5 *Chlamydia trachomatis*

A 19-year-old woman was seen by her doctor for a routine gynecologic examination and complained about some mid-cycle bleeding. She had been with her current boyfriend for a year, had a pregnancy termination 2 years previously, and was taking birth control pills.

Internal examination revealed a **mucopurulent** discharge at the external cervical os (**Figure 5.1**). The cervix was friable and bled easily. The doctor suspected chlamydial infection and collected an endocervical swab specimen for a *Chlamydia* test. The woman returned for the results and was told that the test for *Chlamydia* was positive.

The doctor prescribed a course of doxycycline for 1 week and explained to the patient that this treatment is sufficient and effective in more than 95% of cases, and no repeat testing to prove the eradication of this infection is necessary. However, due to the high risk of re-infection in sexually active young adults, the doctor recommended her to return for a follow-up visit in 6 months.

He further stated that a timely cleared chlamydial infection does not normally lead to infertility, although around a 10% probability of ectopic pregnancy remains. The woman was given a leaflet on chlamydial infection and on other sexually transmitted infections (STIs).

The patient was counseled regarding safe-sex practices. The doctor also advised her to contact the local genitourinary clinic to be tested for other STIs including HIV and made all necessary arrangements according to the national guidelines.

As a part of the disease management, the doctor carried out all the appropriate actions recommended in the national guidelines to notify the patient's partner and to advise him to visit a genitourinary clinic or to see his doctor for *Chlamydia* and other STI tests. The couple were advised to abstain from sex until both were cleared of the infection.

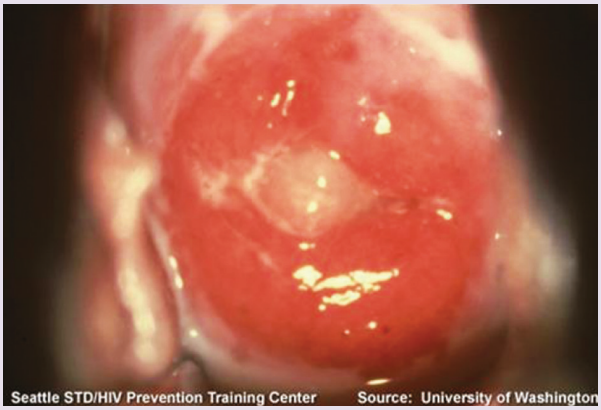

Figure 5.1 Mucopurulent cervical discharge caused by chlamydial infection, showing ectopy and edema. *Courtesy of the Seattle STD/HIV Prevention Training Center, University of Washington, Seattle.*

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

This patient was infected with *Chlamydia trachomatis*, which belongs to the Family Chlamydiaceae of the Order Chlamydiales. The Chlamydiaceae Family consists of two genuses – *Chlamydia* and *Chlamydophila*. Bacteria of this Family are obligate intracellular human and animal pathogens. The term "Chlamydia" is derived from the word "chlamys," which means cloak (Khlamus) in Greek, an appropriate name reflecting the cloak-like chlamydial inclusion around the host cell nucleus (see below).

Chlamydiaceae are some of the most widespread bacterial pathogens in the world and there are several species that infect a variety of hosts based on a wide range of tissue tropism. Two species, *Chlamydia trachomatis* and *Chlamydophila pneumoniae*, are human pathogens and are responsible for various diseases that represent a significant economic burden. *Chlamydophila psittaci* and *C. pecorum* are mainly bird/animal pathogens, although **zoonotic** transmission of the former to humans can occur resulting in the disease psittacosis.

Chlamydiaceae species share some important structural features. Like other gram-negative bacteria, they have inner and outer membranes, but have a specific **lipopolysaccharide** (**LPS**) that differs from that of other bacteria. The extracellular osmotic stability of Chlamydiaceae is provided by several complex disulfide cross-linked membrane proteins, the main ones being a 40 kDa major outer-membrane protein (MOMP, a product of the *ompA* gene); a hydrophilic cysteine-rich 60 kDa protein (OmcA); and a low molecular weight cysteine-rich

lipoprotein (OmcB). The Chlamydiaceae are thought to have little or no muramic acid, the hallmark constituent of peptidoglycan (PG). No transfer RNAs were identified in chlamydial cells, thus confirming the parasitism of this bacterium.

There are two human biological variants (biovars) of *C. trachomatis*: **trachoma** and lymphogranuloma venereum (LGV), and one biovar *C. pneumoniae* infecting mice, and causing mouse pneumonitis (which will not be discussed here).

Fifteen serologic variants (**serovars**) have been identified in the trachoma biovar (A–K, Ba, Da, Ia, and Ja). These mostly infect columnar and squamo-columnar epithelial cells of mucous membranes (see below). Serovars D–K, Da, Ia, and Ja typically infect genitourinary tissues, but were also found in the mucous membranes of the eye conjunctiva and epithelial tissues in the neonatal lung. Serovars A, B, Ba, and C generally infect the conjunctiva and cause trachoma. The LGV biovar consists of four serovars (L1, L2, L2a, and L3), which predominantly infect monocytes and macrophages passing through the epithelial surface to regional lymphoid tissue. Proteomic analysis of the pathogen is very difficult since Chlamydiaceae species are all obligate intracellular parasites and cannot grow in a cell-free system. As a result, most of the data obtained on the structural and functional proteins and biochemical pathways utilized by Chlamydiaceae are derived from gene sequencing and indirect evidence. The genome of *C. trachomatis* (serovars A, D, and L2) has been fully sequenced.

Chlamydiaceae species have a more complicated biphasic developmental life cycle (**Figure 5.2**) than other bacteria in that they have two different forms, a metabolically inert infectious elementary body (EB) and a larger noninfectious reticulate body (RB). Interestingly, the term "elementary body" belongs to the virology world and is derived from the time when Chlamydiaceae were initially considered to be viruses. The EB form of the bacterium survives outside the host cell whereas the RB form lives and replicates in a specialized vacuole of the host cell called an inclusion (another virology-derived term).

ENTRY INTO THE BODY

The infectious EB form of the majority of *Chlamydia* strains is typically 0.2–0.3 µm in diameter. MOMP makes up 60% of its cell wall. Due to their rigid outer membrane, EBs are able to survive outside the eukaryotic host cells. *C. trachomatis* infecting genital tissue usually does so through small abrasions in the mucosal surfaces. The EBs infect nonciliated columnar, cuboidal or transitional epithelial cells, but can also infect macrophages. The infection process is multivalent. EBs bind to the epithelial cells directly via cellular proteoglycans

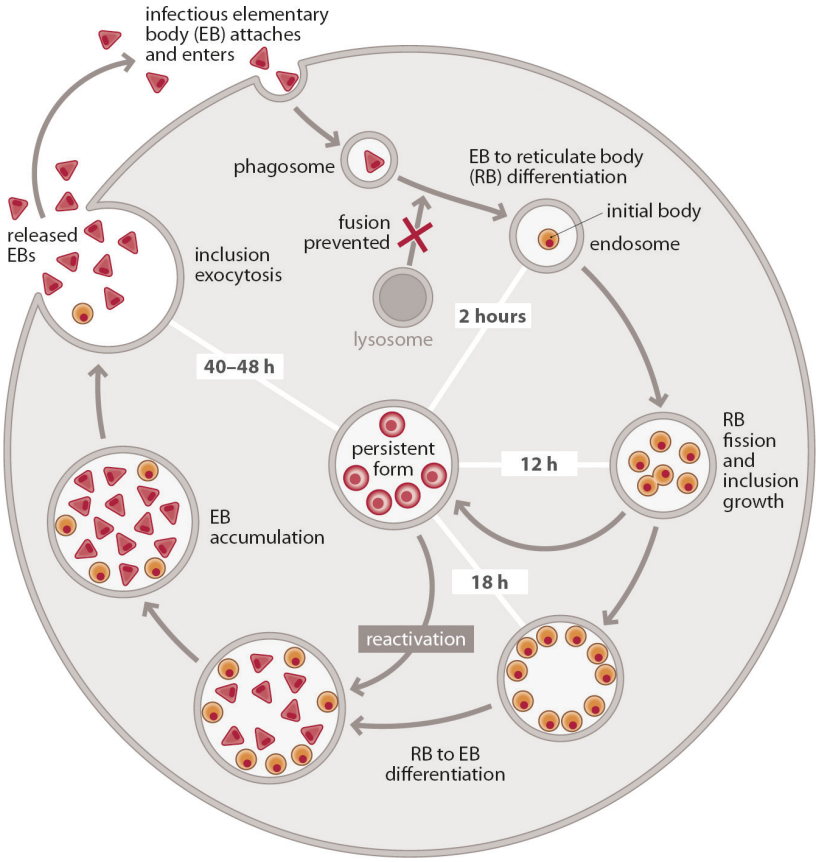

Figure 5.2 Biphasic developmental cycle of *C. trachomatis*. *From Brunham R & Rey-Ladino J (2005) Nat Rev Immunol 5:149-161. https://doi.org/10.1038/nri1551. With permission from Springer Nature.*

with heparan sulfate (HS) moieties (HSPGs) mainly through electrostatic interactions. Depending on the serovar, the degree of HS involvement varies. Additionally, *C. trachomatis* EB form is able to adhere to and enter cells indirectly through binding with fibroblast growth factor 2 (FGF2). This complex then interacts with the FGF2 receptor, which mediates EB internalization into cells. The EB-FGF2 complex may involve synergistic interactions with the EB membrane bacterial protein OmcB, which, in turn, interacts with HSPGs. This facilitates chlamydial entry through **phagocytosis**, receptor-mediated **endocytosis**, and **pinocytosis**.

Early intracellular phase (0–2 hours after infection). Once inside the cell, EB-containing vacuoles move toward the perinuclear region. The vacuole membrane phospholipids promote homotypic fusion of EB vacuoles with each other, but not with **lysosomes** – an important feature that helps the pathogen to avoid intracellular destruction. The homotypic fusion is specific to *C. trachomatis* only and not to other *Chlamydia* species. This results in the formation of a single fusion vacuole (a nascent inclusion) containing several EBs. Some of these vacuoles may contain different serovars such as F and E, leading to the possibility for genetic exchange to occur.

The inclusions then move toward the microtubule organization center where they are supplied with nutrients via the host-cell Golgi apparatus. Bacterial proteins are directly secreted into the host cell cytosol.

Inclusion development (2–40 hours after infection). The EB forms now undergo a lengthy and complex development using host-cell ATP and nutrients as a source of energy. With a reduced genome, *C. trachomatis* is dependent on its host for survival and hijacks host-cell metabolism particularly glycolytic enzymes, aldolase A, pyruvate kinase, and lactate dehydrogenase which are enriched at the *C. trachomatis* inclusion membrane during infection. The increased requirement for glutamine, important for the growth of C. *trachomatis* in infected cells is achieved by reprogramming the glutamine metabolism.

Still remaining in the inclusion, the EB forms now transform into RB forms, which are typically 0.8–1.0 μm in diameter. RBs multiply by binary fission so that the resulting inclusions may contain 500–1000 progeny RBs and occupy up to 90% of the cell cytoplasm. After several rounds of replication, RB forms revert to the infectious EB forms. In tissue culture, the productive infectious cycle of *Chlamydia* lasts about 48–72 hours depending on the serovar. Eventually, the EBs are released to infect other adjacent cells. *C. trachomatis* is able to regulate host cell **apoptosis** throughout the early and productive growth stages. Nonreplicating forms of *Chlamydia* inhibit apoptosis through interfering with the *TP53* tumor suppressor gene. However, late in the life cycle, the pathogen produces a caspase-independent pro-apoptotic Bax protein, which facilitates apoptosis of the host cell thus freeing the secondary EB forms.

Under conditions unfavorable for a pathogen, such as the presence of **interferon-γ** (**IFN-γ**), lack of nutrients or drug treatment (see Section 5) *Chlamydia* may enter a nonreplicating mode called persistence (**Figure 5.2**). During persistent infection, the developmental cycle is lengthened or aborted and RB forms are produced that do not divide or differentiate back into the EB forms. Persistent infection with *C. trachomatis* may lead to serious clinical conditions that are difficult to treat. However, dormant forms can revert to metabolically active forms if the unfavorable conditions are removed.

SPREAD WITHIN THE BODY

EBs of *C. trachomatis* infect cervical columnar epithelial cells, but the bacteria can spread by ascending into the endometrium and the fallopian tubes, causing **pelvic inflammatory disease** (**PID**), ectopic pregnancy, and infertility (see Section 3). Sexually transmitted *C. trachomatis* serovars D–K can also lead to conjunctivitis through autoinoculation or ocular–genital contact.

PERSON-TO-PERSON SPREAD

Genital Tract Infections

C. trachomatis (serovars D–K) is sexually transmitted in vaginal fluid or semen containing the EB form, through vaginal intercourse but occasionally via oral and anal sex. These serovars can also be vertically transmitted from mother to child during birth through an infected birth canal, causing conjunctivitis (**ophthalmia neonatorum**) or chlamydial **pneumonia**.

C. trachomatis (biovar LGV) is also sexually transmitted. In some parts of Africa, Asia, South America, and the Caribbean it is largely found in heterosexuals. In outbreaks in industrialized countries, the cases are mostly confined to men who have sex with men (MSM) with multiple sexual partners.

Ocular Infections

C. trachomatis (serovars A–C) is found predominantly in areas of poverty and overcrowding. Infection can be transmitted from eye-to-eye by fingers, shared cloths or towels, by eye-seeking flies, and by droplets (coughing or sneezing). The latter route is possible because *C. trachomatis* can exist in the nasopharynx and external nasal exudates of children with trachoma.

Importantly, the undiagnosed and untreated children can contribute to a so-called "age-reservoir effect" responsible for the continuous transmission within the community. These must be identified and treated to prevent further spread of the pathogen in communities.

EPIDEMIOLOGY

C. trachomatis (serovars D–K) is the leading bacterial cause of STIs, with over 50 million new cases occurring yearly worldwide and 4 million new cases each year in the US. The highest infection rates are detected in African Americans, American Indian/Alaska Natives, and Hispanics. Two-thirds of new chlamydial infections occur among youth aged 15–24

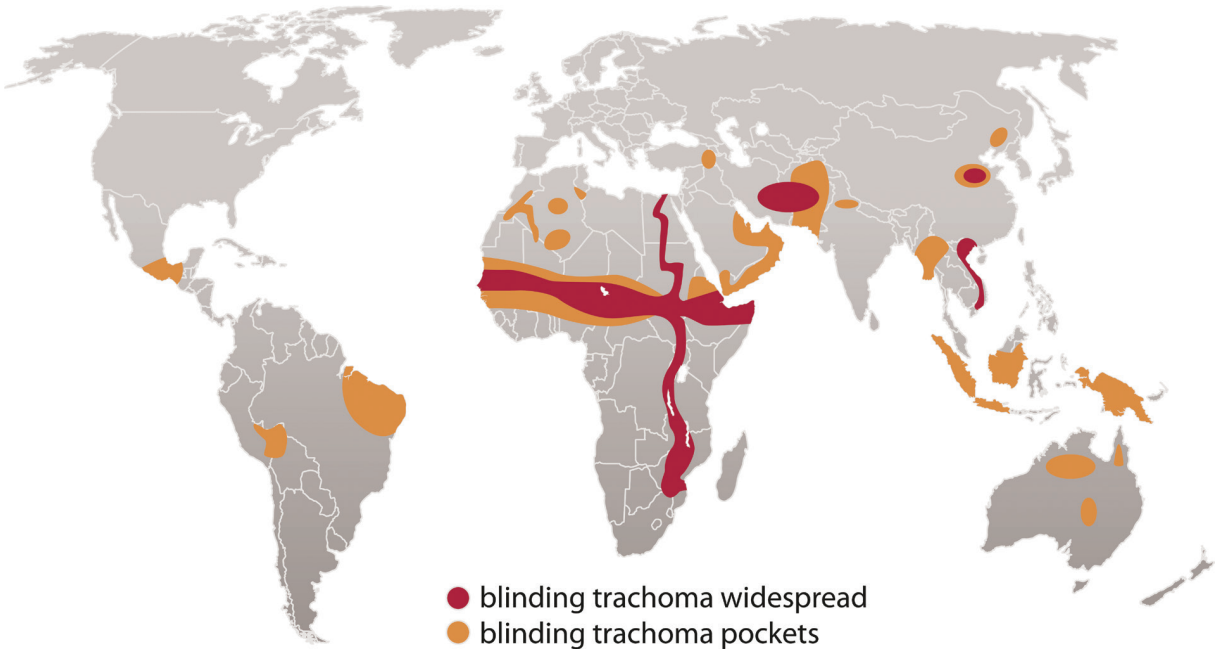

Figure 5.3 World distribution of trachoma according to the WHO. *C. trachomatis* (biovar trachoma) is endemic in large areas of Africa and the Middle East, and focal areas of disease are found in India, South-East Asia, and Latin America. As per 2020 WHO data, trachoma affects some 140 million people worldwide, and about 1.9 million people suffer visual loss and blindness. *From Belland R, Ojcius D & Byrne G (2004) Focus: Chlamydia Nat Rev Microbiol 2:530. https:doi.org/10.1038/nrmicro931. With permission from Springer Nature.*

years. In general practice, around 1 in 20 sexually active women aged less than 25 years may be infected. Among MSM worldwide incidence of rectal chlamydial infection range from 3.0% to 10.5%, and pharyngeal chlamydial infection from 0.5% to 2.3%.

Interestingly, there was a 30% decline in Chlamydia cases as well as in all major STIs in England in 2020 compared to 2019, most likely due to the COVID-19 lockdowns.

Every year *C. trachomatis* (serovars A–C) is a major cause of 500 000 cases of trachoma worldwide (**Figure 5.3**). Active trachoma affects some 85 million people, more than 10 million have **trichiasis** (turned-in eyelashes that touch the eye globe, **Figure 5.4**), and about 6 million people suffer visual loss and blindness. Active disease is most commonly seen in children and, in adults, the prevalence of trichiasis is about three times higher in women than in men. Trachoma is **endemic** in large areas of Africa and the Middle East, and focal areas of disease are found in India, South-West Asia, Latin America, and Aboriginal communities in Australia. The disease is generally found in clusters in certain communities or even households, indicating the existence of local risk factors in addition to the generally accepted poverty and lack of water and sanitation.

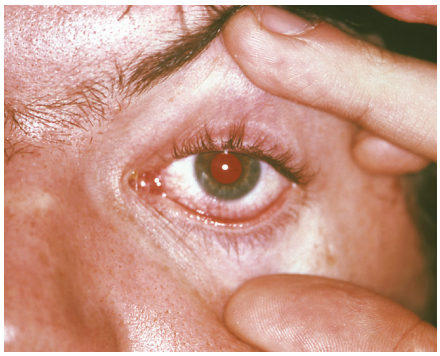

Figure 5.4 Trachomatous trichiasis: at least one eyelash rubs on the eyeball. *Courtesy of the Centers for Disease Control, Atlanta, Georgia. Image is found in the Public Health Image Library #4076. Additional photographic credit is given to Joe Miller, PhD, who took the photo in 1976.*

C. trachomatis (biovar LGV – serotypes L1–L3) causes sexually transmitted disease that is prevalent in parts of Africa, Asia, South America, the Caribbean, and increasingly in Europe and the US. Humans are the only natural host, with MSM being the major reservoir of the disease. In the US, the incidence is 300–500 cases per year.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

INNATE IMMUNITY

Both innate and adaptive immune responses are induced during *C. trachomatis* infection. However, these responses are often insufficient, providing only partial clearance of the pathogen from the body. This can lead to protracted chronic infections and chronic inflammation contributing to pathogenesis.

Innate immune responses. Chlamydial infection initially induces an influx of polymorphonuclear cells and macrophages into the infection site as a part of acute inflammation. This is facilitated by the release by the infected epithelial cells of **cytokines** and **chemokines**, particularly **interleukin-8** (**IL-8**), which is a powerful neutrophil attractant. Infiltration with neutrophils and macrophages is followed by accumulation of B cells, T cells, and **dendritic cells** (**DCs**) in submucosal areas launching T-cell responses and antibody production.

Chlamydial PAMPs (pathogen-associated molecular patterns) are recognized by multiple PRRs (pattern recognition receptors), particularly TLR2 which was shown to be activated by MOMP and heat shock protein 60 (Hsp60), during the productive infection, although the direct ligand for TLR2 in this infection is still unknown.

Antibody responses. A humoral response is invoked resulting in production of mucosal secretory **IgA** and circulating **IgM** and **IgG** antibodies. These antibodies are mostly specific for MOMP and Hsp60. Anti-chlamydial IgG antibodies are also found in ocular secretions in trachoma patients. The effectiveness of these antibodies in damaging or blocking further entry of EBs to the cells adjacent to the infected ones is unclear. However, it appears, that IgG antibodies bound to the bacterial MOMP invoke antibody-dependent cellular cytotoxicity (ADCC) by **natural killer** (**NK**) cells.

Cellular responses. It is likely that at least some professional antigen-presenting cells (APCs) at the site of infection engulf EBs, process and present Chlamydia peptides via the major histocompatibility complex (MHC) class II-mediated pathway leading to the activation of CD4+ T cells. DCs in response to the infection produce IL-12 and drive Th1-cell development and hence **production of interferon γ** (**IFN-γ**) – the major inhibitory cytokine for *Chlamydia* (see below). Recent data suggests that in order to counteract this, *C. trachomatis* up-regulates expression of PD-L1 on the DCs present in the uterus. Its interaction with PD-1 receptor on T cells might lead to blocking of T-cell responses.

That both *Chlamydia*-specific CD4+ and CD8+ T cells are involved in controlling *C. trachomatis* infection is indicated by their expansion with memory phenotype in the endocervix during the infection. Patients with *C. trachomatis* infection had the highest levels of T-cell recruiting cytokines out of major STI, a factor associated with higher risk of HIV co-infection which may occur in patients with untreated Chlamydia infection. In epithelial cells, which do not express MHC class II molecules, recognition of *Chlamydia* peptides may occur via the MHC class I presentation pathway, which activates CD8+ T cells. These can recognize proteins present in the host-cell cytosol or cytosolic domains of membrane proteins. MOMP is one of the potential antigenic targets for CD8+ CTLs. However, no evidence has been found so far of CD8+ T-cell-mediated killing of the infected cells that would disrupt pathogen replication and intracellular survival.

It appears, therefore, that the production of the **Th1** cytokine IFN-γ is one of the most powerful anti-chlamydial T-cell-mediated immune mechanisms. IFN-γ inhibits the growth of *Chlamydia* in cell culture and, in experimental models, disruption of its production enhances host susceptibility to *Chlamydia* infection. It is thought that IFN-γ can potentially limit *C. trachomatis* infection by the following mechanisms:

- activation of macrophages and their phagocytic potential;
- up-regulation of the expression of MHC molecules by professional and nonprofessional APCs;
- expression of **indoleamine 2,3-dioxygenase** (**IDO**), a host enzyme that degrades intracellular tryptophan essential for *C. trachomatis* growth;
- up-regulation of **inducible nitric oxide synthase** (**iNOS**), which catalyzes the production of nitric oxide (NO) and other reactive nitrogen intermediates, that can enhance damage to intracellular pathogens;
- down-regulation of transferrin receptor on infected cells, resulting in an intracellular iron deficiency that may limit *C. trachomatis* replication.

Even although IFN-γ plays a role in protective immunity to *C. trachomatis* infection, its overall effect is limited. Infection does not stimulate long-lasting immunity and repeated re-infections are common, which results in a prolonged inflammatory response and subsequent tissue damage (see Section 3). One of the reasons for poor anti-chlamydial immunity is the ability of the pathogen to evade immune responses.

HOW DOES *C. TRACHOMATIS* EVADE THE HOST IMMUNE RESPONSES?

This bacterium employs several strategies to evade the host immune response.

- Its intracellular location protects it from antibodies and complement.
- It down-regulates the expression of MHC class I molecules on the surface of infected cells, blocking recognition and MHC class I-restricted CD8+ T-cell-mediated cytotoxicity. The pathogen uses a protease-like activity factor (CPAF) able to degrade host transcription factors required for MHC gene activation.
- Fusion of the pathogen-containing **phagosome** with host cell **lysosomes** is prevented.
- *Chlamydia*-infected macrophages induce apoptosis of T cells, by paracrine effects and **tumor necrosis factor-α** (**TNF-α**).

PATHOGENESIS

Tissue damage is the result of the host inflammatory response to the persistent infection as well as direct damage to infected cells by the bacteria. Various species of *Chlamydia* produce cytotoxins that can deliver immediate cytotoxicity of host cells if infected with large doses of the pathogen.

With unsuccessful initial elimination by the innate and adaptive immune systems leading to persistence of the pathogen, the site of infection becomes infiltrated with macrophages, plasma cells, and eosinophils. The continuous production of cytokines and chemokines results in development of lymphoid follicles and tissue scarring due to **fibrosis**. It is believed that IFN-γ production by T cells at the site of infection is reduced as the bacterial load decreases. This, in turn, supports replication of *C. trachomatis* and the inflammatory process resumes, entering into a vicious circle. Chronic inflammation leads to many of the clinical symptoms seen with *C. trachomatis*. Trachoma is characterized by the conjunctival lymphoid follicle formation, which contain germinal centers consisting predominantly of B lymphocytes, with CD8+ T lymphocytes in the parafollicular region. The inflammatory infiltrate contains plasma cells, DCs, macrophages, and polymorphonuclear leucocytes. Inflammatory infiltrates taken from patients with scar tissue are characterized by the expansion of CD4+ T lymphocytes.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

Chlamydia is known as the "silent epidemic", since it may not cause any symptoms sometimes for months or years before being discovered. There are several conditions caused by various biovars and serovars of *C. trachomatis* (**Table 5.1**).

UROGENITAL INFECTIONS

Most people with *C. trachomatis* infection do not have any symptoms and are unaware of the infection. However, when symptoms develop, treatment is urgently required to prevent complications.

Males. When the symptoms develop, the patient may suffer from nongonococcal **urethritis** (**NGU**), which can result in discharge from the penis or pain and burning sensation when urinating. If not treated, it can lead to inflammation near the testicles with considerable pain. Spread to the testicles may cause **epididymitis** and, rarely, sterility. *Chlamydia* causes more than 250 000 cases of epididymitis in the US each year. Post-gonococcal urethritis may occur in men infected with both *Neisseria gonorrhoeae* and *C. trachomatis* who receive antibiotic treatment effective solely for gonorrhoea. MSM men could also develop rectal infection.

Females. The incubation period is usually 1–3 weeks after which the symptoms of urethritis and **cervicitis** may develop: **dysuria** and **pyuria**, cervical discharge or vaginal spotting, and lower abdominal pain. Physical examination reveals yellow or cloudy mucoid discharge from the os (see **Figure 5.1**). If untreated, *Chlamydia* may spread through the uterus to the fallopian tubes, causing salpingitis. In the US, chlamydial infection is the leading cause of first trimester pregnancy-related deaths. Women infected with *Chlamydia* have a three- to five-fold increased probability of acquiring HIV due to the increased behavioral risk.

Complications. More than 4 billion US dollars are spent annually on the treatment of the most common and severe complication of the sexually transmitted *C. trachomatis* PID. Over 95% of women with uncomplicated and effectively treated chlamydial infection will not develop tubal infertility. Chlamydia can also cause subclinical inflammation of the upper genital tract, so called "subclinical PID". Some patients develop perihepatitis, or "Fitz-Hugh-Curtis Syndrome", an inflammation of the liver capsule and surrounding peritoneum, causing pain in the right upper quadrant.

More than 85% of women with PID remain fertile. However, an approximate 10% risk of ectopic pregnancy – a potentially life-threatening condition – remains after both clinical and subclinical PID due to the permanent damage to

Table 5.1 *C. trachomatis* serovars and associated human diseases

Serovars	*Human disease*	*Method of spread*	*Pathology*
A, B, Ba, and C	Ocular trachoma	Hand to eye, fomites, and eye-seeking flies	Conjunctivitis and conjunctival and corneal scarring
D, Da, E, F, G, H, I, Ia, J, Ja, and K	Oculogenital disease	Sexual and perinatal	Cervicitis, urethritis, endometritis, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, neonatal conjunctivitis, and infant pneumonia
L1, L2, and L3	Lymphogranuloma venereum	Sexual	Submucosa and lymph node invasion, with necrotizing granuloma and fibrosis

Reproduced with permission from Brunham RC and Rey-Ladino J. Immunology of Chlamydia infection: implications for Chlamydia trachomatis vaccine. (2005). Nature Reviews Immunology, 5: 149–161.

the fallopian tubes, uterus, and surrounding tissues. Women with PID often suffer later from abdominal pain and may require a hysterectomy. It is difficult to give a precise prognosis of infertility until a patient tries to have a child.

In both sexes, an asymptomatic infection may be present in either the throat or rectum if the patient has had oral and/or anal intercourse.

INCLUSION CONJUNCTIVITIS

This condition is caused by *C. trachomatis* (serovars D–K) being associated with genital infections. It is often transferred from the genital tract to the eye by contaminated hands. The main symptom is a sensation of a foreign body in the eye, redness, and irritation. Other symptoms include mucosal discharge later replaced by purulent discharge, large lymphoid follicles, and papillary hyperplasia of conjunctiva, corneal infiltrates, and vascularization. Corneal scarring is rare and happens mostly in the chronic stage followed by epithelial **keratitis**. Ear infection and **rhinitis** can accompany the ocular disease.

INFANT PNEUMONIA

Infants vertically infected with *C. trachomatis* (serovars D–K) from their mother at birth can develop pneumonia presented by staccato cough and **tachypnea** often preceded by conjunctivitis.

LYMPHOGRANULOMA VENEREUM (LGV)

The causative agent is *C. trachomatis* biovar LGV. The first symptom of the infection is the development of a primary lesion – a small painless **papule** or ulcer at the site of infection, often the penis or vagina. Several weeks after the primary lesion, patients develop painful inguinal and/or femoral **lymphadenopathy**. In the case of extragenital infection, the lymphadenopathy can occur in the cervix. Patients develop a fever, headache, and **myalgia** followed by inflammation of the draining lymph nodes. As a result, the lymph nodes become enlarged and painful and may eventually rupture. **Elephantiasis** of the genitalia, more often in women, can develop due to obstruction of the lymphatics. In females, lymphatic drainage occurs usually in perianal sites and can involve **proctitis** and **recto-vaginal fistulae**. In males, proctitis develops from anal intercourse or from lymphatic spread from the urethra.

OCULAR INFECTIONS

Trachoma is the most serious of the eye infections caused by *C. trachomatis*. The word "trachoma" in Greek means rough (trakhus) and reflects the roughened appearance of the conjunctiva. Repeated re-infection with the ocular serovars A, B, Ba, and C results in chronic **keratoconjunctivitis**. Following infection there is an incubation period of 5–12 days, after which, the symptoms start to appear and include a mild conjunctivitis and eye discharge. Initial infection is often self-limiting and heals spontaneously. A repeated infection, however, leads to the development of chronic inflammation (see Section 2), characterized by swollen eyelids and swelling of lymph nodes in front of the ears. Years of re-infection and chronic inflammation may result in fibrosis and in scarring in the upper subtarsal conjunctiva. Scarring is more frequent in young adults, particularly in women. The progress of scarring over many years causes distortion of the lid margin and the lashes turn inward and rub against the cornea. This is called trichiasis (**Figure 5.4**). If untreated, persistent trauma can result in ulceration of the cornea, corneal opacity, and blindness.

Inflammation and scarring in the eye may block the natural flow of tears, which represent an important first line of defense against bacteria. This can facilitate secondary re-infection with *C. trachomatis* as well as infection with other bacteria or fungi.

OCULAR LYMPHOGRANULOMA VENEREUM

Ocular infection with *C. trachomatis* biovar LGV can lead to conjunctivitis and **preauricular lymphadenopathy**.

REACTIVE ARTHRITIS

This can occur in men and women following symptomatic or asymptomatic chlamydial infection, sometimes coupled with urethritis and conjunctivitis and painless mucocutaneous lesions. Formerly, this complication was referred to as Reiter's Syndrome.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

CLINICAL DIAGNOSIS OF GENITAL INFECTIONS

The following clinical indicators are used for the diagnosis and screening of chlamydial infection in women:

- less than 25 years of age, sexually active;
- more than one sexual partner;
- mucopurulent vaginal discharge (**Figure 5.1**);
- burning sensation when passing urine;
- friable cervix or bleeding after sex or between menstrual periods;
- lower abdominal pain, or pain during sexual intercourse.

Because of the possibility of multiple STIs, all patients with any STI should be evaluated for chlamydial infection and offered an HIV test.

CLINICAL DIAGNOSIS OF OCULAR INFECTIONS

Examination of an eye for the clinical signs of trachoma involves careful inspection of the lashes and cornea, then aversion of the upper lid and inspection of the upper tarsal conjunctiva using binocular loupes. Clinical diagnosis is best made based on investigation of the history of living in a trachoma-endemic environment, in combination with clinical signs.

CLINICAL DIAGNOSIS OF LYMPHOGRANULOMA VENEREUM

The clinical symptoms may initially be unclear since they overlap with those of other STIs. Some men may have had treatment for a range of conditions including inflammatory bowel disease, **Crohn's disease**, and so forth. Diagnosis is largely based on the history of the disease, physical examination, and laboratory tests. The clinical course of LGV is divided into three stages.

1. Primary painless lesion, which develops after incubation of 3–30 days. A papule or ulcer can be found on the genitalia (glans of the penis, vaginal wall, labia, or cervix) or, in some cases, in the oral cavity.
2. Secondary lesion/**lymphadenitis**. It is a regional dissemination causing inguinal and femoral lymphadenopathy and possibly bubo formation that ulcerates and discharges pus. Lymphadenopathy is usually unilateral involving the retroperitoneal lymph nodes in women and the inguinal lymph nodes in men.
3. Tertiary stage/genito-ano-rectal syndrome. The majority of patients will recover from the second stage without sequelae. However, a few may develop proctitis and fibrosis that may result in chronic genital ulcers or fistulas, rectal strictures, and genital elephantiasis. Early symptoms of LGV **proctocolitis** include anal **pruritus** and discharge, fever, rectal pain, and **tenesmus**.

LABORATORY DIAGNOSIS

Sample collection. For genital infections, swabs are collected from the cervix or vagina of women or the urethra of men. Self-collection of swabs should be taken from the throat or rectum if there is a possibility of infection there.

With the use of **nucleic acid amplification tests** (**NAATs**) (see below) a noninvasive urine test can be used for screening for *Chlamydia* with sufficient sensitivity instead of swabs. It is particularly useful in asymptomatic cases where genital examination and sampling may not be justified.

For suspected LGV infections in the primary stage, a swab of the lesion can be taken. In the secondary stage, bubo pus, saline aspirates of the bubo, swabs of the rectum, vagina, urethra, urine, serum, or biopsy specimens of the lower gastrointestinal (GI) tract are used.

For conjunctival specimens, epithelial cells are collected by rubbing a dry swab over the everted palpebral conjunctiva.

The following tests can be made on the samples collected.

Cytology is used to detect the inclusion bodies in stained cell scrapings (**Figure 5.5**), but this method lacks sensitivity and is time-consuming.

Cell culture. For many years, cell culture has been the gold standard for the diagnosis of *C. trachomatis* and is very specific for this pathogen, but NAATs are more sensitive and represent a new gold standard (see below). Cultured cells are stained with Giemsa or iodine, or by fluorescent antibodies and examined for the presence of iodine-staining inclusion bodies. Iodine stains glycogen, which is only found in the inclusions of *C. trachomatis*, not in other *Chlamydia* species.

NAATs are now recommended to replace culture techniques. They include first of all the **polymerase chain reaction** (**PCR**), but also transcription-mediated amplification (TMA). NAATs are characterized by high sensitivity (> 89%) and specificity (95–99.5%) for endocervical, urethral, and conjunctival samples. NAATs are the only testing techniques currently recommended by the English National Chlamydia Screening Programme. Certain NAAT test platforms have been cleared by FDA for non-genital sites. NAATs can be used for detection of the bacterium as well as for the quantification of the bacterial load. The wider use of NAATs worldwide in screening strategies is, however, limited by the costs and complexity of the equipment required.

Direct hybridization probe tests have been used in the past, but now are largely replaced by NAATs. They include Gen-Probe PACE 2 test, which uses a synthetic single-stranded DNA probe complementary to the chlamydial rRNA region, and Digene HC-II-CT-II probe based on **enzyme immunoassay** (**EIA**) for the detection of the RNA probe binding to the single-stranded bacterial DNA.

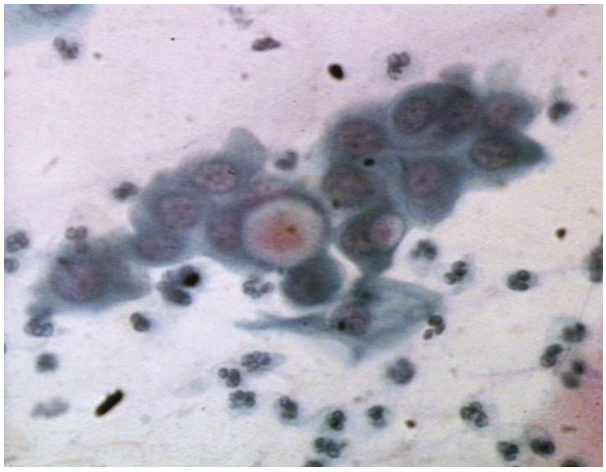

Figure 5.5 Chlamydial inclusions, which may contain 100–500 RB progeny. *From Mabey DCW, Solomon AW & Foster A (2003) The Lancet 362: 223-229. https://doi.org/10.1016/S0140-6736(03)13914-1. With permission from Elsevier.*

Antibody-based laboratory techniques include direct fluorescent antibody (DFA) detection and **enzyme-linked immunosorbent assay** (**ELISA**). The latter can be used for determination of the serovars. Usually diagnostic antibodies target group-specific LPS or serovar-specific outer-membrane proteins (OMPs). However, ELISA-based technology is widely used in screening programs and is of great help in reducing the overall chlamydial infection and incidence of PID and NGU.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of genital chlamydial infection includes gonorrhea, *Ureaplasmaurealyticum*, *Mycoplasma genitalium*, *M. hominis*, and *Trichomonas vaginalis*, as well as urinary tract infections and bacterial vaginosis. Some other conditions such as periurethral **abscess**, **endometriosis**, urethral/vaginal foreign body, other causes of PID, prostatitis, and **epididymo-orchitis** must also be ruled out.

In the case of trachoma, a differential diagnosis can be made with the following conditions: adult inclusion conjunctivitis; other bacterial infections, especially with *Moraxella* species and *Streptococcus pneumoniae*; viral infections including adenovirus, herpes simplex virus, and molluscum contagiosum; *Pediculosis palpebrarum*; toxic conjunctivitis, which is secondary to topical drugs or eye cosmetics; Axenfield's follicular conjunctivitis; Parinaud's oculoglandular syndrome; and vernal conjunctivitis.

For LGV and inguinal **adenopathy**, differential diagnosis of the genital ulcer includes chancroid, herpes, syphilis, and donovanosis. For proctitis, the differential diagnosis includes inflammatory bowel disease.

The differential diagnosis is mostly based on the laboratory findings, particularly PCR analysis, supplemented by clinical observations.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

In 2021, the CDC introduced new guidelines for the treatment of all STIs.

Urogenital Infections

For urogenital infections, the antibiotic doxycycline is the drug of choice and 100 mg is taken twice daily for 7 days. This is effective in 95% of cases but can interfere with the contraceptive pill and can cause photosensitivity and stomach irritation. Doxycycline is also available in a delayed-release 200-mg tablet formulation, which requires once-daily dosing for 7 days.

An alternative CDC-recommended regimen includes azithromycin, 1 g orally in a single dose or levofloxacin 500 mg orally once daily for 7 days. Azithromycin is a derivative of erythromycin and is characterized by improved bioavailability and ability to maintain high tissue concentrations, particularly at sites of inflammation. Azithromycin has also been proven to be neonatally safe. Erythromycin is no longer recommended due to the GI side effect. In the past, it was given to the infected woman if pregnant or lactating, as 500 mg four times a day for 7 days or twice daily for 14 days. Pregnant women are given a single 1 g dose of azithromycin orally or amoxicillin 500 mg orally 3 times a day for 7 days. Neonates – erythromycin base or ethylsuccinate, 50 mg/kg body weight/day orally, divided into 4 doses daily for 14 days. The same regimen is used for infants. However, in addition, they can be prescribed an azithromycin suspension, 20 mg/kg body weight/day orally, 1 dose daily for 3 days.

Since genital chlamydial infections often have no symptoms, particularly in women, the sexual partner could have become infected months ago. In case of multiple sexual partners, all of them should be tested for chlamydial infection and treated if positive. The patients should also be tested for other STIs. Having sex is not recommended during treatment and for at least a week after the completion of the treatment, particularly if both partners are infected (see Case Study).

A high prevalence of *C. trachomatis* infection has been observed post-treatment, due to re-infection caused by failure of sex partners to receive treatment or initiation of sexual activity with a new infected partner, indicating a need for improved education and treatment of sex partners. Repeat infections confer an elevated risk for PID and other complications among women. As a preventive measure, men and women who have been treated for chlamydia should be retested approximately 3 months after treatment, or whenever persons next seek medical care during the first year after initial treatment.

Trachoma

For the treatment of active trachoma, two antibiotic regimens are currently recommended: tetracycline ointment applied twice daily for 6 weeks or one 20 mg kg^{-1} dose of azithromycin can be used instead. It must be noted that the application of the ointment is inconvenient in children. Azithromycin is also effective for treating extraocular reservoirs of chlamydial infection, although antibiotic resistance could eventually develop.

Currently, annual mass treatment for 3 years is recommended by the WHO in districts and communities where the prevalence of follicular trachoma in children aged 1–9 years is equal to or greater than 10%. Surgical correction of trichiasis to fix eyelid deformities and to prevent vision loss, coupled with post-operational one dose of azithromycin, is applied. Due to the severe damage of eyes in trichiasis, cornea transplantation is not considered.

PREVENTION

Prevention of genital chlamydial infections involves safe sexual practices, using a condom during sexual intercourse, and prompt treatment of infected patients and their sexual partners. It is recommended to have a *Chlamydia* test under the following conditions:

- after sex with a new or casual partner;
- immediately if symptoms occur (see Section 3);
- if a sexual partner has *Chlamydia* or symptoms of *Chlamydia*.

The best way to avoid becoming infected with *Chlamydia* and other STIs is to have sexual contact or to be in a long-term, mutually monogamous relationship with a partner who is not infected.

Flies acting as physical vectors for transmission of *C. trachomatis* transmit ocular infection, particularly in children in areas with poor sanitation. The presence of cattle pens has been associated with trachoma in some African countries. Crowded living conditions in the family unit constitute another factor increasing the risk of trachoma. Therefore, special socioeconomic measures have been recommended to reduce the risk of transmission of ocular *C. trachomatis* infection. These include increasing access to water, fly control interventions, education, and improved living conditions.

VACCINE

Due to its high incidence, relapses, prolonged bouts of infection, and significant morbidity, *C. trachomatis* is an important target for vaccine development. A safe vaccine administered prior to adolescence and effective through child-bearing age would have a significant impact on the spread of the disease. The attempts of using inoculation of whole inactivated bacteria, however, led to the exacerbation of inflammatory disease and subsequent re-infection. This shifted the focus of vaccine development toward MOMP-based subunit vaccines. **Monoclonal antibodies** to MOMP neutralize *C. trachomatis* infection *in vitro* and *in vivo*.

The results of clinical trials were reported in 2019 on a multivalent vaccine, CTH522 incorporating MOMP proteins. The vaccine gave promising results by significantly increasing the levels of antigen-specific mucosal IgG and IgA, antibody neutralization, and the production of IFNγ. Further human clinical trials are required to fine-tune this vaccine, which is likely to include recombinant protein combination with adjuvants.

Since 2013, vaccine development against *C. trachomatis* has been intensified and various delivery platforms have been suggested, such as nanoparticles, Hepatitis B core antigen and *Neisseria lactamicaporin* B as career molecules, adenoviral and mRNA vectors.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- *Chlamydia trachomatis* belongs to the family Chlamydiaceae, which contains two genuses: *Chlamydia* and *Chlamydophila*. Bacteria of this family are obligatory intracellular human and animal pathogens.
- *Chlamydia trachomatis* and *Chlamydophila pneumoniae* are human pathogens. *C. trachomatis* can cause sexually transmitted urogenital infections, neonatal conjunctivitis and pneumonia, ocular trachoma, and urogenital infection associated with lymphadenopathy and lymphadenitis (LGV). *C. pneumoniae* can cause bronchitis, sinusitis, and pneumonia, and accelerates atherosclerosis.
- All Chlamydiaceae species have specific lipopolysaccharides and envelope proteins: 40 kDa major outer-membrane protein (MOMP), a hydrophilic cysteine-rich 60 kDa protein, and a low molecular weight cysteine-rich lipoprotein.
- *C. trachomatis* contains two human biological variants (biovars): trachoma and LGV, and one biovar infecting mice. Fifteen serologic variants (serovars) have been identified in trachoma biovar. MOMP confers serovar specificity.
- Chlamydiaceae species are characterized by a biphasic developmental cycle. The infectious EB form is typically 0.2–0.6 µm in diameter and resistant to unfavorable environmental conditions outside of their eukaryotic host cells.
- EBs bind to the epithelial cells directly via cellular proteoglycans with heparan sulfate (HS) moieties (HSPGs). Additionally, *trachomatis* EB form is able to adhere to and enter cells indirectly through binding with fibroblast growth factor 2 (FGF2). This complex then interacts with the FGF2 receptor, which mediates EB internalization into cells.
- EB entry is followed by translocation of EB-containing endosomes to the perinuclear region and their homotypic fusion with each other forming a fusion vacuole – a nascent inclusion. EBs accumulate in an inclusion where they use a supply of nutrients via Golgi apparatus and mature into the RB form.
- *C. trachomatis* is dependent on its host for survival and hijacks host-cell metabolism particularly glycolytic enzymes, aldolase A, pyruvate kinase and lactate dehydrogenase, which are enriched at the *C. trachomatis* inclusion membrane during infection. The increased requirement for glutamine, important for the growth of *C. trachomatis* in infected cells is achieved by reprogramming the glutamine metabolism.
- The RB form (up to 1.5 µm in diameter) multiplies by binary fission. After several rounds of replication during 48–72 hours, the RB form reverts to the infectious EB form. Eventually, the EBs are released through the extrusion of the inclusions by reverse endocytosis or through apoptosis or are released after cell lysis.

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- *C. trachomatis* (serovars D–K) is sexually transmitted in the EB form, usually through vaginal intercourse but occasionally can be transmitted by oral and anal sex. Spread to the fallopian tubes in females, it can lead to pelvic inflammatory disease (PID). It can also lead to conjunctivitis through autoinoculation. Genital infection of *C. trachomatis* (serovars D–K) is the leading bacterial cause of sexually transmitted infection with over 50 million new cases occurring yearly worldwide.
- Ocular infection of *C. trachomatis* (serovars A–C) is spread in areas of poverty and overcrowding. Every year *C. trachomatis* is a major cause of 500000 cases of trachoma worldwide, approximately 85 million people worldwide have active trachoma, more than 10 million have trichiasis (inturned eyelashes that touch the globe), and about 6 million people suffer visual loss and blindness.
- Infection can be transmitted from eye-to-eye by fingers, shared cloths or towels, by eye-seeking flies, and by droplets (coughing or sneezing).
- *C. trachomatis* (biovar LGV) causes sexually transmitted disease that is prevalent in Africa, Asia, and South America. Humans are the only natural host.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- Both innate and adaptive immune responses are induced during *C. trachomatis* infection, but they are usually inefficient at controlling the infection. This may lead to the partial clearance of the pathogen from the body resulting in bacterial persistence, chronic inflammation, tissue damage, and severe clinical symptoms.
- Chlamydial PAMPs are recognized by multiple PRRs, particularly TLR2 which was shown to be activated by MOMPs and heat shock protein 60 (Hsp60), during the productive infection. Infection with *Chlamydia* induces production of secretory IgA and circulatory IgM and IgG antibodies mostly directed to MOMP and Hsp60. IgG antibodies bound to the bacterial MOMP invoke antibody-dependent cellular cytotoxicity (ADCC) by **natural killer** (**NK**) cells.
- Dendritic cells (DCs) in response to the infection release IL-12 and drive Th1 cell development and hence **production of interferon γ** (**IFN-γ**) – the major inhibitory cytokine for *Chlamydia* (see below). Recent data suggests that to counteract this, *C. trachomatis* up-regulates expression of PD-L1 on the DCs in the uterus. Its interaction with PD-1 receptor on T cells might lead to blocking of T-cell responses.
- One of the most powerful anti-chlamydial T-cell-mediated immune mechanisms is production of the Th1 cytokine IFN-γ. IFN-γ can limit *C. trachomatis* infection by activating macrophages, up-regulating expression of MHC, suppressing tryptophan production in the host cells, enhancing production of nitric oxide, and down-regulating transferrin receptor on the host cells.
- Infection does not stimulate long-lasting immunity and repeated episodes of infection are common. Re-infection results in an inflammatory response and subsequent tissue damage.
- One of the reasons for poor anti-chlamydial immunity is the ability of the pathogen to evade or block host immune responses by intracellular location, down-regulation of MHC class I molecules, prevention of the formation of phagolysosome, and induction of apoptosis of cytotoxic T cells.
- Pathogenesis is a result of a direct killing of infected cells and of chronic inflammation. The chronic inflammation induced by *C. trachomatis* infection leads to episodes of PID or conjunctivitis resulting in tubal infertility or blindness, respectively.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Urogenital infection in men causes urethritis, which can produce a discharge from the penis or pain and burning sensation when urinating. If not treated, it can lead to epididymitis and, rarely, sterility.
- Urogenital infection in women leads to urethral discharge, dysuria and pyuria, urethritis, and cervical discharge and friability. If untreated, the chlamydial infection may spread through the uterus to the fallopian tubes, causing salpingitis, infertility, or ectopic pregnancy.
- A severe complication of the sexually transmitted *C. trachomatis* is PID.
- Inclusion conjunctivitis is associated with genital infections transferred from the genital tract to the eye by contaminated hands. The main symptom is a sensation of a foreign body in the eye, redness, irritation.
- Infants infected with *C. trachomatis* vertically from their mother at birth can develop pneumonia, which presents as a tachypnea, staccato cough and is often preceded by conjunctivitis.
- The first symptom of LGV is the development of a primary lesion, often in the penis or vagina. Several weeks after the primary lesion, patients develop painful inguinal and/or femoral lymphadenopathy, fever, headache, and myalgia followed by inflammation of the draining lymph nodes.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Clinical diagnosis of genital chlamydial infection in women is based on age, sexual activity, more than one sexual partner, mucopurulent vaginal discharge, burning when passing urine, friable cervix or bleeding after sex or between menstrual periods, lower abdominal pain, or pain during sexual intercourse.
- Clinical diagnosis of LGV is based on the clinical presentation and the course of the disease assisted by differential diagnostic and laboratory tests.

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- Eye examination for the clinical signs of trachoma involves careful inspection of the lashes and cornea, then reversion of the upper lid and inspection of the upper tarsal conjunctiva. A grading system is used by the WHO for the assessment of the prevalence and severity of trachoma.
- Nucleic acid amplification tests (NAATs), including polymerase chain reaction (PCR), are now increasingly used for diagnosis and differential diagnosis of chlamydial infections, although their wider use in screening strategies worldwide is limited by the costs and complexity of the equipment required.
- Cell culture has traditionally been the gold standard for the diagnosis of *C. trachomatis* and is specific for this pathogen. However, it is now replaced by NAATs.
- Antibody-based laboratory techniques – immunofluorescence and enzyme-linked immunosorbent assay (ELISA) – are widely used for determining the serovars in screening programs.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- For urogenital infections, the antibiotic doxycycline (Doryx®, Vibramycin®) is given 100 mg twice daily for 7 days. Alternative CDC-recommended regimen include azithromycin, 1 g orally in a single dose or levofloxacin. 500 mg orally once daily for 7 days. Pregnant women are given a single 1 g dose of azithromycin orally or amoxicillin 500 mg orally 3 times/day for 7 days. Neonates – erythromycin base or ethylsuccinate 50 mg/kg body weight/day orally, divided into 4 doses daily for 14 days. The same regimen is used for infants. In addition, they can be prescribed an azithromycin suspension, 20 mg/kg body weight/day orally, 1 dose daily for 3 days.
- All sexual partners should be tested for chlamydial infection and treated if positive. Patients should also be tested for other sexually transmitted infections.
- Environmental improvement includes removing the risk factors for transmission of infection such as flies, the presence of cattle pens, and crowded living conditions.
- Safe sexual practices, using a condom during sexual intercourse, and prompt treatment of infected patients and their sexual partners can prevent genital infections with *C. trachomatis*.
- Repeat infections confer an elevated risk for PID and other complications among women. As a preventive measure, men and women who have been treated for chlamydia should be retested approximately 3 months after treatment, or whenever persons next seek medical care during the first year after initial treatment.
- The major target for vaccine development currently is the multivalent MOMP-based vaccine in combination with adjuvants. For the delivery platforms nanoparticles, Hepatitis B core antigen and Neisseria lactamicaporin B as career molecules, adenoviral and mRNA vectors are considered.

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Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.