Chapter 2

Literature Review
2. LITERATURE REVIEW

2.1 HISTORY

Human diseases caused by *Chlamydia trachomatis* have been recognized since antiquity (Schachter and Dawson, 1978). The disease of trachoma was described in Egyptian papyrus some 3000 years ago as well as in old Chinese writings and in the work of Hippocrates. Lymphogranuloma venereum, a systemic disease was probably first described in the 18th century. Within a few years of Neisser’s discovery of the gonococcus in 1879 it had been realized that many men with acute urethritis showed no evidence of infection by *Neisseria gonorrhoeae*. The etiology of non-gonococcal urethritis (NGU) was unknown at that time. In 1907, Halberstaedter and von Prowazek described cytoplasmic inclusion bodies in epithelial scrapings from orangutans inoculated with materials from trachoma cases. Thinking they were protozoan, they named them "chlamydozoa" or "mantle bodies" because reddish elementary particles appeared to be embedded in a blue matrix or mantle (The word *chlamys* is Greek for "cloak draped around the shoulder." This describes how the intracytoplasmic inclusions caused by the organism are "draped" around the infected cell's nucleus). Shortly thereafter, similar inclusions were seen in infants with inclusion blenorrhoea, in cervical scrapings from their mothers and also in the urethral scrapings of men with non-gonococcal urethritis.
T'sang and colleagues (1957) in China were the first to isolate the trachoma agent by a yolk sac inoculation technique and distribute the organism to workers in other countries. The agent of inclusion conjunctivitis was isolated by Jones and colleagues (1959). Chlamydiae were thought to be ‘big viruses’ that time. The organism was described as “TRIC virus” (TRIC, an acronym for TRachoma, Inclusion Conjunctivitis, and virus because chlamydiae were then considered to be large viruses as they grew only in cells). However once the properties of bacteria were defined and the differentiation between viruses and bacteria was clearly made, it became clear that chlamydiae were bacteria that are restricted to an intracellular growth cycle.

The dramatic increase in research on trachoma that was brought about by the ability to grow the organism became important to research in sexually transmitted diseases (STDs). The reports of isolation of trachoma agent were followed by reports of isolation of the organism from the cervices of mothers of infected babies and then from urethras of men with non-gonococcal urethritis. By 1964, a number of studies suggested that there was broad genital reservoir for C. trachomatis that was more important than occasional cases of conjunctivitis would indicate (Jones, 1964; Dunlop et al., 1964; Rose and Schachter, 1964).

Gordan and Quan in 1965 successfully devised cell culture technique for the culture of Chlamydia. With this technical breakthrough, epidemiologic studies of the association of C. trachomatis with STDs could be performed (Barnes, 1989). It was in 1977, Mardh and associates showed the evidence that C. trachomatis was the major cause of pelvic inflammatory disease (PID). Studies followed later found the
association of this organism with tubal factor infertility and ectopic pregnancy, and thus *C.trachomatis* was recognized as an important infectious agent of wide spectrum of human diseases and a major public health problem.

2.2 CHARACTERISATION OF THE MICROORGANISM

Although chlamydiae were originally thought to be protozoa and later viruses, it became clear that chlamydiae had all the requisite properties of bacteria. Molecular evolution of rRNA sequences confirms that chlamydiae are eubacteria, but with only very distant relationships with other eubacterial orders (Weisburg *et al.*, 1986). Chlamydiae have been placed in their own order, *Chlamydiales*, with one family *Chlamydiaceae*, and a single genus, *Chlamydia*. They are non-motile, gram-negative bacterial pathogens. There are four recognized species of the genus *Chlamydia*: *C.trachomatis*, *C.psittaci*, *C.pneumoniae* and *C.pecorum*. Within the *C.trachomatis* species, three biovars namely trachoma, LGV and murine biovars based on their etiology potential are distinguished. Currently, there are 18 distinct serotypes of *C.trachomatis* and additional genovariants (Paavonen and Eggert-Kruce, 1999; Beagley and Timms, 2000). Serovars A, B, Ba, and C infect mainly the conjunctiva; serovars D, Da, E, F, G, H, I, Ia, J, and K are predominantly isolated from the urogenital tract; and serovars L1, L2, L2a, and L3 can be found in the inguinal lymph nodes and are responsible for systemic disease, lymphogranuloma venereum.

The entire genome of *C.trachomatis* was recently sequenced and this revealed that the *C.trachomatis* genome consists of a 1,042,519-bp chromosome and a 7,493bp
plasmid (Stephens et al., 1998). All the serovars of *C.trachomatis* have the plasmid, which remains functionally cryptic. Studies showed that all plasmids from human isolates of *C.trachomatis* are highly conserved (less than 1% nucleotide sequence variation), almost in size at 7500 nucleotides and encode significant ORFs greater than 100 amino acids. Two encoded gene products (CT582 and CT583) in chlamydial chromosome were homologous to proteins encoded by ORF7 and ORF8 of the chlamydial plasmid. Characterisation of plasmid functions and of chlamydial genes in general has been greatly impeded by the lack of a genetic system for *Chlamydia* (Thomas et al., 1997).

Analysis of the genome revealed in the identification of 894 likely protein coding genes. Enzymes were identified in the genome to account for the essential requirements for DNA replication, repair, transcription, and translation. Chlamydiae have substantial capacity for DNA repair and recombination, including mismatch repair (MutL, MutS, and three paralogous MutY proteins, the excinuclease UvrABCD complex, transcription-repair coupling factor (TRCF), and a number of proteins implicated in recombination-coupled repair such as RecA, RecBCD and RecJ. The phylogenetic mosaic of chlamydial genes implies a highly complex evolution for adaptation to obligate intracellular existence (Stephens et al., 1998).

Other than the genomic material, the organism contains 40-50% protein and lipids (Pearlman and McNeeley, 1992). One of the major components of the cell is the major outer membrane protein (MOMP). The MOMP is a variable 40 kDa protein which is the major surface protein of *C.trachomatis*, making up to 50-60% of the
outer membrane (Brunham and Peeling, 1994). The MOMP gene encodes for nine distinct amino acid sequences or regions. Five of these are highly conserved across C. trachomatis serovars while remaining four regions are known as variable domains (VDs). Sequence differences in these variable domains account for the different serovars that have been identified. The domains designated VD-1, VD-2 and VD-4 are exposed on the surface of the organism and contain documented epitopes (Brunham and Peeling, 1994). To date, MOMP sequence variation is found only in C. trachomatis and not in other species of Chlamydia (Bavoil et al., 2000). The gene for MOMP (omp1 or ompA) consists an 1182 bp ORF encoding a 394 amino acid protein, which contains 8 cysteine residues. C. trachomatis contains a lipopolysaccharide (LPS), which lies close to the MOMP on the bacterial surface. The chlamydial LPS contain genus specific epitopes. The antibody to LPS is usually elicited as a result of natural infection.

Until recently, the outer membranes of chlamydiae were known to consist only of MOMP and LPS. The genome sequences encoded a number of new outer membrane proteins, including at least one predicted 39 kDa porin (ompB) in addition to the major outer membrane protein (MOMP/ompA). A family of polymorphic outer membrane proteins (Pmps) has been also identified. The comparative analysis of genome sequences of C. trachomatis and C. pneumoniae revealed that C. trachomatis genome encodes nine Pmps (PmpA–PmpI) whereas the C. pneumoniae genome encodes 21 Pmp paralogs (Kalman et al., 1999). The functional role of Pmps remains unknown. Several potential virulence-associated proteins have been characterized. The type III secretion virulence system required for host invasion by several
pathogenic bacteria is found in three chromosomal locations in the genome of *C. trachomatis* (Stephens et al., 1998). Protein predations from chlamydial genomic information have also been useful for greatly expanding the number of inclusion membrane (Inc) proteins (Stephens and Lammel, 2001), which are associated with chlamydial inclusion membrane. The molecular information derived from genomic sequencing data has revolutionized the approaches to study these unique obligate intracellular pathogens.

2.2.1 Developmental cycle

Chlamydiae have a unique biphasic developmental cycle with dimorphic forms that are functionally and morphologically distinct. An extracellular elementary body (EB), which is the infectious but metabolically inert form, initiates the growth cycle. Having gained the entry into the host cell after endocytosis, the EBs reside in a membrane bound vesicle and transform themselves into metabolically active and larger vegetative forms namely reticulate bodies (RB). The reticulate body multiplies by binary fission, which makes the phagosome enlarged called as an inclusion body. Some of the reticulate bodies after maturity again condense into EBs and they are subsequently released from the inclusion body rupturing the host cell, infecting the adjacent cells repeating the life cycle (Peeling and Brunham, 1996). The length of the complete developmental cycle, as studied in cell culture models, is 48 to 72 hours and varies as a function of the infecting strain, host cell, and environmental conditions (Beatty et al., 1994).
2.3 PATHOGENESIS AND IMMUNOLOGY OF INFECTIONS

The pathogenesis of early chlamydial disease begins with serial infection of surface epithelial cells. This is effected by receptor-mediated endocytosis. Once inside the cell, chlamydiae are contained within host derived endosomes. These coalesce within a cytoplasmic vacuole thus evading the threat of lysosomal activity. The productive developmental cycle of chlamydiae causes the death of the epithelial cells. The dying epithelial cells release cytokines (Rasmussen et al., 1997), which promote increased blood supply with increased permeability of basal membranes. This allows the migration of lymph node cells. These enter local tissues by diapedesis from venules. At the same time, the causative chlamydiae travel through the lymphatics to the relevant lymph nodes. Some EBs freed by the dying epithelial cells are completely phagocytosed by neutrophils. Antigen-activated T-lymphocytes undertake a variety of defence roles including phagocytosis and production of gamma interferon (IFN-γ).

Epithelial infection by Chlamydia is associated with upregulation and secretion of proinflammatory cytokines such as interleukin (IL)-8, growth related protein (GRP), granulocyte/macrophage colony stimulating factor (GM-CSF) and IL-6. The chlamydial LPS induces IL-1 release from monocytes and macrophages, which in turn induces the release of tumor necrosis factor (TNF), a potent initiator of inflammatory process. IL-1 release associated with dying cells also stimulates adjacent non-infected epithelial cells to produce additional cytokines, which are potent activators for neutrophils, monocytes and T-lymphocytes (Morton and Kinghorn, 1999).
The immunologic response to *C. trachomatis* infection appears to play a major role in inducing immunopathology as well as in providing immune protection (Brunham and Peeling, 1994). The precise interaction between *C. trachomatis* and host immunologic response is uncertain. Three main observations like DNA sequence polymorphism of 40 kDa MOMP, evidence for the genetic susceptibility to the disease and the role of a 60 kDa heat shock protein Chsp60 reveals to some extent the pathogenesis of this infectious agent. The MOMP induces both neutralizing antibody and T-cell mediated immune responses. However, polymorphism of MOMP either through point mutations or through recombinational events contributes to the immune evasion and this have obvious implications for the vaccine design (Peeling and Brunham, 1996). The extent to which such polymorphism actually serves as a means of immune escape in vivo is an important unanswered question. There seems to be a genetic restriction of immune responses to *C. trachomatis* infection in selected patients with PID and in trachoma; HLA class I antigen was common in these cases according to recent studies (Stamm, 1999).

The histopathological response to *C. trachomatis* infection offers insight into the pathogenesis of immune mediated tissue damage. Characteristically, the epithelial surface is damaged with cell loss and vaculisation often in juxtaposition to intraepithelial lymphocytes. Epithelial cell degeneration occurring in close approximation to lymphocytes has been suggested as the immunological hallmark of tissue destruction due to *C. trachomatis* infection (Cohen and Brunham, 1999). Diffuse, dense infiltration with mononuclear cells occurs beneath the epithelium into the lumen. Overall, the histopathologic features are characterized by neutrophilic
exudation into the mucous layer associated with infiltration of the lamina propria by activated T and B lymphocytes, histocytes, macrophages, and dendritic cells. Both CD8 and CD4 T cells are present. B lymphocytes and lymphoblasts can be found as discretely organized lymphoid follicles or germinal centers. This may be more common among individuals with complications of chlamydial infection such as upper reproductive tract infection than among individuals with uncomplicated chlamydial cervical infection. Lymphoid follicle formation appears to be a correlate of upper genital tract chlamydial infection. Finally, diffuse plasma cell infiltration of lamina propria without marked T-lymphocyte infiltration appears characteristic of the late fibrotic stages of chlamydial fallopian tube formation (Brunham et al., 1992).

2.3.1 Role of heat shock proteins in pathogenesis

Chlamydial heat shock proteins known to be activators of immunopathologic mechanisms which contribute to human disease. Considerable attention has focused recently on the C. trachomatis 60 kDa heat shock protein (Chsp60). This protein is a member of Gro-EL family and is found both in elementary bodies and reticulate bodies. It is constitutively expressed and its transcription is upregulated by heat stress. Chsp60 is thought to be a major target antigen that stimulates a pathogenic inflammatory response. Responses to Chsp60 have been associated with a sequelae of upper genital tract diseases including PID, ectopic pregnancy, tubal infertility, perihepatitis etc. (Peeling et al., 1997; Sziller et al., 1998; LaVerda et al., 2000). The central role of Chsp60 in Chlamydia-associated inflammation is supported by the findings of multiple studies. These studies show that the severity of the chlamydial
disease in women correlate with high levels of antibodies against Chsp60 (Brunham and Peeling, 1994; Paavonen and Lehtinen, 1996).

There are three possible mechanisms that could explain the involvement of Chsp60 in inflammation. Firstly, the antibodies may be indicative of a chronic infection by *C. trachomatis* that is undetected and the inflammation results from the direct anti-bacterial response. A study done by Cotter *et al.*, (1997) demonstrated that apparently cured infections in mice can be reactivated by immunosuppression. Secondly, Chsp60 exhibits ~50% homology with human heat shock proteins and an immune response indicated by chlamydial infection may result in cross reactivity with human cells and tissues. Therefore, as a result of this ‘*molecular mimicry*’, anti-hsp responses induced by a primary *C. trachomatis* infection may lead to chronic inflammation in the genital tract tissues where the target antigen is actually human hsp60. In a study by Yi *et al.*, (1993), antibodies against peptides from human hsp60 have been demonstrated in sera from women with *C. trachomatis* associated pregnancy. Thirdly, there appears to be a genetic restriction for the Chsp60 antibody response in humans. Genetic restriction in immune responses to Chsp60 may partially explain the heterogeneity in disease outcome following genital chlamydial infection. In a mouse model, a genetic predisposition to Chsp60 antibody formation has been demonstrated. The responses to hsp60 are in part, genetically determined and have been mapped to the MHC locus (Zhong and Brunham, 1992).

Additional antigens that may participate in immunological response to chlamydiae have not been well characterized. A recent study done by LaVerda *et al.*, [insert citation]...
demonstrated that seroreactivity to chlamydial hsp10 (Chsp10) correlated with the severity of human genital tract disease. Further research is warranted to more precisely define the potential contribution of Chsp10 and other conserved chlamydial antigens to the immunopathologic process.

2.3.2 Immunity to *C. trachomatis* infection.

2.3.2.1 Cell mediated immunity

Little is known about the human cell mediated response to *C. trachomatis* infection, and most of our knowledge is derived from animal or in vitro models. Much of the research data points to the cell mediated immune response as the major defense against a genital *C. trachomatis* infection. T-lymphocyte responses are central to host resistance to chlamydial infection. The heterogeneity in helper T-lymphocyte responses may underlie chlamydial immunity versus immunopathology (Abbas *et al.*, 1996). Results from animal models as well as from human patient populations, point to CD4 T cells as the primary mediators of immunity with debatable contributions of CD8 T cells. Conflicting evidence in mice supports the role of CD4 and or CD8 T cells in protective immunity to *C. trachomatis*. Using a gene knockout mouse model, the study done by Morrison and colleagues (1995) demonstrated that MHC class II restricted T cell responses were absolutely required for the development of protective immunity to *C. trachomatis*, whereas the MHC class I restricted T cell pathway was not required. Another study done suggested that CTL or cytokine (IFN-γ) secreting CD8 T cells might limit the establishment of chlamydial infection (Starnbach *et al.*, 1994). Much less human data is available regarding CD8 T cell responses to
chlamydial antigens. Initial studies reported that cell mediated cytotoxicity toward
*Chlamydia trachomatis* infected target cells was not detected among individuals who had
lymphoproliferative responses to chlamydial antigen (Qvigstad and Hirschberg, 1984). Later Holland *et al.*, (1994) readdressed the issue of CD8 cytotoxic T cell
(CTL) activity using target cell capable of supporting *Chlamydia trachomatis* growth, namely,
autologus fibroblasts. Thus it appears that CD8 T cells can be induced during
chlamydial infection. Type 1 delayed hypersensitivity and Th1-like responses (IFN-γ production)
have been identified as the major protective mechanism against
chlamydia1 infection (Yang *et al.*, 1996). Although evidence is stronger for CD4 T
cells (Cohen and Brunham, 1999), both CD4 and CD8 T cell restricted mechanisms
are likely to be important in *Chlamydia trachomatis* associated immunity and disease, affecting
different stages of pathogenic pathway.

### 2.3.2.1 Humoral immunity to *Chlamydia trachomatis* infection.

Of all, the immune effectors, the role of antibody in immunity to chlamydial
infections has been the most extensively studied. Antibodies and B-lymphocytes are
readily detectable during and after *Chlamydia trachomatis* infection (Wang and Grayston,
1974). The protective role of antibodies against genital *Chlamydia trachomatis* infection is
controversial. In many studies, high serum antibody titers correlate with the
complications of chlamydial disease. As such it appears that high levels of serum
antibodies do not protect against the complications of chlamydial infection.
In contrast to serum antibodies, immunoglobulin A (IgA) in mucosal secretions may more directly participate in immunity. Animal studies have demonstrated a role for antibody, particularly mucosal IgA, in protection against clearance of vaginal *C. trachomatis* infections and ascension of infection to the upper genital tract following vaginal infection (Beagley and Timms, 2000). Local antibody mediated neutralization of infectivity seems probable since secretory IgA antibody *C. trachomatis* in cervical mucus has been inversely correlated with quantitative shedding of the organism in the genital tract, and antibody to MOMP can neutralize the organism in vivo (Brunham *et al.*, 1983; Williams *et al.*, 1997).

### 2.4 NATURAL HISTORY

The natural history of genital infection with *C. trachomatis* is poorly defined. In particular the duration of untreated infections remains unknown. The duration of infectivity is important to assess the burden of chlamydial disease and to determine the extent to which the disease spreads in a population. Many chlamydial infections are asymptomatic or not easily recognized on clinical grounds alone. Asymptomatic carriage in both men and women may be prolonged, further increasing potential transmission. As many as 25% of men and 70% of women with *C. trachomatis* infection do not show signs or symptoms of infection (Cates and Wasserheit, 1991). Human studies to understand the natural history of genital chlamydial infections have been hampered by many limitations. Subjects’ onset of infection is generally unknown, re-exposure to infection is common and most of the reported results reflect the findings of a single follow up culture.
In a study done by Rahm et al., (1986), 109 young asymptomatic women with genital *C. trachomatis* infection were followed up. During the 10 to 12 week follow up period, 16 women (15%) developed symptoms that required treatment. Of 85 asymptomatic untreated women available for repeat culture, 68 (80%) remained culture positive. 10 of 17 culture negative women after retesting within six months remained as culture negative. More recently, Parks et al., (1997) retrospectively studied 69 symptomatic patients. They identified instances in which patients were untreated and retested within 45 days. Most study subjects (86%) were recultured within 20 days. At the time of repeat testing, 28% of women were both culture and PCR negative, suggesting that the infections were spontaneously cleared.

Many case series and case reports document that many women remain infected for longer periods. Schachter et al., (1975) reported that 25 of 33 untreated women (76%) retested 2-28 weeks after an initial exposure remained culture positive, when retested. McCormack and colleagues (1979), in their follow-up study of 14 untreated asymptomatic infected women found that 50% had evidence of infection, when retested after 16 to 18 months. Studies in symptomatic men, untreated and inadequately treated subjects, suggest that most of them remain infected for more than 3 weeks and that spontaneous clearance can occur (Golden et al., 2000). Chlamydial urethritis has been reported to persist for as long as one year (Schachter et al., 1975). The extent to which prolonged infections reflect the natural history of single, persistent chlamydial infection as opposed to a result of serial exposures and recurrent infections is uncertain.
2.5 RECURRENCE

Recurrences have long been recognized as common in all forms of chlamydial infections. Studies show that recurrent chlamydial infection in women may increase the risk for serious reproductive sequelae, including PID, ectopic pregnancy, and infertility (Hillis et al., 1997). High rate of recurrence among sexually active populations especially adolescent women has been well documented. A three year follow up study done by Blythe et al., (1992) in sexually adolescent females revealed a recurrence rate around 38%. Recurrence rate is also high in men with non-gonococcal urethritis of chlamydial etiology (Morton and Kinghorn, 1999). Attempts to minimize recurrences have been only partially successful.

Many factors could play a role in inducing recurrent chlamydial infections. Re-exposure from the untreated partners may be one reason for subsequent recurrent disease. It is thought that recurrence may be also due to the reactivation of the disease by some stimuli like super infections with other microorganisms. The possibility of reactivation or unmasking of subclinical chlamydial infection by gonococcal infection has been suggested (Oriel and Ridgway, 1982; Morton and Kinghorn, 1999).

Determining whether the high rate of recurrent disease is due to reinfection or to persistent infection with same organism has been difficult. In studies utilizing serotyping of C.trachomatis strains, it has been shown that recurrences may occur with either the same or a different serovar (Barnes et al., 1986). Whereas recurrences
with a different serovar clearly represent reinfection, same-serovar recurrences may also represent persistent infection (Dean et al., 2000).

2.6 **PERSISTENT INFECTION AND CHLAMYDIAL DISEASE**

"Persistence" describes a long-term association between chlamydiae and their host cell in which these organisms remain in a viable but culture-negative state (Beatty et al., 1994). Persistence may represent a deviation from the typical development of chlamydiae, resulting in delayed intracellular growth under the influence of exogenous factors that may not be as "typical" as cell culture growth conditions. These conditions generally delay reticulate body (RB) maturation, inhibit differentiation to infectious elemental body (EB), and are associated with gross morphological alterations of RBs typified by markedly enlarged, atypical chlamydial forms (Beatty et al., 1994).

Persistent infections are those in which *Chlamydia* has entered a metabolically quiescent and noninfectious state (Somani et al., 2000). Some hosts fail to clear chlamydial infection and remain persistently infected or susceptible to frequent reinfection. It is hypothesized that chlamydiae may have evolved strategies for evading host defence mechanisms in order to establish and to maintain a persistent infection (Zhong et al., 2001). There is limited evidence supporting chlamydial persistence in animal models. In a murine model of *C. trachomatis* cervical infections, persistent chlamydial forms were observed by electron microscopy in epithelial cells months after the initial infection (Philips and Burillo, 1998). Similarly persistent
Chlamydial DNA and antigens were found in the upper genital tract tissue after treatment in the macaque model of chlamydial salpingitis (Patton et al., 1997). Persistence of \textit{C.trachomatis} was also demonstrated in in vitro systems. In cell culture systems, it has been demonstrated that IFN-\(\gamma\), penicillin, or amino acid deprivation can induce culture negative persistence of chlamydial infection characterized by the overproduction of stress proteins like Chsp60 and down regulation of structural proteins like MOMP and LPS. Withdrawal of these factors resulted in viable chlamydiae being seen once again (Beatty et al., 1994; Beatty et al., 1995). In the events of natural infections, chlamydial persistence may evolve into overt disease, depending on the balance of the host-microbe interaction (Stamm, 2001).

Persistently infected individuals appear to be those who develop the long-term disease sequelae of chlamydial disease. In humans, persistent infection is best documented among individuals with \textit{C.trachomatis} tubal infertility. Chlamydia-specific DNA and antigens have been found in the upper genital tracts of infertile women, providing suggestive evidence for persistent chlamydial infection. In one study, 9 (56%) of the 16 individuals and in another, 19 (79%) of 24 individuals with \textit{C.trachomatis} tubal infertility had chlamydial nucleic acid or antigen detected in tubal tissue (Campbell et al., 1993; Patton et al., 1994). In almost all cases of late chlamydial disease, chlamydiae are not in a replication-competent form recoverable by cell culture. Recent evidence using using RT-PCR suggests this persistence reflects viable infection (Gerad et al., 1998). However more studies are needed to clearly establish the extent to which chlamydiae establish long standing upper genital tract infections and whether these infections are indicative of transmissible infection.
Studies of chlamydial persistence in humans are limited by the inability to control for re-exposure to untreated partners. A recent study has provided evidence for long-term cervical persistence of *C. trachomatis* by omp1 genotyping. Dean et al., (2000) studied 7 women who had ≥ 3 recurrent same serovar *C. trachomatis* cervical infections that occurred during a 2-5 year time span. This study suggested that cervical infections with C class serovars can persist for years.

Persistent infections are important, because they may be an unrecognized source for transmission of the organisms, may induce immunopathologic fibrosis and scarring and may require alternative therapeutic approaches. Understanding the clinical implications and molecular mechanisms of chlamydial persistence is currently an important area of research.

2.7 CLINICAL SPECTRUM OF GENITAL CHLAMYDIAL INFECTIONS

*C. trachomatis* causes a variety of clinical syndromes in men, women and infants. Since most of the genital infections are ‘clinically silent’, this leads to serious clinical sequelae and costly outcomes in women and men.
2.7.1 Clinical manifestations of *C. trachomatis* infection in men

Among heterosexual men, chlamydial infections are usually urethral and up to 50% are asymptomatic (Stamm and Cole, 1986; Zelin *et al.*, 1995). When symptoms do occur, usually 1 to 3 weeks following exposure, they are indistinguishable from those of gonorrhea (urethral discharge and/or pyuria). However, compared with gonococcal urethritis, chlamydial urethritis is more likely to be asymptomatic (Black, 1997). The number of heterosexual men with asymptomatic chlamydial infections is larger than the number of men with gonorrhoea since the symptoms due to the former are often absent or mild.

*Non-gonococcal urethritis*: NGU is the most common clinical syndrome seen in the male (Pearlman and McNeeley, 1992). This syndrome is defined by exclusion, that is, the failure to find *Neisseria gonorrhoeae* in urethral specimens from a man with urethritis. Approximately 50-60% of the cases with NGU are caused by *C. trachomatis*. The incubation period is between 1 and 3 weeks and symptoms associated are urethral discharge, dysuria, itching etc. A common method of diagnosing NGU is to demonstrate a significant number of polymorphonuclear leukocytes (PMNs) in either a first-catch urine specimen or a smear prepared from a urethral swab. If a gram stain of discharge shows many PMNs but no intracellular diplococci, a presumptive diagnosis of NGU is made.
Chlamydial urethritis is presumptively diagnosed by history, urethral discharge, and the presence of four or more PMNs per oil immersion field of a gram-stained urethral smear or pyuria noted on urinalysis.

Postgonococcal urethritis: Postgonococcal urethritis is a special subset of NGU. It is seen in men who have been successfully treated for gonococcal infection and either develop symptoms shortly after therapy or remain symptomatic. *C. trachomatis* is the leading cause of this condition, being responsible for 70% to 90% of cases. B-Lactam drugs in dosages used to treat gonorrhea are largely ineffective against chlamydiae. Because of the high (more than 20%) double infection rate, the Centers for Disease Control and Prevention (1993) has recommended that all cases of gonorrhea in heterosexual men be treated presumptively for chlamydial infections.

Epididymitis: Epididymitis is the infection of spermducts of the testicles. The primary symptom is unilateral scrotal pain and common clinical signs are scrotal swelling, tenderness and fever. *C. trachomatis* is the leading cause of epididymitis in sexually active young men under 35 years of age with no underlying genitourinary pathology (Black, 1997). Evidence of a major role for *C. trachomatis* in epididymitis has been provided by studies in which the agent has been isolated directly from epididymal aspirations. In older men, epididymitis is more often due to other etiologies associated with urinary tract abnormalities or instrumentation rather than sexually transmitted origins. Unilateral scrotal pain is the primary symptom, and common clinical signs of this infection include scrotal swelling, tenderness, and fever.
If urethral symptoms are also present, a sexually transmitted bacterial etiology is likely (Black, 1997).

**Proctitis**: Among homosexual men, 4-8% of the infections are seen in the rectum. Rectal infections are generally asymptomatic, but may cause symptoms characteristic of proctitis. When they do occur, symptoms of rectal infection in men and women who practice receptive anal intercourse may include rectal discharge and pain during defecation (Jones et al., 1985). In homosexual men with chlamydial proctitis, the same symptoms are found, together with tenesmus, diarrhea, and rectal bleeding. A presumptive diagnosis of proctitis is based on history, swelling and friability of rectal mucosa, numerous PMNs in gram-stained rectal specimens, or, if indicated, rectal biopsy specimens that show PMN infiltration into the lamina propria (Pearlman and McNeeley, 1992).

2.7.2 Clinical manifestations of *C. trachomatis* infection in women

The main impact of disease caused by *C. trachomatis* is on the female reproductive tract. Although most infections in women are asymptomatic, clinical manifestations include mucopurulent cervicitis, urethritis, endometritis, PID, ectopic pregnancy and tubal infertility. Chlamydial infection can cause apparently more severe tubal immunopathology than other agents inspite of the absence of overt symptoms. This is mostly due to the subclinical or chronic nature of chlamydial infection.
**Mucopurulent cervicitis (MPC):** *C.trachomatis* is the major cause of mucopurulent cervicitis. The affected site is the endocervix. This condition is characterized by a mucopurulent endocervical discharge, often accompanied by easily induced bleeding and edema within a zone of ectopy. Simple objective criteria for the presumptive diagnosis of MPC include an increased number (≥ 10 per high power field) of PMNs in cervical smears, a positive swab test, and increased erythema, edema, and induced mucosal bleeding in the area of ectopy and transformation zone (Paavonen, 1992). A significant proportion of women having MPC due to *C.trachomatis* may develop upper genital tract infections.

**Female urethral syndrome:** *C.trachomatis* frequently infects the female urethra, often coexisting with cervical infection. In case of women who are apparently asymptomatic may manifest mild, intermittent dysuria. The syndrome of frequency and dysuria without bacteriuria (≥ 105 colony forming units/ml) has been associated with urethral chlamydial infection in up to 60% of the women (Pearlman and McNeeley, 1992).

**Pelvic inflammatory disease (PID) and sequelae:** "Pelvic inflammatory disease" refers to infection of the endometrium, fallopian tubes, and contiguous structures. Although it is often caused by multiple infectious organisms, pelvic inflammatory disease results most frequently from the ascent of sexually transmitted chlamydial or gonococcal infection from the cervix into the upper genital tract (McCormack, 1994). PID, which result from the ascending infection, is responsible for most of the morbidity and cost resulting from *C.trachomatis* infection.
Lower abdominal pain, usually bilateral, is the most common presenting symptom. Pain may be associated with an abnormal vaginal discharge, abnormal uterine bleeding, dysuria, dyspareunia, nausea, vomiting, fever, or other constitutional symptoms. While the gonococcal PID tends to have a more abrupt onset and more dramatic symptoms of fever and peritoneal irritation, chlamydial PID may be subtle and run a chronic course (Hillis and Wasserheit, 1996). Women with subclinical *C. trachomatis* infection may experience pelvic, uterine or adrenal pain.

In all forms of pelvic inflammatory disease, complications are common and often irreversible or even fatal. A single pathophysiologic mechanism - scarring - is largely responsible for the principal consequences: *infertility, ectopic pregnancy, and chronic pelvic pain*, which occur in approximately 20%, 9%, and 18% of women with symptomatic pelvic inflammatory disease, respectively (Westrom *et al.*, 1992). Extraluminal scarring is associated with chronic pelvic pain. Intraluminal scarring leads to ectopic pregnancy when occlusion is partial and to tubal-factor infertility when it is complete. PID is associated with 5-7 fold risk for ectopic pregnancy, which a leading cause of maternal morbidity and mortality. After a single episode of PID, approximately 15% of women will be infertile as a result of peritubal adhesions or tubal occlusion. The risk for infertility doubles with each successive episode of pelvic inflammatory disease (Paavonen and Lehtinen, 1996). Studies of the long-term complications of pelvic inflammatory disease underscore the impact of "silent" or atypical disease. Most women who have tubal infertility have never been diagnosed as having chlamydial infection or PID. A large proportion of *C. trachomatis* infections of the fallopian tubes are asymptomatic or subclinical which suggests that silent
infections are the most common cause of tubal infertility (Paavonen and Eggert-Kruse, 1999).

For pelvic inflammatory disease, laparoscopic visualization is considered the gold standard. Inflamed fallopian tubes and other pelvic structures can be visualized. In the absence of laparoscopy, the Centers for Disease Control and Prevention recommends the triad of lower abdominal pain, cervical-motion tenderness, and bilateral adnexal tenderness as the minimal clinical criteria for diagnosing pelvic inflammatory disease (Hillis and Wasserheit, 1996).

Postpartum endometritis: The prevalence of C.trachomatis infection in pregnant women ranges from 2 to 35 % (Ryan et al., 1990; Black, 1997). C.trachomatis cervical infection in pregnant women has been associated with the development of postpartum endometritis, with onset more than 48h after delivery in approximately one-third of cases. Martin et al., (1982) were the first to report an increased risk for stillbirth and neonatal death occurring 10 times more frequently in Chlamydia-infected women.

2.7.3 Clinical manifestations of C.trachomatis infection in infants

Infant conjunctivitis: C.trachomatis is the most common cause of neonatal conjunctivitis. 15-25% of the infants born to Chlamydia-infected mothers and exposed at birth develop conjunctivitis. Although the conjunctivitis may be
subclinical, symptoms usually begin with 1 week of delivery and consist of edema of eyelids, conjunctival erythema, and a mucopurulent discharge.

**Infant pneumonia:** 3-16% of the infants who acquire the infection during birth develop pneumonia (Black, 1997). Most of *C. trachomatis* pneumonia occur within 2 months after delivery. The infants will often have a prodrome of rhinitis, and many will have had conjunctivitis. Affected infants are usually afebrile, are markedly tachypneic and occasionally apneic, and have staccato cough. These conditions are occasionally difficult to treat and infants with chlamydial pneumonia are at increased risk for later pulmonary dysfunction and chronic respiratory disease.

2.7.4 Lymphogranuloma venereum (LGV)

LGV is a systemic disease both in men and women caused by *C. trachomatis* serovars L1 to L3, which are more invasive than other serovars resulting in infection of epithelial layers and underlying soft tissue. Lymphogranuloma venereum has three stages. In its primary stage, the disease is more likely to be detected in men; it may go unnoticed in women. After an incubation period of four to 30 days, a small painless ulcer or blister develops in the genital area. Second-stage LGV develops between one and six weeks later. In this stage, the chlamydial organisms further invade and live within macrophages, which carry the organism to the local and regional lymphatic of the genital region. This leads to inflammation and swelling of the inguinal lymph nodes (Perine and Osoba, 1990). The secondary stage of disease is characterized by systemic symptoms including fever, chills, anorexia, myalgia and arthralgia
(Pearlman and McNeeley, 1992). Untreated infections can lead to ulceration and hypertrophy of the genitalia, arthritis and fistula formation involving rectum, bladder, vagina or vulva. Third-stage LGV, which is sometimes called *anogenitorectal syndrome*, develops in about 25% of patients. In men, this stage is usually seen in homosexuals. Third-stage LGV is marked by rectal pain, constipation, a discharge containing pus or bloody mucus, and the development of strictures (narrowing or tightening of a body passage) in the rectum or vagina.

Prompt treatment of the early stages of LGV is essential to prevent transmission of the disease as well as fertility problems and other serious complications of the later stages. Chronic LGV can be reactivated in patients who become infected with the AIDS virus. These patients develop open ulcers in the groin that are difficult to treat.

2.7.5 Other syndromes associated with *C. trachomatis*

The other major syndromes associated with *C. trachomatis* are adult inclusion conjunctivitis, reactive arthritis and perihepatitis (Fitz-Hugh-Cartis syndrome) in women.

*Adult inclusion conjunctivitis*: Adult inclusion conjunctivitis in both male and the female is an acute follicular conjunctivitis with edema, mucopurulent exudates, and erythema (Schachter and Dawson, 1978). Infection is thought to occur from infected
genital tract secretions via hand-to-eye contact. The illness is self-limited, although treatment will shorten the clinical course.

*Reactive arthritis:* A small proportion of the patients present with the symptoms of urethritis or cervicitis later may develop a persistent and disabling disease. This illness is now termed as reactive arthritis rather than the older term, Reiter's syndrome, which involves a triad of reactive arthritis, conjunctivitis and urethritis. Evidence implicating *C. trachomatis* as a cause of sexually acquired reactive arthritis (SARA) is strongest (Gaston, 2000). This disease is more common in males.

*Perihepatitis:* A complication of salpingitis is perihepatitis (Fitz-Hugh-Curtis syndrome), assumed to be caused by peritoneal spread of the infection. Serologic studies have implicated *C. trachomatis* as the etiological agent in most of these cases, and the organism has been recovered from the surface of the liver (Schachter, 1999).

The involvement of non-LGV strains of *C. trachomatis* in other conditions is less well documented. Recent studies have shown that *C. trachomatis* infection is associated with cervical carcinomas and suggested its possible cofactor role in inducing squamous metaplasia and metaplastic cell atypia (Paavonen, 2001).

2.8 CHLAMYDIAL INFECTION AND HIV INFECTION

The association between HIV with *C. trachomatis* has been demonstrated in several studies (Ghinsberg and Nitzan, 1992; Laga *et al.*, 1994; Brunham *et al.*, 1996;
Ghys et al., 1997) and it seems that active *C. trachomatis* infection increases the risk of HIV infection. There are several possible explanations of the relationship between the two infections. In the first place, STDs including HIV and *C. trachomatis* have the same behavioural risk factors, for example, inconsistent condom use and frequency of sexual intercourse. One epidemiological study by Ohshige et al., (2000) showed that sexual behavioural risk factors had some mutual relationship with HIV infection but acute *C. trachomatis* infection had an association with HIV infection independent of the sexually transmissible risk factors. Secondly, there are two possible relationships between HIV infection and *C. trachomatis* infection: (1) immunological changes due to HIV infection may favour *C. trachomatis* infection (Miller et al., 1992; Cohen et al., 1995); (ii) *C. trachomatis* infection may facilitate HIV infection, through a change in vaginal flora or a histological change in the vaginal epithelium (Kiviat et al., 1990; Miller et al., 1992).

The biological plausibility of the relationship between the two infections is based on important factors that influence infectiousness and susceptibility. These include but are not limited to (i) damage to the epithelial layer (i.e., increased friability, viral egress, and entry through exposed and damaged blood vessels); (ii) CD4+ inflammatory cells in lesions (these cells are targets for viral infection or a source of HIV shedding); and (iii) inflammatory cytokines induced by other sexually transmitted diseases. Neutrophils cocultivated with *Chlamydia* infected cells secrete soluble substances that induce replication of HIV in quiescent cells (Hitchcock, 1999). However, more work is needed on the biological mechanisms that contribute to the synergism between these pathogens.
2.9 DIAGNOSIS OF GENITAL CHLAMYDIAL INFECTIONS

The various diagnostic methods currently used for *C. trachomatis* are isolation in cell culture, antigen detection by direct fluorescent antibody (DFA) staining or by enzyme immunoassays (EIAs), serological tests, nucleic acid probe assays, nucleic acid amplification tests etc. Regardless of the methodology employed for detecting *C. trachomatis*, the importance of collecting an adequate specimen cannot be overemphasized. Both sensitivity and specificity of the diagnostic techniques are directly related the adequacy of specimens. Specimen inadequacy may many times lead to underdiagnosis of genital chlamydial infection.

**Isolation in cell culture:** Many cell lines like McCoy, Hela 229, BGMK etc., have been used for the isolation procedures. Culture is performed by inoculating specimens in to cell culture monolayers. A centrifugation step is essential to facilitate the infectivity of chlamydiae to the cell layers. The inclusions can be visualized following 48-72 hrs of incubation by staining with iodine, giemsa or an immunofluorescent stain. Iodine staining can be used to distinguish *C. trachomatis* inclusions (which stain light to dark brown) from inclusions of other *Chlamydia* species. The preferred method for identification of inclusions or EBs is to stain the infected cell monolayers with species-specific, anti-MOMP fluorescein labeled monoclonal antibodies.

Isolation of the organism in cell culture had been the gold standard for the diagnosis because it has a specificity that approaches 100%. However, because of its labor-intensive methodology, requirement of technical expertise, stringent transport
conditions and relative insensitivity (70% -85%), its use is limited to only research laboratories. Hence, culture has been mostly replaced by other non-culture tests like antigen detection methods, enzyme immunoassays (EIA), direct fluorescent antibody (DFA) technique etc., because of their ease and as they are less time consuming.

**Antigen detection methods:** Antigen detection is done by using DFA tests or EIAs. DFA testing remains one of the useful diagnostic techniques available. DFA tests use either MOMP monoclonals or anti-LPS antibodies for the capture of the chlamydial antigen. With the use of monoclonal antibody against MOMP of *C. trachomatis*, the sensitivity of DFA is 80-90% and the specificity is 98-99% relative to culture (Black, 1997). The DFA is the only test, which permits simultaneous assessment of specimen adequacy by visualization of columnar epithelial cells. Inspite of higher sensitivity, specificity and rapidity of DFA tests, the microscopic evaluation of specimens is laborious and requires technical expertise to avoid false and non-specific fluorescence.

Although screening of large number of specimens is possible, the enzyme immunoassays (EIAs) lack sensitivity and specificity. Low specificity of these assays is due to cross reactivity of chlamydial LPS with other gram-negative bacteria. Some assays use a confirmation test with a blocking antibody or alternatively use a DFA test along with these assays.

**Serological methods:** Serological tests are generally not useful in the diagnosis of genital tract infections caused by *C. trachomatis* as antibodies elicited due to these
infections are long lived and these tests will not distinguish between the past and current infection (Black, 1997). Complement fixation is usually insufficiently sensitive to detect antibodies stimulated by uncomplicated genital infections, but has an acceptable place in the diagnosis of lymphogranuloma venereum. (Taylor-Robinson, 1996). Microimmunofluorescence (MIF) test developed by Wang and Grayston in 1970 is the most sensitive test of serologic tests for Chlamydia species. It is the only serologic test that detects species and serovar specific responses. MIF is also useful in detecting IgM antibodies in first episode infections of the female genital tract, although subsequent infections may fail to show IgM responses. The MIF test is the diagnostic test of choice for chlamydial pneumonia in infants by the detection of IgM antibodies. The limitation of commercially available serological ELISA tests is that these tests mostly fail in species specificity as chlamydial LPS is used as the antigen. Serological test with a single serum specimen will not provide a conclusive evidence of an active infection with C. trachomatis (Black, 1997). While serology is not reliable for the diagnosis of acute infections, it may be useful in identifying the chlamydial etiology in ascending upper genital tract infections, where direct tests may fail to detect the presence of organism (Bas et al., 2001).

**Nucleic acid probes:** Nucleic acid hybridization tests use a direct nucleic acid probe that hydrolyse a species-specific sequence of chlamydial 16S rRNA and detect C. trachomatis by chemiluminescence. The performance of these assays is similar to that of available EIAs, with sensitivities of around 75-80% and specificities to > 99%. Perhaps no more needs to be said about the straightforward DNA probes because they have been left in the wake of the amplified DNA methods.
**Nucleic acid amplification tests (NAATs):** The development of nucleic acid amplification tests (NAATs) transformed the diagnostic power to the sensitivity of almost 100%. The most widely used techniques currently are polymerase chain reaction (PCR) and ligase chain reaction (LCR). Both use primers directed at the *C. trachomatis* cryptic plasmid, which is usually present at 10 copies per cell. Primers directed at MOMP gene are not frequently used for diagnosis as the sensitivity is usually 10 fold lesser than plasmid-based PCR systems, but are mostly used for confirmatory testing in research laboratories. Transcription mediated amplification (TMA) is another technique which uses reverse transcriptase and a T7 RNA polymerase to amplify the RNA. This methodology does not require the use of a thermal cycler as this reaction is 100% thermal. Strand displacement amplification (SDA) is an isothermal NAAT that utilizes a restriction enzyme to ‘nick’ one DNA strand at a modified site. Currently PCR, LCR, TMA and SDA are approved by the Food and Drug Administration (FDA) for the diagnosis of *C. trachomatis* genital infections (Hammerschlag, 2001).

NAATs are more sensitive than culture for detection of *C. trachomatis* in genital specimens in adults, detecting an additional 25–30% over culture. Multiple studies have demonstrated that each has a sensitivity of >80% to 100% versus 65% to 88% for culture while maintaining high specificities (95-100%). In the case of DNA amplification tests, false negatives due to inhibitors of DNA polymerase are more of a
problem than false positives and these are present more in urine and cervical specimens.

Because nucleic acid amplification is exquisitely sensitive, theoretically capable of detecting as little as a single gene copy, and highly specific, it offers the opportunity to use noninvasive urine samples for the screening of asymptomatic population. The testing of mailed home-obtained specimens makes it possible to extend screening beyond the traditional settings (Morre et al., 1999a).

**Rapid or 'point-of-care' tests:** Rapid tests, also called ‘point-of-care tests’ or ‘near to patient’ tests, for *C. trachomatis* employ EIA technology in formats based primarily on membrane capture or latex diffusion. These tests are used mostly in clinical settings. Even though these tests offer the advantage of immediate results, lack of specificity is the problem. The results of a rapid test should always be considered presumptive and should be confirmed by a sensitive and specific laboratory performed test.

**Issues regarding gold standard**

The ‘gold standard’ for evaluating *Chlamydia* diagnostic tests has changed over these years. Traditionally, culture had been the reference standard for the evaluation of diagnostic tests for genital *C. trachomatis* infections. However, culture
alone is an inadequate standard for diagnosis in that detect only 75 to 85% of infections in excellent laboratories (Black, 1997). For evaluating various diagnostic tests for *C. trachomatis*, many investigators and the FDA now use an expanded definition of a true positive result based on the results of a combination of culture and non-culture tests (Schachter et al., 1992).

The expanded standard of positivity is important to use particularly when evaluating highly sensitive nucleic acid amplification tests. The definition of true positive has been expanded further to include an alternate target DNA amplification test (Black, 1997). Recently the approach of using a combination of tests (culture and NAATs) for evolving an ‘expanded gold standard’ is being suggested by many workers (Newhall et al., 1999; Johnson et al., 2000; Van Dyck et al., 2001).

2.10 EPIDEMIOLOGY OF GENITAL CHLAMYDIAL INFECTIONS

2.10.1 Global epidemiology

*C. trachomatis* infections are the most prevalent bacterial sexually transmitted infections recognized throughout the world (Paavonen and Eggert-Kruse, 1999). The World Health Organization (WHO) estimated that 92 million new cases of *C. trachomatis* infection have occurred worldwide in 1999 alone (WHO, 2001). The prevalence of this infection is high in the western industrialized countries. In the United States, it is the most frequently reported infection, and its annual incidence is estimated to be approximately 4-5 million cases (Centers for Disease Prevention and
2.6 million affect women, 1.8 million affect men and the rest resolve infants. Reporting is fragmentary, and testing is inadequate, making underestimation likely. The reported rates for females are seven times the male rates, because women are more likely to be screened. These infections are found in all strata of the society, although the highest infection rates are found among the young and the poor (Schachter, 1999).

In Europe, 3 million cases occur annually (Peeling and Brunham, 1996). In UK, the number of new cases of *C. trachomatis* infection in men and women has remained between 33,000 and 37,000 since 1989 (Robinson and Ridgway, 1996). A population-based study in Britain using a stratified probability sample survey method, estimated the prevalence of genital chlamydial infections around 2% in men and women. The study demonstrated that the prevalence was higher in men (2.3%) than women (1.5%) and stressed the importance of involving men in the *Chlamydia* screening programmes (Fenton *et al.*, 2001). The prevalence of chlamydial infection amongst pregnant women ranges from 2.7% in Italy to 8% in Iceland, with low prevalence and incidence rates in the Nordic countries, following wide scale screening programmes in the 1970s (WHO, 2001). A nationwide sentinel survey of *C. trachomatis* infections in Finland reported the prevalence rates of 9% and 5% respectively in STD clinics and general clinics (Hiltunen-Back *et al.*, 2001). About 3% of asymptomatic *C. trachomatis* infection was observed in an inner city population of Netherlands using mailed home obtained urine samples (Van Valkengoed *et al.*, 2000). In Denmark, the prevalence of *C. trachomatis* in young women from the general population was found to be 7% (Munk *et al.*, 1999). In Hungary, a study done
in different groups of patients, showed the following prevalence rates: 6% in asymptomatic groups of men and women; 15% in gynaecological patients and 21-25% in STD patients (Tchoudomirova et al., 1998).

Surveillance data from Australia showed that the prevalence of genital infections with *C. trachomatis* remained stable at 2% to 14% in STD clinics and 5% in family planning clinics over a period of 10 years (Mulhall et al., 1995). Prevalence studies from Latin America and Caribbean show rates from 9% amongst teenagers in Chile, 2.1% amongst pregnant women in Brazil, and 12.2% amongst attendees to family planning clinics in Jamaica (WHO, 2001).

Reports from African countries also reveal a higher prevalence of *C. trachomatis* infection in several populations. Studies amongst pregnant women have revealed a prevalence rate from about 6% in Tanzania to 13% in Cape Verde (WHO, 2001). A recent study has shown a high prevalence rate (6%) of asymptomatic *C. trachomatis* genital infections in a rural South African community (Colvin et al., 1998).

WHO estimated figures (WHO, 2001) show alarming incidence rates in South and South East Asia where majority of infections occurred in 1999. The incidence has been shown to be increasing (40 million in 1995 to 42 million in 1999). 4 to 6% of asymptomatic infection due to *C. trachomatis* was reported in Thailand (Kilmarx et al., 1998; Chandeying et al., 2000). A Malaysian report showed 10% and 11% respectively for high-risk groups of men and women (Gaydos et al., 1996).
Reports from other Asian countries also place genital *C. trachomatis* infections to be a highly prevalent STD.

2.10.2 Epidemiology of genital chlamydial infections in India

The epidemiological picture of genital *C. trachomatis* infections is not very clear in the Indian subcontinent. So far, prevalence estimates in India were derived from limited studies done in patients attending antenatal clinics or high-risk populations like STD patients. The prevalence of these infections in the general population is not yet known. With few exceptions, most of the reported prevalence studies have used conventional serologic or antigen detection methods, thereby likely underestimating the prevalence rates.

A report from Mumbai has shown prevalence around 23% in high-risk group of women attending STD clinic (Divekar *et al.*, 2000). Joshi *et al.*, (1994) reported 15% positivity for chlamydial infections in young women attending gynecological clinic. A study by Paul *et al.*, (1999) has shown the prevalence of *C. trachomatis* infection in mid-pregnancy and at labour to be 17% and 18.6% respectively. A follow-up study in pregnant women revealed a positivity of 21.3% in the study group and has shown the association of *C. trachomatis* infection with the adverse outcomes of pregnancy (Rastogi *et al.*, 1999). Shrikhande *et al.*, (1995) observed an overall positivity rate of 33% for *C. trachomatis* infection in PID patients. A seroprevalence study demonstrated a high prevalence of 74% antichlamydial antibodies in women with tubal infertility (Tyagi and Singh, 1998). In a study by Rastogi *et al.*, (2000), the
detection rate of *C. trachomatis* among spontaneous abortion patients was 15.6%, while that in women undergoing induced abortion was 4% only.

Some of the studies by using culture and PCR have shown high prevalence rates of genital chlamydial infection. A single report of cell culture showed a prevalence of chlamydial infection to be about 40% in symptomatic women (Mittal *et al.*, 1993). A recent study from Delhi showed 50% positivity among the 50 STD women patients tested using PCR and indicated that the frequency of genital chlamydial infections may be higher in this group of patients (Gopalakrishna *et al.*, 2000). More recently, Singh *et al.*, (2002) studied symptomatic women attending a gynaecology clinic in Delhi and observed a prevalence of 43.1% by using PCR. While above-mentioned studies from North India show high prevalence rates, there is a paucity of information about genital chlamydial infections in the southern part of the country. There is, so far, only one report from South India, which shows a low prevalence rate of 3.3% in pregnant women (Alexander *et al.*, 1993).

Understanding the quantum of genital chlamydial infections and the disease burden in the population is necessary to control the spread of these infections and the associated sequelae. As the molecular methods of screening improved the knowledge on the epidemiology of these infections, better need based prevention strategies can be adopted.
2.11 PREVENTION AND CONTROL

Since many of the chlamydial infections are asymptomatic and diagnosis at an early stage would prevent serious complications, these considerations suggest that periodic screening for high-risk populations for chlamydial infection should be an effective control measure. An approach known as universal screening has frequently been implemented and has been highly successful in western countries (Stamm, 1999). In case of settings with low prevalence of infection, approaches to selective screening have been developed among young women. The effectiveness of selective screening for C. trachomatis infection has been demonstrated in a number of recent studies in US and Sweden. One randomized controlled trial done at Group Health Cooperative, a large health maintenance organization (HMO) in Seattle has provided strong evidence that intervention with selective screening for chlamydial infections effectively reduces the incidence of PID (Scholes et al., 1996). It has been suggested recently, that men should always be included in a screening programme and not just as 'partners of infected women' (Duncan and Hart, 1999). This would probably identify the asymptomatic reservoir of infection for women more effectively.

Recent technological advances should further enhance efforts to prevent chlamydial infection. The single-dose therapy using azithromycin and the use of non-invasive urine specimens tested by DNA amplification techniques now provides the opportunity to test these approaches and such studies are ongoing. Use of urine specimens or self-collected vaginal swabs also afford the opportunity to develop programs for community-based screening. Thus prevention of these sexually
Transmitted asymptomatic infections is necessary to arrest the rapid spread in the community. The interventions could be achieved equally through broad based screening programs in public settings and public awareness sensitization programmes.

2.12 TREATMENT

Treatment of chlamydial genital infection is always indicated to avoid complications and to prevent the spread of infection (Ridgway, 1997). There are many variables that effect the decision of which treatment is best for which patient. These include age, pregnancy status, allergies and question of compliance. Infections with *C. trachomatis* have long been effectively treated with appropriately chosen antibiotics; i.e., various tetracycline analogues or with erythromycin. Penicillin, cephalosporins, spectinomycins and amino glycosides have not been proved efficient for the treatment of chlamydial infections. Tetracylines have been the mainstay of therapy. For pregnant or nursing women, children and adolescents the therapy of choice is erythromycin and tetracyclines are contraindicated.

2.12.1 Newer Medications

Azithromycin, a new antimicrobial agent has come to be used in the treatment of chlamydial infections. Until recently, eradication of chlamydial infections has required multidose therapy for at least 7 days. New guidelines for the treatment of patients with sexually transmitted chlamydial infection have been recently published (Centers for Disease Control and Prevention, 1998). Recommended regimens are
azithromycin 1g orally in a single dose or doxycycline 100mg orally twice daily for 7 days. Alternative regimens include erythromycin 500mg orally four times daily for seven days, or ofloxacin 300mg orally twice daily for 7 days. Compared with conventional therapy, azithromycin has excellent pharmacokinetic characteristics such as increased bioavailability; lower incidence of gastrointestinal tract side effects and increased concentration in mucus, macrophages and tissues with a half-life of 5 to 7 days (Paavonen and Eggert-Kruise, 1999). The single-dose azithromycin treatment demonstrated efficacy in the treatment of chlamydial cervical infection and non-gonococcal urethritis equal to that of 7 days traditional doxycycline (Stamm et al., 1995). Cost effective analyses also indicate that the markedly improved compliance with single-dose regimen compared with the poor compliance seen with 7 day regimen of doxycycline results in an overall reduction in subsequent morbidity and cost among those treated with azithromycin (Magid et al., 1996). At present, limited data are available on the use of single-dose therapy during pregnancy, and for syndromes such as PID. Treatment of PID is more complicated and requires multiple drugs.

Other treatments include amoxycillin, a penicillin derivative (500 mg twice a day for 7 days) which can be used instead of erythromycin. Trovafloxacin, a new quinolone antibiotic is also shown to be highly active against C.trachomatis and N.gonorrhoeae. A recent double blind multicentric study compared the efficacy of trovafloxacin with that of doxycycline suggested the use of trovafloxacin (200 mg twice for 5 days) for uncomplicated chlamydial infection (McCormack et al., 1999).
It has been suggested that early treatment of chlamydial infection is successful, whereas antibiotic therapy during chronic phase generally might be less effective. Antimicrobial resistance of *C. trachomatis* has been documented in few reports. Jones *et al.*, (1990) were the first to report resistance to tetracycline and erythromycin. However, resistance to these antibiotics was not associated with apparent treatment failure. Recently, Somani and coworkers (2000) have reported multiple drug resistant *C. trachomatis* strains and have documented clinical and microbiological treatment failures in association with increased MICs of azithromycin and doxycycline. This seems to be an emerging problem and may profoundly influence the clinical management of chlamydial infections in future.

2.13 VACCINE DEVELOPMENT

One of the primary goals of chlamydial immunology research is the development of a vaccine that would resolve and/or protect against disease. As yet there is no consensus as to what constitutes a protective immune response against genital chlamydial infection (Beagley and Timms, 2000). Studies in animal models have shown that cell mediated immunity both Th1-driven macrophage activation and cytotoxic T-cell responses, as well as antibody to mediate protection at different stages of the chlamydial developmental cycle. A successful vaccine would probably need to elicit both cell mediated immunity as well as antibody to limit/restore established infections and a mucosal IgA response to reduce bacterial shedding leading to spread of infections to partners of infected individuals.
The research data accumulated over these past years suggest a paradigm for chlamydial immunity that has great relevance for vaccine design. The paradigm posits that Th1 CD4 T cell responses play a dominant role in protective immunity against chlamydial infection, whereas Th2 cytokine responses, especially IL10, may be associated with immunopathologic responses (Brunham et al., 2000). Th2 cells appear to accelerate tissue fibrosis and granulomatous reactions, fail to localize to areas of chlamydial infection, and by definition do not secrete cytokines such as IFN-γ that inhibits the growth of chlamydiae. Furthermore, Th1, not Th2 effector cells appear to have the appropriate surface receptors that allow them to home infected cells. Therefore, an effective chlamydial vaccine will need to induce a strong CD4 Th1 response if it is to protect against chlamydial infection and prevent tissue pathology.

Modern tools and genetic recombinant technology tend to throw light on possible chlamydial vaccines. DNA vaccines are an exciting new tool in engendering protective immunity to chlamydial antigens. It was shown that C.trachomatis MOMP DNA immunization induced Th1 immune responses that correlated with faster clearance of the organism (Zhang et al., 1997). Although MOMP DNA vaccination was found to be promising, several known and potential limitations exist with this approach. DNA vaccination induces only partial protection and not sterile immunity when used as single gene constructs. Secondly, Th1 immune responses and antibody responses evoked by this method of vaccination are generally weaker than those evoked by infection and immunity (Brunham et al., 2000). Moreover, MOMP is a
polymorphic protein among human strains of *C. trachomatis* and immunity engendered by it may be immunotype specific.

The recent chlamydial genome project has suggested several new candidate chlamydial antigens that might be useful for chlamydial vaccine evaluation. Outer membrane surface components predicted other than MOMP, predicted from the genome sequence are also vaccine candidates, including the newly identified polymorphic membrane protein (Pmp) family (Stephens *et al.*, 1998). Many other predicted surface proteins have also been identified by the genome sequence and may prove useful for eliciting neutralizing antibodies. Of importance is the type III secretion system (TTS) found in *C. trachomatis*. The proteins of TTS are essential virulence determinants mediating intracellular invasion and pathogenesis and represent additional new targets for vaccine testing. Of particular interest, are the effector proteins delivered by the type III secretion system. These proteins are likely delivered to host cell cytoplasm and hence should be presented by MHC class I on the surface of infected cells and stimulate CD8 T cells to secrete inhibitory cytokines (e.g., interferon-γ) and directly kill infected cells (Lambe *et al.*, 1998; Stephens, 2000). The genomic information provides opportunity to try out multiple candidate antigens to elicit both effective humoral and subsequently an effective cell mediated immune response. Studies with whole cell (EB) *C. trachomatis* vaccines demonstrated that protective immunity could be induced by parenteral immunization in humans (Grayston and Wang, 1978). However these vaccines were of limited value as they also contained antigens that elicited hypersensitivity in primed individuals. Animal studies have shown that animals repeatedly exposed to *C. trachomatis* develop more
intense inflammation and tissue damage than do animals exposed for the first time. Antigens such as the 60 kDa heat shock protein (Chsp60) are shown to exacerbate inflammation through molecular mimicry antibody responses (Beagley and Timms, 2000). Therefore antigens like Chsp60 and others that may induce inflammatory responses must be avoided as vaccine antigens.

A live attenuated chlamydial vaccine also may be a possibility in the light of new knowledge of chlamydial metabolism derived from the genome studies. However this has to go a long way, as much more understanding of antigen specific basis for immunopathology is needed for the directed deletion of harmful antigen genes from an attenuated Chlamydia strain. This remains an important research priority. Vaccine development for C.trachomatis seems more possible now than in the past based on the genomic information and the recent knowledge regarding the molecular and cellular basis of chlamydial immunity and pathology.