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Review

Current problems of perinatal *Chlamydia trachomatis* infections

Kei Numazaki*

Address: Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Japan

Email: Kei Numazaki* - numazaki@sapmed.ac.jp

* Corresponding author

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Abstract

Chlamydia trachomatis has been recognized as a pathogen of trachoma, nongonococcal urethritis, salpingitis, endocervicitis, pelvic inflammatory disease, inclusion conjunctivitis of neonates, follicular conjunctivitis of adults, infantile pneumonia and associated conditions. Chlamydial infections during pregnancy may also cause a variety of perinatal complications. Different antigenic strains of *C. trachomatis* from endocervical, nasopharyngeal and conjunctival origins have been associated with different clinical conditions. Control programs emphasizing early diagnosis, targeted screening, and effective treatment will lead to an eventual decline in the incidence of perinatal chlamydial infection. This review focuses on current problems of perinatal *C. trachomatis* infections in the aspects of microbiological and immunological pathogenesis.

Introduction

Chlamydiae are obligate intracellular bacteria that have been associated with a wide spectrum of human diseases. Currently they can be divided into four groups; *C. trachomatis, C. psittaci, C. pneumoniae* and *C. pecorum*. *C. trachomatis* is the causal agent of trachoma which is an important cause of blindness and affects approximately 500 million people, mainly in developing countries. *C. trachomatis* has been recognized as a pathogen of nongonococcal urethritis (NGU), salpingitis, endocervicitis, pelvic inflammatory disease (PID), lymphogranuloma venereum (LGV), inclusion conjunctivitis of neonates, follicular conjunctivitis of adults, infantile pneumonia and associated conditions. Psittacosis is a systemic infection caused by *C. psittaci* and is common in apparently healthy birds and domestic animals. *C. pneumoniae* is a common etiological agent causing acute infection of the respiratory tract and has also been associated with coronary artery disease and atherosclerosis.

The developmental cycle of Chlamydiae is unique. Infectious extracellular form, but metabolically inactive elementary bodies (EB), attach to the host cell and are taken up by endocytosis. Within 6 to 8 hours EB become noninfectious, metabolically active reticulate bodies (RB) which replicate by binary fission. Both EB and RB are totally dependent on host nucleotide pools as they are incapable of de novo nucleotide biosynthesis. They also can synthesize their own proteins by using the host cell's energy-generating apparatus.

Pneumonia due to *C. trachomatis* is a disease limited for the most part to infants under 6 months of age. [1,2]*C. pneumoniae* usually causes pneumonia and other respiratory infections in children, adolescents and adults. [3] It has been suggested that *C. trachomatis* infection in pregnant women may be related to premature labor and to perinatal death. Although transmission of the organism from mothers to their infants generally occurs at the time

of delivery with passage of the infant through the infected cervix, the possibility of intrauterine infection at late pregnancy has been reported. [4]

Genital or ophthalmic chlamydial infections still have been recognized as a major public health problem throughout the world. This review focuses on current problems of perinatal *C. trachomatis* infections.

Immune responses to *C. trachomatis*

Studies in trachoma-endemic areas have found that the duration of untreated infection is shorter in older people, which suggests that acquired immunity has a role in the recovery of infection. [5] As cultures of lung biopsies from infants with *C. trachomatis* pneumonia have frequently failed to yield the organism, immunological reactions of the host to these agents appear to be more important than the direct effects of *C. trachomatis* or *C. pneumoniae* in the pathogenesis of chlamydial pneumonias. [6]

Cellular immune response to chlamydial antigens of the Th1 type is important. [7,8] Chlamydial infections induce inflammatory changes that might induce modulation of secretion of cytokines. The Th1 cytokine interferons inhibit chlamydial replication in vitro by inducing the degradation of tryptophan, resulting in a state of chlamydial latency, with developmental arrest at the reticulate-body stage. [9]

It was also postulated that activation of specific suppressor/cytotoxic CD8+ cells might play a part in the persistence of chlamydial infections. [10,11] Some degree of differentiation may be necessary for permissive infection of phagocytic cells with Chlamydiae. It is likely that specific cellular interactions as well as secretion of cytokines are important for the pathogenesis of chlamydial infections.

Chlamydiae, intracellular organisms, survive and grow in both epithelial and phagocytic cells. *C. trachomatis* serovars associated with endemic trachoma (A, B, Ba or C-complex) preferentially infect mucosal columnar epithelial cells of the genital tract and eye. In contrast, the LGV serovars primarily infect lymph nodes causing more systemic infections. LGV is caused by serovars L1, L2, and L3 which are more virulent in animal models than the more prevalent serovars A to K of *C. trachomatis*, and more invasive in humans. The LGV serovars infect predominantly monocytes and macrophages, pass through the epithelial surface to regional lymph nodes, and may cause disseminated infection. *C. pneumoniae* is a common etiological agent in respiratory-tract infections, including pneumonia. [12]

Although the elevated serum antibodies and the presence of circulating *Chlamydia* – specific immune complexes have been found in several chronic infections, the role of mononuclear phagocytes in the pathogenesis of chlamydial infections has yet to be clarified. Despite the various pathogenic effects of Chlamydiae, there is only limited direct evidence that chlamydial infections occur to a significant extent in monocytes and macrophages. It is likely that mononuclear phagocytes also play an important role in the persistence of chronic chlamydial infections and act as reservoirs and vehicles for chlamydial dissemination in the infected hosts.

Alveolar macrophages are thought to be the major immune response-regulating cells of the lung. The limitation of occurrence of *C. trachomatis* pneumonia to early infancy and of *C. pneumoniae* pneumonia to children more than 2 years-old, adolescents and adults might be due in part to the possible maturational or functional difference between alveolar or peripheral blood macrophages of infants and adults. [13] Theoretically, Chlamydiae might enter mononuclear phagocytes in three ways: nonspecific phagocytosis, specific receptor-mediated binding of Chlamydiae to the cell membrane and subsequent fusion, or by receptor-mediated endocytosis of antibodies complexed with Chlamydiae. Chlamydial receptor-mediated binding involves a sulphated glycosaminoglycan (GAG)-dependent mechanism of microbial infection for mammalian cells. [14] Chlamydiae appear to mimic heparan sulphate that is the naturally occurring ligand for GAG. Heparan sulphate-like-mediated interactions between *C. trachomatis* and eukaryotic cells are essential for infectivity.

C. trachomatis can be utilized as intracellular microbial targets to characterize the antimicrobial mechanisms of the human monocytes and activated macrophages. It was speculated that interferons also might play a role in producing or perpetuating persistent chlamydial infection by maintaining the organisms as immature forms within intracytoplasmic inclusions. The infection and persistence of LGV biobar of *C. trachomatis* in monocytes-macrophages may have critical roles in the pathogenesis and immunological reactions in systemic infections. [15] Organisms from the LGV biovar survived in mononuclear phagocytes infected after 8 days or more in culture, whereas those from the trachoma biovar continued to be killed by such cells. [16] Macrophages derived from human peripheral blood mononuclear cells (PBMC) may not kill *C. trachomatis* L and other LGV strains, but may kill trachoma serovars.

The chlamydial 60-kDa heat-shock protein (CHSP 60) may also have some roles in inducing nonspecific hypergammaglobulinemia, delayed-type hypersensitivity reac-

tion, and autoimmune reaction associated with chlamydial infections. [17,18] Serum antibodies to hsp60 are not only associated with the presence of conjunctival scarring but also with PID, ectopic pregnancy and tubal infertility in human beings. [19] Whether the immune response to this protein has a role in the pathogenesis of scarring, or whether serum antibody to hsp60 is merely a marker of persistent infection that itself is more likely to give rise to scarring, is not clear.

Recent findings in areas of *C. trachomatis* immunopathogenesis further delineate the complex pathogen-host relationship in disease and may have implications for vaccine design. [12] A 57 kDa chlamydial protein was identified as a heat shock protein of the GroEL family of stress proteins. Polymorphism of the major outer membrane protein (MOMP) showed the evidence for the genetic susceptibility to the disease and the association of antibody response to a 60 kDa chlamydial heat shock protein (CHSP 60) may develop adverse sequelae following chlamydial infections. The risk factors associated with CHSP 60 antibody response may be similar to those for repeated chlamydial infections. Polymorphism of MOMP are actually thought to be associated with immune escape and allelic variances.

At present, it remains unclear whether antibody response to CHSP 60 is involved in the pathogenesis of chlamydial ocular infections or a marker of persistent chlamydial infections. T cell responses to chlamydial antigens, including CHSP 60, were more depressed in patients with trachoma than in those who recovered from infection without sequelae. In adequate responses of memory T-cells in mucosal immune system may be related in the pathogenesis of *C. trachomatis* infections.

Different pathogenicities between serovars of *C. trachomatis*

Eighteen serovars of *C. trachomatis* were classified by the microimmunofluorescence (MIF) test. [20] The epitopes that distinguish serovars reside principally on MOMP. The sequences of the MOMP gene which includes four variable domains (VDs) have been determined for 15 of 18 serovars. [21] The serovars D though K have generally been isolated from the genital tract. The polymerase chain reaction (PCR) to amplify a large part of the MOMP gene (*omp1*), including four VDs, and restriction fragment length polymorphism (RFLP) can be used to determine the serotypes of *C. trachomatis*. [22,23] However, the polymorphism in the *omp1* gene was considerable. [24]

The method of PCR-RFLP for serotyping also allows quick and objective identification of *C. trachomatis*. Theoretically, application of similar approach for identification and typing to *C. pneumoniae* or *C. psittaci* serovars will likely prove fruitful. However, the choice of a gene with demonstrated heterogeneity, such as MOMP of *C. trachomatis* will be necessary. The genome of the organism has been sequenced. [15] Trachoma strains but not genital isolates carry a deletion or frame shift mutation in a variable region encoding genes for tryptophan synthesis.[16]

C. trachomatis strains of differing in infection organ-tropism correlated with inactivating mutations in the pathogen's tryptophan synthase (trpBA) genes. Serovar B isolated from the genital tract were found to possess a functional trpBA provided further persuasive evidence of this association. [25]. These results argue that there is an important host-parasite relationship between chlamydial genital strains and the human host that determines organotropism of infection and the pathophysiology of disease. It was speculate that this relationship involves the production of indole by components of the vaginal microbial flora, allowing Chlamydiae to escape IFN-gamma-mediated eradication and thus establish persistent infection.

The relationship between serotypes and clinical manifestations is controversial. Serotype E has most frequently been associated with asymptomatic infection. Stability in *omp1* sequences of serotypes E and F has been reported.[26,27] In subjects infected with serotype E, a T-cell epitope in VD 3 is recognized significantly less often than in subjects infected with other serotypes.[28] Serotype E has reached an equilibrium state with its host in which optimum epitope arrangements have been reached, and further changes do not result in a transmission advantage. [29] There may be inherent differences in the antigenic flexibility of the serotypes, because serotypes D, G, and J are more variable than E and F.

Manifestations of ocular disease due to infection with *C trachomatis* depend on the age of the host. Infection of serovars of urogenital origin of an infant's eyes during delivery leads to neonatal conjunctivitis (ophthalmia neonatorum). Adults infected with serovars of urogenital tract-origin can develop a self-limiting follicular conjunctivitis (adult inclusion conjunctivitis).

Although Japan was thought to be belong to an endemic area of trachoma, the serovars that we identified were similar to those reported in other studies from non-trachoma-endemic areas [30,31] These identified serovars were thought to be urogenital tract-origin. Chlamydial pneumonias of these Japanese infants were speculated to be caused by mother-to-infant transvaginal transmission of *C. trachomatis*.

Serotyping using monoclonal antibodies recognizing antigenic determinants located on MOMP is also standard

method for characterization of *C. trachomatis* clinical isolates. We found the presence of unclassified serovars of *C. trachomatis* both by PCR-RFLP and the reactive pattern by MIF using monoclonal antibodies obtained from Japanese infants and neonates. [32,33]

The sequences of MOMP gene for all 15 serovars allowed the construction of restriction endonuclease cleavage-site maps that confirm the fragment-size patterns observed by electrophoresis. [20] Sequencing the entire MOMP gene and cataloguing the sequences of VDs of all serovars has confirmed the molecular basis of serotyping procedure and provided a method for determining serovars by PCR-RFLP. [22] Not only 15 classical serovars but also at least four serovariants (Da, Ia, L, and Ga) have been described. Genovariants have been also reported for most of serovars. [30] There is no clear distinction between the serovars of endemic trachoma from those associated with STD.

Antigenic variations of *C. trachomatis* were also considered among the strains from nasopharyngeal and conjunctival origins. Only limited number of variants by serological methods has been reported. [34] A larger study involving many more clinical isolates and a battery of restriction enzymes may be necessary to catalog unclassified serovars. Characterization of unclassified variants will allow more detailed epidemiological studies of perinatal *C. trachomatis* infections.

C. trachomatis infection and perinatal complications

Chlamydial infections during pregnancy may also cause a variety of perinatal complications. It was reported that the rates of seropositivity to *C. trachomatis* during pregnancy were significantly higher in mothers who had given birth to infants with complications than in matched control. [35,36] Several investigators have reported that 2 to 20 % of pregnant women have *C. trachomatis* in their endocervix. Pregnant women who carry *C. trachomatis* in their genital tract may suffer from a general disturbance of immunoregulation. It has been suggested that *C. trachomatis* infection in pregnant women may be related to premature labor and to perinatal death.

Although transmission of the organism from mothers to their infants generally occurs at the time of delivery with passage of the infant through the infected cervix, the possibility of intrauterine infection at late pregnancy has been reported. [4] Chorioamnionitis is a frequent finding in prematurity and respiratory insufficiency in premature babies and may be attributable to intrauterine infection. *C. trachomatis* can lead to chorioamniotic infection. [37] The frequency of chorioamnionitis and meconium-stained amniotic fluid was also higher in the anti *C. trachomatis* IgM antibody-positive pregnant women. [35] Gencay et al. [35] reported that the rates of seropositivity for IgM to *C. trachomatis* during pregnancy were significantly higher in mothers who had given birth to infants with complications than in matched controls. Low-birthweight infants and premature rupture of membranes occurred more frequently in women infected with *C. trachomatis*. The fact that neonates having the symptoms of chronic lung diseases also manifest elevated serum IgM levels suggested that these respiratory-tract disorders arise from infections during late pregnancy [1,38]

In their article on factors associated with recurrence of preterm delivery, Adams et al. [39] conclude that recurrence of preterm delivery contribute a notable portion of all preterm deliveries, especially at the shortest gestation. They also report that short cervical length, the detection of fetal fibronectin, and bacterial vaginosis during pregnancy increase the risk of spontaneous preterm delivery. Carey et al. [40] report on the largest randomized trial of antibiotics for the prevention of preterm delivery. They conclude that the treatment of asymptomatic bacterial vaginosis with metronidazole does not reduce the occurrence of preterm delivery or other adverse perinatal outcomes.

On the other hand, Lamont [41] comments that preterm labor is either physiologic, with a normal initiating factor occurring too early in pregnancy, or pathologic, occurring because of abnormal initiating factor, such as infection. Holzman et al. [42] suggest that an early maternal inflammatory response, linked to an increased risk of preterm birth, may manifest itself as a rise in maternal immunoglobulin production in mid-trimester. They report that IgM concentrations greater than the median in maternal serum at 15–19 weeks of pregnancy are strongly associated with delivery before 29 weeks. It was also reported that a maternal inflammatory response directed at a single antigen seems unlikely produce large changes in concentrations of total immunoglobulin isotypes.

The etiology of preterm delivery and whether recurrent preterm delivery share the same etiology as incident preterm deliveries remain elusive. Other factors, other than common vaginal or intrauterine and perinatal chlamydial infections, may contribute to produce high concentrations of serum immunoglobulins and cytokines associated with early preterm delivery. Early diagnosis and appropriate treatment of chlamydial infections may reduce these complications. [43,44] Although further studies in large number of populations are definitely necessary, detection of serum IgG and IgA antibodies to *C. trachomatis* during late stage of pregnancy is considered to permit more laboratories to diagnose perinatal chlamydial infections and also to be useful for the screening of infection.

Current aspects of chlamydial eye diseases

Serological tests are usually not useful in the diagnosis of ophthalmologic infection caused by *C. trachomatis*. This is because serum antibodies elicited by chlamydial infections are long lived and a positive antibody titer will not distinguish current infection from past one. However, high seropositivity of IgG and IgA antibodies in patients with active trachoma was considered as a result of recurrent infection of *C. trachomatis*. In *C. trachomatis* infection, immunopathology causes scarring of the conjunctivae as a consequence of reinfection and the delayed hypersensitivity has been implicated in the pathogenesis of blindness from trachoma. The exact mechanism by which trachoma is spread remains unclear.

Active trachoma is most commonly seen in children, and the complications leading to visual loss and blindness in adults, with several times excess risk for women.[45,46] The characteristics of households affected by trachoma are that they have young children and poor living conditions, specifically inadequate access to water and sanitation. Recent studies have shown that children younger than 5 years of age have the highest ocular chlamydial loads, and even those younger than 1 year old constitute a significant reservoir of infection. [47]

Repeated episodes of chlamydial infection associated with moraxella or other bacteria result in signs of chronic inflammation. Vascular infiltration of the upper cornea (pannus) is common but rarely progresses to affect vision. Such signs of active disease are seen mainly in young children, but also occur in older children and some adults. Lietman et al. [48] report that in areas where trachoma is moderately prevalent (<35% in children), it should be treated annually, but hyperendemic areas (>50% in children), it should be treated biannually. In less-developed countries, young children are the reservoir of infection, so some researchers have recommended treating only children under the age of 10 years.

Activities to control trachoma are interventions undertaken with the community, rather than treatment for individuals in medical facilities [47,48]. The aim of trachoma control can be to prevent visual loss and blindness; decrease the level of infection so that trachoma is no longer a public-health problem; or eliminate trachoma from a population. The "SAFE" strategy is used for the control of trachoma: surgery for in-turned lashes, antibiotics for active disease, facial cleanliness, and environmental improvement. By means of the SAFE strategy, WHO and its partners aim to eliminate trachoma as a public-health problem by the year 2020. Flies are suggested to be important vectors of trachoma. [49,50] In hyperendemic are, eye-to-eye transmission of *C. trachomatis* is speculated to be main route of transmission of trachoma.

Any serovar of *C. trachomatis* including urogenital tract-origin can cause inclusion conjunctivitis and the clinical manifestations of trachoma are thought to be due to the complex pathogen-host relationship in disease. Presence of both ocular and urogenital cycles of *C. trachomatis* infections were speculated. Repeated reinfection over many years causes dense scarring of the upper eyelid. The resultant inversion of the lashes abrades the eyeball, and the abrasion leads to corneal opacification and visual impairment. In hyperendemic areas, severe disease leading to scarring and blindness may be the result of frequent reinfection of different serovars of *C. trachomatis* including extraocular and urogenital tract-origin and mixed infection of bacteria.

Schachter et al. [51] reported that community-wide treatment with oral azithromycin markedly reduced *C. trachomatis* infection and clinical trachoma in endemic areas and might be an important approach to control of trachoma. They also reported that extraocular infections of *C. trachomatis* could be a source for reinfection of the eye. For the elimination of trachoma effective disease control program for extraocular especially urogenital chlamydial infections is also necessary. [52]

Conclusions

C. trachomatis sometimes causes serious disease in neonates who acquire the organism transvaginally or in utero. Perinatal *C. trachomatis* infection mainly refers to infection acquired during delivery through exposure to infected maternal genital secretions. Control programs emphasizing early diagnosis, targeted screening, and effective treatment will have led to an eventual decline in the incidence of chlamydial infections. Entirely new approaches to prevention and treatment of chlamydial infections in infants seem to be necessary, including antimicrobial interventions and the development of a vaccine strategy.

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