

T lymphocyte immunity in host defence against *Chlamydia trachomatis* and its implication for vaccine development

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Chlamydia trachomatis is an obligate intracellular bacterial pathogen that causes several significant human infectious diseases, including trachoma, urethritis, cervicitis and salpingitis, and is an important cofactor for transmission of human immunodeficiency virus. Until very recently, over three decades of research effort aimed at developing a *C trachomatis* vaccine had failed, due mainly to the lack of a precise understanding of the mechanisms for protective immunity. Although most studies concerning protective immunity to *C trachomatis* have focused on humoral immune responses, recent studies have clearly shown that T helper-1 (Th1)-like CD4 T cell-mediated immune responses play the dominant role in protective immunity. These studies suggest a paradigm for chlamydial immunity and pathology based on the concept of heterogeneity (Th1/Th2) in CD4 T cell immune responses. This concept for chlamydial immunity offers a rational template on which to base renewed efforts for development of a chlamydial vaccine that targets the induction of cell-mediated Th1 immune responses.

Key Words: Cell-mediated immunity, *Chlamydia trachomatis*, Cytokine, Th1/Th2, Vaccine

Immunité des lymphocytes T dans la défense de l'hôte contre *Chlamydia trachomatis* et son implication pour la création d'un vaccin

RÉSUMÉ : *Chlamydia trachomatis* est un organisme pathogène bactérien intracellulaire obligé qui provoque plusieurs infections importantes chez l'être humain, y compris le trachome, l'urétrite, la cervicite et la salpingite et il est un important cofacteur de transmission du virus de l'immunodéficience humaine. Jusqu'à très récemment, trois décennies de recherches visant la mise au point d'un vaccin contre *C. trachomatis* avaient échoué, principalement à cause d'une mauvaise compréhension des mécanismes de l'immunité. La plupart des études ayant porté sur l'immunité contre *C. trachomatis* se sont attardées sur les réponses de l'immunité humorale, or, de récentes études ont clairement démontré que la réponse immunitaire à médiation cellulaire par les CD4 T de type Th1 jouent un rôle prédominant dans l'immunité. Ces études suggèrent un paradigme de l'immunité et de la pathologie liée au *Chlamydia* sur la base du concept d'hétérogénéité (Th1/Th2) dans les réponses immunitaires des cellules CD4 T. Ce concept d'immunité chlamydiale offre un modèle rationnel sur lequel fonder de nouveaux efforts pour la mise au point d'un vaccin anti-*chlamydia* qui ciblerait l'induction des réponses immunitaires à médiation cellulaire Th1.

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Chlamydia trachomatis is a globally important human bacterial pathogen that causes several significant infectious diseases. Trachoma, a problem mainly restricted to certain developing areas, is the world's leading cause of preventable blindness, with a prevalence of over 500 million infected people, among whom approximately 7 million are blind (1). In developed countries, genital tract disease is the dominant form of chlamydial infection and leads to significant reproductive disability, especially among women. *C trachomatis* is ranked as the most common sexually transmitted bacterial infection in North America. Globally, *C trachomatis* must also rank among the most common serious bacterial infections, although exact incidence and prevalence data are lacking. *C trachomatis* infectious diseases, while not fatal, are nonetheless severe because of the effects of maternal blindness due to trachoma on infant mortality and because of the adverse effects of genital infections on reproduction. Recently, genital *C trachomatis* infection has also been identified as a potent cofactor, facilitating the transmission of human immunodeficiency virus (HIV).

Because *C trachomatis* is a pathogen of public health importance, great efforts have been aimed at developing a chlamydial vaccine. More than three decades of research have been devoted to this goal, although little practical progress has been made (2,3). One major obstacle has been the lack of a precise understanding of the mechanism(s) for protective immunity of *C trachomatis* infection. Recent research, mainly in nonhuman animal models of infection, has made important advances in elucidating the immunobiology of chlamydial infection. These results and related studies in humans are the primary focus of this review. This review complements other recent reviews that we have prepared on the major protein antigens of *C trachomatis* (2) and on emerging aspects of human chlamydial infection (4). It is anticipated that the advances in the immunological understanding of *C trachomatis* infection will guide vaccine development for this pathogen.

For many years, studies concerning protective immunity to chlamydial infection focused on understanding humoral immune responses. These studies were based on the simple notion that neutralizing antibodies to surface-exposed antigenic sites on the organism constituted immunity. Great efforts were devoted to investigating antibody responses following immunization or infection and on localization and characterization of neutralization epitope(s) on chlamydial antigens (5-13). The data that supported the notion of antibody-mediated immunity arose from observations that serum and monoclonal antibodies specific for *C trachomatis* are able to neutralize the organism in tissue culture (14,15). However, the relationship between neutralizing antibodies and protective immunity has been remarkably difficult to demonstrate in vivo. The most provocative human data indicated an inverse correlation between the abundance of organism shed and the prevalence of *C trachomatis*-specific local immunoglobulin A (IgA) antibody in cervical mucus (16). However, no confirmatory data that directly correlate neutralizing antibodies with protective immunity in humans are available (2).

Because of difficulties in assaying specific human T lymphocyte responses to *C trachomatis*, data on the relationship

between T cell immunity and protective immunity against chlamydial infection were slow to accumulate. However, recent experiments, mainly in mice, have clearly shown the dominant role that T lymphocytes play in chlamydial immunity. These data, when combined with recent epidemiological data from human chlamydial infection, suggest that a new paradigm for chlamydial immunity is needed – one that is based on the concept of heterogeneity of CD4 T cell immune responses (17,18). This paradigm posits that states of polarized activation of T helper-1 (Th1)-like immune responses correlate with *C trachomatis* immunity, whereas states of polarized activation to Th2-like immune responses correlate with *C trachomatis* immunopathology.

Before describing the specific data on the immunology of *C trachomatis* infection it is necessary to provide some basic description of the microbiology of *Chlamydia* species.

CHLAMYDIA TRACHOMATIS AS MICROORGANISMS

Chlamydia species are obligate intracellular bacterial pathogens with a characteristic developmental cycle, and two morphologically and metabolically distinct cellular forms (19,20). The infectious form of the organism, termed the elementary body (EB), transmits infection from one cell (or host) to another, and the replicative form, termed the reticulate body (RB), is the intracellular form that multiplies by binary fission within an intracytoplasmic vacuole or inclusion (21). The developmental cycle starts with entry of EBs into a host cell, proceeds through reorganization of EBs to RBs followed by replication of RBs and differentiation of RBs back to EBs and, finally, the release of EBs into the extracellular environment. From an immunological perspective, how chlamydial antigens enter the major histocompatibility complex (MHC) class I and class II processing and presentation pathways from their intracellular vacuole within an infected cell remains a major unanswered question (22). Understanding this process will be important because recognition of chlamydial antigens by T lymphocytes is determined entirely by whether and how antigen enters class I and class II presentation pathways in antigen presenting cells and in infected cells.

Chlamydia species largely resemble Gram-negative bacteria in that they have an inner cytoplasmic membrane and an outer membrane in which a single major outer protein (MOMP) accounts for 60% of the total protein mass of the outer envelope and which contains a rough type lipopolysaccharide (23,24). Chlamydial lipopolysaccharides have three antigenic domains, one of which is unique to *Chlamydia* species and two of which are shared with other Gram-negative bacteria (25,26). Different from typical Gram-negative bacteria, however, *Chlamydia* species lack detectable peptidoglycan between the inner and outer membranes of the cell envelope (27).

Chlamydia species possess both RNA and DNA, with a genome consisting of a single double-stranded DNA molecule of 1045 kb, containing enough information to encode for about 900 different proteins (28). Therefore, it is among the small prokaryotic genomes. The downsized genome of *Chlamydia* species is likely due to its obligate parasitic lifestyle, whereby many genes for de novo synthesis of metabolic precursors that

are easily obtained from the host cell have been deleted. Many but not all strains of *Chlamydia* species also contain an extra-chromosomal plasmid of approximately 7.4 kb in size that is of unknown function.

The chlamydia genus belongs to an ancient bacterial phylum, which is entirely parasitic in its ecology and is classified into four species – *C trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumonia* and *Chlamydia pecorum* (29). *C trachomatis* and *C pneumonia* are mainly human pathogens (30-32), while *C psittaci* and *C pecorum* are usually pathogens of birds and mammals (33). *C trachomatis*, the organism on which this review will focus, can be divided into three biological variants (biovars) based on the natural host species of the organism and the range of diseases that they induce. The three *C trachomatis* biovars are trachoma, lymphogranuloma venereum (LGV) and mouse pneumonitis (MoPn) (34). The preferred sites of infection are different among the three biovars, with trachoma biovar infecting human squamocolumnar epithelial cells, LGV infecting human lymph node cells and MoPn infecting mouse mucosal cells. Except for the MoPn biovar, each *C trachomatis* biovar is composed of several serological variants (serovars) based on antigenicity of the MOMP. Serovars A, B, Ba and C cause trachoma, while serovars D to K are the major causes of genital infection. The LGV biovar includes L1, L2 and L3 serovars, which cause LGV (35).

THE MOUSE MODEL OF *C TRACHOMATIS* INFECTION

Animal models have been especially helpful in defining the immunobiological features of *C trachomatis* immunity. The models include, but are not limited to, nonhuman primates, rabbits, guinea pigs and mice (10,36-38), among which guinea-pig and mouse models have been studied most extensively. The mouse model has been particularly informative, largely because of the ready availability of immune reagents for studies in mice. All three *C trachomatis* biovars can infect the mouse via a variety of routes of inoculation (36,38-41), although the MoPn biovar is most commonly used. MoPn was originally isolated from mouse lung tissue and is thought to be a natural pathogen in the mouse (42,43). Hence, it offers an evolutionarily adapted pathogen for analyzing host-pathogen interactions during *C trachomatis* infection. Experimental MoPn infections include ocular, respiratory and reproductive tract infections, inducing diseases such as conjunctivitis, pneumonitis, vaginitis and salpingitis (38,41,44-46). Of the potential routes of infection, respiratory and genital infections have been the most comprehensively studied.

Murine immunity against *C trachomatis* infection is much more dependent on T cells than on B cells: Williams et al (38) first established the murine MoPn pneumonia model and demonstrated the role of T cells in resolution of chlamydial infection. Athymic nude mice (nu/nu), lack a mature thymus and peripheral T cells, and were much more susceptible to MoPn pulmonary infection than their heterozygous (nu/+) littermates. Transplantation of the thymus to nude mice conferred resistance to MoPn infection. In contrast, transfer of immune sera to nude mice only slightly delayed death, without a sig-

nificant change in overall mortality (47,48). These results indicated a strong T cell dependence of protective immunity to chlamydial infection.

T cell-mediated immunity was subsequently demonstrated to be of primary importance for host defence against respiratory chlamydial infection in mice. Williams et al (49) and Williams and Schachter (50) demonstrated that heterozygous mice developed both delayed-type hypersensitivity (DTH) and lymphocyte-proliferative responses to MoPn during chlamydial infection and that immune T cells adoptively transferred protective immunity from heterozygous to nude mice.

Similarly, the importance of T cells in host defence was also documented in MoPn models of genital infection. More than 15 years ago, Barron et al (44) showed that female mice could be readily infected by intravaginal inoculation with MoPn. Rank et al (51) subsequently found that athymic nude mice developed chronic vaginal infection lasting as long as 265 days postinfection. In comparison, infections in heterozygous mice cleared in approximately 20 days.

Studies using B cell-deficient mice generated by repeated anti- μ antibody treatment demonstrated, more convincingly, the dominant role of T cell-mediated immunity in host defence against *C trachomatis* infection. Although previous studies, especially T cell adoptive transfer experiments, indicated the important role of T cells in resolution of chlamydial infection, these studies could not delineate whether T cells functioned via cell-mediated immunity or as helper cells for antibody production. By using B cell-deficient mice, this distinction could be made. Using anti- μ treated mice, Williams et al (52) and Ramsey et al (53) demonstrated separately that B cell-deficient mice were able to resolve primary respiratory and genital chlamydial infection at a rate comparable with that of normal control mice. As well, B cell-deficient mice that recovered from primary genital infection were resistant to rechallenge. The authors concluded that, in mice, antibody responses are not required for either resolution of primary infection or resistance to secondary chlamydial infection.

CD4 and CD8 T cell subsets are involved in protective immunity to *C trachomatis*: Further experimental studies involved adoptive transfer of T cell lines or clones. These studies provided insight into the T cell subpopulations that are involved in protective immunity to *C trachomatis*. Adoptive transfer of one T cell line, especially the CD4 T cell component, generated from MoPn-infected heterozygous littermates to nude mice, resolved chronic MoPn genital infection (54). Similarly, significant protection was conferred to infected nude mice by adoptive transfer of a *C trachomatis*-specific CD4 T cell clone (55). Severe combined immunodeficiency mice lack both functional T and B lymphocytes and have a more profound immune defect than nude mice, which only lack functional T cells. They were also protected by transfer of the same CD4 T cell clone following respiratory infection (56).

Adoptive transfer experiments, while demonstrating an important role for CD4 T cells in chlamydial immunity, also suggested a role for CD8 T cells. For more than a decade, the role for CD8 T cells in chlamydial immunity has been debated. The controversy stems primarily from the failure to readily demon-

strate cytotoxic T cell activity to *C trachomatis* (57,58), and an inability to understand how intravacuolar growth of *C trachomatis* allows chlamydial antigen to enter the cytoplasmic processing and presentation pathway to MHC class I molecules for CD8 T cell recognition (59). Recently, however, CD8 T cell immunity in *C trachomatis* infection has been demonstrated unequivocally. In particular, adoptive transfer of a predominantly CD8 T cell line (97% CD8 T cells) resolved MoPn genital infection in the nude mouse with chronic MoPn genital infection (54). More impressively, Igietsme et al (60) generated a CD8 T cell clone (100% CD8) that led to resolution of chronic genital infection in over 50% of mice following adoptive transfer.

Classical CD8 cytotoxic T lymphocytes (CTLs) targeted to *C trachomatis*-infected cells have also been demonstrated. Starnbach et al (40) characterized a CTL line derived from mice infected intraperitoneally with *C trachomatis* serovar L2. The CTL line was able to lyse infected target cells specifically in vitro. Moreover, the antigenic epitope recognized by the CTL line was presented by a classical MHC class I molecule, H-2 L^d. Adoptive transfer of the CTL line into naive mice conferred partial protection, which was abolished by administration of anti-interferon gamma (IFN γ) monoclonal antibody in vivo. In a separate study, Beatty and Stephens (61) also detected CD8 CTL activity during murine chlamydial infection, especially when the infected target cells were transfected in vitro with intercellular adhesion molecule type 1 (ICAM-1).

A common problem in interpreting results obtained from adoptive transfer of T cell lines or clones in infectious disease is that, although the results suggest a potential role of a given cell population in protective immunity, they may not reflect the relative contribution of each cell population during naturally acquired immunity to infection. This point should be considered when evaluating the relative roles of CD4 and CD8 T cell populations in protective immunity to *C trachomatis* infection. Thus, studies involving in vivo depletion or transfer of freshly isolated polyclonal T cell subpopulations provide a more representative picture of protective immunity during natural infection. Indeed, depletion of CD4 T cells in vivo significantly exacerbated murine chlamydial salpingitis and increased the number of organisms recovered from the oviducts of infected cells (62). Similarly, mice depleted of CD4 T cells showed a significantly higher mortality rate following pulmonary infection than control mice. CD8 T cell-depleted mice had mortality rates comparable with those of control mice (63). Moreover, adoptive transfer of freshly isolated polyclonal CD4 but not polyclonal CD8 T cells obtained from mice recovered from a primary genital infection conferred significant immunity to naive mice (64). These studies indicate that, although both CD4 and CD8 T cells play a role in protective immunity to *C trachomatis*, the CD4 T lymphocyte predominantly mediates protection.

Cytokines that are involved in protective immunity to *C trachomatis*: The expression of a number of cytokines during murine chlamydial infection, including interleukin-1a (IL-1a), IL-2, IFN γ , IL-5, IL-6, tumour necrosis factor-alpha (TNF α), lymphotoxin (TNF β), colony-stimulating factors (CSFs) and

IL-10, has been documented. Because IL-1 is inhibitory to chlamydial growth in vitro (65) and is a mediator of inflammation (66) and fibroblast proliferation (67), the finding of IL-1 production following pulmonary MoPn infection in vivo (68) suggests that IL-1 may be an effector in host defence and/or in the pathogenesis of fibrosis that can be associated with chlamydial disease sequelae. TNF α has also been found to be inhibitory for chlamydial growth both in vitro (65,69,70) and in vivo (71). TNF α is produced during chlamydial infection, and neutralization of TNF α in vivo with polyclonal antibody significantly increased mouse mortality and MoPn growth in the mouse lung, suggesting it may contribute to host defence in *C trachomatis* infection (71). Magee et al (72) demonstrated the production of CSFs in murine MoPn lung infection by both T cell-dependent and -independent mechanisms. Because CSFs are capable of increasing production and effector functions of phagocytes, Magee et al (72) proposed that CSFs may also play a role in host defence to chlamydial infection.

IFN γ is the key cytokine in host defence against *C trachomatis* infection. It is produced by CD4 (55) and CD8 (60) T cells and by natural killer cells (73) following chlamydial infection. The inhibitory effect of IFN γ on chlamydia replication was first demonstrated by studies in vitro in the early 1980s (74-77). Byrne et al (78) and Williams et al (79,80) reported production of IFN γ following pulmonary MoPn infection in vivo and the exacerbation of chlamydial infection by neutralization of IFN γ activity with anti-IFN γ antibody. In addition, the role of IFN γ in control of chlamydial infection in vivo was shown in infections with other *C trachomatis* serovars through experiments involving neutralization of endogenous IFN γ in vivo (81) or following administration of exogenous recombinant IFN γ (82).

IFN γ can inhibit chlamydial growth by induction of targeted gene expression in different cell types, such as the gene for indoleamine 2,3-dioxygenase, which causes tryptophan depletion, an essential amino acid for several strains of chlamydia (83) or for nitric oxide synthase, which produces the microbicidal molecule nitric oxide (37,84,85). IFN γ also induces the expression of class II molecules on cells that normally do not express class II molecules, such as epithelial cells. Loading of *C trachomatis* peptides onto the IFN γ -induced class II molecules could allow effector CD4 T cells to recognize chlamydia-infected cells specifically. Igietsme et al (86) and Igietsme (87) found that IFN γ produced by chlamydia-specific T cell clones induced epithelial cells to produce nitric oxide. They also showed a quantitative relationship between the amount of nitric oxide produced and the extent of chlamydial growth inhibition. Thus, induction of nitric oxide production appears to be one of the final effector mechanisms for anti-chlamydial T cell action in mice. In addition to these mechanisms, IFN γ may also play a role in chlamydial inhibition via activation of macrophage phagocytosis, phagolysosomal fusion and lysosomal degradation of the organism (58).

IL-5 and IL-6 have been shown to be the important cytokines in regulating IgA production (88-90), and Magee et al (68) first demonstrated the production of IL-6 during *C trachomatis* infection. Because IgA may be a key antibody response in protection, we analyzed the correlation among these cytokines

and *C trachomatis*-specific IgA antibody responses (personal communication). We observed that IL-6 but not IL-5 production correlated with serum and local IgA production following MoPn pulmonary infection. Interestingly, C57BL/6 mice, which were high IFN γ producers, also produced higher amounts of IL-6 and showed higher IgA production than Balb/c mice, which were higher IL-5 producers. Measurement of secretory antibody and cytokine levels in the lung lavage of infected mice also showed a qualitative correlation among secretory IgA antibody, IL-6 and IFN γ levels. Thus, we observed a stronger correlation between IL-6 production and IgA responses than between IL-5 production and IgA in this model system. Together, these findings suggest that high IFN γ production and strong DTH responses can coexist with high levels of IL-6 and local IgA production. A similar finding of a correlation between IFN γ and IgA production was recently reported in a murine salmonella model (91).

Heterogeneity in helper T cell function correlates with *C trachomatis* immunity: Arguably, the description of T lymphocyte functional diversity has exerted the single greatest influence on contemporary thinking about immune regulation and host defence against infectious diseases (17,18,92). It was first reported by Mosmann and Coffman (93) that long term cultured murine CD4 T cell clones can be grouped into Th1 or Th2 subsets based on their cytokine production patterns. Th1 clones, which produce IL-2, IFN γ and lymphotoxin but not IL-4, IL-5 or IL-10, mainly mediate cellular immune responses, including DTH, whereas Th2 clones, which produce IL-4, IL-5, IL-10 and IL-13 but not IL-2 and IFN γ , facilitate humoral immunity. Subsequently, different patterns of cytokine production were demonstrated in a variety of models of mouse and human infectious diseases, and cytokine patterns were strongly correlated with resolution or exacerbation of the disease state (94-97). Th1 and Th2 immune responses were observed to be mutually antagonistic in vitro and, thus, were suggested to be capable of generating highly polarized immune states in vivo. As a consequence of this knowledge, the Th1-like and Th2-like patterns of cytokine production have become a major template for conceptualizing the mechanisms of immune resistance to *C trachomatis* infection.

The previously described results show that cell-mediated immunity and IFN γ production are correlated with resolution of and resistance to chlamydial infection in mice, and suggest that Th1-like responses are important for protective immunity. Indeed, it was reported that intravaginal infection with MoPn induced a local Th1 response (98) and that adoptive transfer of a MoPn-specific CD4 Th1 cell clone resolved chronic MoPn vaginal infection in nude mice (55). Recently, we (99) reported that differences in IL-10 and IFN γ responses among MoPn-infected mice correlated with differences in susceptibility or resistance to *C trachomatis* infection in different inbred strains of mice. We observed that immune responses and cytokine production by spleen cells were correlated with the growth of MoPn in the lungs of C57BL/6 and Balb/c mice. Specifically, Balb/c mice had higher IL-10 production, higher serum IgG1 antibody responses, less IFN γ production, less intense DTH responses and were significantly slower to clear

MoPn infection than C57BL/6 mice, which produced higher IFN γ , stronger DTH and less IL-10. Moreover, neutralization of IL-10 in Balb/c mice in vivo significantly increased the DTH response and enhanced the clearance of MoPn infection. The data confirm the importance of IFN γ and T cell-mediated immunity in clearance of chlamydial infection and demonstrate the inhibitory role that IL-10 has on IFN γ production and DTH responses (100-102). The study clearly demonstrated the effect of Th1-like (IFN γ predominant) versus Th2-like (IL-10 predominant) CD4 T cells in modulating immune responses to and host defences against murine *C trachomatis* infection.

Cell to cell interaction between immune T cells and infected epithelial cells appears to be important in the cell-mediated immune response to *C trachomatis*: The classic Mackaness cell-mediated immune (CMI) response involves T lymphocyte activation of macrophages. However, the *C trachomatis* protective CMI response involves different cellular players. Antigen-specific T cells have been observed to be directly inhibitory to *C trachomatis* growth in epithelial cells in experiments using a polarized epithelial-lymphocyte coculture system. With this in vitro model, Igietsme et al (103) demonstrated that activated *C trachomatis*-specific T cell lines and clones produce IFN γ and TNF α , and could inhibit chlamydial growth in cultured polarized epithelial cells. In particular, they found that close proximity between epithelial cells and T cells was required for inhibition. The authors speculated that immune T cells need to act at very close distances to the epithelial cell in order to inhibit chlamydial growth, possibly due to the short-range nature of cytokine action. Impressively, the study also showed that immune T cells could function even when the epithelial cells had been infected for 24 h before T cells were added to the culture.

Adhesion molecules, including ICAM-1 and leukocyte function antigen-1, are involved in cell to cell interaction between antigen-specific T cell clones and epithelial cells, and have been shown to be important for epithelial cell production of nitric oxide and inhibition of *C trachomatis* growth by T cells (86). The demonstration of involvement of adhesion molecules in epithelial cell to T cell interaction for chlamydial growth inhibition has physiological significance because when *C trachomatis* infects mucosal epithelial cells, T cells are recruited to and remain localized within the intraepithelial microenvironment. The interaction of T cells and epithelial cells via adhesion molecules is probably beneficial in arresting infection in situ. This may also explain discrepancies among some adoptive transfer studies. It appears that, among transferred CD4 or CD8 T cell clones specific for chlamydial antigens, only some are protective, even though they all produce IFN γ (55,60). Expression of adhesion molecules on T cell clones may differ and may be critical for homing to infected epithelial surfaces.

STUDIES OF CHLAMYDIAL IMMUNITY USING GENE KNOCKOUT MICE

Development of techniques for generating targeted gene knockout animals has greatly advanced studies of many physiological and pathological processes in vivo. Mutant ani-

mals offer a powerful means for identifying functions unique to a particular cell population or biological molecule. The gene targeting technique was originally developed by Capecchi (104). Briefly, the generation of gene knockout mice involves construction of a targeting vector that includes one or more selection markers and a sequence homologous to the target gene that contains a partial deletion of the coding sequence; disruption of the specific gene via homologous recombination in embryonic stem cell clones by gene targeting with the targeting vector; injection of mutant ES cell clones into early stage mouse blastocysts and subsequent implantation of the blastocysts into the uterine cavity of female mice (newborn animals will be chimeras and some of them will be germline chimeras); and intercross of mutant germline heterozygotes to produce mutant homozygotes. This technique has been powerfully exploited for the molecular analysis of the murine immune system. Thus far, MHC class I, MHC class II, CD4 T cell, alpha-beta T cell, gamma-delta T cell and recombinase-activating gene 1 (RAG-1)-deficient mice have been used to delineate their possible roles in protective immunity to *C trachomatis*. Studies using gene knockout mice for chlamydial immunity studies have confirmed the predominant role that CD4 T cells play in host defence.

Chlamydial infection in MHC class I and MHC class II mutant mice: MHC class I-deficient mice ($2m^{-1}$) are generated by inactivation of the gene for beta-2-microglobulin (105), which is required for cell surface expression of MHC class I molecules. MHC class II-deficient mice are generated by inactivation of the I-A beta gene (106). Because the maturation of CD8 and CD4 T cells in the thymus is dependent on MHC class I and class II molecules, respectively, class I-deficient mice are also deficient in CD8 T cells and class II mutants are deficient in CD4 T cells. Using such mice, Morrison et al (107) demonstrated that MHC class II-restricted immune responses are the primary protective mechanism in *C trachomatis* genital infection. Specifically, class I-deficient mice resolved genital infection at a rate comparable with that of wildtype control mice (complete clearance of the organism at 30 to 35 days postinfection), while class II mutants failed to resolve infection up to 70 days postinfection. Analysis of humoral and cell-mediated immune responses in gene knockout mice showed that class II mutants were severely impaired in both cell-mediated immunity and local antibody production, as indicated by negative DTH and vaginal IgA antibody responses. Secondary challenge of wildtype and class I-deficient mice demonstrated that they acquired immunity to reinfection. Magee et al (63) also observed that class II-deficient mice were significantly slower than wildtype or class I mutant mice to clear lung infection. Thus, in both respiratory and genital models, MHC class II-restricted CD4 T cell responses play the predominant role in protective immunity to chlamydial infection. However, differences between the two infection models were also observed. In particular, although class I mutants resolved genital infection at a rate comparable with that of wildtype controls, class I-deficient mice with respiratory infection showed significantly higher chlamydial growth and mortality rates than wildtype mice, although they were not as severely affected as class II knockouts (63).

When results of experiments using gene knockout mice are combined with those generated by antibody depletion of T cell subsets in vivo (63,107), it is apparent that the protective role of CD8 cell is consistently demonstrated for the respiratory *C trachomatis* infection model but not for the genital infection model. This difference may reflect the relative importance of the infected sites to essential host physiological processes and the susceptibility of the local microenvironment for organism growth and/or may reflect the necessity of different effector cell populations at different sites of chlamydial infection. Thus, it may be that CD8 T cells represent an ancillary defence mechanism, bolstering CD4 T cell dependent protective immunity to chlamydial infection, especially at pulmonary sites.

T lymphocytes with the alpha-beta T cell receptor are important in *C trachomatis* immunity: T lymphocytes can be divided into alpha-beta and gamma-delta T cells based on the type of antigen receptors expressed. Alpha-beta T cells include CD4 and CD8 T cells and represent the major component of the peripheral mature T cell repertoire, while gamma-delta T cells lack CD4 and CD8 molecules and comprise a minor portion of the peripheral T cell repertoire. In general, gamma-delta T cells are much more prevalent at epithelial surfaces than in peripheral blood. Both alpha-beta and gamma-delta T cells have been shown to play a role in immunity to several infectious diseases (108-111). We have examined the roles of alpha-beta and gamma-delta T cells in the resolution of MoPn pulmonary infection by using alpha-beta T cell-deficient and gamma-delta T cell-deficient mice generated by alpha (112) and delta (113) gene disruption, respectively, and comparing their responses with those of control wildtype mice together with T and B lymphocyte-deficient mice generated by RAG-1 disruption (114). The results show that alpha-beta T cell-deficient mice, when compared with wildtype control or gamma-delta T cell-deficient mice, have dramatically higher mortality and growth of *C trachomatis* in vivo (personal communication). Alpha-beta T cell mutants were as susceptible to MoPn infection as RAG-1 mutants, confirming the extreme importance of alpha-beta T cells in resistance to chlamydial infection. Moreover, both alpha-beta T cell-deficient and RAG-1 mutant mice failed to mount DTH to MoPn and had undetectable IFN γ production by splenocytes upon restimulation with chlamydial antigen in vitro. In contrast, gamma-delta T cell-deficient mice exhibited intact DTH responses and levels of IFN γ production that were even higher than those of wildtype controls. These data indicate that alpha-beta T cells are the major cell type responsible for host defence against chlamydial infection and that gamma-delta T cells may play a role in regulating the magnitude of alpha-beta T cell responses.

Our data are at variance with a recent report by Williams et al (115), in which gamma-delta T cell-deficient mice (B129xC57BL/6) showed increased chlamydial growth in vivo at early (three and seven days postinfection) stages of infection compared with control mice that had a different genetic background (B6129F2/J). An advantage of our study was that mutant and wildtype mice of the same genetic background (C57BL/6J) were used, thus facilitating an isogenic comparison between wildtype and mutant mice. This is particularly

important because host susceptibility to chlamydial infection in mice is tightly controlled at the genetic level (99,116-118).

HUMAN IMMUNE RESPONSES TO *C TRACHOMATIS* INFECTION

The precise conclusions derived from the mouse model of *C trachomatis* infection can be used to guide interpretation of new observations concerning the human immune response during chlamydial infection. Human cell-mediated immune responses to *C trachomatis* infection were initially documented during the 1970s by Hanna et al (119). They found that classical DTH (Frei test) and lymphocyte transformation response to *C trachomatis* antigens in vitro could be detected in humans. Subsequent studies showed that lymphocyte transformation responses were readily correlated with current chlamydial infection in men and with current or past chlamydial infection in women (120). However, for more than a decade, the role of T cell-mediated immunity in protection against human chlamydial infection remained largely unexplored.

Recent epidemiological and clinical investigations carried out in Gambia and Kenya have enriched our understanding of protective immunity to human *C trachomatis* infection. The Gambian studies focused on individuals with trachoma and the Nairobi studies on women with genital chlamydial infection. The Gambian studies correlated IgG antibody responses and cell-mediated immunity with clearance of infection and ocular tissue damage (121-123). The investigators observed that *C trachomatis* IgG antibodies in tears increased the incidence and duration of clinical trachoma, whereas local IgA appeared to have the opposite effect. They suggested that local IgG antibodies might actually enhance infection. As well, they (124) tested responses of peripheral blood mononuclear cells (PBMC) to several chlamydial antigens in vitro (including the MOMP and heat shock protein 60 [hsp60]) in subjects with severe conjunctival scarring and age-, sex- and community-matched controls who lacked evidence of conjunctival scarring but who presumably had prior healing trachoma. They found that individuals with conjunctival scarring showed reduced lymphocyte proliferative responses to chlamydial antigens when compared with matched controls. They also observed that serum antichlamydial antibody titres were significantly higher in subjects with scarring trachoma than in matched controls. More recently, the same investigators (125) reported a further study in which subjects with conjunctival scarring due to trachoma and age-, sex- and community-matched controls who lacked conjunctival scarring were compared for cytokine production profiles by PBMCs in response to chlamydial antigens. They found that PBMC incubation with chlamydial antigens resulted in increased IL-4 secretion from subjects with scarring disease but increased IFN secretion from controls without scarring disease. IL-4 mRNA was only detected in antigen-stimulated PBMCs of patients with scarring disease. These data suggest that reduced CMI and high levels of Th2-like responses are correlated with conjunctival scarring and evidence of persistent chlamydial infection in individuals with trachoma.

High titres of serum antibodies to *C trachomatis*, including antibodies to the chlamydial hsp60 have also been documented consistently among women with the sequelae of genital tract infection due to *C trachomatis* (2,126,127). Although CMI responses were not analyzed in these studies, the typical inverse relationship between DTH and antibody responses in other antigen systems (128,129) suggests that the individuals with reproductive tract sequelae of *C trachomatis* may also have depressed cell-mediated immunity. To test the concept that intact T cell immunity is particularly important in human genital chlamydial infection, we studied the risk of chlamydial infection among female sex workers in Nairobi, Kenya by comparing rates among women with and without HIV infection. Specifically, we observed that the incidence of chlamydial cervical infection among HIV infected women was greatly increased compared with HIV uninfected women (130) and that the risk of chlamydial salpingitis was inversely correlated with the number of CD4 T cells among HIV infected women (131). These data indirectly suggest that CD4 T cell-mediated immunity is central to protection from sexually transmitted chlamydial infection and disease similar to that observed among individuals with trachoma.

Observations of *C trachomatis* infection in both mouse and human systems are highly reminiscent of immune correlates observed in other infectious diseases that exhibit a spectrum of host responses such as leishmaniasis and leprosy (95,132). The demonstration of impaired cell-mediated immunity together with high serum levels of IgG antibody in individuals with scarring trachoma and chlamydial salpingitis strongly suggests that CMI is involved in the resolution of chlamydial infection. In aggregate, human studies suggest that Th1-like CD4 T cells are important in immunity to *C trachomatis* infection and disease and that Th2-like CD4 T cell responses are associated with the pathological sequelae of persistent chlamydial infection.

CONCLUSIONS

New data show that protective immunity to *C trachomatis* infection centrally involves T cell populations. Alpha-beta T lymphocytes, which recognize MHC class II-presented *C trachomatis* peptides, play a pivotal role in mediating host defence against infection. CD4 T cell-mediated immunity and Th1-like cytokine production are the predominant effectors of protective immunity. CD8 T cells may play an additive role via production of cytokines such as IFN and/or TNF and perhaps by cytolysis of infected cells, especially in cases of relatively severe and/or systemic chlamydial disease. Gamma-delta T cells may also play a role in modulating the magnitude of the alpha-beta T cell IFN response.

Effector T cells in CMI against *C trachomatis* infection appear to act through two or more mechanisms. First, effector T cells directly inhibit chlamydial replication via secretion of cytokines or by cytolysis of infected cells (86,103). Second, effector T cells may work indirectly by activating other host cells (eg, macrophages) via cytokines (58). Because macrophages are not accumulated abundantly in mucosal epithelia and the direct inhibition of chlamydial growth in epithelial cells by

IFN γ -producing T cell clones has been clearly demonstrated, it appears that the predominant protective mechanism is direct inhibition of chlamydial growth by effector T cells. From this perspective, the role of adhesion molecules involved in T cell homing to and interacting with infected epithelial cells deserves more detailed analysis. Studies of the mechanisms for constitutive and induced MHC class I and II expression and antigen processing and presentation function by infected epithelial cells will also provide a rich source of information of immunological interest and help elucidate how antigen-specific T cells are able to recognize *C trachomatis*-infected epithelial cells.

Even though serum antibodies are poorly correlated with protective immunity, they may still play a protective role via collaboration with cell-mediated immunity, including antibody-dependent cell-mediated cytotoxicity and improvement of macrophage function (49). Indeed, Wyrick and Brownridge (133) reported that antibody-pretreated *Chlamydia* species were more readily destroyed in macrophage phagolysosomes than nonopsonized organisms. Moreover, Williams et al (48) showed that locally delivered antibody enhances protective immunity in T cell intact heterozygous (nu/+) mice but not in nude (nu/nu) mice. Among the possible types of B cell responses, local (secretory IgA) antibody production to *C trachomatis* infection is likely to be most relevant to protective immunity. Although the studies in B cell-deficient mice indicate that antibody is not essential for resolution of respiratory and genital infection or for resistance to reinfection, the results do not exclude the possibility that antibody is a component of protective immunity in humans. In fact, studies using the guinea pig model of *C psittaci* infection demonstrated a correlation between local antibody production and clearance of chlamydial infection (9,10). As well, clinical observations demonstrating a strong correlation between the prevalence of secretory IgA antibody and quantity of *C trachomatis* shed-

ding during human endocervical infection support this concept (16). In the mouse respiratory model of *C trachomatis* infection, high levels of IgA production are correlated with strong DTH responses, high levels of IFN γ production and rapid clearance of a chlamydial infection. Such observations suggest that a dominant Th1-like response with a strong mucosal IgA response is also likely to be important in protective immunity during human chlamydial infection.

Progress in understanding the nature of protective immunity to *C trachomatis* infection offers a basis for rational development of a chlamydial vaccine. The data described above indicate that a highly protective vaccine should possess the characteristics of delivering chlamydial peptides via MHC class II and predominantly activating CD4 T cells with a Th1 phenotype. Cytokines such as IL-12 that promote the early development of Th1-like cells may be helpful as adjuvants (134,135). When administered as protein or DNA constructs, vaccine antigens have the capacity to orchestrate the types of immune response they elicit. Carefully chosen doses of protein antigen can selectively induce cell-mediated Th1 immune response (128) and may be important for a *C trachomatis* vaccine if recombinant protein is used. DNA vaccines also elicit strong CD4 Th1 immune responses (136,137). The ease in constructing multivalent DNA vaccines makes it possible to deal with the challenge of multistrain immunity for *C trachomatis* in a way that is difficult for recombinant protein vaccines. With the knowledge that a vaccine should induce strong T cell immunity, it is feasible to renew efforts dedicated to development of a vaccine for *C trachomatis* (138).

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