Chlamydia trachomatis is an obligate intracellular bacteria characterized by a biphasic development cycle of replication. The organism is now recognized the major cause of sexually transmissible human bacterial infections throughout the world. Paralleling this rise in chlamydial infection during recent decades, the prevalence of pelvic inflammatory disease (PID), ectopic pregnancy and tubal infertility has undergone a steady increase. Definitive control of Chlamydia trachomatis sexually transmitted diseases (STDs) is possible through the development of a safe and effective vaccine. A better understanding of the protective immunity and immunopathology of Chlamydia has emerged in recent years from studies using a mouse model of chlamydial genital tract infection. The important progress in our knowledge of functional immunobiology of Chlamydia has established the essential immunologic parameters for vaccine selection and evaluation, including the obligatory requirement for a vaccine to induce a T-helper Type 1 immune response that controls chlamydiae. Recent advances in chlamydial genomics should facilitate identification of likely chlamydial gene products that fulfill the antigenic requirements of putative vaccine candidates. Further studies are however needed in the development of novel and effective delivery systems, vehicles and adjuvants. This review summarizes the status of contemporary Chlamydia immunobiology, immunopathology and vaccine research. Also, in this article we review data generated from other studies on chlamydial genital infections in veterinary medicine such as those into Chlamydophila abortus, the most frequent cause of infectious abortion in sheep (ovine enzootic abortion, or OEA) and goat. This organism is also considered an important zoonotic agent cause of severe, life threatening disease in pregnant women.

KEY WORDS: Chlamydia trachomatis/ Chlamydophila abortus/ Immunity/ Immunopathology/ Vaccines.
INTRODUCTION

Chlamydiae are obligate intracellular Gram-negative bacteria that are responsible for a diverse range of diseases in humans, other mammals, and birds. Following reclassification of the order Chlamydiales in 1999, the family Chlamydiaceae is now divided into two genera, Chlamydia and Chlamydophila\(^\text{2}\). The genus Chlamydia comprises the species Chlamydia trachomatis (pathogen of man), Chlamydia suis (swine), and Chlamydia muridarum (hamsters and mice). The Chlamydophila genus contains the species Chlamydophila pneumoniae (pathogen of man), Chlamydophila abortus (ruminants and swine), Chlamydophila pecorum (ruminants, swine, and marsupials), and Chlamydophila psittaci (birds and poultry) and Chlamydophila caviae (guinea pigs).

Chlamydia trachomatis, one of the three major species within the genus Chlamydia, is an etiological agent for a group of common genital tract syndromes, including urethritis and epididymitis in men and urethritis, cervicitis and pelvic inflammatory disease (PID) in women. C. trachomatis is now recognized as one of the most common sexually transmissible bacterial infections among persons under than 25 years of age living in industrialized nations such as the United States, where the rate of prevalence runs at 4.2\%\(^\text{2}\). Similar prevalence rates have also been documented in a recent population-based study in Britain. Sub-Saharan Africa and Southern and Southeast Asia have particularly high burdens of disease, with an estimated 15 million new cases occurring in Africa and 45 million new cases in Southern Asia every year. All these data highlight a universal feature of C. trachomatis: that infection is mainly observed in adolescents and young adults\(^\text{2}\).

Chlamydial urogenital tract infections are readily cured with antibiotics, but measures based on antimicrobial chemotherapy alone are hampered by the frequency of asymptomatic infections and delayed diagnosis. In addition, a number of studies have documented that within a year after treatment of a previous chlamydia infection, 13-26\% of individuals show persistent or recurrent infection. In fact, Burstein and colleagues\(^\text{2}\) have estimated that the mean time of reinfected is 6-7 months in a sexually active adolescent population. Therefore, considering the high rate of reinfected, screening programs must be extremely aggressive and frequent, in order to reduce the prevalence of chlamydial infection. The definitive control of C. trachomatis sexually transmitted diseases (STDs) will only be possible through the development of a safe and effective vaccine that induce an adequate immune response, avoiding immunopathological consequences. Progress toward the development of an effective vaccine has been disappointingly modest, as it has been for vaccines against other sexually transmitted pathogens that infect the genital tract mucosa. The strict tropism for mucosal epithelial cells, the complex biology and antigenic structure, and the predilection to cause persistent infection have presented formidable challenges to chlamydial vaccine development. A better understanding of protective immunity to C. trachomatis urogenital infection has emerged in the past decade from studies using a mouse model of chlamydial genital tract infections. The insights are of considerable interest because they offer promise for the development of an effective chlamydial vaccine.

This review focuses on the progress made and summarizes the current understanding of the immunological and pathological basis of C. trachomatis genital tract infections. This will provide a rational foundation for the design of a vaccine against infection of this organism. We also review data from similar studies concerning animal chlamydial genital infections, including Ovine Enzootic Abortion (OEA) disease, which is associated with Chlamydophila abortus that causes abortion in both ruminants and humans.

THE INTRACELLULAR DEVELOPMENT CYCLE OF CHLAMYDIA

The key to understanding the pathophysiology of genital tract disease caused by C. trachomatis, is the biphasic development cycle of these organisms (Fig. 1). The bacteria exist in two development forms: the infectious extracellular elementary bodies (EBs) which attach to the host-cell and are internalized in an entry vacuole that avoids fusion with host-cell lysosomes. Within 8-10 h, the small EB (0.2-0.3 \(\mu\)m) differentiate into the second form, the larger (0.5-1.6 \(\mu\)m) non-infectious metabolically active, reticulate bodies (RBs), which proliferate within the same membrane-bound vacuole. After several divisions by binary fission, the RB differentiate back into EB towards the end of the cycle (24-48 h, depending on species) and the EBs are released from the infected cell by lysis or exocytosis to begin a new cycle of infection\(^\text{4}\). The EB, in contrast to the RB, is structurally rigid, as a result of extensive disulphide linkages between various cysteine-rich proteins in, or associated with, the outer membrane. This rigidity results in EBs being resistant to both chemical and physical factors and therefore they are adapted for prolonged extracellular survival, an important factor in terms of chlamydial pathogenesis and treatment of chlamydial infections. The host-cell death observed at the end of the infection cycle could thus be involved in the release of EB from the host cell and could partially contribute to the inflammatory response of the host, since macrophages undergoing apoptosis secrete inflammatory...
cytokines and cells dying necrotically stimulate inflammation\(^4\).

Deviating from this typical development cycle, a third persistent form exits in vitro (i.e., in cell-culture systems), where enlarged aberrant RBs, have been experimentally induced by a variety of stimuli, including IFN-\(\gamma\), antibiotics and nutrient deprivation (Fig. 1). Mention should be made of the ability of these stimuli, particularly IFN-\(\gamma\), to alter Chlamydia to adopt a non-infectious, non-replicating form (aberrant form) that retains viability (persistence). Persistent forms can redifferentiate into infectious EBs upon removal of IFN-\(\gamma\) and subsequent replenishing of intracellular strain. Alternatively, even in an IFN-\(\gamma\)-rich environment, strains of chlamydiae that posses a functional tryptophan synthase (i.e., genital strains) may use indole (perhaps produced by local microbial flora) as a substrate for tryptophan synthesis to counter the growth inhibitory effects of IFN-\(\gamma\).

**GENITAL TRACT CHLAMYDIAL INFECTION**

*C. trachomatis* is the most common cause of STD\(^6\) with approximately 90 million new cases estimated to occur worldwide each year. In young women, the majority of genital tract infections are asymptomatic and so remain undetected and untreated. This leads to persistent infections, which in a large number of cases can result in PID, leading to chronic pelvic pain, ectopic pregnancy or infertility.

*C. trachomatis* EBs normally infect the single-cell columnar layer of the epithelium in the endocervix of women and the urethra of men. At the site of mucosal infection, intense inflammation characterized by redness, oedema and discharge can occur, resulting in the syndrome of mucopurulent cervicitis in women and non-gonococcal urethritis in men. Asymptomatically infected women can show signs of disease as mucopurulent endocervical discharge, hypertrophic cervix, and friability. Clinical symptoms include dysuria, abnormal vaginal discharge, abnormal menstrual bleeding, postcoital bleeding and lower abdominal pain. In some untreated women (20-40%), infection ascends the endometrial epithelium to the fallopian tubes, where *C. trachomatis* can establish persistent infection and cause PID. Overall, 11% of women with PID develop tubal factor infertility and 9% develop ectopic pregnancies. Moreover, this risk seems to be higher for those suffering PID caused by infection with
C. trachomatis than PID caused by other factors, such as infection with Neisseria gonorrhoeae.

THE IMMUNOBIOLGY OF C. TRACHOMATIS

Currently, there are 18 serovars of C. trachomatis (Table I) based on immunoeptope analysis using monoclonal antibodies directed against the major outer membrane protein (MOMP) of chlamydiae. The MOMP comprises approximately 60% of the EB envelope. Among the serovars, the DNA sequence homology of omp1, the gene for MOMP, is greater than 80%, with most of the variance found within the variable domains. The four variable domains are flanked by five constant domains. Many of the C. trachomatis serovars can cause genital infection in humans, in particular serovars D though K. The most commonly isolated serovars from infected individuals are D, E and F. Knowledge of the immune response against C. trachomatis is essential for developing a vaccine. A vaccine needs to induce a protective immune response and avoid responses associated with persistence of infection or immunopathology. A mouse model of vaginal infection, using C. muridarum, has been used to analyse the innate and adaptive responses to infection with C. trachomatis, since both seem to closely mimic acute infection of the genital tract in women.

The Innate Immune Response in Chlamydial Infection

Generally, female genital tract (FGT) contains few leukocytes and must recruit immune cells capable of eradicating infections from the central circulatory system. One early mechanism that appears to reduce the number of organisms early after infection is an influx of neutrophils, which have the capacity to destroy chlamydiae, as demonstrated in vitro. Likewise, in mice that were depleted of neutrophils by antibody treatment, the number of organisms isolated from the FGT were approximately 10-fold greater the day after infection. However, neutrophils are not critical for the eradication of C. trachomatis genital infection since all the mice were able to resolve the infection within the same time frame. Thus, neutrophils appear to play a role in reducing the initial amplification of C. trachomatis and possibly in limiting the spread locally within the FGT.

Natural killer (NK) cells, as well as γδ T lymphocytes have also been implicated in the initial control of clamydial infections. Tseng and Rank reported that mononuclear cells isolated from the FGT of infected mice showed YAC-1 cell cytotoxicity in vitro, which is a measure of NK cell function. Although the depletion of NK cells did not reduce the number of organisms isolated from the FGT during the first week after infection, continued depletion throughout the course of infection resulted in the delayed clearance of Chlamydia. Interestingly, NK cells appear to be necessary for the development of a Th1 protective response against Chlamydia. The authors indicated that NK cells are responsible for the early production of IFN-γ, which would suggest that the primary role of early IFN-γ production by NK cells is to down-regulate the Th2 response, thereby allowing expression of a strong Th1 response which has been shown to be essential for resolution of the infection. In contrast, studies on the role of γδ T cells have shown that these cells play a modest positive role in host defense early in C. trachomatis infection.

Role of DTH in Protective Immunity against and in the Pathology of Chlamydial Infection

The Delayed Type Hypersensitivity (DTH) response has long been considered as both potentially protective and pathological, and this is represented the functional differences in Th1 and Th2- type DTH responses in chlamydial infection. Chlamydia is able to induce both Th1 and Th2 DTH responses each being associated with its respective cytokines which are related with either the clearance of infection or immunopathology. Many groups have shown that DTH and Th1 responses (IFN-γ production) are the major protective mechanisms against chlamydial infection. Paradoxically, immunopathological responses (mucosal scarring) to

**TABLE I. Chlamydia trachomatis serovars and their associated human diseases**

<table>
<thead>
<tr>
<th>Serovars</th>
<th>Human disease</th>
<th>Method of spread</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Ba and C</td>
<td>Ocular tracoma</td>
<td>Hand to eye, fomites and eye-seeking files</td>
<td>Conjunctivitis, and conjunctival and corneal scarring</td>
</tr>
<tr>
<td>D, E, F, G, H, I, Ia, J, Ja, K</td>
<td>Oculogenital disease</td>
<td>Sexual and perinatal</td>
<td>Cervicitis, urethritis, endometritis, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, neonatal conjunctivitis and infant pneumonia</td>
</tr>
<tr>
<td>L1, L2 and L3</td>
<td>Lymphogranuloma venereum</td>
<td>Sexual</td>
<td>Submucosa and lymph-node invasión, with necrotizing granulomas and fibrosis</td>
</tr>
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Chlamydia have also been found to be mediated by DTH (20, 21). Yang et al. (22) studied the protective and immunopathological immune response to *C. trachomatis* using IL-10 gene knockout (KO) and IFN-γ KO mice. The results indicated that, in the absence of IFN-γ, mice were still capable of mounting a significant CD4 T cell-mediated DTH to chlamydial infection, which is associated with Th2 cytokine production and characterized by eosinophil infiltration. The Th2 type DTH response in these mice induced by chlamydial infection was similar in cellular pattern and kinetics to the type 2 cytokine-associated inflammatory reactions in other intracellular models systems (23). Therefore, this Th2 type DTH in IFN-γ KO mice was not protective in controlling local chlamydial infection and preventing its dissemination. The study by Yang et al. (22) suggested that DTH is a double-edged sword in *Chlamydia*-induced immune responses. Although DTH type 1 is critical for host defense against chlamydial infection because of providing protection, DTH type 2 is not protective and may even promote patholgy. The existence of the distinct types of DTH response may be one of the reasons for the dual role of DTH in chlamydial protective immunity and immunopathology.

Cytokines
Recent progress in elucidating the development basis for Th1/Th2 differentiation resulted from the finding that microenvironental cytokines are key factors that influence the behaviour of Th cell precursors to become Th1 or Th2 cells. In particular, the early presence of IFN-γ and IL-12 favors Th1 polarization, whereas the early presence of IL-4 and IL-10 is a potent stimulus for Th2 commitment. In general, the source of these cytokines during the early phases of the immune response often depends on the innate defense mechanisms mobilized by the pathogen.

Given the intracellular development cycle of *Chlamydia*, there is evidence for the involvement of cell-mediated immunity (CMI) (Th1-pathway) and its associated cytokines as IFN-γ, IL-2, and IL-12, in resolving a chlamydial infection. After infection with *Chlamydia* spp., epithelial cells produce various pro-inflammatory mediators, including CXC-chemokine ligand 1 (CXCL1), CXCL8 (also known as IL-8), CXCL16, granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-1β, IL-6 and tumor necrosis factor α (TNFα). Also, an upregulate expression of the chemokines CXC-chemokine ligand 5 (CCL5) and CXCL10 is produced, and the epithelial infected cells secrete cytokines that promote the production of IFN-γ, including IFN-α, IFN-β and IL-12. Infected fibroblasts secrete IFN-γ, IFN-β and nitric oxide, whereas infected macrophages produce TNF-α and IL-12 (24).

Previous studies showed that Th1 CD4+ T cell responses play the dominant role in protective immunity against chlamydial infection, whereas Th2 cytokine responses, especially IL-10, may be associated with immunopathological responses. The Th2 cells appear to accelerate tissue fibrosis and granuloma reactions, fail to reach areas of chlamydial infection and by definition do not secrete cytokines such as IFN-γ that inhibit the growth of *Chlamydiae* (25). Recent studies have provided data that confirming the extreme importance of IL-10 in mediating host susceptibility to chlamydial infection and the development of pathological changes. However, besides IL-10, other cytokines may be also involved in granuloma formation and fibrosis in the course of chlamydial infection. For example, transforming growth factors-β (TGF-β), a cytokine produced predominantly by Th3 cells (25, 26), and other cell types can promote the growth of fibroblasts and induce the synthesis of extracellular matrix proteins (27). Moreover, TGF-β1 is able to alter the antigen presenting cells (APC) thus polarizing T cell responses towards a Th2 type immune response (20).

Carefully regulated cytokine production is crucial to successful immune responses to intracellular pathogens. Deficiencies or overstimulation in the production or activity of these pro-inflammatory cytokines may mediate with failure of protective immunity or harmful inflammation. The pathways trough which acute chlamydial infection is resolved or, alternatively, progresses to chronic infection with severe pathology, appears to be varied and depend on a closely entwined interplay between host and pathogen. Are still to be resolved the means by which a particular immune pathway (humoral or cell-mediated immunity) is adopted, strengthened, or shifted away from balance in favour of one, as demonstrated in other infections (29), all under the influence of changing cytokine patterns, and the degree to which each of these effect the outcome of infection.

One obstacle to understanding the significance of cytokines in infection is the difficulty of extrapolating from animal to human models (30). The contribution of IFN-γ to chlamydial resistance has become less clear due to variations in cytokine susceptibility across different *C. trachomatis* strains (31) and genetic differences between mouse strains (32).

Role of Dendritic Cells in the Immune Response to Chlamydial Infection
The Th cells are activated by recognition of antigenic peptides presented with histocompatibility complex (MHC) class II molecules by APCs, including dendritic cells (DCs), macrophages and B cells. A recent hypothesis states that differential expression and engagement of Toll Like Receptor (TLR) family members at the surface of DCs influences...
the type of immune response that is induced by a microbial pathogen (33). Given the high level of expression of TLRs by DCs and the ability of DCs to polarize immune responses, identification of the role of DCs in Chlamydia-specific immune response is crucial for understanding the type of immune response that is elicited and therefore also for designing a vaccine against infection by C. trachomatis.

The capacity of DCs to present chlamydial antigens has been demonstrated by in vitro and in vivo studies with controversial results. Waalen and colleagues (39) found that DCs were much more potent than monocytes in presenting C. trachomatis antigens. They also found that the DCs from different sources all expressed high levels of MHC class II molecules and that the DC-induced T cell responses to chlamydial antigens could be inhibited by antibodies against HLA-DQ and HLA-DR molecules. The data from this study indicate that DCs may play an important role in presenting chlamydial antigens to T cells in rheumatoid inflammation.

Ojcius and colleagues (34) found that chlamydiae were internalized by DCs in a non-specific manner through macropinocytosis followed by the fusion of the macroinosomes with DC lysosomes which express MHC class II molecules. DCs which have internalized chlamydial organisms can present chlamydial antigens and activate chlamydia-specific CD4+ T cells. Detailed data on the uptake and processing of chlamydial antigens by human DCs (35) showed that the entry of C. trachomatis was mediated by the attachment of the organism to heparin sulfates, which could be blocked by heparin. Infection of DCs with C. trachomatis led to the activation of these APCs, which produce IL-12 and TNF-α but not IL-10. Besides their ability to present chlamydial antigen to CD4+ T cells, infected DCs were also found to be able to activate chlamydia-specific CD8+ T cells (36).

Several studies have been performed to study the role of DCs in inducing an anti-chlamydial immune response in vitro using bone marrow (BM)-derived DCs. Lu and Zhong (37) showed that vaccination with murine BM-derived DCs pulsed with heat-killed Chlamydia could induce protective immune responses in a genital infection mouse model. The protective immune response induced by Chlamydia-pulsed DCs was correlated with a Chlamydia-specific Th1 type immune response, similar to that immunized by live chlamydial strains. They also found that BM-derived DCs could efficiently phagocytose Chlamydia, secrete IL-12 and present chlamydial antigen to infection-sensitized CD4+ T cells in vitro. A very interesting study reported by Shaw and colleagues (38) showed that DCs pulsed with a recombinant chlamydial MOMP antigen had a different role on activating CD4+ T cells in vitro and in vivo experiments. The authors found that, although DCs pulsed with recombinant MOMP secreted IL-12 and stimulated infection-sensitized CD4+ T cells to proliferate and secrete IFN-γ in vitro, the adoptive transfer of the pulsed DCs to naïve mice unexpectedly generated a Th2 rather than Th1 anti-MOMP immune response. These findings suggest that the immunological properties of ex vivo pulsed DCs are not necessarily predictive of the immune response generated in vivo following adoptive transfer. Although there have been substantial studies on in vitro DCs cultures, the recruitment and characteristics of DCs in a natural infection circumstance has yet to be well documented.

Studies (34) reported that immunity to C. trachomatis lung infection induced by vaccination with live organisms was correlated with early production of GM-CSF and IL-12 and the maturation of DCs. The immune response in mice vaccinated with viable organisms included high levels of organism-specific DTH reaction, IFN-γ production and IgA responses. In contrast, vaccination with inactivated organisms mainly induced IL-10 production and IgG1 antibody response without IgA or DTH reaction. The results suggest that early production of pro-inflammatory cytokines and the recruitment of DCs may be the key mechanism by which live-organism vaccination induces active immunity to C. trachomatis infection.

Recently, Rey-Ladino and colleagues (39), in an interesting study using murine BM-derived DCs to examine DC maturation and immune effector function induced by live and UV-irradiated C. trachomatis EBs, confirmed that the level of protection induced by UV-EB-pulsed DC in mice was significantly less than achieved using DC pulsed ex vivo with viable EBs. Thus, exposure of DC to live EBs of C. trachomatis resulted in a mature DC phenotype which was able to promote protective immunity, while exposure to UV-EBs generates a semimature phenotype with less protective potential. This result may explain in part the differences in protective immunity induced by natural infection and immunization with whole inactivated organisms. This study indicates that future directions for rational vaccine design require the use of strategies that cause full DC activation and that the ideal antigen-adjuvant combination would mimic the DC maturation effects induced by live EBs.

The CD4+ and CD8+ T Cells in the Immune Response to Chlamydial Infection

In animal models, the transfer of T lymphocytes from infected or immunized mice can facilitate the clearance of infection in T cell-deficient mice, and this effect has been demonstrated for both CD4+ and CD8+ T lymphocytes (40-44). In humans, both T cell subsets can be detected at the site of C. trachomatis infection, but most work on defining the immune response to C. trachomatis in humans has concerned...
CD4+ T cells. A number of *C. trachomatis* antigens which can be recognized by human CD4+ T cells have been identified, including MOMP\(^{60}\), the 60-kD cysteine-rich outer membrane protein 2 (Omp2)\(^{46}\), polymorphic outer membrane protein D (POMP-D)\(^{67}\), heat shock protein 60 (hsp 60)\(^{46}\), the histone-like protein Hcl and enolase\(^{47}\); several epitopes have been mapped within these antigens. In contrast, much less is known about the antigenic specificity and roles of CD8+ T lymphocytes during *C. trachomatis* infection. In recent years human CD8+ T cells able to recognize MOMP\(^{49,50}\) or Hsp60\(^{48}\) have been isolated from infected humans, but the approach in each case was to identify peptides in *C. trachomatis* proteins which could be predicted to bind to common class I HLA alleles and to determine whether infected subjects had CD8+ T cells able to recognize these peptides.

Cellular toxicity and cytokine-mediated functions are two possible mechanisms that may participate in the CD4+ Th1 immune response that is essential for host resistance to chlamydial genital tract infection. The Th1 cytokine IFN-γ and TNF-α are essential for optimal clearance of infection from genital tract tissues\(^{61,35,51,56}\) in humans and in experimental animals. The effector role for IFN-γ in mediating chlamydial clearance could include both immunoregulatory and non-regulatory functions. An immunoregulatory function for IFN-γ production CD4+ Th1 cells would be in the activation of antigen-specific cytotoxic CD8+ T cells, although such a mechanism seems unlikely because CD8+ T cells are not required for immunity\(^{57,59}\). Although the effector mechanisms of IFN-γ mediated in vivo infection with *C. trachomatis* are not completely understood, it is well established that IFN-γ limits the in vitro growth of *C. trachomatis* through inducing production of tryptophan-decylizing enzyme indoleamine 2,3-dioxigenase (IDO). Activation of IDO by IFN-γ leads to the degradation of tryptophan, and lack of this essential aminoacid causes the death of *C. trachomatis* through tryptophan starvation\(^{61}\) (Fig. 1). Recently, it has been showed that genital, but not ocular, serovars of *C. trachomatis* can use indole as a substrate to synthesize tryptophan in the presence of IFN-γ. This finding suggest that genital strains of *C. trachomatis* might escape IFN-γ mediated eradication in the genital tract by using indole provided by the local microbial flora of the FGT\(^{62}\). Another IFN-γ inducible host cell function in chlamydial immunity is the induction of inducible nitric oxide synthase, although the production of bactericidal nitric oxide free radicals is not a plausible mechanism because mice genetically deficient in inducible nitric oxide synthase resolve both primary and secondary chlamydial infections with kinetics similar to those of wild type mice\(^{63}\).

In passive transfer experiments, CD4+ T cells which produce IFN-γ have been shown to provide protection against *C. trachomatis* in rodents but clearance of the pathogen was slower than in animals to which both CD4+ and CD8+ T cells were transferred, suggesting that additional IFN-γ might be produced by the CD8+ T subset\(^{46,65}\). The *C. trachomatis*-specific CD8+ T cells also play a role in the activation of DC. Matyszak and Gaston\(^{64}\) showed that clones of CD8+ when co-cultured with infected DCs, induced a rapid increase in IL-12 production by DC. Since IL-12 is critical for stimulating the Th1 response and, therefore the expansion of IFN-γ-producing CD4+ T cells, the effects of CD8+ in addition to their own ability to produce IFN-γ, may be to obtain an appropriate Th1-polarized CD4+ T-cell response to *C. trachomatis*, although the CD8 T cells might have a contributory role in limiting infection with *Chlamydia* spp., mainly via cytokines, they are apparently not essential for the clearance of chlamydial infection\(^{57,59}\). Some observations suggest that antigen-specific cytotoxic T cells may contribute more to the pathogenesis of chlamydial infection than to protective immunity. For example, chlamydiae-specific T cell-mediated cytolysis requires high lymphocyte-to-target cell ratios and the lysis of infected targets occurs late in the chlamydial development cycle, at a time when the majority of organisms have differentiated into EBs. This finding does not support a mechanism that would favour inhibition of intracellular growth and argues that cytolytic T cells could potentially contribute more to the pathology or spread of infection than to its eradication\(^{65}\).

**Role of Humoral Immune Response in Chlamydial Immunity**

A definitive role for anti-chlamydial antibodies has been more difficult to demonstrate than for a cellular immune response. However it is increasingly clear that a specific humoral immune response, including secretory and systemic antibodies, appears to play a role in protective chlamydial immunity, facilitating memory response and augmenting the primary CMI during reinfection\(^{58,65,66}\). Several immunobiological studies in a murine model of chlamydial genital and pulmonary infection revealed that efficient clearance of reinfections and the development of a protective memory response were dependent upon a competent humoral immune response with an intact B-cell function and antibody production\(^{58,65}\). Interestingly, mice that lack Fc receptors suffer more severe secondary infection with *C. muridarum* than wild-type mice, owing in part to impaired cellular immune responses, which indicates that B cells and antibodies might also be important for enhancing protective effector T-cell responses\(^{67}\). Possible mechanisms for how B cells contribute to immunity to re-infection include antibody-mediated neutralization and opsonization, as well as enhanced...
antigen presentation to T cells, possibly following Fc-receptor-mediated uptake of antigen-antibody complexes\(^{(59, 68)}\). However, these mechanisms may be more important in preventing the dissemination of chlamydiae to distant sites rather than in resolving infection of the mucosal epithelium. Antibodies might also contribute to the resolution of intracellular infection by an antibody-dependent cellular cytotoxicity (ADCC) mechanism. The potential role for an ADCC mechanism in immunity to chlamydial infection comes from studies demonstrating that an immunoglobulin A (IgA)-dependent CD4\(^+\) T cell ADCC mechanism functions in immunity to other intracellular bacterial pathogens such as Salmonella and Shigella\(^{(68\text{-}71)}\). In addition, B cells are important antigen presenting cells in the activation of memory Th cells and function by promoting the clonal expansion of high frequencies of antigen-specific memory Th cells\(^{(72)}\).

Thus, current findings support the operational paradigm that a potentially efficacious chlamydial vaccine should elicit high levels of both mucosal and systemic Th1 responses, as well as a humoral response that rapidly foments Th1 activation following reinfection.

**VACCINE DEVELOPMENT**

From the above discussion, based on current knowledge, an ideal vaccine against *C. trachomatis* genital infection would need to induce both a systemic CMI response, to deal with *C. trachomatis* as an intracellular pathogen, plus a local mucosal IgA response to reduce bacterial shedding and the resulting spread of infection. At this moment, this represents a daunting challenge for several reasons: little knowledge exists on regulation of the immune response to *C. trachomatis* in the FGT which seems influenced by sex hormones\(^{(73, 74)}\), the lack of adjuvants that target vaccines to the genital mucosa and our limited knowledge concerning which *C. trachomatis* antigens induce protective immune response. The observation that the immune response is directly or indirectly involved in the pathogenesis of disease caused by *Chlamydia* spp. further complicates the vaccine development process. The successful design and delivery of a future chlamydial vaccine will depend on a better understanding of these factors and how they can be manipulated to achieve optimal vaccine efficacy.

**Antigens**

The form, structural and immunobiochemical properties of an antigen selected as potential vaccine determine its capability to induce the required immune effectors that provide protective immunity against chlamydiae. Experimental vaccine selection efforts using outer membrane fractions, recombinant proteins, naked DNA and *ex vitro* antigen-pulsed DCs in models of chlamydial genital, respiratory and ocular infections have revealed promising vaccine candidates\(^{(75\text{-}78)}\).

Because immune protection against *C. trachomatis* infection is probably mediated by immunization with targets protein of CD4\(^+\) and possible CD8\(^+\) T cells, identification of such proteins is particularly important. In fact, recent years have seen the characterization of eight *C. trachomatis* proteins that are target for T-cell recognition\(^{(43, 46, 48, 50, 77, 78)}\). However, the most studied and most promising vaccine candidate is *C. trachomatis* MOMP, which contains serovar-specific epitopes, and five constant domains which are highly conserved among the different serovars and which contain several conserved CD4\(^+\) and CD8\(^+\) T-cell epitopes. Another vaccine candidate is *C. trachomatis* Omp2 which is also an immunodominant antigen that contains CD4\(^+\) and CD8\(^+\) T-cell epitopes, this could provide protection against the different *C. trachomatis* serovars because it has more highly conserved in amino-acid sequence than MOMP\(^{(79)}\). The entire genome of *C. trachomatis* strain D has suggested several new candidate chlamydial antigens that contain known T-cell epitopes, including Hsp60, YopD homologue (homologue of *Yersinia pseudotuberculosis*), enolase and POMP\(^{(76, 80)}\). Among the many exciting findings was the identification of genes encoding the components for a complete type III secretion system (TTSS), the principal virulence mechanism of the organism\(^{(80)}\). As for the outer membrane proteins, the effector proteins secreted by TTSS are possible immunotherapeutic targets. Possible candidates for secretion via the TTSS include the Inc proteins which lack classical amino terminal signal sequences but which have been shown to be localised in the inclusion membrane\(^{(81)}\). Indeed, one such protein known as CrpA, has been shown to be targeted by CD8\(^+\) T cells and to confer partial protection to mice infected with *C. trachomatis*\(^{(49)}\).

It is also important to consider that some *C. trachomatis* antigens contain epitopes that might be associated with pathogenic responses. For example, *C. trachomatis* Omp2 or Hsp60 have been found in patients with reactive arthritis triggered by previous infection with *C. trachomatis*\(^{(47)}\).

**Preference for Subunit Vaccines**

Following immunization with the whole organism, poorly protected individuals who were re-exposed to chlamydiae developed a more severe disease than individuals who were not immunized\(^{(82)}\) (Table II). Mounting evidence suggests that the Hsp60 may account for the negative effects observed during vaccination trials and for some of the long-term sequelae that may result from chlamydial infection\(^{(80)}\). In recent years attention has turned to DNA vaccination as a way of inducing a protective response against chlamydial...
genital infection\(^{[84]}\). However in the search for a vaccine against human chlamydial infection this method has generally been more successful at eliciting a protective response in the murine respiratory model than in the equivalent genital tract model\(^{[85]}\). In general, DNA vaccination has been shown to be more effective in mice than in humans or large animals\(^{[86]}\). As a consequence, the focus of \textit{C. trachomatis} vaccine research has now turned to the production of subunit vaccines that are based on individual \textit{C. trachomatis} protein antigens, which are administered with adjuvant or other delivery vehicles. In this respect, recent studies in chlamydial genomics have predicted several immunogenic proteins that may serve as potential chlamydial vaccines\(^{[87-90]}\). In addition, the formerly serologically defined and molecularly characterized chlamydial antigens of the MOMP are identifying additional vaccine candidates\(^{[67-92]}\). However, vaccine effectiveness based upon MOMP has been limited, due in part, to poor immunogenicity and consequently producing only partial immunity, as measured by a reduction in the infectious burden or pathology. The lack of satisfactory protective immunity with MOMP-based vaccine regimens would suggest that either MOMP alone is inadequate as a vaccine (calling for multisubunit vaccines), or that more effective delivery systems are needed to optimize the effect of MOMP and potentially other emerging single subunit vaccine candidates. Interestingly, some of the POMPs have been shown to induce antigen-specific T cell response against chlamydia antigens\(^{[47]}\).

Comparative structural and immunological analysis of these antigens could well lead to the judicious selection of a combination of immunogens for a multisubunit vaccine. A major advantage of the multiple subunit approach is the potential synergistic immunological benefit of a combination of epitopes from multiple antigens, which could likely induce a higher frequency of immune effectors that ensures an effective long-lasting immunity. In addition, the associated epitopes required for optimal Th1 activation may depend on antigen conformation. Additional studies are therefore needed to clarify the role of antigen conformation in the induction of protective T-cell immunity against chlamydiae.

**Vaccine Delivery Systems**

The focus on a chlamydial subunit vaccine against \textit{C. trachomatis} requires the development of safe and effective delivery vehicles, such as adjuvants and vectors, or biological manipulations techniques capable of boosting the Th1 response and targeted to the genital mucosa, which is the major goal in chlamydial research. There have been a limited number of vaccine studies that have investigated the role of adjuvants in the protective immune response against chlamydial infection in humans. To date, only aluminium salts, liposomes and MF59 have been approved for use in humans\(^{[93]}\). Aluminium adjuvants alone are probably not suitable for controlling primary chlamydial infections as they induce a Th2 humoral response, whereas MF59 and ISCOM matrix are perhaps more appropriate.

<table>
<thead>
<tr>
<th>Immunogen and adjuvant(^*)</th>
<th>Route of challenge</th>
<th>Protection level</th>
<th>Immune response</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOMP DNA</td>
<td>Intranasal</td>
<td>(10^3) less IFUs in lungs</td>
<td>Th1-like, enhanced DTH and IgG2a and IFN-(\gamma) production</td>
<td>84</td>
</tr>
<tr>
<td>MOMP DNA (\text{(ISCOMs)})</td>
<td>Vaginal</td>
<td>No effect</td>
<td>Weak DTH and antibody production</td>
<td>85</td>
</tr>
<tr>
<td>MOMP (rVCGs)</td>
<td>Intranasal</td>
<td>(10^3) less IFUs in lungs</td>
<td>Th1-like, enhanced DTH and IgG2a and IFN-(\gamma) production</td>
<td>85</td>
</tr>
<tr>
<td>Conformational MOMP (Freud’s adjuvant)</td>
<td>Upper genital tract</td>
<td>(70%) reduction in IFUs in vagina</td>
<td>Th1-like, increased IgG2a in serum and IFN-(\gamma) production by splenocytes</td>
<td>24</td>
</tr>
<tr>
<td>MOMP (CT and CpG-containing ODNs)</td>
<td>ND</td>
<td>ND</td>
<td>Mixed Th1/Th2</td>
<td>99</td>
</tr>
<tr>
<td>MOMP (OspA of \text{Borrelia burgdorferi})</td>
<td>Vaginal</td>
<td>(50%) reduction in IFUs in vagina</td>
<td>Mixed Th1/Th2</td>
<td>98</td>
</tr>
<tr>
<td>MOMP and OMP2 (\text{(rVCGs)})</td>
<td>Vaginal</td>
<td>(80%) of animal protected</td>
<td>Th1, IgG2a in serum and IFN-(\gamma) production by splenic T cells</td>
<td>24</td>
</tr>
</tbody>
</table>

\*Adjuvant(s) shown in parentheses. CT, cholera toxin; DTH, delayed-type hypersensitivity; IFN-\(\gamma\), interferon-\(\gamma\); IFU, inclusion-forming unit; ISCOM, immunostimulating complex; MOMP, major outer membrane protein; ND, not determined; ODN, oligodeoxynucleotide; OMP2, outer membrane protein 2; OspA, outer surface protein A; rVCG, recombinant Vibrio cholerae ghost; Th, T helper.
as they can induce a Th1 cellular response(94). In a murine model of C. trachomatis genital tract infection following intramuscular immunization MOMP incorporated into ISCOMs has been shown to induce a local genital mucosal Th1 response and protection(95).

Vector-mediated immunization with naked DNA has received much attention, with delivery of MOMP and Hsp60 genes showing some promising results(96,97). However, this approach has been successful in the murine lung model not in the genital tract(98). Other approaches have investigated the use of bacteria and bacterial antigens as delivery vehicles and adjuvants. Co-administration of the outer surface protein, OspA, of Borrelia burgdorferi with C. muridarum MOMP offered significant protection in mice against chlamydial genital challenge(99). Recent efforts in designing multisubunit proteins on the epitheliotropic experimental ghost vaccines, by expressing select chlamydial genes showing some promising results(100,101). However, this approach has been successful in the murine lung model not in the genital tract(102).

Intramuscular immunisation with recombinant VCG-MOMP has been found to induce elevated local genital mucosal and systemic Th1 responses in a murine model of C. trachomatis genital tract infection(103). This option has the advantage that the VCGs are non-toxic, possess intrinsic adjuvant properties, maintain the structural functional and immunological integrity of expressed antigens, adequately target the primary APCs and are likely to induce mucosal immune responses since Vibrio cholerae ghosts (VCG) have also provided attractive results. Intramuscular immunisation with recombinant VCG-MOMP has been found to induce elevated local genital mucosal and systemic Th1 responses in a murine model of C. trachomatis genital tract infection(104). This option has the advantage that the VCGs are non-toxic, possess intrinsic adjuvant properties, maintain the structural functional and immunological integrity of expressed antigens, adequately target the primary APCs and are likely to induce mucosal immune responses since Vibrio cholerae ghosts (VCG) have also provided attractive results. Intramuscular immunisation with recombinant VCG-MOMP has been found to induce elevated local genital mucosal and systemic Th1 responses in a murine model of C. trachomatis genital tract infection(105). However, this approach has been successful in the murine lung model not in the genital tract(106).

As regards OEA, adult ewes are infected as a result of the contamination of lambing pens or pasture by fetal membranes and discharges. Few or no clinical signs are observed in non-pregnant animals until, during a subsequent pregnancy, ewes abort. Abortion always appears in the last weeks of gestation, regardless of the moment of infection(107).

**Immune response against Chlamydia abortus**

As in the case of C. trachomatis, mouse models have been widely used to study the pathogenesis and the immune response in C. abortus infections(108,109). In experimental murine infections the systemic spread of C. abortus is followed by the establishment of an effective immune response capable of eliminating the infection from every organ except placenta where the bacteria multiply, inducing abortion. Studies from our laboratory have described the kinetic of C. abortus colonization in placenta using a mouse model(110) and natural host(111).

The important role of innate immunity, especially associated with neutrophils, has been shown(112) in the early stages of a primary infection, when it contributes to establish a specific immunity through the secretion of different cytokines. Neutrophils also influence the recruitment of other leukocyte populations, especially CD8+ T cells(113) in a primary response, although these cells have been shown to be of limited relevance in a secondary infection(114). Another component of innate immunity, the natural killer (NK) cells, has been studied in C. abortus infection by Buendía and colleagues(115). The authors demonstrated the relationship between the NK cells and early IFN-γ production in the control of infection of C. abortus, as well as the complex and close relationship with neutrophils, which produce cytokines that are chemotactic and activators for NK cells.

Although innate immunity plays an important role, C. abortus infection is mainly controlled by a specific Th1 immune response which is, at least partly, IL-12-independent and characterized by the early production of high concentrations of IFN-γ(116) and the presence of T cells, particularly CD8(109,113) in contrast with C. trachomatis.
Inmunología bn 72p  26/10/05  10:45  Página 308

immune response(57-60). In fact, unpublished findings(117) suggest an additional regulatory role for CD8+ T cells in the primary response to C. abortus infection.

The balance of the specific immune response is a complex feature. Some reports have shown that the exacerbated production of cytokines in response to C. abortus infection can induce pathological changes(116,118) and abortion has been associated with the detrimental effect of inflammatory cytokines (IFN-γ, TNF-α) induced by the infection in the placenta(119).

The role of humoral immunity against C. abortus has been poorly studied, although we have demonstrated that the passive transfer of anti-MOMP monoclonal antibodies protect pregnant mice against chlamydial abortion(107). We also studied the role for B cells during C. abortus infection(110) and found that B cells are not essential for controlling the multiplication of C. abortus in a secondary infection but could have some part in controlling the exacerbated inflammatory response induced by C. abortus primary infection.

**Vaccination against Chlamyphila abortus infection**

The goal for the prevention of OEA is to obtain an effective vaccine against C. abortus infection. Inactivated vaccines prepared from egg-grown or cell cultures have formed the basis for the prevention of the infection since the early 1950s(120). However, efficiency varies, since cell culture yields of C. abortus are poor, the purification of MOMP oligomer from(107) in contrast to C. trachomatis. However, since cell culture yields of C. abortus are poor, the purification of MOMP oligomer from the bacteria is very difficult and prohibitively expensive for an ovine vaccine. Furthermore, vaccine studies performed in ewes to examine the efficacy of different forms of recombinant MOMP against experimental infection have been disappointing(126). Finally, all the vaccination attempts in mouse models for C. abortus with different DNA vaccines including the genes of Dnak (Hsp70) and MOMP have failed in the induction of a protective immune response(127).

The experimental mouse model is a useful tool for validating commercial or experimental vaccines against C. abortus(107,127-131). Our group demonstrated that none of the inactivated vaccines commercially available in Spain provides an acceptable degree of protection against the bacteria(130). Therefore, in the same murine model, our lab tested different vaccine-production procedures in order to design new inactivated vaccines against C. abortus(132). The results showed that two experimental vaccines (QS-21 and Montanide ISA 773 adjuvanted vaccines) induced good protection and elicited an adequate degree of the cellular immune response required for the clearance of infection. Finally, the selected vaccines were seen to prevent abortion and C. abortus shedding at delivery in pregnant mice.

One important aspect of vaccination against OEA is to choose specific adjuvants that help activate the effector cells or cytokines, polarizing the immune response towards a Th1 type. Related with this fact, in a study on the influence of a Nippostrongylus brasiliensis parasite infection before or after C. abortus vaccination(132), using QS-21 and aluminium hydroxide as adjuvants, we determined that the best protection was offered by QS-21 vaccine. However, the efficacy offered by the vaccine adjuvanted by aluminium hydroxide, an adjuvant usually included in vaccines for veterinary medicine, was reduced when the Th2 response induced by N. brasiliensis was established just prior to infection with C. abortus. In field conditions it is usually not possible to prevent parasitic infections before C. abortus infection, so the authors concluded that care should be taken in choosing the most effective adjuvant or type of vaccine being used to avoid the deleterious immune effects of a high parasitic burden. Recently(133), our group tested these vaccines in sheep and compared the results with those obtained with two representative commercially available vaccines in the natural host. The results showed that the new inactivated vaccines notably increased the protection conferred and minimized C. abortus shedding at delivery.

Finally, the recent publication of the complete genome sequence of C. abortus(134), as it is already available for C. trachomatis, will undoubtedly contribute to the identification of other potential protective antigens that could serve as candidates in experimental vaccines against ovine enzootic abortion.
CLOSING REMARKS

In human and veterinary medicine, contemporary immunological, antigenic and immunomodulatory paradigms guiding vaccine design against Chlamydiae are and well grounded on reproducible and experimental evidence. Experimental findings in animal models are being extended and substantiated by more recent results from clinical studies in humans, which have confirmed the crucial role of the Th1 response in anti-chlamydial immunity. The complementary role of antibodies is appealing in that they contribute to chlamydial immunity by facilitating an enhanced Th1 response.

Vaccine capable of eliciting high levels of Th1 response and the complementary CMI-associated IgG2a and IgA antibodies are the current focus of anti-chlamydial vaccine research. More recent approaches involving the use of recombinant antigens and peptides have been relatively unsuccessful, and so current research aims to improve the presentation of the chosen antigens, and thus the protective efficacy of the vaccine, through the careful selection of appropriate adjuvants, delivery vehicles and routes of inoculation. Chlamydiae vaccine research will continue to focus on the identification of additional antigens that induce protective T cell responses, more feasible now that the complete sequence of the chlamydial genome is available, and on the mechanisms that promote protective immunity in FGT, including the role of DCs in antigen uptake and presentation and the role of pro-inflammatory cytokines in influencing the Th1/Th2 response bias. Further data are required to understand the mechanisms that downregulate the immune response in the FGT, including the effects of sex hormones and the menstrual cycle.

Ideally, suitable vaccines should also limit the shedding of infectious organisms and the spread of infection, but also address the spread of infection, but also

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