



Review

The Immunology of *Chlamydia trachomatis*

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Abstract. *Chlamydia trachomatis* (*C. trachomatis*) is one of the most common sexually transmitted bacterial agents. What distinguishes it from other organisms is its intracellular reproductive cycle. Up to now, four antigens have been identified in the *Chlamydia* genus: genus-specific antigen as well as species-specific, type-specific and subspecies-specific. *C. trachomatis* is a powerful immunogen which stimulates the host's immunological processes. The intracellular parasitism of the bacteria is the basis for both symptomatic or asymptomatic infection as well as for chronic ones. The primary infection leads to a local inflammatory reaction due to penetration and reproduction of the bacteria in the epithelial cells and to IgA secretory antibody production. In most cases the host's reaction to the primary infection is transient and does not cause tissue damage. In the course of chronic infection or reinfection, the most important processes are those of delayed hypersensitivity, which lead to a fast and intense immunological reaction of specifically sensitized Th1 lymphocytes. This reaction leads to progressive damage of the epithelial cells and to cicatrization and fibrosis, which means irreversible complications. Interferon γ is of special importance in the process of *C. trachomatis* infection. High concentrations of it inhibit the bacteria's reproductive cycle, while lower concentrations promote the development of atypical, non-contagious forms of *Chlamydia* of diminished metabolic activity and altered antigenicity. The chlamydial heat shock proteins are considered to be of great importance lately. Their molecular weights of 60 and 10 kDa are a powerful stimulant of immunological reactions and show significant homology (40–90%) to human and other bacterial heat shock proteins.

Key words: *Chlamydia trachomatis*; primary infection; latency; reinfection.

Introduction

Chlamydia trachomatis (*C. trachomatis*) is one of the most common sexually transmitted bacterial agents. Each year 90 million *C. trachomatis* infections appear in the world, including over 4 million in the United States of America and 5.5 million in Europe²⁰. The wide spread of these infections and their chronic oligo-symptomatic or asymptomatic course pose a great

clinical and epidemiological problem. These “silent infections” may lead to dangerous, frequently irreversible complications both in women and men whose treatment is extremely expensive⁴⁰. What distinguishes *Chlamydia* from other bacteria is their intracellular parasitism, responsible for the chronic and asymptomatic course of infection. The immunological events in the infected host have not been fully elucidated. Multicenter studies have been continuously performed to determine the role

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of the respective immunological mechanisms in acute and chronic *C. trachomatis* infections and their distant sequelae.

Development Cycle of *Chlamydia*

The unique development cycle of *Chlamydia*, lasting from 48 to 72 h, unusual for other bacteria, is based on the co-existence of two morphological forms, the so-called elementary body (EB) and the reticulate body (RB)⁴⁰. The EB, granular in shape and 0.2–0.4 µm in diameter, is an infectious form, metabolically inactive and unable to replicate. The RB or initiating body, oval in shape and 0.8–1.3 µm in diameter, is a vegetative, non-infectious, metabolically active intracellular form. The developmental cycle can be divided into several stages (Fig. 1):

- EB binding with a host cell,
- EB penetration into a host cell via endocytosis with the involvement of membrane adhesion proteins,
- formation of intracytoplasmic inclusions (after 4–8 h),
- conversion of EB into RB (after 8–12 h),
- replication of RB by binary fission (after 20 h),
- conversion of RB into EB (after 30–40 h),
- release of EB via cell lysis or exocytosis (after 48–72 h).

Antigenic Structure of *C. trachomatis*

C. trachomatis is a strong immunogen that stimulates immunological processes of the host. The immune

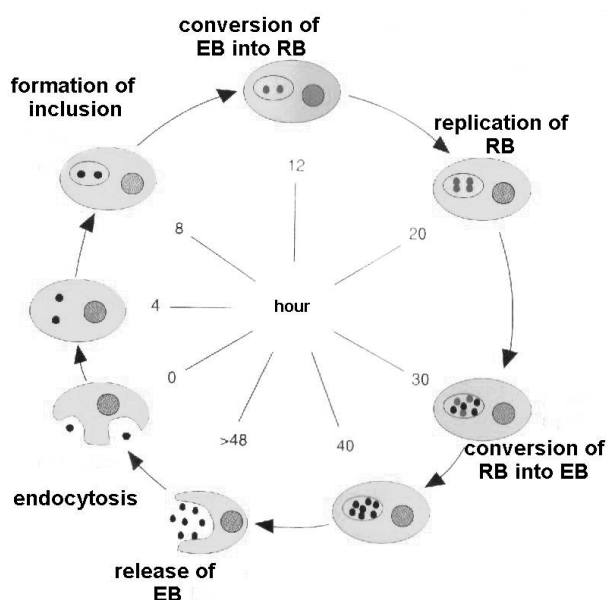


Fig. 1. Development cycle of *Chlamydiae*

response involves a number of bacterial cell antigens which are not fully known yet. At present, four antigen groups are distinguished within the *Chlamydia* genus (Fig. 2). Group-specific antigen, shared by all *Chlamydia* species, the best is known. Its main component, a thermostable polysaccharide complex, contains two constituents: lipopolysaccharide (LPS) and glycolipid (GLXA)^{6,33}. In chemical structure, molecular weight and function, the group-specific antigen resembles the LPS antigen of certain Gram-negative bacteria and yeast-like fungi. Species-specific antigens, varying according to the *Chlamydia* species, have a protein structure and are thermolabile. They contain a few types of proteins of various molecular weight, of which the most important are the major outer membrane protein (MOMP) of 38–42 kDa, constituting 60% of all outer membrane proteins; 60–62 and 155 kDa proteins; and the so-called chlamydial heat shock proteins (C-HSP) of 10 and 60 kDa³⁵. The third antigen group is of type-specific antigens, characteristic of the respective serotypes within the *C. trachomatis* species, most likely polypeptides of 30 kDa and thermolabile by nature³². The last type of antigen, also of polypeptide structure, refers to subspecies-specific antigens³⁴. Based on their presence, the respective serotypes of *C. trachomatis* have been categorized as two major subspecies, the so-called group B and group C.

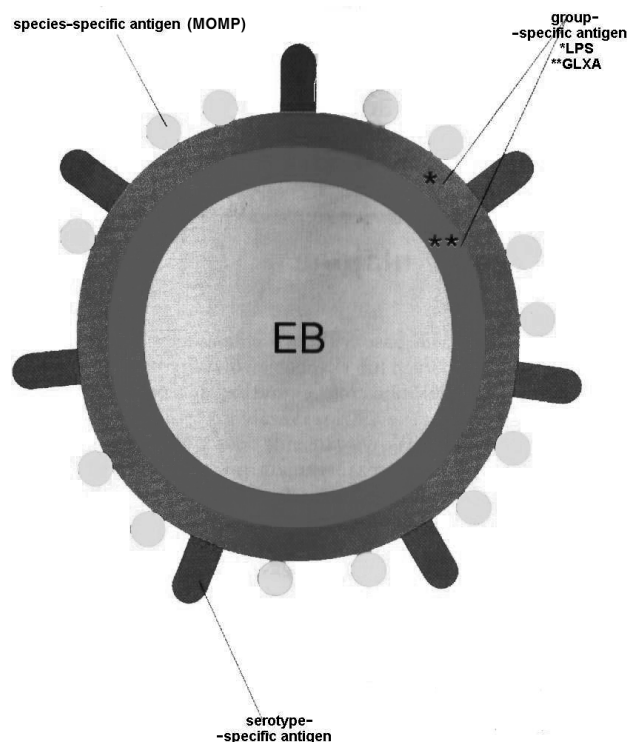


Fig. 2. Antigenic structure of *C. trachomatis*

Primary Infection

Chlamydia infection involves mechanisms of both nonspecific and specific, humoral and cellular resistance, of varied intensity depending partially on the type of infection.

Primary infection of *C. trachomatis* usually manifests itself as urethritis in men and/or cervicitis in women⁴⁰. Its course is most frequently oligosymptomatic or asymptomatic. In most cases the infection is accompanied by the presence of polymorphonuclear leukocytes in the secrete from the urethra or uterine cervix. During the early phase of infection, the bacteria are found in approximately 70% of patients. In 32% of men with detected but untreated chlamydial infection, PARKS et al.²⁶ found no microorganisms in a urethra swab after 45 days, suggesting the possibility of idiopathic extinction of the infection.

During primary infection a serial infection of mucosal cells may occur. The infected epithelial cells and those being damaged secrete numerous proinflammatory chemokines and cytokines, including interleukin (IL)-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), growth regulated oncogene, and tumor necrosis factor (TNF)- α ^{18, 19, 31}. Contrary to the rapid secretion of cytokines in infections induced by extracellular microorganisms, in the case of *Chlamydia* the secretion is delayed up to 20–24 h after infection onset¹⁹. The released cytokines cause vasodilation, increased endothelial permeability, activation and migration of neutrophils, monocytes and, to a lesser extent, macrophages and T lymphocytes, elevated expression of adhesion molecules, and stimulation of other cells to secrete cytokines. The local inflammatory reaction is most intense on days 2–4 after infection onset. A special role has been ascribed to IL-1, which is secreted earliest by undamaged cells and stimulates the secretion of other cytokines by non-infected adjacent cells, and TNF- α release inductor, a strong initiator of the inflammatory process³¹. The maximum concentration of this cytokine is observed 48 h after infection onset, persists for 4 days and is then gradually reduced. At the same time, *Chlamydia* pass via lymphatic vessels to local lymph nodes, which in many cases become enlarged.

Some elementary bodies, released by decaying epithelial cells, are phagocytized by neutrophils, probably through the fusion of phagosomes and granulocyte lysosomes and the formation of the so-called phagolysosomes. The bactericidal action of neutrophils is associated with the production of hydrogen peroxide and secretion of myeloperoxidase³⁹. An important role dur-

ing the early phase of the infection has been ascribed to T lymphocytes, mainly T helper (Th)1, which, due to *Chlamydia* antigen-induced activation, secrete, for example IFN- γ , necessary for infection regression¹⁵. IFN- γ increases the potential of various phagocytes, especially monocytes and macrophages, to destroy *Chlamydia* and stimulates the secretion of other cytokines, including IL-1³⁷. IL-1, in turn, by stimulating the secretion of IL-2 by Th1 lymphocytes, causes increased replication of cytotoxic lymphocytes and natural killer cells. The production of secretive immunoglobulin A class antibodies also plays a role in the neutralization of primary infection. Locally produced antibodies limit the spread of chlamydial infection, but do not eliminate the bacteria completely. In most cases, the immune response of the host to the primary infection is transitory and is not associated with tissue damage.

Chronic Infection, Recurrent Infection, Reinfection

Chronic infection, associated with the persistence of the bacteria in host cells, recurrent infection or reinfection are much more dangerous. The inflammatory state develops in a considerably shorter time and is more intense, which is associated with the presence of specifically sensitized Th1 lymphocytes. A long-term or recurrent stimulatory action of *Chlamydia* antigens results in a delayed hypersensitivity reaction or the more seldom type III hypersensitivity reaction (Arthus reaction)^{23, 27}. Antibodies are not involved in the delayed (cellular) type reaction developing within 24–48 h due to antigen interaction with specifically sensitized Th1 lymphocytes. In a type III reaction, immune complexes are formed in the circulation or tissues as the result of interactions of soluble antigen with a specific antibody. Processes which occur during these immune reactions lead to tissue damage, fibrosis and cicatrization within the affected organs. The consequences can be severe and often irreversible, e.g. trachoma (conjunctiva cicatrization, blepharon deformation, loss of sight), pelvic inflammatory disease (PID) leading to mechanical infertility, ectopic pregnancy, chronic pelvic pains, and chronic urethritis (urethrostenosis)^{7, 40}. Since chlamydial infection is frequently asymptomatic, the sequelae are common and pose serious health and economic problems. Epidemiological data indicate that every year approximately one million cases of salpingitis appear in Europe, including 600,000 of chlamydial etiology, of which 120,000 cases result in mechanical infertility¹⁰. After a single episode of salpingitis about 1 patient in

10 becomes infertile because of tubalocclusion. After two or three episodes, infertility ensues in about 35 and 70% cases, respectively¹⁰. In chronic infections in women with salpingitis, *Chlamydia* can be more frequently detected in the material collected from the salpinx or endometrium (40–60%)²⁵ than from the uterine cervix (up to 20%)¹¹. A substantial disproportion between slight clinical symptoms in PID patients and considerable changes observed during laparoscopy is a characteristic feature of *C. trachomatis* infection.

Chronic infection may result from lack of treatment or improper therapeutic management. The role of penicillin and its derivatives has been emphasized¹³. Dietary factors, such as an insufficient supply of tryptophan, L-isoleucine, and cysteine in the diet, as well as certain cytokines, e.g. interferon (IFN)- γ , TNF- α and transforming growth factor (TGF)- β , play a significant role^{1, 4, 36}. They all lead to disorders in the developmental cycle of the bacteria, including RB maturation delay or inhibition of their differentiation into infectious EB and, thus, to the growth of atypical forms of *Chlamydia*⁵. The atypical forms are larger, non-infectious, have reduced metabolic activity, and do not replicate, yet remain alive. They display a different antigenic structure, characterized by a reduced number of MOMP and LPS and invariable amounts of the pathogenic C-HSP 60 kDa, a strong stimulator of immune reactions. The role of the C-HSP 10 kDa, in the pathogenesis of complications in *C. trachomatis* infection has been emphasized lately^{8, 12, 16, 28}. Irreversible sequelae occur mainly due to the delayed hypersensitivity reactions induced by this strong antigen or as a result of an autoimmune reaction associated with a similarity between the chlamydial and human HSP (about 50% homology)³⁸. In patients with complications, a decrease in the production of anti-MOMP antibodies with protective properties and an increased production of pathogenic anti-C-HSP antibodies are frequently observed²⁴.

Chronic and occult infections pose a number of diagnostic and therapeutic problems³. Because of the variable antigenic structure of the bacteria, the routine diagnostic methods used to treat chlamydial infections (ELISA, DIF, cell culture) do not always identify the atypical forms. Therefore, the role of molecular methods which detect the genetic material of *Chlamydia* and the contribution of serological methods have been emphasized. Persistent forms are also slightly antibiotic-sensitive, which seems to be associated with reduced MOMP count and thus decreased transport of antibiotics to the cell. Therefore, in the case of chronic infections, therapy frequently results in failure.

Recurrence and reinfection constitute another problem. Reinfection is due to repeated infection, while recurrence is caused by the presence of a *Chlamydia* reservoir in the lymph nodes and spleen. Determination of the serotypes of patients infected with *C. trachomatis* and their partners can help differentiate between these two cases. Reports on a large number of recurrences (20–39%) observed both in men and in women with chlamydial infection a few months after therapy may sound alarming^{14, 21}. A special role in disease recurrence has been ascribed to macrophages, in which *Chlamydia* can persist even for 10 days. They circulate with macrophages round the body, finding a temporary shelter in the lymph nodes, spleen and serous cavities. Affected by agents which induce chronic infection, e.g. IFN- γ , the bacteria are “hypnotized” in macrophages and their genetic material is transmitted onto further generations of these cells¹⁹. Attempts to limit the number of recurrences in humans have brought about only partial success. Recurrences in the cases of trachoma or lymphogranuloma venereum occur even after repeated or long-term treatment.

In the course of chlamydial infection the phenomenon of acquired protective immunity remains a controversy¹⁷. *C. trachomatis* infections both of the vision organ and urogenital tract affect mainly young people. The less common spread of the infection in older people may be caused by low exposure to *Chlamydia* and by physiological changes which reduce sensitivity but, on the other hand, it can be due to resistance acquired during natural exposure. Observations of patients with trachoma showed that recurrences were more frequent in the younger age group and the active period of the disease was prolonged compared with older patients, who showed more intensified changes². In acquired immunity, cellular immunity with late complications plays the major role. Humoral response is of minor significance³⁰. Although the anti-MOMP antibodies in the serum and/or tears of trachoma patients inhibit the growth of bacteria in cell culture, they play only a slightly protective role in reinfection^{9, 22, 29}, hence the lack of efficacy of the vaccine based on the major outer membrane antigens.

Despite many years of study of immunological phenomena in the course of *C. trachomatis* infection, many problems still remain unexplained. Further investigations into correlations between protective mechanisms and pathological phenomena in infected patients seem necessary. The attempts to generate a vaccine hitherto have led to irreversible damage to the infection site. A future vaccine may eliminate the dangerous sequelae of *C. trachomatis* infection.

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