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Immunity, immunopathology, and human vaccine development against sexually transmitted Chlamydia trachomatis

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This review examines the immunity, immunopathology, and contemporary problems of vaccine development against sexually transmitted Chlamydia trachomatis. Despite improved surveillance and treatment initiatives, the incidence of C. trachomatis infection has increased dramatically over the past 30 years in both the developed and developing world. Studies in animal models have shown that protective immunity to C. trachomatis is largely mediated by Th1 T cells producing IFN-γ which is needed to prevent dissemination of infection. Similar protection appears to develop in humans but in contrast to mice, immunity in humans may take years to develop. Animal studies and evidence from human infection indicate that immunity to C. trachomatis is accompanied by significant pathology in the upper genital tract. Although no credible evidence is currently available to indicate that autoimmunity plays a role, nevertheless, this underscores the necessity to design vaccines strictly based on chlamydial-specific antigens and to avoid those displaying even minimal sequence homologies with host molecules. Current advances in C. trachomatis vaccine development as well as alternatives for designing new vaccines for this disease are discussed. A novel approach for chlamydia vaccine development, based on targeting endogenous dendritic cells, is described.

Introduction

Chlamydia trachomatis is the most common sexually transmitted bacterial infection in the world with more than 92 million new cases reported per annum and with more than two-thirds of these occurring in developing countries.¹,² Pelvic inflammatory disease (PID), ectopic pregnancy, and infertility are the major adverse clinical outcomes of public health importance.³,⁴ Chlamydia control programs aimed at reducing the incidence, prevalence and morbidity of disease have been implemented in many developed countries. These programs involve the screening and detection of infected individuals, antimicrobial treatment and partner notification. Over the past 30 y the incidence of sexually transmitted chlamydia has dramatically increased in various countries including the USA,²,⁵ Canada,⁶ Sweden,⁷ Norway, and Finland.⁸ In addition, the World Health Organization (WHO) has identified high levels of transmission in Sub Saharan Africa and South East Asia.⁹ Whether this increase correlates with a net increase in chlamydia disease or whether it is the result of more widespread screening and improved diagnosis is controversial. It is clear however, that the highest rates of chlamydia world-wide occur among young adults, particularly women aged 15–24 y old from lower socio-economic backgrounds.¹⁰

Sexually transmitted C. trachomatis infects the endocervical epithelia of women and the urethral epithelia in men.³ Infection remains subclinical in a large proportion of infected individuals (70–90% of women; 30–50% of men) who can still transmit infection.³ Among untreated women, C. trachomatis can ascend along the endometrial epithelium to the fallopian tubes where it can establish persistent infection causing pelvic inflammatory disease (PID) (Fig. 1). Symptoms of PID range from none to severe abdominal pain, fever, dyspareunia, prolonged menstruation, and inter-menstrual bleeding.

Studies in humans and animal models have identified some correlates of anti-chlamydial immunity. However, recent studies have also demonstrated that immune responses to chlamydial infection can be a significant source of pathology and disease. Vaccine development will need to ensure that immunisation does not cause exacerbation of inflammatory mechanisms which may lead to further tissue and organ damage.¹¹⁻¹³

A safe and reliable vaccine is considered to be the best approach to reduce global prevalence of C. trachomatis infection. While considerable progress has been made in recent years, an efficacious C. trachomatis vaccine remains elusive. Identification of correlates of immunity studies in both animal models and human studies, and clarification of protective immune mechanisms to infection, have impaired progress. The discovery of a number of new C. trachomatis and C. muridarum vaccine candidate antigens (Table 1) are encouraging and has inspired renewed efforts to design a human vaccine. However, suitable adjuvants that help stimulate T cell-
mediated immunity against intracellular bacteria are lacking. Likewise, a vaccine delivery system to induce and sustain the immune response to *C. trachomatis* is needed. Research in cancer immunotherapy and experimental vaccines for intracellular infections provides valuable insight for the design of a new vaccine against *C. trachomatis* infection. Vaccines that target endogenous immune cells, such as dendritic cells (DCs), have shown a significant enhancement of T-cell immunity in cancer.\(^{16}\) This review examines various aspects of chlamydia immunity and immunopathology in the context of current vaccine development and explores the possibility of utilizing a DC targeting approach to design new *C. trachomatis* vaccines.

**Immunity to *C. trachomatis***

The last two decades have seen significant progress in the understanding of immunity to *C. trachomatis* genital infection. It is now known that mice or guinea pigs can resist reinfection 30–60 d after primary infection, but these animals can be reinfected after 75 d, although the severity of disease is reduced.\(^{17,18}\) However, the dynamics of susceptibility and host resistance to *C. trachomatis* infection in humans, as compared with animal models, differ substantially. Epidemiological studies, for example, show that under natural conditions T-cell immunity develops slowly perhaps reflecting the silent nature of *C. trachomatis* infection.\(^{19}\)

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**Chlamydia-Specific T cell Immunity**

In both mice and guinea pigs, immunity to *C. trachomatis* is characterized by the development of antigen specific T-cells and antibodies. Antibodies are detectable within 10–14 d after infection. In mice, antibodies produced are long-lasting and can be detected in serum 300 d after infection.\(^{18,20}\) The presence of chlamydia-specific antibodies, however, does not correlate with resolution of infection in humans and, in fact, seem to be more correlated with increased morbidity.\(^{21,22}\) Nevertheless, studies in mice have shown that anti-chlamydia antibodies are more important in the control of re-infection rather than primary infection.\(^{23,24}\) Specifically, B-cell deficient mice with no detectable antibody responses successfully resolved genital infection with *C. muridarum*.\(^{23}\) However, mice depleted of both CD4\(^+\) T cells and CD8\(^+\) T cells, expressing normal anti-chlamydia antibody responses, successfully resisted reinfection.\(^{24}\)

Studies in mice consistently show that development of immunity to genital *C. muridarum* depends on chlamydia-specific CD4\(^+\) T cells and that production of IFN-\(\gamma\) by these cells is required to inhibit chlamydia growth. Gene knockout studies in mice have shown that mice lacking CD4\(^+\) T cells or lacking IFN-\(\gamma\) are impaired to control infection,\(^ {25,26}\) but immunity can be restored by adoptive transfer of CD4\(^+\) Th1 cells.\(^ {27}\) A more recent report showed that CD4\(^+\) T cells are necessary and...
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1Molecular weight in kDa. Abbreviations: MOMP, outer membrane protein; rVCGs, recombinant *Vibrio cholerae* ghost; OspA, outer surface protein A of *Borrelia burgdorferi*; IFUs, inclusion forming units; ReA, reactive arthritis; Cap1, class I accessible protein 1; CrpA, cysteine-rich protein A; PmpD, PmpG, PmpE, polymorphic membrane protein D, G, or E; RpIF, ribosomal protein L6; NrdB, ribonucleoside reductase, small chain protein; CPAF, chlamydial protease-like activity factor; Tarp, type III-secreted effector protein; CrpA, cysteine rich protein A; IFA, incomplete Freund’s adjuvant; ArtJ, arginine binding protein J; ND, not determined.
sufficient for protection against chlamydial reinfection in murine genital mucosa. CD4⁺ and CD8⁺ T cells accumulate in the upper genital tract of guinea pigs and macaque monkeys following *C. trachomatis* genital infection. Presence of both cell types correlated with resistance to reinfection. Furthermore, a study in humans showed that CD4⁺ and CD8⁺ T cells are recruited to the cervix of women with *C. trachomatis* infection and that these cells disappeared following antibiotic treatment. Although these studies suggest that CD4⁺ and CD8⁺ T cells may be involved in the control of *C. trachomatis* infection, the importance of those cell populations has been clearly defined only for CD4⁺ T cells in the mouse where they are essential for protection.

**T-Cell Effector Mechanisms**

IFN-γ produced by T cells is critical to control chlamydia genital infection. In vitro cell culture of human cells have shown that IFN-γ activates infected cells to restrict intracellular growth of *C. trachomatis* by inducing the tryptophan-catabolizing enzyme, indoleamine 2,3-dioxygenase (IDO). Without tryptophan, *C. trachomatis* cannot survive. Thus, mutant cell lines responsive to IFN-γ but deficient in IDO activity, efficiently support *C. trachomatis* growth. Likewise, infected cells cultured in medium with incremental levels of exogenous tryptophan indicated that chlamydial growth requires presence of this essential amino acid. *C. trachomatis* strains isolated from the human urogenital tract can synthesize tryptophan if provided exogenous indol. Therefore, it is possible that indol produced by the microbiota of the female genital tract (FGT) could serve as a source for indol, thus circumventing or diminishing the protective effect of IFN-γ. Additional antimicrobial mechanisms of *C. trachomatis* have also been documented such as the production of nitric oxide and oxygen free radicals by phagocytes, both of which inhibit the chlamydial growth following phagocytosis. In contrast to human epithelial cells, mouse epithelial cells lack IDO, and IFN-γ mediated suppression of *C. trachomatis* growth is thought to be due to a family of IFN-γ inducible p47 GTPases. Moreover, in mice, recent experimental evidence has demonstrated that chlamydia specific CD4⁺ T cell clones can inhibit the growth of *C. muridarum* in epithelial cells by degranulation-dependent mechanisms that appear to directly lyse chlamydial cells. Thus, although IFN-γ inhibits chlamydia growth, the specific effector mechanisms differ substantially in mice compared to in humans. This has implications for vaccine development, as results from the mouse model may not be directly extrapolated to humans and a vaccine developed in mice may require further testing in non-human primates prior to human clinical trials.

**Immunity and Duration of Infection**

A relevant question in the natural history of *C. trachomatis* infection is the amount of time the infected host needs to control an untreated infection. In guinea pigs and mice, primary infection lasts a few weeks before anti-chlamydial immunity and resolution of infection develops naturally. In humans however, the duration of infection can be much more extended. A review of human epidemiological studies on *C. trachomatis* infections reported that in the short-term intervals (weeks) between initial screening and follow-up, spontaneous resolution rates were between 11% and 44%. In a long-term study of 82 women with untreated *C. trachomatis* cervical infection, it was demonstrated that 54% spontaneously resolved infection at one year, 82% at two years, 91% at three years, and 95% at four years. Similarly, another follow-up cohort study has reported that only 45% of *C. trachomatis* positive women cleared infection after one year. From these studies, it is not known for certain if those long duration infections were unresolved infections or if they were re-infections. Nevertheless, under natural conditions, immunity to *C. trachomatis* infection in humans appears to take months to develop. Protracted development of immunity in humans may be due to a number of factors such as low virulence properties of the bacteria at the time of infection, host immunosuppressive abilities of the microbe (reviewed in ref. 4), enhanced survival of chlamydia outside cells, and ability to persist intracellularly.

Once infected, as many as 85–90 percent of *C. trachomatis* infections in men and women remain asymptomatic. At least one third of asymptomatic infected women present local signs of infection. The reason for the low virulence of *C. trachomatis* is not well understood. *C. trachomatis* contains a lipopolysaccharide (LPS) of reduced potency at triggering activation of host immunity. For example, *C. trachomatis* LPS is at least 100 times less potent at activating blood leukocytes than LPS from *Salmonella*. The reduced LPS response may explain, in part, why genital tract infection remains mostly asymptomatic. In addition, *C. trachomatis* downregulates MHC class I and II genes induced by IFN-γ. Together, these observations suggest that by inducing immunosuppressive mechanisms on the host and by not inducing or stimulating sufficient immune responses, *C. trachomatis* has evolved effective mechanisms for survival which should be considered in the design of prophylactic vaccines.

**Human Immunity**

For ethical reasons, studies addressing the dynamics of anti-chlamydial immunity in humans have been difficult to develop. Once diagnosed, patients infected with *C. trachomatis* are required to be promptly treated. Nevertheless, evidence from epidemiological observations has shown that immunity in humans does develop slowly over time. Both the prevalence and intensity of infection among young women are significantly higher than those observed in older women, suggesting development of age acquired immunity. Furthermore, women with reduced CD4⁺ T cell counts, due to human immunodeficiency virus (HIV) infection, possess an increased risk for developing *C. trachomatis* PID, indicating that CD4⁺ T cells are necessary to control infection in humans. *C. trachomatis* infection rates are inversely correlated with duration of the disease in female commercial sex.
Acquired immunity and pathology

Although the most potent host defense mechanism against *C. trachomatis* is based on T-cell immunity, human studies have shown correlation between anti-chlamydial T cell immunity and pathology in the FGT. For example, IFN-γ levels were significantly higher in cervical washes of women with recurrent chlamydial infection as compared with IFN-γ levels observed in women with primary infections.56 These high IFN-γ levels were also associated with infertility.57 Another study showed that cervical cells isolated from *C. trachomatis* infected women secrete IL-1, IL-6, IL-8, and IL-10 in vitro, a cytokine profile more compatible with disease exacerbation rather than immunity.58 In this context, high levels of IL-10 in cervical washes of *C. trachomatis* infected women were linked to infertility.59

Further studies in animals infected with sexually transmitted chlamydia have shown association of T cell-immunity and pathology in the upper genital tract.52,53,54 For example, in macaques, cytotoxic CD8+ T cells and other immune cells infiltrate tubal tissue implants infected with *C. trachomatis*. Infiltrated cells expressed Th1 type cytokines including IFN-γ, IL-2, and IL-6 and accumulation of fibrotic tissue. Moreover, studies confirmed the presence of these CD8+ T cells in the fallopian tubes and their association with pathology in primates and guinea pigs following infection with *C. trachomatis*.55,56

Chlamydia-specific CD4+ T cells have also been implicated in pathology. Protective CD4+ T cells were found to preferentially migrate to the upper genital tract of *C. muridarum* infected mice and their presence was associated with both immunity70 and tubal pathology in the genital tract.71 Interestingly, a recent study has demonstrated that IL-17, produced by Th17 cells, can promote modest immunity in mice infected with *C. muridarum*.72 However, further studies are needed to examine the role of Th17 cells in *C. trachomatis* infection. Together, these observations suggest that a complex interplay of immune responses influences both immune protection and pathology in sexually transmitted *C. trachomatis* infections. Antigens in *C. trachomatis* subunit vaccines should be rigorously examined for specificity, so that potential cross-reactivity can be avoided.

Vaccine Development

Over the past 20 y, significant advances have been made in the development of a *C. trachomatis* vaccine. Firstly, studies in mice and epidemiological observations in humans, consistently show that cellular immunity to genital chlamydia infections depends on chlamydia-specific T cells producing IFN-γ and anti-chlamydia antibodies.73-75 Secondly, a number of protective T cell antigens have now been identified (Table 1)76-85 (reviewed in ref. 86). Immunization of laboratory animals has shown that these antigens are recognized by chlamydia-specific T cells producing IFN-γ and partially protected animals following immunization.75 Importantly, a study has demonstrated that the chlamydial major outer membrane protein (MOMP) antigen alone is insufficient to induce the necessary protection, however MOMP and a second antigen, outer membrane protein 2
(OMP2), can substantially increase resistance to reinfection. This suggests that a vaccine is likely to be based on several, rather than single, protective antigens. Third, it is becoming clear that immune responses in the mucosa occur at local lymph nodes (LNs) and that effector cells migrate via blood to mucosal infection sites. Finally, both locally produced IgA as well as systemic IgG are required to control infection in the genital tract, although only IgG antibodies have been reported to have protective effects against *C. muridarum* in the mouse.

**Vaccine Adjuvants**

A major problem for a *C. trachomatis* vaccine is the lack of suitable non-toxic adjuvants that efficiently promote Th1 type of immunity. Immunization of mice with MOMP DNA without adjuvants, or MOMP with classical adjuvants such as Freund’s showed moderate levels of T cell responses. However, immunization with MOMP incorporated into *Vibrio cholera* ghosts (rVCGs), or Immune Stimulating Complexes (ISCOMS) as adjuvanted delivery technologies, induced robust Th1 responses and protected mice against subsequent chlamydial infection. Likewise, transcutaneous immunization of mice with MOMP incorporated in the delivery system, lipid C, a novel lipid-based matrix originally developed for oral immunization, induced partial protection of both genital and respiratory infections with *C. muridarum*. Interestingly, intranasal immunization with antigen chlamydial protease-like activity factor (CPAF) and IL-12 as an adjuvant, resulted in significantly high levels of anti-IgG2a and anti-IgA antibodies in bronchoalveolar lavage and vaginal fluids. Immunized mice developed Th1 immunity against CPAF, and immunized animals significantly reduced bacterial shedding following challenge and accelerated resolution of infection as compared with non-immunized controls. Overall, these observations showed that vaccine adjuvants are remarkably effective at triggering and sustaining Th1 immunity, leading to increased resistance to infection challenge.

The potential of TLRs-derived adjuvants

TLRs are specialized recognition receptors expressed by immune cells and most DCs. TLRs recognize pathogen-associated molecules known as PAMPs, expressed by pathogens. Binding of TLRs to their PAMP ligands activates DCs, by triggering the NF-κB signaling pathway, resulting in transcription of pro-inflammatory mediators including chemokines, adhesion molecules, matrix metalloproteases, nitric oxide synthase, several enzymes and Th1 type cytokines such as IL-12; all of which contribute to the development of Th1 immunity. For example, experimental vaccines against *Salmonella Typhi*, *Toxoplasma gondii*, and *Leishmania major* have employed CpG-containing oligodeoxynucleotide (CpG-ODN), a TLR9 agonist, as adjuvant in laboratory animals. Immunized animals develop remarkably high levels of Th1 immunity and become resistant to secondary infections. CpG-ODN has been employed in experimental vaccines against *C. muridarum* and *C. caviae* in guinea pigs and in Balb/C mice. These immunization studies showed that CpG-ODN induced potent Th1 type immunity and partial resistance against chlamydial infection.

**Targeting Dendritic Cells (DCs): A Novel Approach for a *C. trachomatis* Vaccine**

Caldwell and collaborators immunized mice with DCs pulsed with heat-killed chlamydia. Immunized mice developed a potent Th1 immunity and resisted chlamydial genital infection. Brunham and co-workers have employed DCs pulsed with chlamydial immunopeptides in immunizations by adoptive transfer. Immunized mice developed Th1 protective immunity and partially resisted chlamydial lung and genital infections. These data show that DCs can be effective inducers of anti-chlamydial immunity and therefore, are attractive tools to design novel T-cell vaccines, including a *C. trachomatis* vaccine.

DCs are professional antigen presenting cells (APCs) with a unique capacity to stimulate naïve T cells. DCs differentiate from bone-marrow CD34+ proliferating progenitors and colonize most peripheral tissues as immature non-dividing cells. DCs have been found in mucosal tissue and are recruited to cervical mucosa during *C. trachomatis* infection. The diverse functions of DCs in immunity depend in part on the state of maturation. Mature DCs optimally prime and induce expansion of both CD4+ and CD8+ T cells producing IFN-γ. Maturation of DCs occurs following detection of microbial PAMPs by TLRs on DCs. Maturing DCs express high levels of the NF-κB family of transcription factors that induce expression of MHC class I and II antigens and co-stimulatory molecules CD80, CD86, CD40, as well as enhanced secretion of IL-12 and IFN-α; all of which contribute to promote Th1 type of immunity.

**Murine DCs Subgroups for Vaccine Targeting**

Two main groups of DC relevant to vaccine targeting have been characterized in the mouse. The first subgroup relevant to vaccine targeting expresses the inhibitory receptor, dendritic cell receptor 2 (DCR2). Antigens delivered to DCR2 DCs are processed in the MHC class II antigen pathway and presented to
CD4+ T cells. A second DCs subgroup expresses the endocytic mannose receptor DEC-205, which is a 205-kDa protein containing C-type lectin domains. Antigens phagocytized by DCs via DEC-205 are presented to both CD4+ T cells and to CD8+ T cells. Remarkably, vaccines delivered via DEC-205 DCs have been shown to be 100–1000 times more efficient at priming peptide-specific CD4+ and CD8+ T cells than a peptide given in Complete Freund’s Adjuvant (CFA). Additionally, vaccines targeting DEC-205 dendritic cells have been tested in experimental vaccines for intracellular infections caused by *Leishmania major* and HIV, with significant high levels of both CD4+ and CD8+ T cell immunity produced.

### Potential Human DCs for Vaccine Targeting

Various human DC markers have been identified. The C-type lectin domain family 9 member A (Clec9A), encodes a type II membrane protein expressed by human blood DCs. In the mouse, Clec9A DCs homologs promote CD4+ and CD8+ T cell responses. An equivalent of the mouse DEC-205 receptor, has been identified in humans and antibodies to human DEC-205 have been developed. In vitro, targeting of human DCs expressing DEC-205 results in induction of CD4+ and CD8+ T cells. Similarly, the human homolog of mouse DCR2, (DCIR), has been identified on human plasmacytoid DCs (pDCs). Antigens targeted to pDCs via DCIR in vitro are efficiently presented to T cells. The DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is another marker expressed on a subset of human DCs. DC-SIGN is abundantly expressed on immature DCs and downregulated during DC maturation. Recent immunization studies showed that DC-SIGN dendritic cells promote induction of antigen-specific CD4+ and CD8+ T-cell responses in DC-SIGN transgenic mice. Together, these results show that simultaneous discovery of complementary DC groups in the mouse and in humans, can facilitate translation of immunization results from mice to humans. Furthermore, targeting DCs with *C. trachomatis* vaccines in the presence of safe TLR-mediated adjuvants such as Pol:IC promise to greatly enhance Th1 type of immunity and resistance to chlamydial infection.

### Future Directions

A number of challenges remain before an efficacious vaccine against *C. trachomatis* can be formulated. The immune regulation of the FGT, which is highly influenced by sex hormones, is poorly understood. Thus, targeting T cell vaccines to the FGT without causing collateral pathology is a major concern. Related to this problem is the fact that a suitable adjuvant that vigorously promotes human T cell-mediated immunity is lacking. Experimental chlamydial vaccines have employed Freund’s adjuvant, *Vibrio cholera* ghosts, and immuno-stimulating complexes, some of which have been tested in human clinical trials. Current testing of TLR mediated adjuvants is encouraging. CpG oligonucleotide, poly I:C as well as monophosphoryl lipid A, are presently used in human vaccines or in clinical trials for other infections. Hence, it is feasible that human *C. trachomatis* vaccine trials employing these or new adjuvants and available candidate antigens could commence in the foreseeable future.

DCs have emerged as attractive targets to develop novel chlamydial vaccines. The diverse functions of DCs in immunity and their unique ability to prime naïve T cells represent excellent opportunity to design novel chlamydial vaccines in the future. Vaccines targeting endogenous DCs with chlamydia antigens promise to induce and expand both CD4+ and CD8+ T cells producing IFN-γ, with the potential to efficiently control *C. trachomatis* infection. An ideal future *C. trachomatis* vaccine would contain various chlamydia antigens targeted to endogenous DCs and a TLR-derived adjuvant as a source of DC maturation signal that would promote optimal DC processing and presentation of T-cell antigens. Targeting specific receptors on DCs requires recombinant antibody molecules and construction of these antibodies is not always feasible. Similarly, TLR-derived adjuvants can be toxic for host cells and chemical modifications are needed before they can be employed in humans. These challenges, which are currently being addressed in various experimental vaccines, will need to be considered when designing a chlamydia vaccine targeting DCs in humans.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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