

IMMUNOLOGY OF *CHLAMYDIA* INFECTION: IMPLICATIONS FOR A *CHLAMYDIA TRACHOMATIS* VACCINE

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Abstract | Sexually transmitted *Chlamydia trachomatis* infections are a serious public-health problem. With more than 90 million new cases occurring annually, *C. trachomatis* is the most common cause of bacterial sexually transmitted disease worldwide. Recent progress in elucidating the immunobiology of *Chlamydia muridarum* infection of mice has helped to guide the interpretation of immunological findings in studies of human *C. trachomatis* infection and has led to the development of a common model of immunity. In this review, we describe our current understanding of the immune response to infection with *Chlamydia* spp. and how this information is improving the prospects for development of a vaccine against infection with *C. trachomatis*.

PELVIC INFLAMMATORY DISEASE (PID). Infection of the upper compartment of the female genital tract, which includes the uterus, fallopian tubes, ovaries and related structures.

ECTOPIC PREGNANCY Pregnancy in which the fertilized egg implants and the fetus begins to develop in tissues other than the normal lining of the endometrium.

SALPINGITIS Inflammatory disease involving the fallopian tubes, which often occurs as a result of infection.

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Worldwide, an estimated 90 million sexually transmitted *Chlamydia trachomatis* infections occur each year¹. More than two-thirds of these cases occur in the developing world, where diagnostic and treatment services are almost absent. 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. The prevalence of infection in Asia might be even higher than this estimate, because a recent study in China concluded that 2.5% of people of 20–64 years of age are infected². Similar prevalence rates (2.1%) have also been documented in a recent population-based study in Britain³. Rates are about twofold higher (4.2%) among a random sample of young adults (18–26 years) in the United States, highlighting a universal epidemiological feature of *C. trachomatis* — that infection is mainly observed in adolescents and young adults⁴.

Sexually transmitted *C. trachomatis* infection is an important public-health concern because of its adverse effects on reproduction¹. In women, infection with *C. trachomatis* causes PELVIC INFLAMMATORY DISEASE (PID) and has long-term consequences — such as infertility, ECTOPIC PREGNANCY and chronic pelvic pain — that are

secondary to scarring of the fallopian tubes (caused by SALPINGITIS) and ovaries. In addition, infection with *C. trachomatis* facilitates the transmission of HIV⁵ and might be a co-factor in human papilloma virus (HPV)-induced cervical neoplasia⁶. Because of public-health concerns, programmes to control *C. trachomatis* have been implemented in many developed countries; these involve the detection of infected individuals through diagnostic testing, which is followed by antimicrobial treatment and tracing of individuals who might have been exposed through sexual contact with the infected person. Although these programmes might control *C. trachomatis* infection, many regions are now showing an increase in the number of infected individuals⁷. This increase might reflect, in part, improvements in diagnostic testing and/or changes in sexual behaviour. Alternatively, the administration of antimicrobial agents might be altering the development of natural immunity to *C. trachomatis* in the population. For example, antimicrobial agents have clearly been shown to blunt the development of immunity to *Chlamydia muridarum* in mouse models of infection⁸. Antimicrobial treatment of infected individuals helps to reduce transmission by shortening the average duration of infection. In the absence of

antimicrobial therapy, *C. trachomatis* infections typically last for many months, but they can undergo spontaneous clearance^{9–11}, which is associated with increasing age and duration of infection and is presumed to be immune mediated^{9,12}.

Why *C. trachomatis* infections take so long to clear is not certain, but it might be a consequence of the many immune-evasion strategies of the organism (BOX 1). Data from animal models of infection indicate that clearance depends on the recruitment of effector T cells and their clonal expansion to a crucial threshold in the genital tract¹³, and it might be that reaching this threshold takes many months in humans. Taken together, these observations indicate that, by shortening the average duration of infection, control programmes that involve antimicrobial treatment might be blunting the development of immunity to *C. trachomatis* and thereby increasing the susceptibility of the population to *C. trachomatis* transmission.

This hypothesis for why the rates of infection with *C. trachomatis* increase in the face of control programmes

needs to be validated, but if it is correct, it has obvious implications for the need for a vaccine to adequately control this infectious disease. Because *C. trachomatis* is such an important pathogen from a public-health perspective and because current programmes for the control of *C. trachomatis* infection are not affordable for much of the developing world and might have an inherent weakness, vaccine development has been identified as essential to controlling infection with *C. trachomatis*.

In general, a vaccine against *C. trachomatis* needs to elicit protective T-cell and B-cell immunity in the genital-tract mucosa. Mouse models of genital infection with *C. muridarum*, which has most of the same genes as the human strains of *C. trachomatis*¹⁴, have provided information on the immune mechanisms of clearance of infection and resistance to re-infection, and these models seem to be useful for analysing immunity to *C. trachomatis* in humans^{12,15}. However, there are several important differences between *C. muridarum* and *C. trachomatis* that might affect the immunobiology of infection. First, *C. trachomatis* infection in humans is much more prolonged than *C. muridarum* infection in mice: mice generally resolve infection after ~4 weeks, whereas in humans, *C. trachomatis* infection can last several months before spontaneous clearance^{9–11}. Second, immune-evasion strategies also differ such that some strains of *C. trachomatis* use tryptophan biosynthesis to escape interferon- γ (IFN- γ)-mediated defence mechanisms of the host (BOX 1), whereas *C. muridarum* does not^{14,16}. Last, *C. trachomatis* shows substantial allelic variation of its dominant surface protein — the major outer-membrane protein (MOMP) — whereas *C. muridarum* has a single allele¹⁴. Because MOMP seems to be an important target of immunity, the specificity of immunity to different serovars (strains) of *C. trachomatis*¹² cannot be studied in the *C. muridarum* model. Although these differences limit the direct extrapolation of findings from *C. muridarum* infection to *C. trachomatis* infection, the mouse model has provided information about the immunobiology of *C. trachomatis* and is guiding the development of a vaccine against infection with this organism.

Here, we review the data generated from studies of *C. muridarum* genital-tract infection of mice and the similar observations obtained from studies of human infection with *C. trachomatis* that have led to our current understanding of the immunology of infection with *Chlamydia* spp. Understanding the immunological basis of immunity to *Chlamydia* spp. and identifying correlates of protective immunity will provide a rational foundation for the design of a vaccine against infection with *C. trachomatis*¹⁷. Because T-cell immunity is central to both mouse and human immunity to *Chlamydia* spp., we describe the antigens derived from *C. trachomatis* and *C. muridarum* that are important for eliciting T-cell responses. Last, we describe how these results, together with recent findings from studies of multisubunit vaccines administered to *C. muridarum*-infected mice, are informing the design of a vaccine against *C. trachomatis* for use in humans.

Box 1 | Immune-evasion mechanisms of *Chlamydia trachomatis*

Enhanced survival outside host cells

- Presence of antigenically diverse surface proteins, such as the major outer-membrane protein and the polymorphic membrane proteins, avoids detection by antibodies¹¹⁶.

Enhanced survival inside host cells

- Replication within a membrane-bound inclusion limits exposure to antibodies and to host-cell antigen-processing and -presentation machinery¹¹⁷.
- Inhibition of mitochondrial release of cytochrome *c*, which is required for caspase-9-mediated apoptosis, inhibits apoptosis of infected host cells¹¹⁸.
- Presence of a particular tyrosyl radical site in the bacterial ribonucleotide reductase is probably responsible for increased resistance to nitric oxide¹¹⁹. *Chlamydia trachomatis* shares this feature with other intracellular pathogens, including *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Tropheryma whippelii*.

Reduced inflammatory responses

- Presence of a lipopolysaccharide (LPS) of reduced potency decreases LPS-mediated activation of host cells. *C. trachomatis* LPS is at least 100-fold less potent at activating host cells than other types of bacterial LPS¹²⁰.

Reduced adaptive immune responses

- Secretion of tumour-necrosis factor by *C. trachomatis*-infected macrophages induces apoptosis of activated T cells *in vitro*¹²¹.
- Cytoplasmic secretion of a *C. trachomatis* protease that degrades transcription factors required for the transcription of MHC genes downregulates interferon- γ (IFN- γ)-induced expression of MHC class I and class II molecules¹²².

Ability to persist as alternative intracellular forms

- Development of persistent, non-replicating forms after exposure of *C. trachomatis* to antibiotics, nutrient deprivation or cytokines (such as IFN- γ) or after infection of monocytes^{84,123}.
- Upregulation of genes involved in intracellular survival, so *C. trachomatis* persistence in response to IFN- γ is controlled at the transcriptional level¹²⁴.
- Maintenance of viability of persistent forms, which can rapidly regain the normal developmental cycle on removal of IFN- γ ^{124,125}.
- Expression of genes encoding tryptophan synthase and a tryptophan repressor by genital strains of *C. trachomatis* suppresses the growth inhibitory effect of IFN- γ if indole is made available¹²⁶.

Table 1 | ***Chlamydia trachomatis* serovars and their associated human diseases**

Serovars	Human disease	Method of spread	Pathology
A, B, Ba and C	Ocular trachoma	Hand to eye, fomites and eye-seeking flies	Conjunctivitis, and conjunctival and corneal scarring
D, Da and E, F, G, H, I, Ia, J, Ja and K	Oculogenital disease	Sexual and perinatal	Cervicitis, urethritis, endometritis, pelvic inflammatory disease, tubal infertility, ectopic pregnancy, neonatal conjunctivitis and infant pneumonia
L1, L2 and L3	Lymphogranuloma venereum	Sexual	Submucosa and lymph-node invasion, with necrotizing granulomas and fibrosis

Chlamydia trachomatis causes ocular trachoma and several sexually transmitted diseases. It has 18 main serovars, as determined by DNA-sequence analysis and immunotyping of the *C. trachomatis* major outer-membrane protein. Serovars A, B, Ba and C cause trachoma, a leading cause of blindness worldwide. Serovars D to K mainly cause sexually transmitted diseases. Serovars L1 to L3 cause lymphogranuloma venereum.

MUCOPURULENT CERVICITIS
Inflammatory disease of the endocervix, which is most often a result of sexually transmitted infection, such as infection with *Chlamydia trachomatis*.

NON-GONOCOCCAL URETHRITIS
Inflammatory discharge from the male urethra, which is most often a result of sexually transmitted infection, such as infection with *Chlamydia trachomatis*.

Infection process

C. trachomatis is an obligate intracellular bacterium that causes several sexually transmitted diseases in humans¹⁸ (TABLE 1). *C. trachomatis* normally infects the single-cell columnar layer of the epithelium in the endocervix of women (FIG. 1) and the urethra of men. Inside epithelial cells, *Chlamydia* spp. undergo a unique developmental cycle that produces infective forms (known as elementary bodies), which then infect neighbouring epithelial cells (FIG. 2). At the site of mucosal infection, intense inflammation that is characterized by redness, oedema and discharge can occur, resulting in the clinical syndrome of MUCOPURULENT CERVICITIS in women and NON-GONOCOCCAL URETHRITIS

in men¹⁹. However, despite initiating local inflammation, *C. trachomatis* infection remains subclinical in a high proportion of infected individuals (70–90% of women and 30–50% of men)¹⁹. Asymptomatically infected women can show signs of disease: in general, mucopurulent endocervical discharge, HYPERTROPHIC CERVICAL ECTOPY and friability (that is, easily induced bleeding of the cervical epithelium)²⁰. Clinical symptoms include dysuria, abnormal vaginal discharge, abnormal menstrual bleeding, postcoital bleeding and lower abdominal pain¹⁹. 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,

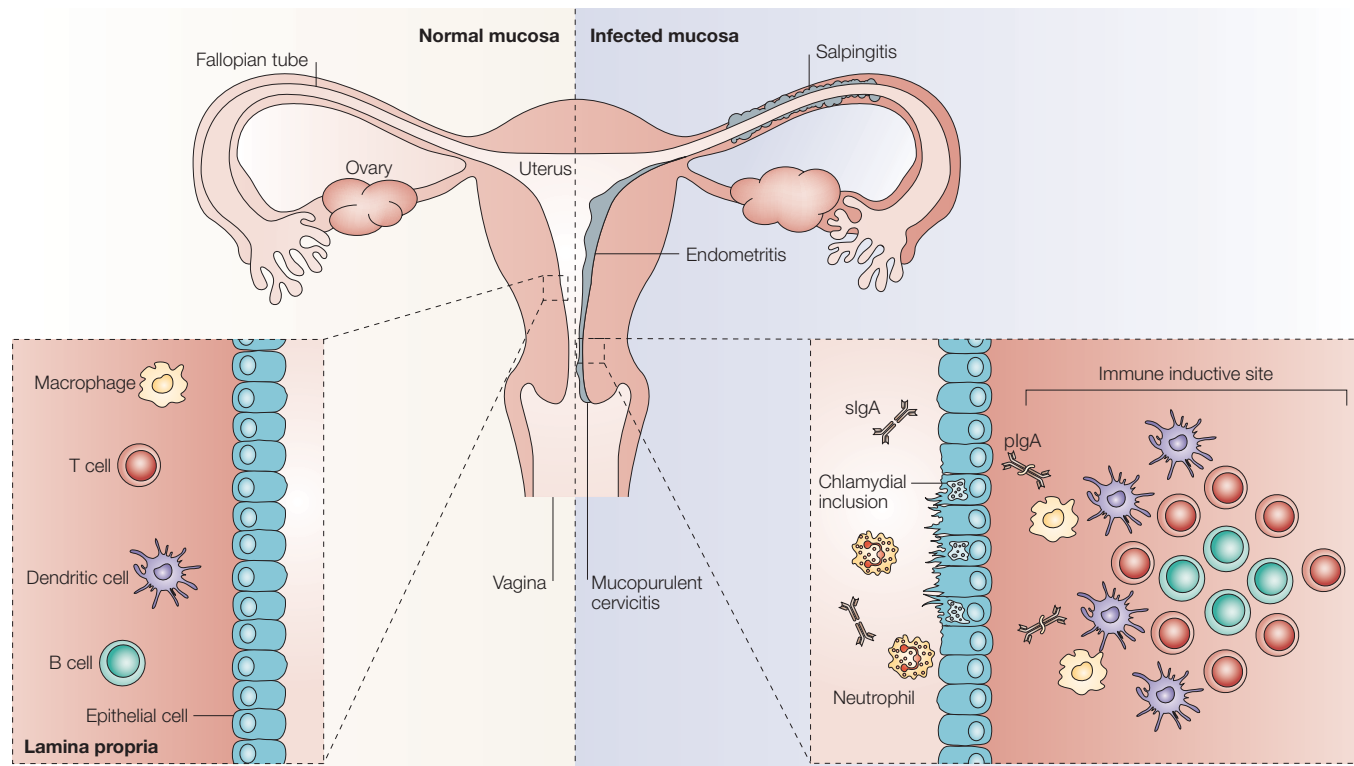


Figure 1 | **Infection of the female genital tract with *Chlamydia trachomatis*.** *Chlamydia trachomatis* elementary bodies infect the columnar epithelial cells of the cervix, which often causes few or no clinical symptoms. The bacteria can ascend to infect the endometrium and the fallopian tubes, causing pelvic inflammatory disease, tubal inflammation (also known as salpingitis), scarring and occlusion, which can lead to infertility or ectopic pregnancy. The inflammatory reaction is characterized by an influx of macrophages and neutrophils and the formation of immune inductive sites in the submucosa. These inductive sites, which contain B cells, T cells, dendritic cells and macrophages, coordinate the initiation of an acquired immune response, including the deployment of a secretory IgA (sIgA) response. pIgA, polymeric IgA.

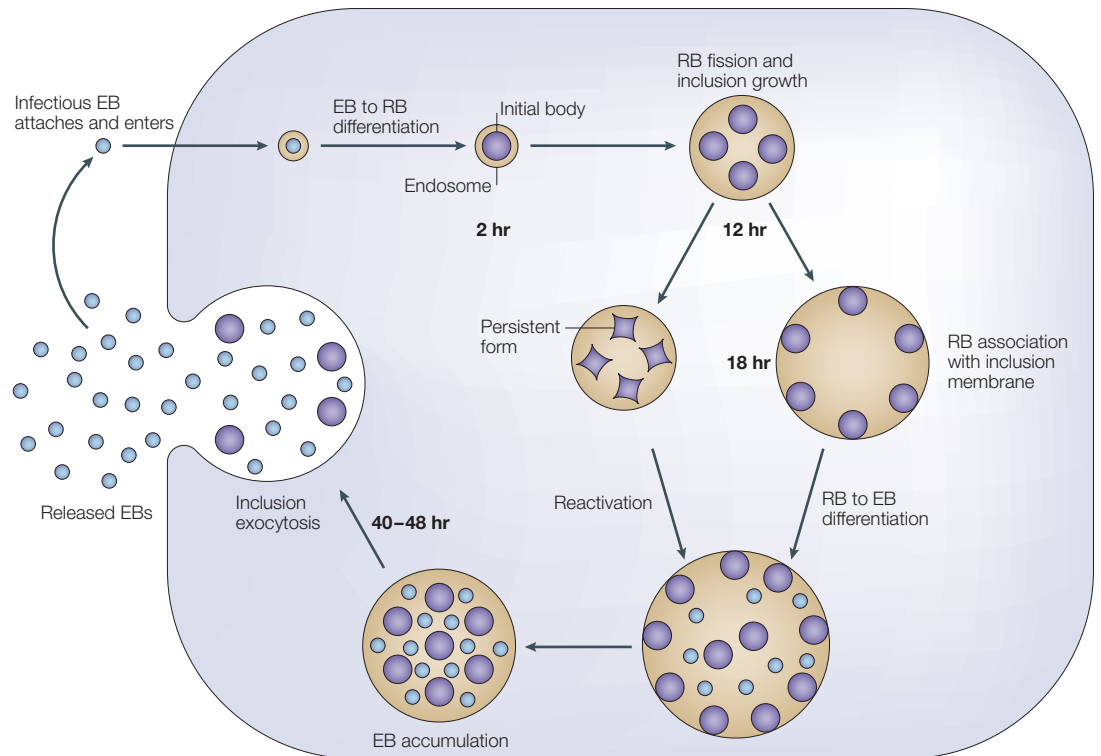


Figure 2 | The developmental cycle of *Chlamydia trachomatis*. *Chlamydia trachomatis* is an obligate intracellular pathogen that resides within a specialized vacuole and has a biphasic developmental cycle¹⁴⁰. An infectious, but metabolically inactive, elementary body (EB) is taken up by mucosal epithelial cells. After internalization, the EB is surrounded by an endosomal membrane to form an inclusion — a vacuole formed from normal endosomal-trafficking pathways — which creates a permissive intracellular niche for the replication of *C. trachomatis*¹¹⁷. Within the inclusion, the EB transforms into a larger metabolically active reticulate body (RB), which divides by binary fission. Within 40–48 hours (hr), the RBs transform back into infective EBs, which are subsequently released from the inclusion vacuole to infect neighbouring cells. In the presence of growth inhibitors, such as interferon- γ , intracellular *C. trachomatis* bacteria acquire a non-replicating, persistent form, and bacteria in this form differentiate back into infectious forms after removal of the inhibitor.

11% of women with PID develop tubal factor infertility and 9% develop ectopic pregnancies²¹. Moreover, this risk seems to be higher for those with PID caused by infection with *C. trachomatis* compared with PID caused by other factors, such as infection with *Neisseria gonorrhoeae*²².

Immunobiology

Elucidating the immunobiology of infection with *Chlamydia* spp. is essential for developing a vaccine. A vaccine needs to induce immune responses that are protective and not responses that are associated with persistence of infection or immunopathology. Establishing immune correlates of protection facilitates the identification of protective antigens in animal models of infection and guides Phase I and Phase II trials of immunogenicity in humans. Identification of immune correlates of protection is an important priority in *C. trachomatis* research, and *C. muridarum* infection models have begun to shed light on immune correlates of protection against infection with *C. trachomatis*. The mouse model of vaginal infection (using *C. muridarum*) has been used to analyse the innate and adaptive responses to infection

with *C. trachomatis*, and it seems to closely mimic acute infection of the genital tract in women^{12,15}.

Cytokines. After infection with *Chlamydia* spp., epithelial cells produce various pro-inflammatory mediators, including CXC-chemokine ligand 1 (CXCL1), CXCL8 (also known as interleukin-8, IL-8), CXCL16, granulocyte/monocyte colony-stimulating factor (GM-CSF), IL-1 α , IL-6 and tumour-necrosis factor (TNF)^{23,24}. Infected epithelial cells also upregulate expression of the chemokines CC-chemokine ligand 5 (CCL5) and CXCL10, and they secrete cytokines that promote the production of IFN- γ , including IFN- α , IFN- β and IL-12 (REFS 24,25). Infected fibroblasts secrete IFN- α , IFN- β and nitric oxide²⁶, whereas infected macrophages produce TNF and IL-6 (REF 27). Most of these are T helper 1 (T_H1)-cell cytokines, which have a role in polarizing the immune response to *Chlamydia* spp. towards a protective T_H1-type response²⁴. By contrast, cytokines such as TNF, IL-1 α and IL-6 might be involved in the pathology associated with infection with *Chlamydia* spp.²⁷ Together, these cytokines trigger inflammation and promote the recruitment of immune cells, thereby actively contributing to the development of innate and adaptive immune responses.

HYPERTROPHIC CERVICAL ECTOPY
Distinctive oedema of the columnar epithelium in the female endocervix. This is usually a feature of mucopurulent cervicitis and is often a result of sexually transmitted infection, such as infection with *Chlamydia trachomatis*.

PEYER'S PATCHES

Specialized lymphoid follicles localized in the submucosa of the small intestine and appendix.

COMMON MUCOSAL IMMUNE SYSTEM

It has been proposed that specialized dynamics of immunity occur in the mucosal compartment. This model considers, for example, that lymphocytes that originate in mucosal inductive sites will home to mucosal effector sites.

LAMINA PROPRIA

Connective tissue that underlies the epithelium of the mucosa and contains various myeloid and lymphoid cells, including macrophages, dendritic cells, T cells and B cells.

Toll-like receptors and dendritic cells. Toll-like receptors (TLRs) detect microbial infection and have an essential role in the induction of innate and adaptive immune responses²⁸. A recent hypothesis states that differential expression and engagement of TLR-family members at the surface of dendritic cells (DCs) influences the type of immune response that is induced by a microbial pathogen²⁸. Infection with *C. muridarum* has been shown to stimulate DCs to produce IL-12 (a cytokine that polarizes immune responses to T_H1-type responses)^{29,30} and CXCL10 (a chemokine that recruits T cells) and to express CC-chemokine receptor 7 (CCR7; a chemokine receptor that is required for the migration of DCs to local lymph nodes)³¹. And, although it is not confirmed which particular TLRs expressed by DCs are engaged by *Chlamydia* spp., TLR2 might have an important role in the activation of DCs by *Chlamydia pneumoniae*³². Furthermore, signalling through TLR2, but not TLR4, is associated with increased fallopian-tube pathology in *C. muridarum*-infected mice²⁷, indicating that engagement of TLR2 is a potential common pathway in both the immunity and immunopathology induced by *Chlamydia* spp. Given the high level of expression of TLRs by DCs and the ability of DCs to polarize immune responses, the identification of the role of DCs in *Chlamydia*-specific immune responses is crucial for understanding the type of immune response that is elicited and therefore also for designing a vaccine against infection with *C. trachomatis*.

DCs have been found in mouse vaginal and cervical mucosae³³ and are recruited to the site of inflammation in response to infection with *Chlamydia* spp.³⁴ Evidence indicates that sampling of microbial antigen across the epithelia of the vagina is accomplished by migratory DCs that carry antigens to peripheral lymph nodes, where antigen is presented to naive T cells³⁵. Mature DCs are highly effective at presenting

antigen and priming protective adaptive immune responses. Accordingly, adoptive transfer of DCs pulsed with *C. muridarum* elementary bodies protects mice against subsequent infection²⁹. Live and inactivated *C. muridarum* induce different levels of DC maturation, and adoptive transfer of DCs pulsed with live *C. muridarum* has been shown to be even more effective at providing protective immunity than DCs pulsed with inactivated bacteria³⁶. These observations might help to explain why vaccination with whole inactivated *C. trachomatis* was only partially protective in human trials³⁷.

Immature DCs and regulatory DCs have also been described to be associated with immune tolerance³⁸ and therefore might have a role in promoting disease pathogenesis, although this has not yet been studied for *Chlamydia* spp. Studies of DCs that reside in the genital tract will be essential to enable the design of vaccines against infection with *C. trachomatis*.

Inductive sites. Although the female genital tract (FGT) mucosa lacks the organized lymphoid structures that are found at other mucosal sites (BOX 2), such as the PEYER'S PATCHES in the intestine, after infection with *Chlamydia* spp., immune cells are recruited to the inflammatory site in the FGT in response to chemokines that are secreted by infected epithelial cells. This results in the subsequent accumulation of lymphocytes and other immune cells and the formation of immune inductive sites (FIG. 1), in which naive B and T cells are clonally selected and expanded³⁹. In FGT infection with *C. muridarum*, these sites form perivascular lymphoid clusters that mainly contain CD4⁺ T cells⁴⁰. In women who have a genital-tract infection with *C. trachomatis*, the inductive sites form lymphoid follicles that mature into germinal centres⁴¹. By contrast, in primates with trachoma — an ocular disease caused by *C. trachomatis* — inductive sites take the form of lymphoid follicles that contain plasma cells, B cells, T cells, DCs, macrophages and neutrophils⁴². Importantly, systemically circulating lymphocytes also seem to be recruited to the FGT during infection with *Chlamydia* spp., because the chemokines (CCL5, CCL7 and CXCL10) that attract lymphocytes are abundantly secreted by *C. muridarum*-infected epithelial cells²⁴ and because the adhesion molecules MADCAM1 (mucosal vascular addressin cell-adhesion molecule 1) and VCAM1 (vascular cell-adhesion molecule 1), which are required for lymphocyte homing from mucosal and systemic inflammatory sites, are highly expressed in fallopian-tube epithelia that are infected with *C. trachomatis*⁴³. So, it seems that *Chlamydia*-specific adaptive immune responses occur not only at mucosal immune inductive sites but also at more distant secondary lymphoid structures, such as regional lymph nodes, and immune cells at these sites then migrate to the local inflammatory site^{41,44,45}.

CD4⁺ and CD8⁺ T cells. Studies of animal models have clearly established that T cells have a crucial role in the resolution of infection with *Chlamydia* spp. Accordingly, nude mice cannot control infection, and adoptive transfer

Box 2 | Unique immunological features of the FGT mucosa

The female genital tract (FGT) mucosa has unique immunological features that differentiate it from the COMMON MUCOSAL IMMUNE SYSTEM of the lungs or the intestine^{88,127,128}.

- The function of the FGT is regulated by sex hormones, which create a balance between tolerance to infection and immunity to infection. Sex hormones and the menstrual cycle both influence the immune response¹²⁹.
- The FGT contains two immunologically different environments: the lower genital tract, which is non-sterile¹³⁰; and the upper genital tract, which is essentially sterile¹²⁷.
- The proportion and quality of the IgA produced in the FGT is different from the IgA produced at other mucosal sites¹³¹. Most IgA in the intestine is produced locally from IgA-secreting plasma cells in the LAMINA PROPRIA. IgA in the genital tract is produced locally by plasma cells and is also transported from the serum¹²⁷. IgA in respiratory-tract and upper intestinal-tract secretions is mostly IgA1, whereas equal proportions of IgA1 and IgA2 are found in FGT secretions¹³¹. Because IgA1, but not IgA2, is susceptible to IgA proteases that are secreted by pathogens, such as *Neisseria gonorrhoeae*, IgA in the FGT is more likely to remain functionally active than IgA at other mucosal sites.
- The FGT mucosa lacks organized lymphoepithelial structures — such as Peyer's patches, which are found in the intestinal mucosa^{35,127} — but contains discrete lymphoid aggregates¹³².

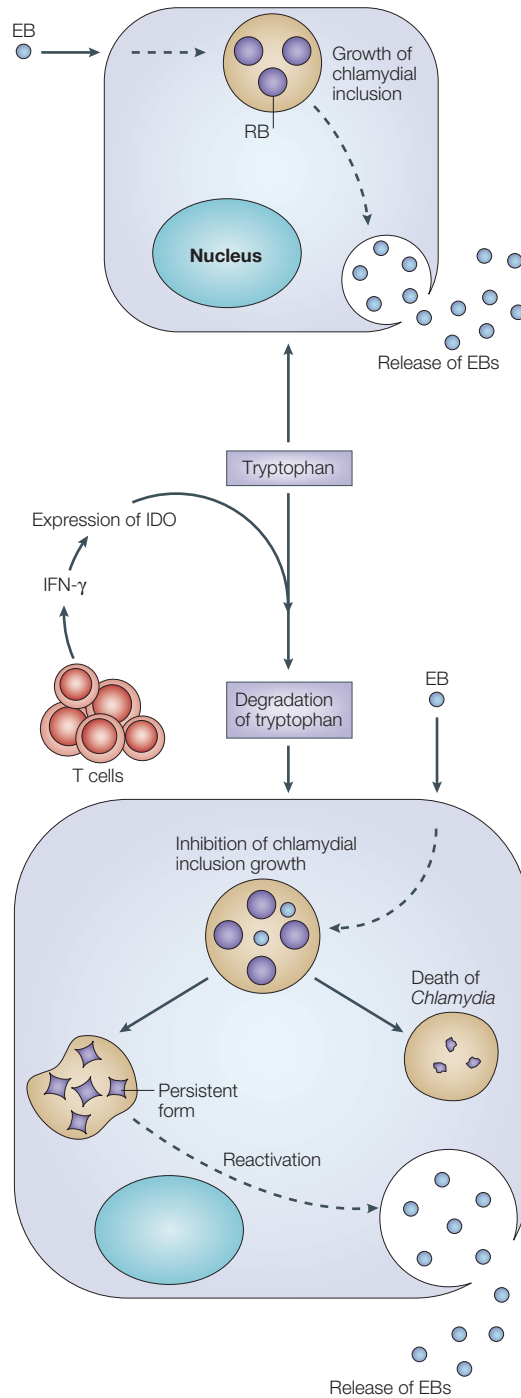


Figure 3 | **Inhibition of chlamydial growth by interferon-γ.** Interferon-γ (IFN-γ) produced by T cells induces the expression of cellular indoleamine-2,3-dioxygenase (IDO), which degrades tryptophan, thereby resulting in reduced levels of intracellular tryptophan^{58,123}. The lack of tryptophan leads to the death of *Chlamydia* spp. through tryptophan starvation. However, a population of *Chlamydia* spp. reticulate bodies (RBs) responds to the lack of tryptophan by acquiring a non-replicating but viable, persistent form. After removal of IFN-γ and replenishment of tryptophan, the persistent forms of *Chlamydia* spp. bacteria rapidly redifferentiate into infectious elementary bodies (EBs). Some strains of *Chlamydia* spp. (genital serovars) have a functional tryptophan synthase that can use indole to synthesize tryptophan and therefore bypass this growth-inhibitory mechanism^{16,59}.

of CD4⁺ or CD8⁺ *Chlamydia*-spp.-specific T-cell lines allows these mice to successfully control infection^{46,47}. Specifically, protection in the *C. muridarum*-infection model seems to be mediated by CD4⁺ T cells that produce IFN-γ^{15,48,49}, as mice deficient in MHC class II molecules⁵⁰, CD4 (REFS 50,51), IL-12 (REF. 52), IFN-γ⁴⁹ or the IFN-γ receptor⁵³ and mice depleted of *C. muridarum*-specific CD4⁺ T cells⁵¹ all have a marked inability to control infection. Furthermore, adoptive transfer of *C. muridarum*-specific CD4⁺ T_H1-cell clones, but not T_H2-cell clones, protected nude mice against infection with *C. muridarum*⁵⁴.

The role and effector mechanism of *Chlamydia*-specific CD8⁺ T cells are less clear. MHC class I peptide presentation to CD8⁺ T cells is not essential for clearance of infection with *Chlamydia* spp.: mice deficient in β₂-microglobulin resolved infection as efficiently as wild-type mice^{50,51}, and mice deficient in perforin or CD95 (also known as FAS) — which are crucial cytolytic effector molecules of CD8⁺ T cells — effectively cleared infection with *C. muridarum*⁵⁵, implying that CD8⁺ T cells are not essential for clearance of infection with *Chlamydia* spp. However, *C. muridarum*-specific CD8⁺ T cells efficiently lysed *C. muridarum*-infected cells when cells were transfected with intercellular adhesion molecule 1 (ICAM1), indicating that, in some situations, CD8⁺ T cells might be important for the elimination of cells infected with *Chlamydia* spp.⁵⁶ Also, adoptive transfer of CD8⁺ T-cell lines specific for serovar L2 of *C. trachomatis* protected mice against infection with *C. trachomatis* through a mechanism involving production of IFN-γ⁵⁷. So, CD8⁺ T cells might have a supporting role in limiting infection with *Chlamydia* spp.

Considerable *in vitro* and *in vivo* evidence shows that production of IFN-γ by *C. muridarum*-specific T cells is essential for clearance of *C. muridarum* from the genital tract¹⁵. Although the effector mechanisms of IFN-γ-mediated control of *in vivo* infection with *C. trachomatis* are not completely understood, it is well established that IFN-γ controls the *in vitro* growth of *C. trachomatis* through inducing production of the enzyme indoleamine-2,3-dioxygenase (IDO)⁵⁸. Activation of IDO by IFN-γ leads to the degradation of tryptophan, and lack of this essential amino acid causes the death of *C. trachomatis* through tryptophan starvation⁵⁸ (FIG. 3). Recently, it has been shown that genital, but not ocular, serovars of *C. trachomatis* can use indole as a substrate to synthesize tryptophan in the presence of IFN-γ, which might allow genital strains of *C. trachomatis* to escape IFN-γ-mediated eradication in the genital tract by using indole provided by the local microbial flora of the FGT^{16,59}. Additional immune effector mechanisms that are induced by IFN-γ include the induction of nitric-oxide production, which inhibits the growth of *C. muridarum*⁶⁰, and the promotion of T_H1-type protective immune responses, which downregulate non-protective T_H2-type responses⁴⁹.

B cells. The importance of antibodies in immunity to *C. trachomatis* was indicated by an early epidemiological observation of an inverse correlation between the

MOLECULAR MIMICRY

When a microbial protein has structural and sequence similarity to a host protein, the immune response can trigger a crossreactive autoimmune attack.

amount of IgA in cervical secretions and the amount of *C. trachomatis* recovered from the cervix of infected women⁶¹. *In vitro*, antibodies specific for *C. trachomatis* can neutralize infection in tissue culture⁶². However, high titres of *C. trachomatis*-specific antibody do not correlate with resolution of infection in humans and, in fact, are more strongly correlated with increased severity of sequelae of infection, such as tubal infertility in women⁶³. Moreover, mice that lack B cells do not show a markedly altered course of primary genital infection with *C. muridarum*⁶⁴. By contrast, B cells are probably important for resistance to secondary infection, because mice that have normal numbers of B cells but are depleted of CD4⁺ and CD8⁺ T cells successfully resolve secondary infection^{51,65}. 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 responses⁶⁶. These results indicate that, although B cells do not have a decisive role in resolution of primary infections, they might be required to control re-infection. 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^{51,67,68}.

Overall, these data show that *Chlamydia*-specific CD4⁺ T_H1 cells, and to a more limited extent CD8⁺ T cells and B cells, are required to control *C. muridarum* infection of the genital tract in mice^{15,69}. And, observations from humans infected with *C. trachomatis* indicate that similar immune effector mechanisms occur in humans^{12,69}. However, despite the mobilization of many immune effectors, infection with *C. trachomatis* can be recurrent and/or prolonged, which probably reflects the array of immune-evasion mechanisms of this pathogen (BOX 1). Immune-avoidance mechanisms might also contribute to pathogenesis and tissue damage, by inducing persistent infection and by enhancing susceptibility to re-infection.

Models of pathogenesis

The pathogenesis of *C. trachomatis* disease is not completely understood, and mouse models (using infection with *C. muridarum*) have been less helpful in this area than they have been in elucidating the basis of immunity. Part of this discrepancy might result from the dependence of *C. trachomatis* pathogenesis on prolonged infection, as *C. muridarum* does not typically cause long-term infections.

Circumstantial evidence from studies of animals infected with *Chlamydia* spp. and from observations of humans infected with *C. trachomatis* repeatedly shows a strong correlation between *Chlamydia*-specific immune responses, such as antibodies and T cells specific for heat-shock protein 60 (HSP60) from *Chlamydia* spp., and PID and fallopian-tube pathology^{70,71}. There are two main hypotheses of pathogenesis, and these are not

mutually exclusive: first, the immunological hypothesis states that immune responses induce collateral tissue damage that is central to pathogenesis⁷⁰; and second, the cellular hypothesis states that pro-inflammatory cytokines that are produced by persistently infected cells are the direct cause of tissue damage⁷².

The immunological hypothesis is supported by the following evidence: first, protective CD4⁺ T_H1 cells preferentially home to the infected fallopian-tube tissue, where they can confer immunity, as well as cause tissue damage^{25,73,74}; second, T_H2 cells that are generated in response to infection with *Chlamydia* spp. might downregulate the protective T_H1-type immune responses, thereby promoting persistent infection^{49,75–77}; third, host and *Chlamydia*-derived antigens (such as HSP60) are recognized by autoreactive T and B cells through MOLECULAR MIMICRY^{78–80}; and fourth, *C. trachomatis*-specific CD4⁺ and CD8⁺ T-cell epitopes are often identified in *C. trachomatis*-associated chronic infections, such as reactive arthritis^{81,82}.

By contrast, the cellular hypothesis (based on the deleterious effects of some cytokines) is supported by the finding that pro-inflammatory cytokines, such as transforming growth factor- β , TNF, IL-1 α and IL-6, are secreted by cells infected with *Chlamydia* spp.^{23,24} So, persistent infection might induce the secretion of pro-inflammatory cytokines, leading to chronic inflammatory cellular responses and tissue damage^{83,84}.

An alternative proposal that might reconcile the two competing hypotheses stems from the observation that IL-10-deficient mice are more resistant to infection with *C. muridarum* and have a shorter course of infection than wild-type mice^{76,77}. Regulatory T cells produce IL-10 (REFS 85,86) and might be important in the pathogenesis caused by *Chlamydia* spp. For example, T cells that are reactive to *C. trachomatis* HSP60 and produce IL-10 have been found in infertile women⁸⁷ and therefore might be involved in the suppression of *C. trachomatis*-specific responses, which could contribute to the ability of *C. trachomatis* to persist. Although T cells with regulatory properties have been described in the mouse FGT⁸⁸, the effect of regulatory T cells has not been examined in infections with *C. trachomatis*, and this warrants further investigation.

Vaccine development

Developing a vaccine against *C. trachomatis* remains a challenge. In part, this results from our poor understanding of the regulation of the immune response in the FGT (which seems to be highly influenced by sex hormones) (BOX 3), the lack of adjuvants that target vaccines to the genital mucosa, our limited knowledge of which *C. trachomatis* antigens induce protective immune responses and the absence of tools to genetically manipulate *Chlamydia* spp.⁸⁹ The observation that the immune response is directly or indirectly involved in the pathogenesis of disease caused by *Chlamydia* spp. also introduces further complexity to the vaccine-development process⁹⁰. Nonetheless, the substantial progress that has been made in elucidating the immunobiology of *C. muridarum* infection is greatly facilitating a renewed effort to design a vaccine

Box 3 | Influence of the menstrual cycle and sex hormones on immune responses in the FGT

Sex hormones modulate both innate and adaptive immune responses in the female genital tract (FGT). For example, in the FGT, levels of the antimicrobial compound lactoferrin are affected by the concentration of oestradiol and the stage of the menstrual cycle¹³³. In the human uterus, natural killer cells accumulate in early pregnancy but progressively disappear after mid-gestation, and their function seems to be controlled by oestradiol¹³⁴. The high levels of oestradiol and progesterone that are present during the secretory phase of the menstrual cycle downregulate the activity of cytotoxic T lymphocytes in the uterus¹³⁵. Furthermore, high levels of progesterone at the fetal–maternal interface potently induce T-helper-2-cell-type cytokines¹³⁶.

The immune response to infection with *Chlamydia trachomatis* is also affected by the presence of sex hormones. In guinea pigs that are infected with *Chlamydomphila caviae*, chronic inflammation and fibrosis in the fallopian tubes is observed in the middle of the menstrual cycle but not at the beginning or the end¹³⁷. Mice that are in the oestrous state or are injected with oestrogen seem to be more resistant to infection with *Chlamydia muridarum*⁶⁹. By contrast, rats that are treated with progesterone are more susceptible to infection with *C. trachomatis*. Progesterone forces the animals into an anoestrous state, which makes the genital epithelium more susceptible to infection^{69,138}. Consistent with the immune-suppressive activities of progesterone is the observation that local vaginal immunization of women during the follicular (high oestrogen), but not the luteal (high progesterone), phase of the menstrual cycle induces the strongest local IgA responses¹³⁹.

against infection with *C. trachomatis*. Selection of defined antigens for a recombinant subunit vaccine that stimulates CD4⁺ T_H1 cells is central to the current design strategy.

Subunit vaccines. Initial human vaccine trials involved intramuscular administration of whole inactivated *C. trachomatis* elementary bodies³⁷, which led to the development of partial short-lived protection. However, in some individuals, the vaccine seemed to exacerbate disease during re-infection episodes^{37,90}. As a consequence, the focus of *C. trachomatis* vaccine research has now turned to the production of subunit vaccines that are based on individual *C. trachomatis* protein antigens, which are administered with adjuvant or other delivery vehicles. As described earlier, T-cell-mediated immune responses are the main requirement for controlling *C. trachomatis* infection, and several antigens that trigger T-cell responses have been identified in humans and in mice. *C. trachomatis* proteins that are recognized by CD4⁺ or CD8⁺ T cells in various *C. trachomatis*-related infections^{57,82,91–97} are shown in TABLE 2.

Selecting antigens. Because immune protection against infection with *C. trachomatis* is likely to be mediated by immunization with *C. trachomatis* proteins that are targets of CD4⁺ and possibly CD8⁺ T cells, identification of such proteins is particularly important. Considerable progress has been made during the past seven years in the characterization of eight *C. trachomatis* proteins that are targets for T-cell recognition (TABLE 2). When the inclusion-membrane-associated protein CrpA (cysteine-rich protein A), which contains *C. trachomatis*-specific CD8⁺ T-cell epitopes, is injected into mice, partial protection against challenge with *C. trachomatis* is observed⁵⁷. Similarly, Cap1 (class I accessible protein 1) — another *C. trachomatis* inclusion-membrane-associated protein, which has high homology among the human *C. trachomatis* serovars — also contains T-cell epitopes, thereby making it a potential vaccine candidate⁹⁷.

However, the most studied and most promising vaccine candidate is *C. trachomatis* MOMP. MOMP constitutes ~60% of the total protein mass of the bacterial outer membrane and is 84–97% identical (at the amino-acid level) between the many *C. trachomatis* serovars⁷⁰. MOMP has four variable domains, which contain serovar-specific epitopes, and five constant domains, which are highly conserved between the different serovars and which contain several conserved CD4⁺ and CD8⁺ T-cell epitopes⁹⁸. Another vaccine candidate is *C. trachomatis* outer-membrane protein 2 (OMP2). OMP2 is also an immunodominant antigen that contains CD4⁺ and CD8⁺ T-cell epitopes (TABLE 2). It is more highly conserved in amino-acid sequence among different *C. trachomatis* serovars than MOMP⁹⁹; therefore, in a vaccine, it could provide protection against the different *C. trachomatis* serovars. Recent experiments have shown that inclusion of OMP2 considerably improves the protective potential of MOMP-based vaccines (discussed later). Other potential protective antigens that contain known T-cell epitopes include HSP60, YopD homologue (homologue of *Yersinia pseudotuberculosis* YopD), enolase and PmpD (polymorphic membrane protein D) (TABLE 2). However, whether these T-cell antigens provide immune protection remains to be determined.

Other *C. trachomatis* T-cell antigens for potential incorporation in a vaccine include secreted components of the *C. trachomatis* TYPE III SECRETION SYSTEM¹⁰⁰ — the principal virulence mechanism of the organism. Because proteins secreted by this system enter the cytosol, they are likely to enter the MHC class I antigen processing and presentation pathway and be targets for recognition by CD8⁺ T cells. Further candidate vaccine antigens might also be revealed by analysis of peptides eluted from MHC class I and class II molecules expressed by DCs pulsed with *Chlamydia* spp.^{36,101}

For the design of a vaccine against infection with *C. trachomatis*, it is also important to consider that some *C. trachomatis* antigens contain epitopes that might be associated with pathogenic responses that occur through

TYPE III SECRETION SYSTEM
A specialized molecular machine present in some bacteria that allows translocation of bacterial proteins into host cells.

Table 2 | *Chlamydia trachomatis* proteins that are recognized by human or mouse T cells

Protein	Molecular weight*	Localization	Type of T cell that recognizes protein	Source of T cells	Reference
CrpA	15	Inclusion	CD8 ⁺ T-cell clones (pooled)	T cells from <i>C. trachomatis</i> -infected mice	57
Cap1	30	Inclusion	CD8 ⁺ T-cell clones (pooled)	T cells from <i>C. trachomatis</i> -infected mice	97
MOMP	40	Membrane	CD8 ⁺ T-cell clones (pooled)	PBMCs from patients with trachoma	93
			CD8 ⁺ T-cell clones (pooled)	PBMCs from <i>C. trachomatis</i> -infected patients	94
			CD8 ⁺ T-cell clones (pooled)	PBMCs from <i>C. trachomatis</i> -infected patients	95
OMP2	60	Membrane	CD8 ⁺ T-cell clone	PBMCs from <i>C. trachomatis</i> -exposed human	91
			CD4 ⁺ T-cell clone	Synovial fluid from <i>C. trachomatis</i> -infected patients with ReA	82
			CD4 ⁺ and CD8 ⁺ T cells	Splenocytes from mice immunized with OMP2-derived peptide	92
HSP60	60	Cytoplasm	CD4 ⁺ T cells	PBMCs from patients with ReA	96
			CD8 ⁺ T-cell clones (pooled)	PBMCs from patients with trachoma	93
YopD homologue	ND	Membrane	CD4 ⁺ T-cell clone	Synovial fluid from <i>C. trachomatis</i> -infected patients with ReA	82
Enolase	46	ND	CD4 ⁺ T cell clone	Synovial fluid from <i>C. trachomatis</i> -infected patients with ReA	82
PmpD	160	Membrane	CD4 ⁺ T-cell clone	Synovial fluid from <i>C. trachomatis</i> -infected patients with ReA	82

*Molecular weight in kDa. Cap1, class I accessible protein 1; CrpA, cysteine-rich protein A; HSP60, heat-shock protein 60; MOMP, major outer-membrane protein; ND, not determined; OMP2, outer-membrane protein 2; PBMCs, peripheral-blood mononuclear cells; PmpD, polymorphic membrane protein D; ReA, reactive arthritis; YopD homologue, homologue of *Yersinia pseudotuberculosis* YopD.

GHOSTS

Lysis of the cytoplasmic membrane of Gram-negative bacteria while maintaining the outer membrane intact generates bacterial ghosts that are useful for antigen delivery.

molecular mimicry. For example, *C. trachomatis*-specific T cells that recognize *C. trachomatis* OMP2 or HSP60 have been found in patients with reactive arthritis (an autoimmune condition in which HLA-B27-restricted responses are thought to have a role) that was triggered by previous infection with *C. trachomatis*⁸² (TABLE 2). In addition, responses to an OMP2-derived peptide were found to be associated with autoimmune heart disease in a mouse model of *C. muridarum*-induced myocarditis⁹². However, this 'pathogenic' epitope is found in the leader peptide of the pro-protein⁹² and is not likely to be presented to the immune system during natural infection, indicating that an OMP2 protein that lacks the signal sequence might be an acceptable vaccine candidate.

Adjuvants and delivery systems. Because sexually transmitted infections with *C. trachomatis* are restricted to the genital-tract mucosa, to be effective, a vaccine might need to target the genital mucosal inductive sites or the associated secondary lymphoid tissues. In general, the mucosa-associated lymphoid tissue has been regarded as a compartmentalized immune environment containing inductive sites that interact with effector sites in the same compartment (that is, other mucosae)¹⁰². So, mice that were pre-infected intranasally with *C. muridarum* had enhanced T_H1-type protective immunity compared with mice that were infected orally or subcutaneously, and these mice were resistant to re-infection of the genital tract¹⁰³. By contrast, mice that were immunized intramuscularly with MOMP formulated with the adjuvant ISCOMs (immunostimulating complexes) were found to be better protected against *C. muridarum* infection of the genital tract than mice that were immunized intranasally with MOMP and ISCOMs¹⁰⁴. These results indicate that an appropriate combination of antigen and adjuvant can be successful even if the vaccine is delivered to a non-mucosal site.

Adjuvants and multisubunit vaccines. MOMP has been extensively used in *C. trachomatis* and *C. muridarum* vaccination studies, together with a diverse range of adjuvants and vaccine delivery systems, and these studies have shown varying levels of protection^{105–112}. TABLE 3 lists the results of some *C. muridarum* vaccination studies. For example, immunization with MOMP DNA has been shown to be highly protective in the lung model of *C. muridarum* infection¹¹³ but not in the genital-tract model¹¹⁰, although at present the reasons for this are not understood. Priming the immune response with MOMP DNA followed by boosting with MOMP protein (formulated with ISCOMs) was found to be highly protective in the lung model of *C. muridarum* infection¹⁰⁸. Although DNA vaccines are a useful experimental tool, their application to human vaccines is uncertain: it has been observed that DNA vaccines are better expressed by transcriptionally active cells of young animals; therefore, they might not be as effective in older humans as in young mice¹¹⁴.

The success of a MOMP-based vaccine might depend on several factors, including the presence of the MOMP epitopes in the correct conformation¹⁰⁵, the availability of an appropriate vector to deliver the MOMP antigen and the presence of other antigens in addition to MOMP¹⁰⁶. This latter possibility is supported by the finding that, although adoptive transfer of DCs pulsed with whole *C. muridarum* elementary bodies protected mice against infection of the genital tract, adoptive transfer of DCs pulsed with MOMP alone did not^{29,115}. More recently, it was shown that mice immunized with *Vibrio cholerae* GHOSTS expressing both MOMP and OMP2 were better protected than mice immunized with *V. cholerae* ghosts expressing MOMP alone¹⁰⁶ (TABLE 3). Mice immunized with both antigens also had a higher frequency of T_H1 cells. These results confirm that MOMP alone is probably not sufficient for providing protection, and they support

Table 3 | Recent results of vaccination trials using *Chlamydia muridarum* major outer-membrane protein as antigen

Immunogen and adjuvant*	Mouse strain	Route of immunization	Route of challenge	Protection level	Immune response	Refs
MOMP DNA	BALB/c	Intramuscular	Intranasal	~10 ³ less IFUs in lungs	T _H 1-like, enhanced DTH and IgG2a and IFN-γ production	108,112
MOMP DNA	BALB/c	Intramuscular	Vaginal	No effect	Weak DTH and antibody production	110
MOMP DNA (ISCOMs)	BALB/c	Intramuscular	Intranasal	~10 ⁶ less IFUs in lungs	T _H 1-like, enhanced DTH and IgG2a and IFN-γ production	108
Conformational MOMP (Freund's adjuvant)	BALB/c	Intramuscular	Upper genital tract	~70% reduction in IFUs in vagina	T _H 1-like, increased IgG2a in serum and IFN-γ production by splenocytes	105
MOMP (rVCGs)	BALB/c	Intramuscular	ND	ND	CD4 ⁺ T cells protected naive mice on adoptive transfer	106,107
MOMP (CT and CpG-containing ODNs)	BALB/c	Transcutaneous	Vaginal	~50% reduction in IFUs in vagina	Mixed T _H 1/T _H 2	109
MOMP (OspA of <i>Borrelia burgdorferi</i>)	C3H/HeN	Intramuscular and subcutaneous	Vaginal	~50% reduction in IFUs in vagina	Mixed T _H 1/T _H 2	111
MOMP and OMP2 (rVCGs)	C57BL/6	Intramuscular	Vaginal	80% of animals protected	T _H 1, IgG2a in serum and IFN-γ production by splenic T cells	106

*Adjuvant(s) shown in parentheses. CT, cholera toxin; DTH, delayed-type hypersensitivity; IFN-γ, interferon-γ; 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; T_H, T helper.

the idea that an effective vaccine is likely to be based on several *C. trachomatis* antigens¹⁰⁶.

Conclusions and future prospects

The protective immune response to infection with *Chlamydia* spp. is highly dynamic and involves both innate and adaptive immune responses. Infection of mice with *C. muridarum* has shown that CD4⁺ T cells, and possibly CD8⁺ T cells, producing IFN-γ, as well as B cells, are required to clear infection and to prevent re-infection. However, immune responses that are associated with persistent infection with *C. trachomatis* seem to induce pathology as a result of chronic inflammation and tissue damage. So, a fine balance between protective immunity and immune-associated disease pathogenesis characterizes the host response to infection with *C. trachomatis*, and this has an impact on the future design of vaccines.

The search for a vaccine against infection with *C. trachomatis* continues to be a complex task. Nevertheless, progress has been achieved in the past few years and has led to the identification of various protective *C. trachomatis* antigens as potential vaccine candidates. Although immunization regimens involving

priming with DNA vaccines and boosting with protein-based vaccines have been found to be highly protective in mice, their practical application in humans remains unclear. Given that multisubunit protein vaccines seem to be more effective than vaccines based on single antigens, in future, *C. trachomatis* vaccine candidates are likely to include various antigens.

C. trachomatis vaccine research will continue to focus on the identification of additional *C. trachomatis* antigens that induce protective T-cell responses and on the mechanisms that promote protective immunity in the FGT, including the role of DCs in antigen uptake and presentation and the role of pro-inflammatory cytokines in influencing the T_H1/T_H2 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, as well as the possible regulatory effect of particular T-cell populations. Finally, a better definition of human immune-response correlates with *C. trachomatis* protective immunity and disease pathogenesis needs to remain an important research priority if we are to develop a vaccine against *C. trachomatis* infection that has protective and not deleterious effects.

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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