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Microreview

Immune-mediated control of *Chlamydia* infection

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Summary

Infection with the bacterium *Chlamydia trachomatis* can lead to a variety of diseases, including ectopic pregnancy, infertility and blindness. Exposure of the host to *C. trachomatis* stimulates multiple innate and adaptive immune effectors that can contribute towards controlling bacterial replication. However, these effectors are often insufficient to resolve the infection and prevent re-infection, and the continued presence of *C. trachomatis* within the host may induce immune effectors to chronically produce inflammatory cytokines. This may eventually lead to the tissue pathologies associated with the infection. Reducing the incidence and sequelae of infection will ultimately require the development of a *C. trachomatis* vaccine that can stimulate sterilizing immunity while avoiding immune-mediated pathology.

Introduction

Chlamydia trachomatis is a Gram-negative obligate intracellular bacterium that infects over 90 million people each year (WHO, 2001). The human diseases associated with *Chlamydia* infection are largely due to the inflammation and ensuing damage of infected mucosal tissues. Chronic inflammation of infected tissues is thought to be mediated by immune effectors that are constantly attempting but unable to eliminate the *Chlamydia* organisms from the host. Although *Chlamydia* infections can be readily cured with antibiotics such as azithromycin or doxycycline, infection often results in 'silent' or asymptomatic disease. Therefore, the challenge in controlling *Chlamydia*-associated diseases is to identify and treat infected individuals before irreversible tissue damage occurs. An even more effective approach to control infections is the development of an effective *Chlamydia* vaccine.

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The *C. trachomatis* species is divided into serovars based on reactivity of patient serum to the major outer membrane protein (MOMP). Certain serovars are associated with infection of ocular tissues while other serovars are associated with infection of genital tissues (Wang *et al.*, 1973; Grayston and Wang, 1975). Serovars A–C infect the conjunctival epithelium and can give rise to trachoma, the leading cause of preventable blindness worldwide (World Health Organization, 2001). Serovars D–K are associated with urogenital tract infections. In the USA, more cases of sexually transmitted disease are caused by *C. trachomatis* than any other bacterial pathogen. Complications arise primarily in women, and include salpingitis and pelvic inflammatory disease which can lead to chronic pain, infertility and ectopic pregnancy. Serovars L1–L3 also establish infection in the urogenital tract but can additionally spread into the draining lymph nodes to cause a relatively rare systemic disease called lymphogranuloma venereum.

Although the different *C. trachomatis* serovars exhibit different tissue tropisms, they all primarily infect epithelial cells, in which the organisms undergo a similar intracellular developmental cycle. The bacterium alternates between two developmental forms, the elementary body (EB) and the reticulate body (RB) (Fig. 1) (Moulder, 1991). EBs are small (300 nm), able to survive in the extracellular environment, and display little or no metabolic activity. It is the EB form of the organism that is infectious, and upon encounter with host cells, EBs induce their own uptake into a vacuolar compartment termed an inclusion. Within 2 h, EBs differentiate into the metabolically active but non-infectious RB form. The RBs then replicate by binary fission within the expanding inclusion. After about 18 h, the RBs begin to differentiate back into EBs. Depending on the *Chlamydia* serovar, lysis and release of EBs into the extracellular space occurs approximately 36–72 h post infection (Moulder, 1991).

Host responses to C. trachomatis

Although *C. trachomatis* has evolved to survive intracellularly within the host cell, the host has in turn evolved an elaborate system to detect as well as to control infection. The immune response to *C. trachomatis* is a co-ordinated event where innate immune cells, B cells, and T cells act

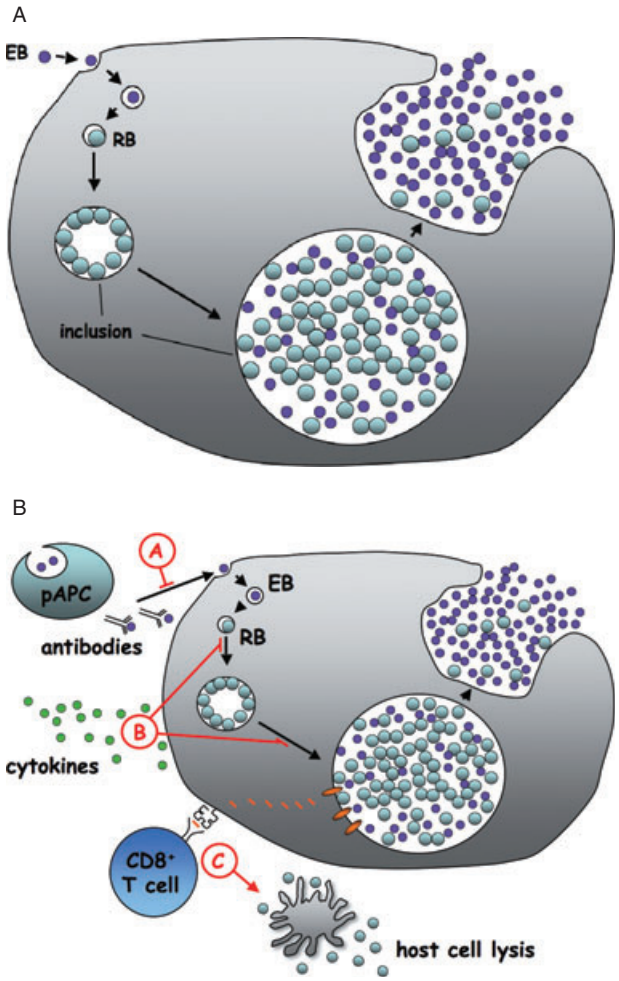

Fig. 1. (The top panel) *C. trachomatis* undergoes a unique developmental cycle. The infectious form of the organism, the elementary body (EB), enters into host cells. Following entry, the EB converts into a metabolically active but non-infectious form of the organism, the reticulate body (RB). The RB replicates within a vacuolar compartment termed the inclusion. Towards the end of the developmental cycle, the RBs differentiate back into EBs. The developmental cycle culminates in host cell lysis and release of EBs into the extracellular space. (The bottom panel) Multiple immune effector mechanisms limit *C. trachomatis* replication. Several steps in the *C. trachomatis* developmental cycle are subject to disruption by immune effectors. (A) Prior to entry into host cells EBs can be engulfed and destroyed by professional antigen presenting cells (pAPC). EBs also can be bound by antibodies, neutralizing their ability to enter epithelial cells and subjecting them to Fc receptor-mediated destruction. (B) Cytokines produced by cells of the innate and adaptive immune systems not only can recruit and activate additional immune cells, but also can directly inhibit the development and replication of *C. trachomatis* within cells. (C) Inclusion membrane proteins and others with access to the host cell cytosol are processed into peptides and displayed on the host cell surface to $CD8^+$ T cells. The responding T cells produce cytokines such as IFN-γ that contribute to inhibition of replication. CD8+ T cells also have the capacity to lyse host cells in which *C. trachomatis* is replicating. Although it is possible that lysis disrupts the development and replication of *C. trachomatis*, it is unknown whether this contributes to immune control of infection.

in concert and where each of these immune effectors have roles in recognizing different stages of the infection. This review focuses on the mechanisms by which these immune effectors act co-ordinately to effect clearance of *Chlamydia* organisms, and how these same effectors may also contribute to the tissue pathology associated with *Chlamydia* infection.

Innate immunity to C. trachomatis

During genital infection with *C. trachomatis*, the mucosal barrier of the genital tract provides the first line of host defense. The ability of the *Chlamydia* organisms to enter this physical barrier is influenced by the stage of the estrus cycle. Shedding of the endometrial epithelium during certain stages of this cycle can limit the ability of *C. trachomatis* to establish a robust infection (Tuffrey *et al*., 1986; Ramsey *et al*., 1999). Urethral samples of infected individuals also contain defensins that have been shown to inhibit *C. trachomatis* infection *in vitro* (Porter *et al*., 2005).

When *Chlamydia* organisms are able to enter the mucosal lining and establish productive infection, innate immune effectors provide the next line of defense against the bacteria. Although epithelial cells are not classically considered critical players within the innate immune system, they are capable of initiating and propagating innate immune responses (Quayle, 2002). *C. trachomatis* infection of both human and murine epithelial cells can induce the production of pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumour necrosis factor-α (TNF-α) and Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Rasmussen *et al*., 1997; Johnson, 2004). In addition, secretion of chemokines such as IL-8 by infected epithelial cells can recruit classical innate immune cells (Buchholz and Stephens, 2006). These include NK cells and phagocytes such as neutrophils, macrophages and dendritic cells (DCs), which are abundant in the genital mucosa (Parr and Parr, 1990; 1991). The recruited cells can in turn produce more inflammatory cytokines such as TNF-α, leading to the restriction of *C. trachomatis* growth within the infected epithelial cells (Dessus-Babus *et al*., 2002). On the other hand, there is evidence that these inflammatory cytokines may also contribute to the pathology associated with *Chlamydia* infection. For example, lower levels of TNF-α and IL-6 have been correlated with decreased genital tract tissue pathology following *Chlamydia* genital infection (Darville *et al*., 2003).

Another major inflammatory cytokine produced by innate cells is interferon-γ (IFN-γ), which can control *Chlamydia* replication through multiple distinct mechanisms. IFN-γ can upregulate the phagocytic potential of macrophages, thereby promoting the engulfment and destruction of extracellular EBs (Zhong and de la Maza,

1988). IFN-γ can also directly inhibit *Chlamydia* growth within infected cells. Upregulation of inducible nitric oxide synthase in response to IFN-γ treatment can kill intracellular *Chlamydia* organisms in infected cell lines (Chen *et al*., 1996; Igietseme *et al*., 1997). In addition, when human fibroblasts are treated with IFN-γ, indoleamine 2,3-dioxygenase production is induced (Gupta *et al*., 1994). Indoleamine 2,3-dioxygenase can inhibit *Chlamydia* growth by catalysing the catabolism of tryptophan, one of the amino acids that *Chlamydia* scavenges from the host cell. Lastly, IFN-γ-mediated downregulation of the transferrin receptor may also limit *Chlamydia* growth by limiting the intracellular stores of iron available to the organism (Byrd and Horwitz, 1993; Freidank *et al*., 2001).

Interferon-γ has also been shown to be required for *Chlamydia* clearance during experimental animal infections. Both IFN-γ and IFN-γ receptor knockout mice are more susceptible to infection with *C. trachomatis* (Cotter *et al*., 1997a; Johansson *et al*., 1997; Perry *et al*., 1997; Lampe *et al*., 1998; Ito and Lyons, 1999). Furthermore, the increased susceptibility of some mouse strains to *C. trachomatis* infection has been attributed to reduced expression of a member of the p47 family of IFN-γ-inducible GTPases. These GTPases are capable of limiting *C. trachomatis* replication in mice and in primary cells through mechanisms that are yet to be elucidated (Nelson *et al*., 2005; Bernstein-Hanley *et al*., 2006). The effects of the IFN-γ-inducible GTPases appear to be limited to models where human serovars of *C. trachomatis* are used to infect mice. Another commonly used mouse model of *Chlamydia* infection is to inoculate female mice in the vaginal vault with the species *Chlamydia* muridarum, often designated as the mouse pneumonitis agent MoPn. Although *C. muridarum* is not a human pathogen, genital infection of mice with *C. muridarum* phenotypically mimics many aspects of *C. trachomatis* genital infection in humans (Barron *et al*., 1981; Everett *et al*., 1999). Interestingly, *C. muridarum* is largely resistant to the inhibitory effects of IFN-γ in both murine cell lines and *in vivo* mouse models (Rottenberg *et al*., 2002; Nelson *et al*., 2005). How *C. muridarum* survives the inhibitory effects of IFN-γ is unknown, but one consequence of this resistance is that a more robust adaptive immune response may have to compensate for the deficit in innate control of *C. muridarum* of mice as compared with *C. trachomatis* infection of mice. Therefore, the species of *Chlamydia* used for analysis influences how the effects of IFN-γ play out in the innate control of *Chlamydia* replication.

The production of IFN-γ and other pro-inflammatory cytokines in response to *C. trachomatis* infection is enhanced through the recognition of pathogen-associated molecular patterns (PAMPs). The best characterized receptors for PAMPs are the Toll-like receptors (TLRs). Although *C. trachomatis* LPS can be recognized by TLR4 (Prebeck *et al*., 2003), TLR2 appears to be more essential for signalling pro-inflammatory cytokine production following *Chlamydia* infection (Darville *et al*., 2003; O'Connell *et al*., 2006). *Chlamydia* organisms appear to colocalize with TLR2, but the *Chlamydia* PAMP that signals through TLR2 remains to be identified (O'Connell *et al*., 2006).

In addition to producing inflammatory cytokines and destroying *Chlamydia* organisms, a subset of phagocytes, the DCs, is also efficient at processing and presenting *Chlamydia* antigens to T cells (Steele *et al*., 2004). The DCs therefore provide an essential link between innate and adaptive immunity.

B cell responses to C. trachomatis

Although innate immunity provides an early line of defence against *C. trachomatis* replication, cells of the adaptive immune system are necessary to limit the infection and provide protection during a future encounter with *Chlamydia*. Indeed, severe combined immunodeficiency mice, which lack an adaptive immune system, are extremely susceptible to *Chlamydia* infection (Magee *et al*., 1993). One key element of adaptive immunity are B cells, which recognize soluble antigen via the B cell receptor.

The concept that B cells and the antibodies they produce can mediate immunity to *C. trachomatis* originated early, when it was observed that the presence of *Chlamydia*-specific antibodies correlated with protective immunity against *C. trachomatis* infection in humans (Jawetz *et al*., 1965; Barenfanger and MacDonald, 1974). Subsequently, it was demonstrated that monoclonal antibodies against *Chlamydia* MOMP could neutralize *Chlamydia* infection *in vitro* (Peeling *et al*., 1984; Peterson *et al*., 1991) and provide a modest level of protection against infection when passively administered to mice (Cotter *et al*., 1995). In addition to neutralization, there is also evidence that Fc receptor mediated activities of antibodies also play an important role in combating infection (Moore *et al*., 2002; 2003).

However, during primary infection, B cells may not play a critical role in controlling *Chlamydia* infection. Su *et al*. (1997) showed that B cell-deficient mice control primary *Chlamydia* genital infection as efficiently as wild-type mice. However, *Chlamydia* clearance following secondary infection is slightly delayed in the absence of B cells (Su *et al*., 1997; Williams *et al*., 1997), suggesting that B cells may play a somewhat larger role in the memory response to *C. trachomatis*, although this protective effect may not hold true for all *Chlamydia* serovars (Johansson and Lycke, 2001). Recently, a more definitive protective role for B cells was revealed by the observation that B cell-deficient mice depleted of $CD4^+$ T cells are completely unable to control secondary infection with *Chlamydia*, whereas wild-type mice depleted of $CD4^+$ T cells alone only exhibit a slight

delay in clearing secondary infection (Morrison *et al*., 2000). This B cell-mediated protection is due to antibody production because passive transfer of immune serum or *Chlamydia*-specific monoclonal antibodies into B cell-deficient, CD4$^+$ T cell-depleted mice rescues the ability of these mice to control secondary infection (Morrison and Morrison, 2005). The high susceptibility of the CD4$^+$ T cell-depleted, B cell-deficient mice to *Chlamydia* infection suggests that there is a synergy between CD4$^+$ T cells and B cells in immunity to *C. trachomatis*.

T-cell responses to C. trachomatis

Although antibodies can neutralize *Chlamydia* infectivity and enhance phagocytosis of EBs, they are unable to access the *Chlamydia* organisms that have established intracellular infection. At this stage, T cells become crucial for recognizing infected cells and orchestrating *Chlamydia* clearance.

Both CD4$^+$ and CD8$^+$ T cells limit C. trachomatis *infection*

The importance of T cells for controlling *Chlamydia* infection was documented over 20 years ago when it was observed that nude mice, which lack T cells, establish chronic infection with *Chlamydia* whereas wild-type mice clear infection within 20 days (Rank *et al*., 1985). Conversely, transfer of polyclonal *Chlamydia*-specific T cells into *Chlamydia*-infected T cell-deficient mice facilitates bacterial clearance (Ramsey and Rank, 1991; Thoma-Uszynski *et al*., 1998). Studies using gene knockout animals or antibody-mediated depletion of CD4$^+$ or CD8$^+$ T cells demonstrated that both CD4$^+$ and CD8$^+$ T cells can contribute to controlling *Chlamydia* infection, with CD4$^+$ T cells appearing to play a bigger role during natural infection (Landers *et al*., 1991; Magee *et al*., 1995; Morrison *et al*., 1995; Williams *et al*., 1997). Furthermore, it has been demonstrated that CD4$^+$ and CD8$^+$ T cell clones can confer protection against *Chlamydia* infection when transferred into nude mice (Igietseme *et al*., 1993; 1994). Protection has also been observed following transfer of *Chlamydia*-specific CD4$^+$ and CD8$^+$ T cells into immunocompetent mice (Starnbach *et al*., 1994; Su and Caldwell, 1995; Roan and Starnbach, 2006; Roan *et al*., 2006). Collectively, data from multiple groups suggest that both CD4$^+$ and CD8$^+$ T cells can provide protective effects during *Chlamydia* infection *in vivo*.

CD4$^+$ and CD8$^+$ T cells recognize antigens from different compartments

CD4$^+$ and CD8$^+$ T cells recognize antigens that are processed through different pathways. CD4$^+$ T cells typically recognize antigens that are engulfed by professional antigen presenting cells (APCs). These antigens are processed by proteases within the lysosomal compartments of APCs, and the resulting peptides are presented by major histocompatibility complex (MHC) class II molecules to CD4$^+$ T cells (reviewed in Trombetta and Mellman, 2005). *Chlamydia* EBs in the extracellular space within tissues can be phagocytosed by professional APCs and thereby serve as a source of CD4$^+$ T-cell antigen. RB antigens can also be presented to CD4$^+$ T cells by professional APCs that have engulfed infected cells harboring RB organisms. CD4$^+$ T cells may therefore recognize antigens from multiple stages of *Chlamydia* infection.

In contrast, CD8$^+$ T cells typically recognize antigens that have access to the cytosol of infected cells. These cytosolic antigens are processed by the proteasome into peptide fragments, which are eventually shuttled to the cell surface in complex with MHC class I to activate CD8$^+$ T cells (reviewed in Cresswell *et al*., 2005). Although *Chlamydia* are confined to the inclusion during the intracellular stages of the developmental cycle, a number of *Chlamydia* proteins have access to the host cytosol. These include proteins that are secreted into the cytoplasm (Zhong *et al*., 2001; Clifton *et al*., 2004) as well as inclusion membrane proteins that have domains which extend into the cytosol of the host cell (Bannantine *et al*., 2000; Fling *et al*., 2001). *Chlamydia* proteins that have access to the cytosol have been demonstrated to serve as CD8$^+$ T-cell antigens (Fling *et al*., 2001; Starnbach *et al*., 2003).

Effector functions of CD4$^+$ T cells

Activated CD4$^+$ T cells are important producers of effector cytokines. In general, the types of effector cytokines produced by CD4$^+$ T cells are largely dependent upon the nature of the infection and the types of cytokines present in the environment during T-cell differentiation. Classically, the two major lineages of CD4$^+$ effector T cells are Th1 and Th2 cells. Th1 cells produce IFN-γ, a cytokine also produced by innate effectors, as discussed earlier. In addition to its role in directly limiting *Chlamydia* replication, IFN-γ also plays an important role in adaptive immunity as it can enhance the presentation of antigens to both CD4$^+$ and CD8$^+$ T cells (Gaczynska *et al*., 1993; Steimle *et al*., 1994). CD4$^+$ T cell-derived IFN-γ appears to protect against infection as an IFN-γ-producing CD4$^+$ T-cell clone, but not an IL-4-producing CD4$^+$ T-cell clone, protected mice against *Chlamydia* genital infection (Hawkins *et al*., 2002).

In contrast to Th1 cells, Th2 cells produce IL-4, IL-5 and IL-13. They do not appear to protect against *Chlamydia* infection and may even indirectly enhance *Chlamydia* load by inhibiting the development of protective Th1

responses (Brunham and Rey-Ladino, 2005). The inability to completely eliminate *Chlamydia* organisms from hosts with inadequate Th1 responses can result in continuous production of inflammatory cytokines which can lead to tissue destruction. The factors that determine whether a Th1 or a Th2 response develops following *Chlamydia* infection is largely unknown, but will be important to understand in order to develop a vaccine that will stimulate protective and not pathological outcomes. Recent experiments comparing immune responses against different species of *Chlamydia* have suggested that NKT cells may be an important determinant of the Th1/Th2 bias during *Chlamydia* infections (Joyee *et al*., 2007).

In addition to cytokine production, $CD4^+$ T cells are also crucial activators of other immune effectors, such as B cells and $CD8^+$ T cells. Mice deficient in $CD4^+$ T cells therefore have defects in multiple arms of adaptive immunity. This may in part explain why mice deficient in $CD4^+$ T cells are more susceptible to *Chlamydia* infection than mice deficient in only B cells or $CD8^+$ T cells (Morrison *et al*., 1995; Williams *et al*., 1997).

Effector functions of $CD8^+$ T cells

Like $CD4^+$ T cells, $CD8^+$ T cells are also potent producers of effector cytokines such as IFN-γ. $CD8^+$ T cell-derived IFN-γ can contribute significantly towards controlling *C. trachomatis* infection. Whereas adoptive transfer of IFN-γ-producing *Chlamydia*-specific $CD8^+$ T cells into naïve mice confers protection against *C. trachomatis* challenge, *Chlamydia*-specific $CD8^+$ T cells unable to produce IFN-γ do not confer protection (Lampe *et al*., 1998). In contrast to $CD4^+$ T cells, which recognize peptides presented by MHC class II expressed mainly on professional APCs, $CD8^+$ T cells recognize peptides presented by MHC class I, which is expressed on virtually all nucleated cells. Therefore, $CD8^+$ T cells may provide an essential source of IFN-γ in response to *Chlamydia* infection of the genital mucosa, where bacterial replication occurs predominantly in epithelial cells.

In addition to cytokine production, $CD8^+$ T cells also have the ability to directly kill target cells, hence the name cytotoxic T lymphocytes. $CD8^+$ T cell-dependent cytolysis is very specific for cells expressing the appropriate peptide-MHC class I complex, thereby ensuring the elimination of infected cells while sparing neighboring healthy ones. T cell-mediated lysis of *Chlamydia*-infected cells has been observed *ex vivo* (Starnbach *et al*., 1994; Roan and Starnbach, 2006). It has always been tempting to predict that lysis of infected cells should contribute to controlling infection as it could deprive *C. trachomatis* of its intracellular niche and lead to the release of non-infectious RBs. However, a role for lysis in protection against *C. trachomatis* infection *in vivo* has yet to be demonstrated.

Antigen-specific T-cell responses to C. trachomatis

Although it has long been appreciated that both $CD4^+$ and $CD8^+$ T cells respond to *C. trachomatis* infection, it is only recently that investigators have begun to examine how T cells specific for defined *Chlamydia* antigens respond to the infection. We have recently identified *C. trachomatis*-specific T-cell epitopes in mice which have allowed us to enumerate T cells specific for a single antigen over the course of infection (Fling *et al*., 2001; Starnbach *et al*., 2003). Furthermore, we have generated T-cell receptor (TCR) transgenic and retrogenic mice specific for defined *Chlamydia*-specific $CD4^+$ and $CD8^+$ T-cell antigens, allowing us to examine the early response of *Chlamydia*-specific T cells to cognate antigen (Roan and Starnbach, 2006; Roan *et al*., 2006). Both TCR transgenic and retrogenic mice serve as an abundant source of antigen-specific T cells that can be adoptively transferred into wild-type recipient mice, thereby boosting the frequency of these cells so that they can be detected during the early phases of the infection, before the T cells have clonally expanded. By adoptively transferring *Chlamydia*-specific TCR transgenic and retrogenic cells into recipient mice, we have demonstrated that in response to genital infection with *C. trachomatis*, both *Chlamydia*-specific $CD4^+$ and $CD8^+$ T cells are activated and proliferate in the iliac lymph nodes that drain antigen from the genital tract. Furthermore, both subsets of T cells develop the ability to produce IFN-γ before migrating to the infected genital mucosa (Roan and Starnbach, 2006; Roan *et al*., 2006).

In these studies, proliferation of both *Chlamydia*-specific $CD4^+$ and $CD8^+$ T cells is not initiated until several days following genital infection with *C. trachomatis*. The period between *Chlamydia* inoculation and activation of the antigen-specific T cells may define the amount of time required for *Chlamydia* antigens to reach the draining lymph nodes where they can be presented to naïve T cells. The delay in T-cell activation may reflect structural differences between the immune system in genital tissues and those found in other mucosal tissues such as the intestine. The intestinal mucosa contains Peyer's patches in which pathogen-derived antigens can be presented to T cells within hours after infection (McSorley *et al*., 2002). In contrast, the genital mucosa lacks organized lymphoid elements, and the initiation of T-cell responses against genital pathogens must occur outside the genital mucosa in regional lymph nodes such as the iliac lymph nodes. The genital and intestinal mucosa also differ in cell surface adhesion molecules responsible for recruiting lymphocytes (Rott *et al*., 1996; Perry *et al*., 1998). Our ability to now identify and characterize T cells specific for defined *Chlamydia* antigens will allow us to identify homing molecules which direct *Chlamydia*-specific T cells to the genital mucosa.

The *Chlamydia*-specific TCR transgenic and retrogenic mice that have been described are specific for the $CD4^+$ T-cell antigen Cta1, a predicted periplasmic protein, and the $CD8^+$ T-cell antigen CrpA, an inclusion membrane protein (Roan and Starnbach, 2006; Roan *et al*., 2006). We and others have identified a number of other *C. trachomatis*-specific $CD4^+$ and $CD8^+$ T-cell antigens from *Chlamydia*-infected mice using a variety of approaches (Allen *et al*., 1991; Beatty and Stephens, 1992; Ishizaki *et al*., 1992; Su *et al*., 1990; Murphey *et al*., 2006; and our unpub. data). In order to determine how the properties of individual antigens can influence T-cell responses, it will be valuable to determine when during the developmental cycle these *Chlamydia* antigens are expressed, and to correlate this information with the kinetics, magnitude, effector functions and protective capacities of T cells specific for these antigens. T cells recognizing antigens expressed early during the developmental cycle may play a bigger role in controlling infection because they may recognize infected cells before non-infectious *Chlamydia* RBs have differentiated back into infectious EBs. In contrast, T cells recognizing antigens expressed late during the developmental cycle may be ineffective at containing infection because recognition and destruction of *Chlamydia*-infected cells late in the developmental cycle may simply result in the release of infectious EBs which can go on to infect new sites. It will be important to compare T-cell responses against a range of antigens expressed at different stages of the developmental cycle in order to identify the window of time when recognition of infected cells has the largest impact in terms of limiting *Chlamydia* replication and dissemination.

T-cell responses may also be influenced by the subcellular localization of *Chlamydia* protein antigens. In particular, $CD8^+$ T cells typically recognize proteins that have access to the cytosol of non-professional APCs. However, $CD8^+$ T cells can be initially primed against not only cytosolic antigens but also non-cytosolic antigens. Priming of non-cytosolic antigens can occur via cross-presentation, a process whereby professional APCs phagocytose and present antigens from infected epithelial cells to T cells. Although $CD8^+$ T cells specific for non-cytosolic antigens can be primed, during the subsequent effector phase these T cells may never recognize epithelial cells infected with *C. trachomatis* as epithelial cells are unable to cross-present non-cytosolic antigens. As a consequence, these $CD8^+$ T cells specific for non-cytosolic antigens may not recognize the typical cell type infected with *C. trachomatis*, and may therefore not contribute significantly towards containing the infection. The CrpA antigen described above has access to the cytosol (Bannantine *et al*., 2000), and indeed T cells specific for this antigen are protective (Starnbach *et al*., 2003; Roan and Starnbach, 2006). It will be interesting to determine whether $CD8^+$ T cells specific for antigens that do not have access to the cytosol of host cells can provide protection against *Chlamydia* infection. Defining the characteristics of antigens that stimulate protective T-cell responses will be important in the rational design of a *Chlamydia* vaccine.

C. trachomatis *persistence and immunopathology*

Despite the ability of the immune system to detect and respond to *Chlamydia* infection, *Chlamydia* appears to persist within the host. There is evidence of *Chlamydia* persistence both *in vitro* and *in vivo*. *In vitro*, low levels of IFN-γ can induce *Chlamydia* to develop into an aberrant, dormant state that may represent a persistent form of the organism (Beatty *et al*., 1993). Incubation of infected cells under stressful conditions such as nutrient deprivation or in the presence of some antibiotics can also induce *Chlamydia* to differentiate into a phenotypically similar form (Coles *et al*., 1993; Raulston, 1997; Hogan *et al*., 2004). These dormant forms can revert to metabolically active forms following IFN-γ removal or the elimination of stressful conditions, showing that persistence *in vitro* is reversible.

Evidence for *Chlamydia* persistence *in vivo* is indirect. *Chlamydia* antigen, DNA and RNA have been detected in human patients that were culture-negative (Rahman *et al*., 1992; Patton *et al*., 1994; Gerard *et al*., 1998), suggesting the presence of unculturable *Chlamydia* organisms that remain within hosts. In mice, the immune system itself may induce *Chlamydia* organisms into an unculturable form. It has been observed that the immunosuppressant cortisone reactivates *Chlamydia* shedding from culture-negative mice which had recovered from a previous *Chlamydia* genital infection (Cotter *et al*., 1997b).

Chlamydia persistence may lead to the pathological outcome associated with infection. If the organisms are not completely cleared by the immune system, they may persist within the host. As the immune response to the organisms wanes, persistent *Chlamydia* may reactivate and replicate, and in turn restimulate inflammation and recruitment of immune effectors to the site of infection. The inflammatory effectors deployed by the immune system may then drive the organisms back into a persistent form. Repeated cycles of *Chlamydia* persistence alternating with reactivation may stimulate the chronic inflammation associated with *Chlamydia*-induced disease.

Immune evasion by C. trachomatis

Chlamydia persistence may be mediated in part by mechanisms *Chlamydia* has evolved to avoid recognition by the host. *C. trachomatis* can directly downregulate the expression of MHC molecules within infected cells

through the degradation of RFX-5 and USF-1, transcription factors that induce expression of MHC class I and MHC class II respectively (Zhong *et al*., 1999; 2000). Degradation is mediated by CPAF, a *Chlamydia* protease that is secreted into the host cytosol (Zhong *et al*., 2001). Downregulation of MHC expression on infected cells can potentially limit their recognition by *Chlamydia*-specific $CD4^+$ and $CD8^+$ T cells, but may also render the infected cells susceptible to lysis by NK cells. Indeed, human NK cells are able to lyse *Chlamydia*-infected epithelial cells (Hook *et al*., 2004). The role of CPAF in immune evasion *in vivo* remains to be determined. Another class of factors that may play a role in evading T cells are the deubiquitinases (DUBs) encoded by *C. trachomatis* (Misaghi *et al*. 2006). The functions of *Chlamydia* DUBs are unknown, but it is attractive to speculate that the removal of ubiquitin from *Chlamydia* proteins by the DUBs may interfere with targeting of these proteins to the proteasome. This would in turn lead to reduced MHC class I presentation of *Chlamydia* antigens to $CD8^+$ T cells.

In addition to downregulating MHC expression, CPAF has more recently been demonstrated to also degrade pro-apoptotic BH3-only proteins (Pirbhai *et al*., 2006). The ability of *Chlamydia* to degrade BH3-only proteins, as well as sequester pro-apoptotic proteins and induce the expression of antiapoptotic factors, may explain the long-standing observation that *Chlamydia*-infected cells are largely resistant to immune-mediated induction of apoptosis *in vitro* (Byrne and Ojcius, 2004; Miyairi and Byrne, 2006). Evading apoptosis may be important for *Chlamydia* in order to allow the bacteria to complete their replication cycle and prevent the release of non-infectious RBs.

However, the influence of *Chlamydia* on host cell apoptosis is complex as *Chlamydia* has also been documented to induce apoptosis (Perfettini *et al*., 2000). Apoptosis can be mediated through the expression of *Chlamydia* protein associated with death domain, which may promote apoptosis through association with mammalian death receptors (Byrne and Ojcius, 2004; Miyairi and Byrne, 2006). In addition, *Chlamydia* induction of TNF-α may promote apoptosis of both infected as well as bystander cells (Perfettini *et al*., 2000). Indeed, TNF-α produced by *Chlamydia*-infected macrophages has been shown to induce T-cell apoptosis in a co-culture system (Jendro *et al*., 2004).

Although it appears contradictory that *Chlamydia* can both inhibit and induce apoptosis, both these processes may help protect the bacterium against immune effectors *in vivo*. *Chlamydia* may restrict apoptosis long enough to complete the developmental cycle, thereby allowing time for differentiation of RBs back into infectious EBs. Towards the end of the developmental cycle, it may be beneficial for the organism to induce apoptosis in order to avoid necrosis, which can stimulate inflammation and enhance *Chlamydia*-specific immune responses. Apoptosis of *Chlamydia*-infected cells towards the end of the developmental cycle may also aid in the release of infectious *Chlamydia* EBs.

In vivo, the evidence for immune evasion by *C. trachomatis* is indirect. Previous exposure of humans to *C. trachomatis* does not provide robust immunity against re-infection, perhaps because *Chlamydia* infection induces the development of inadequate immune effectors (Brunham and Rey-Ladino, 2005). In our lab we have demonstrated that the *Chlamydia*-specific $CD8^+$ T-cell recall response in mice appears to be diminished relative to $CD8^+$ T-cell recall responses typically observed following infection with well-characterized pathogens such as vaccinia (Loomis and Starnbach, 2006). We have used the *Chlamydia*-specific TCR transgenic and retrogenic mice described above to show that *Chlamydia*-specific T-cell responses are initiated following infection with *C. trachomatis*; however, *Chlamydia*-specific T cells do not appear to develop into memory T cells capable of mounting a robust recall response. We will now be able to use the *Chlamydia*-specific TCR transgenic and retrogenic mice as an abundant source of identifiable and readily manipulable T cells in order to examine the basis of this defective recall response.

Vaccines against C. trachomatis

The development of a *Chlamydia* vaccine could significantly reduce the prevalence of infection, leading to a decrease in *Chlamydia*-associated diseases. This would also lead to a relief in the socio-economic burden associated with diagnosis and treatment of the infection, and a relief in the significant financial burden associated with treatment of *Chlamydia*-induced sequelae such as infertility, pelvic inflammatory disease and ectopic pregnancy. The major challenge in developing an effective *Chlamydia* vaccine is to generate long-lasting and sterilizing immunity while avoiding immunopathology. In the 1960s, a human vaccine trial for trachoma was carried out using non-viable whole organisms. The results of the trial suggested that immunity is serovar-specific and short-lived. In addition, post-vaccination exposure to *Chlamydia* in some individuals resulted in more severe disease than that seen in unvaccinated individuals (Grayston and Wang, 1978).

The disappointing results of this trial has directed attention away from whole organisms towards a focus on a subunit vaccine approach. Most subunit vaccine efforts have focused on MOMP because this protein is abundant (comprising 60% of the total protein mass in the outer membrane), surface-exposed, and elicits T-cell responses and neutralizing antibodies (Caldwell *et al*., 1981). Although immune responses against MOMP can be elicited, protection against *Chlamydia* infection following

MOMP immunization is rather poor (Stagg, 1998; de la Maza and Peterson, 2002). The disappointing results of MOMP as a vaccine candidate may in part be due to the failure to obtain the native conformation of MOMP in these immunization protocols (Pal *et al*., 2001). Regardless, efforts are under way to identify other *Chlamydia* antigens as a subunit vaccine consisting of multiple antigens is more likely to be effective at inducing protective immunity.

An ideal subunit vaccine should elicit immunity superior to that observed following natural infection, as it has long been observed that previous exposure to *C. trachomatis* does not provide significant immunity against re-infection (Brunham and Rey-Ladino, 2005). It would seem that stimulating multiple arms of the adaptive immune system would be the ideal approach to induce robust immunity. A subunit vaccine that stimulates B cells to produce neutralizing antibodies against cell-surface proteins, such as MOMP (Peeling *et al*., 1984; Peterson *et al*., 1991), may decrease the number of *Chlamydia* organisms that are able to establish intracellular infection. But in order to be effective, a subunit vaccine also should also stimulate immune effectors that can recognize the *Chlamydia* organisms once they establish intracellular infection. Stimulating $CD8^+$ T cells specific for antigens that are presented on the surface on infected epithelial cells, the relevant targets for *Chlamydia* infection, may be important for inducing robust immunity. It may be important that these $CD8^+$ T cells recognize antigens expressed before the RBs have differentiated back into EBs, as $CD8^+$ T cell-mediated lysis of an infected cell late in the developmental cycle may have the undesired effect of enhancing infection by facilitating the release of infectious EBs. A subunit vaccine should also stimulate $CD4^+$ T cells, which are important in activating other immune effectors such as B cells and $CD8^+$ T cells. Stimulation of $CD4^+$ T cells can also enhance production of IFN-γ and other cytokines that inhibit *Chlamydia* replication. Identifying appropriate targets for subunit vaccine incorporation will be crucial in eliciting sterilizing immunity against *C. trachomatis*.

Conclusions

Innate immune cells, B cells and T cells all respond to infection with *C. trachomatis*. Some of these responses are effective at controlling infection, whereas others are not. Ineffective responses which fail to eliminate the *Chlamydia* organisms from the host may induce chronic inflammation which can lead to tissue pathology. The balance between protective versus pathological responses determines the final outcome of *Chlamydia* infection. Unfortunately, in many cases the outcome is lack of *Chlamydia* clearance and tissue damage. Factors that may influence whether the immune response to *C. trachomatis* is protective or pathological include the nature of the antigens presented during infection, the effector functions deployed by immune effectors, as well as immune evasion strategies implemented by the *Chlamydia* organisms. A better understanding of characteristics which define protective versus pathological responses will be instrumental for developing an effective vaccine that can generate sterilizing immunity without inducing tissue pathology.

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