measures used to prevent spread of *C. psittaci* infections.

C. psittaci is a zoonotic disease and can be transmitted to humans. In humans, exposure to infected birds can also cause 'chlamydiosis' and infection can range from asymptomatic to a systemic toxic syndrome without respiratory infection. Common clinical signs include fever, chills, muscular aches and pains, and severe headaches. Due to the introduction of bird feed containing antibiotics and a 30 day quarantine period for imported birds, the cases of human *C. psittaci* infections have dramatically decreased, with less than 50 confirmed cases being reported per year.

People most at risk include pet shop and poultry industry workers. Diagnosis is made by either isolation of the organism by culture, which is often not done due to the infectious nature of the organism, or a fourfold increase in *C. psittaci*-specific antibody titers. The treatment of choice is a 10–21 day regimen of tetracycline or doxycycline.

C. abortus

C. abortus infects the genital tracts of ruminants (sheep, cattle, and goats) and mostly causes abortions. Infections by *C. abortus* can also cause stillbirths and the delivery of weak full-term newborns. *C. abortus* infections are the most common cause of abortions in sheep, known as ovine enzootic abortion (OEA) or enzootic abortion of ewes (EAE). The organism is usually transmitted during lambing seasons, as infected animals undergoing abortions secrete large amounts of organisms in placental tissues, uterine discharges, and in feces. In the initial stages of infection, the organism is found in the main organs of the ewe. Infection remains subclinical until the last 4 weeks of pregnancy, when abortions occur. At this time, the placenta becomes infected, while the organism is cleared from the other parts of the animal. The fetus becomes infected, leading to death and subsequent abortion. Infected ewes generally recover with their subsequent fertility unaffected. Diagnosis is made by identifying the bacteria in impression smears of placental cotlyedons, by isolation of the organism by passage in tissue culture cells, and by detection of *C. abortus*-specific antibodies. Infections can be treated with oral chlortetracycline if administered prior to the appearance of the second chlamydemia. However, a more effective treatment plan includes intramuscular injections of oxytetracycline. *C. abortus* infections are controlled by management procedures and by vaccination. Human infection due to exposure to aborting sheep has been documented and can lead to abortion or severe respiratory disease in nonpregnant individuals.

C. felis

Infection by *C. felis* causes conjunctivitis, rhinitis, or pneumonia in cats. Although cats of all ages are susceptible to infection by *C. felis*, infection is more common in young kittens (5–12 weeks old). Clinical signs include fever, depression, sneezing, anorexia, coughing, occasional pneumonia, and serous discharge from the eyes and nose. *C. felis* infections of humans has been documented but they are rare. If left untreated, infections often persist for 6–8 weeks. Treatment usually involves both topical antibiotics in the form of eyedrops or ointment and oral antibiotics for 6–8 weeks. Feline vaccines exist that help reduce the severity of clinical disease.

C. pecorum

C. pecorum infections occur in some species of mammals including cattle, sheep, goats, koalas, and swine. Depending on the specific biotype, clinical symptoms may include conjunctivitis, polyarthritis, pneumonia, abortion, encephalitis, and enteritis. In cattle, clinical signs also include metritis, salpingitis, and infertility. Laboratory diagnosis is difficult because of difficulties in growing the organisms in culture and cross-reactivity of antibodies with *C. abortus*.

C. caviae

The natural host of *C. caviae* is the guinea pig. Clinical signs include conjunctivitis. *C. caviae* infection of the guinea pig is routinely used as an animal model system to study human chlamydial genital tract infections.

C. suis

C. suis is a swine pathogen and is believed to be endemic in pigs. Clinical syndromes include conjunctivitis, pneumonia, genital tract disease, and enteritis. *C. suis* is one of the few animal pathogens that remained within the genus *Chlamydia*. Due to its similarity to human isolates, recent concern has arisen due to the identification of tetracycline-resistant isolates of *C. suis*.

C. muridarum

C. muridarum is a mouse-adapted species that is primarily used in laboratory murine model systems to mimic infections by the human *C. trachomatis* genital tract isolates.

Conclusion

Exciting advances over the last several years have revealed that *Chlamydia* and *Chlamydophila* species target multiple host trafficking and signaling pathways to mediate their intracellular survival and pathogenesis. Despite the fact that there are still no genetic tools with which to manipulate the chlamydial genome, numerous T3SS-independent and -dependent effector molecules that are secreted into the host cytosol and inclusion membrane have been identified and shown to specifically interact with several host proteins. The development of genetic tools and further expression of chlamydial genes in heterologous systems combined with cell biological and functional genomics approaches should help to further clarify the functional role of these chlamydial effectors and how they mediate chlamydial pathogenesis.

See also: Global Burden of Infectious Diseases; Sexually Transmitted Diseases; Subversion of host defences by microbes

Further Reading

Bavoil PM and Wyrick PB (eds.) (2006) Chlamydia: *Genomics and Pathogenesis*. Norfalk, UK: Horizon Bioscience.

Dautry-Varsat A, Subtil A, and Hackstadt T (2005) Recent insights into the mechanisms of *Chlamydia entry*. *Cellular Microbiology* 7: 1714–1722.

Peters J, Wilson DP, Myers G, Timms P, and Bavoil PM (2007) Type III secretion á la *Chlamydia*. *Trends in Microbiology* 15: 241–251.

Stephens RS (ed.) (1999) *Chlamydia: Intracellular Biology, Pathogenesis, and Immunity*. Washington, DC: ASM Press.